ROOM	TOPIC
Conference Hall	Plenary session 1
	1.1 Agricultural and Food Biotechnology
	1.2 Agricultural and Food Biotechnology
	Plenary session 2
	1.3 Agricultural and Food Biotechnology
	1.4 Agricultural and Food Biotechnology
	2.5 Biopharmaceutical and Medical Biotechnology
	11.2 Bio-Digital Convergence Technology
Conference Room 1	2.1 Biopharmaceutical and Medical Biotechnology
	2.2 Biopharmaceutical and Medical Biotechnology
	2.3 Biopharmaceutical and Medical Biotechnology
	10.1 Nanobiotechnology, Biosensors, and Biochips
	9.3 Biocatalysis and Protein Engineering
	7.1 Drugs Delivery and Development
Conference Room 2	3.1 Tissue Engineering and Biomaterials
	6.2 Environmental Microbiology and Biotechnology
	3.3 Tissue Engineering and Biomaterials
	5.1 Biocatalysis and Protein Engineering
	5.2 Biocatalysis and Protein Engineering
	15.1 Bioindustry Promotion and Bioeducation

ROOM	TOPIC
Conference Room 3	6.1 Environmental Microbiology and Biotechnology
	3.2 Tissue Engineering and Biomaterials
	11.1 Bio-Digital Convergence Technology
	2.4 Biopharmaceutical and Medical Biotechnology
	13.1 Clinical and Public Health
	10.2 Nanobiotechnology, Biosensors, and Biochips
	12.2 Systems and Synthetic Biotechnology
Conference Room 4	EFB-AFOB Joint Session
	AFOB Board Meeting
	16.1 Marine Biotechnology
	12.1 Systems and Synthetic Biotechnology
	16.2 Marine Biotechnology
Conference Room 5	14.1 Medical Devices
	14.2 Medical Devices
	9.1 Biocatalysis and Protein Engineering
	9.2 Biocatalysis and Protein Engineering
Conference Room 6	4.1 Bioenergy and Biorefinery
	4.2 Bioenergy and Biorefinery
	8.1 Applied Microbiology
	8.2 Applied Microbiology

ABSTRACT BOOK

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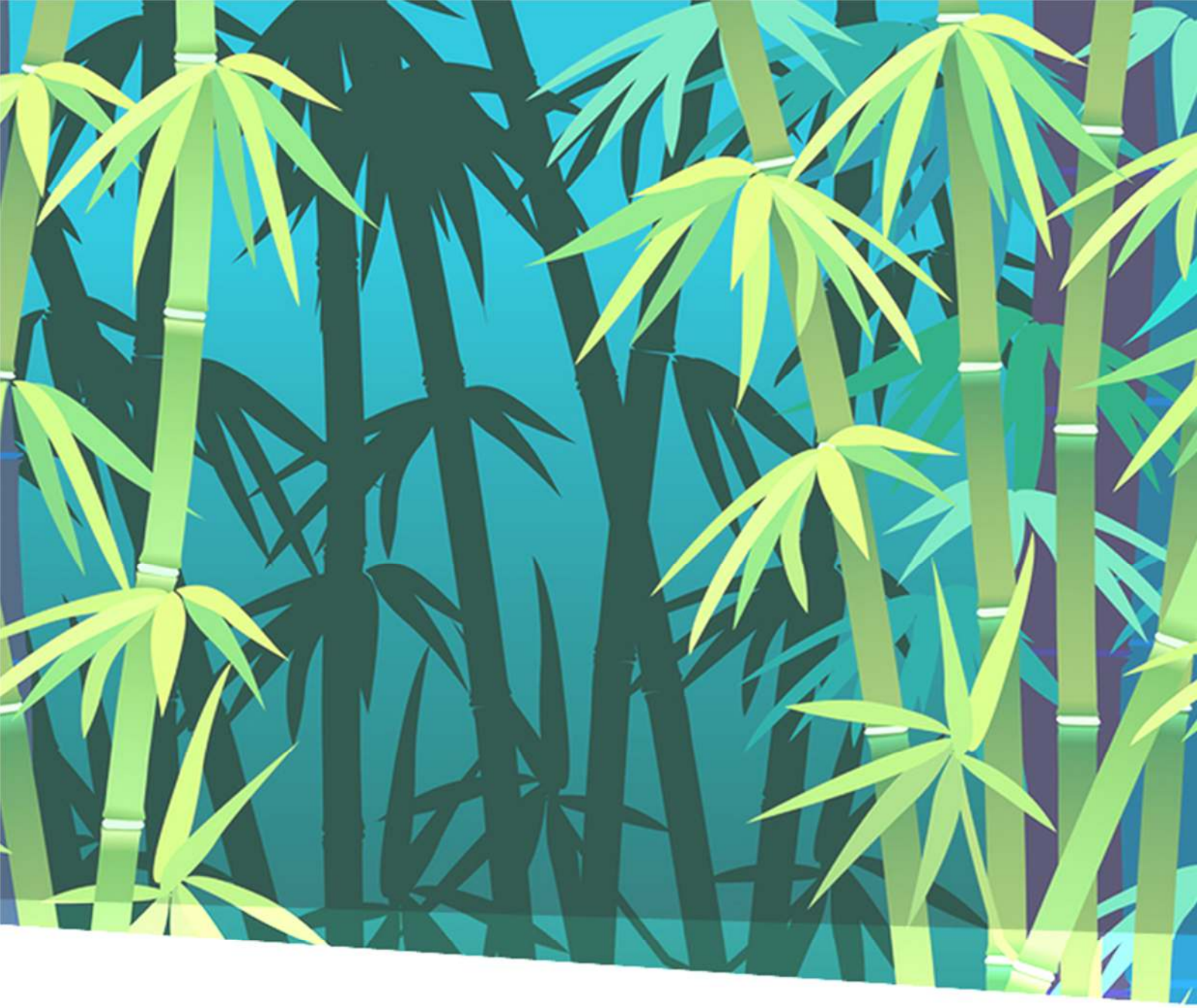
Asian Congress on Biotechnology
Frontiers in Biotechnology

October 15th - 19th, 2023
Biotechnology Center of Ho Chi Minh City,
Ho Chi Minh City, Vietnam



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Vietnam Biotechnology Association (VBA)

Ho Chi Minh City Association of Biology and Biotechnology

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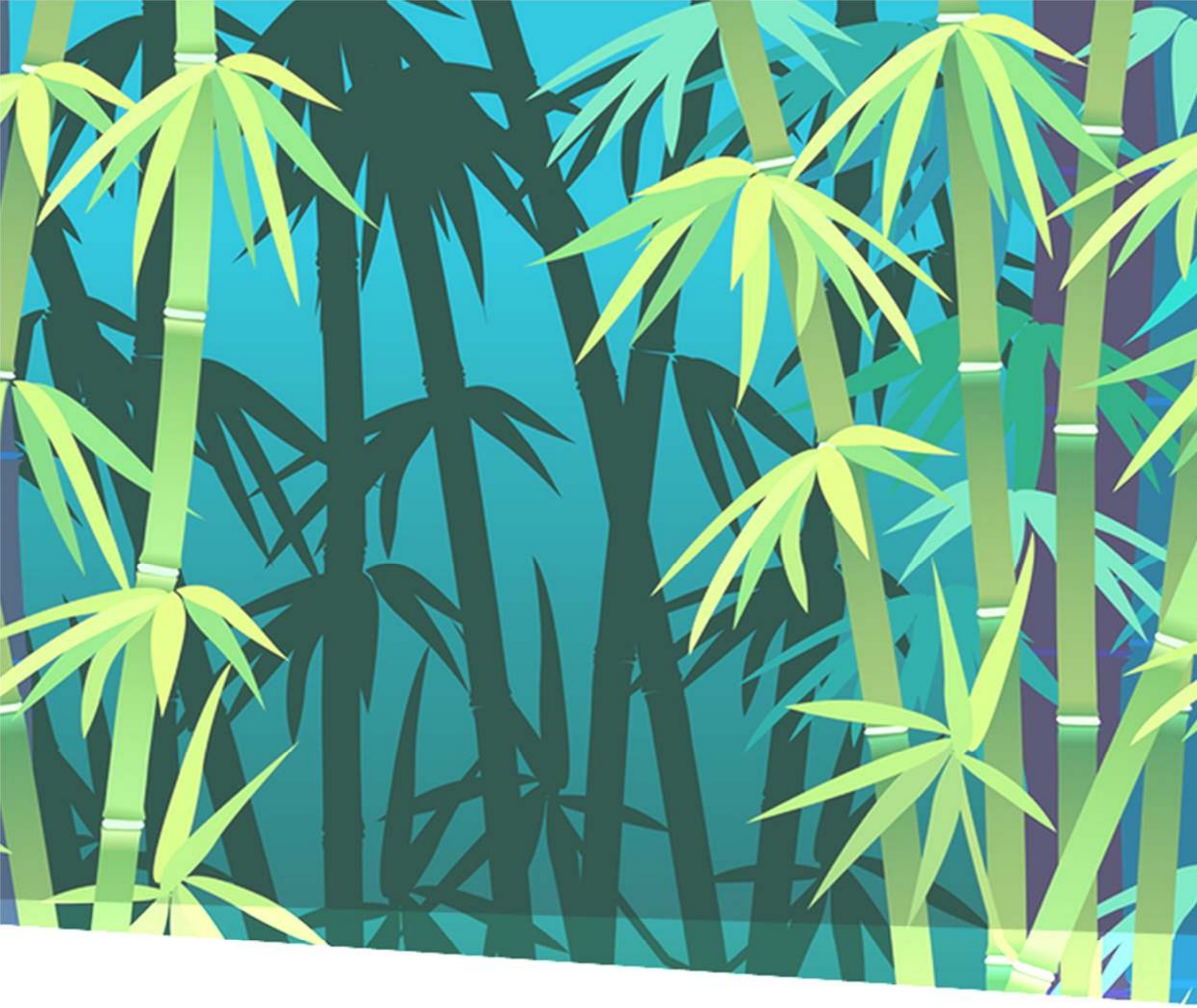


Conference program

GENERAL SCHEDULE
of The 16th Asian Congress on Biotechnology
Frontiers In Biotechnology

Sunday, October 15th, 2023

Time	Program	Venue
13:00 – 18:00	Registration at Hotel lobby	

Monday, October 16th, 2023

Time	Program	Venue
07:30 – 09:00	Shuttle buses from Hotel to HCMBiotech	
09:00 – 09:40	**Registration** **Poster Hanging**	**Lobby**
09:40 – 10:15 *09:40 - 09:55* *09:55 - 10:10* *10:10 - 10:15*	**Opening Ceremony** *Vietnamese traditional performance* *Welcome Speech Delivered by* *+ Representative of the Leaders of Ho Chi Minh City* *+ Prof. Takeshi Omasa, President AFOB* *+ Prof. Tran Linh Thuoc, Chairman of Local Organizing Committee of ACB2023* *Present Thank-you Flowers and Appreciation Certificates to Sponsors*	**Conference hall**
10:15 – 12:05 *10:15 – 10:55* *10:55 – 11:10* *11:10 – 11:50* *11:50 – 12:05*	**Plenary Session 1** Chairman: Prof. Choul-Gyun Lee (Inha University, Korea) Co-Chairman: Prof. Tran Linh Thuoc (Vietnam Biotechnology Association, Vietnam) ***KN01:*** *Title: From Micrografting to Gene Editing against Plant Viruses* *Prof. Le Tran Binh | Vietnam Biotechnology Association | Vietnam* *Break* ***KN02:*** *Title: Sustainable Biorefinery: Harnessing Agrowaste Materials for Enzymes and Value-Added Products* *Prof. Suraini Abd-Aziz | Universiti Putra Malaysia | Malaysia* *Spare time*	**Conference hall**
12:05 – 12:15	**Group Photo**	
12:15 – 13:30	Lunch & Poster session	
13:30 – 15:30	**Oral Presentation Session 1 & EFB-AFOB Joint Session**	**Conference hall** **Conference rooms**
15:30 – 15:45	Break	
15:45 – 17:45	**Oral Presentation Session 2**	**Conference hall** **Conference rooms**
17:45 – 18:15	Shuttle buses from HCMBiotech to the Welcome party venue	
18:30 – 21:00	**Welcome Party**	**Ben Xua Resort**
21:00	Shuttle buses from the Welcome party venue to Hotel	

Tuesday, October 17th, 2023

Time	Program	Venue
08:00 – 09:00	Shuttle buses from Hotel to HCMBiotech	
09:00 – 11:30 *09:00 – 09:40* *09:40 – 10:20* *10:20 - 10:35* *10:35 – 11:15* *11:15 - 11:30*	**Plenary Session 2** Chairman: Prof. Takeshi Omasa (Osaka University, Japan) Co-Chairman: Prof. Le Tran Binh (Vietnam Biotechnology Association, Vietnam) ***KN03:*** *Title: Three Decades of Movement on Plant Biotechnology Application* *Prof. Kazuo N. Watanabe | University of Tsukuba | Japan* ***KN04:*** *Title:* *Assoc. Prof. Chuenchit Boonchird, Ph.D | Mahidol University | Thailand* *Break* ***KN05:*** *Title: Advanced Production of High-Value Products from Renewable Biomass Using Customized Cell Factories* *Prof. Sung Ok Han | Korea University | Korea* *Spare time*	**Conference hall**
11:30 – 13:30	Lunch & Poster session	
11:30 – 13:30	**AFOB Board member meeting**	**Conference room 4**
13:30 – 15:30	**Oral Presentation Session 3**	**Conference hall** **Conference rooms**
15:30 – 15:45	Break	
15:45 – 17:45	**Oral Presentation Session 4**	**Conference hall** **Conference rooms**
17:45	Shuttle buses from HCMBiotech to Hotel	

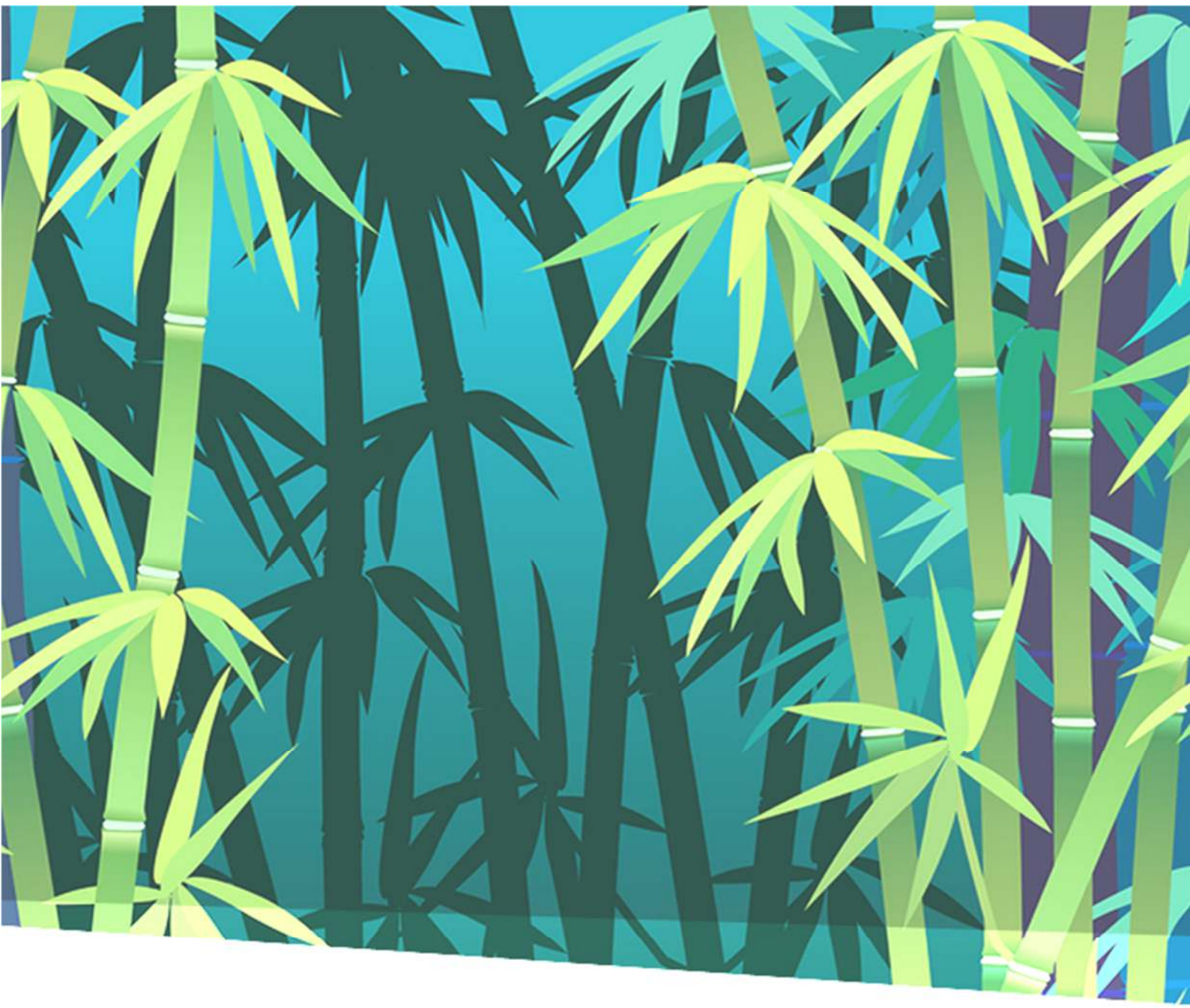
GENERAL SCHEDULE
of The 16th Asian Congress on Biotechnology
Frontiers In Biotechnology

Wednesday, October 18th, 2023

Time	Program	Venue
08:30 – 09:30	Shuttle buses from Hotel to HCMBiotech	
09:30 – 11:30	**Oral Presentation Session 5**	**Conference hall** **Conference rooms**
11:30 – 13:00	Lunch & Poster session	
13:00 - 15:00	**Oral Presentation Session 6**	**Conference hall** **Conference rooms**
15:00 – 15:15	Break	
15:15 – 16:30	**Closing Ceremony**	**Conference hall**
15:15 - 15:45	*Conclusion from Each AFOB Division Chair*	
15:45 - 15:55	*Best Presenter Awards* *Best Poster Awards*	
15:55 - 16:00	*Appreciation Plaque Ceremony*	
16:00 - 16:15	*Closing Speech Delivered by* *+ Prof. Le Tran Binh, Vice Chairman of Local Organizing Committee of ACB2023* *+ Prof. Choul-Gyun Lee, Secretary General of AFOB*	
16:15 - 16:25	*Speech by Representative of the Host Organization of the 17th Asian Congress on Biotechnology*	
16:25 – 16:30	*Asian Research Exchange Award of The Korean Society for Biotechnology and Bioengineering*	
16:30 – 17:30	Shuttle buses from HCMBiotech to Hotel	
18:20 – 18:50	Shuttle buses from Hotel to the Gala dinner venue	
19:30 – 21:15	**Gala Dinner**	**Queen (2nd floor)**
21:15	Shuttle buses from the Gala dinner venue to Hotel	

Thursday, October 19th, 2023

Time	Program	Venue
08:00 – 11:30	**City Tour**	**District 1 and Binh Thanh District, Ho Chi Minh City**
08:00	*Pick up at the hotel by Double-decker bus. Enjoy Acoustic Band (live)*	
09:00	*Stop at Reunification Palace and inside visit*	
10:00	*Visit Notre Dame Cathedral, City Post Office and City Hall*	
10:30	*Transfer to Saigon Pier and Back Dang Park*	
10:40	*Embark Saigon water bus and enjoy the panoramic view along Saigon River*	
10:55	*Disembark at Binh An Pier and transfer back to the hotel*	
11:30	*Drop off at the hotel. Finish*	



Plenary Session

FROM MICROGRAFTING TO GENE EDITING AGAINST PLANT VIRUSES

Le Tran Binh
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Abstract.

Plant viruses are causing serious yield lost in all kind of crop plants and also reducing value of ornamental plants. Plant protection provides various solutions, mostly based on the eliminating transmission vectors such as application of insecticides. The creation of virus resistance always demanding breaking advantage in scientific technological advantages. In this talk a chronological development of technologies for creating virus resistance by my team at the laboratory of Plant Cell Biotechnology will be summarized.

In the seventies Valencia Oranges have been selected and domesticated in Xuan Mai Experimental Station by Cuban agronomists. This orange cultivar seem to be welly adapted to North Vietnamese climate and soil conditions except the susceptibility to greening viruses, by which some time the entire plant, even the whole plantation area has got infected, stopped to grown and start dying. Of course there are no agents available to control this disease. It went so far that there are no more mother plants providing buds for propagation. The only technology available at this time to produce virus-free material is the micro grafting. In this technique micro apical tip in the size of 0.2 mm. According to scientific finding of the virologist the concentration of the virus particles in the fast growing part of the plant is diluted to zero in the apical meristem. Based on this finding, the use of micro shoot tip for grafting allows to produce virus-free plant. In this grafting technique we have combined *micro grafting* with *in vitro root stock culture*, e.g. the root stock in this case is only a piece containing a part of the root and a part of the stem of a 1-week old Graf fruit seedling. After one month of in vitro cultivation, the grafted tips developed to a healthy plantlets which are able to be transferred to soil and grown in a mesh house to a mother plants providing virus-free buds for further clonal propagation.

The antisense technology based on the introduction of a DNA sequence which is constructed in antisense direction to the virus DNA in order to block the translation of the viral gene by forming double stranded RNA molecule after transcription. The first successful application of antisense technology has been conducted in Hawaii by Dr. Dennis Gonsalves against Papaya Ring Spot Virus (PRSV). An ASEAN Biotechnology Network has been established involving researchers from five countries Malaysia, Philippines, Indonesia, Thailand and Vietnam, supported by two stockholder Zeneca and Monsanto and the International Service for the Acquisition of Agri-Biotech Application (ISAAA).

There is no universal antisense construct for all PRSV strains, each country has to isolated its own strain for its own construct. Our Vietnam team, after alignment

DNA from hundred virus samples collected around the country we decided to use information from the Ly Nhan strain for our construct. Successful transformation, positive resistance of transformed papaya proved in green house for at least one year until one strain from Taiwan spread widely in Vietnam.

RNAi (RNA interference) is an active gene inactivation mechanism involving specific sequence-specific double-stranded RNA fragments with the resultant inactivation of genes with corresponding complementary sequences. RNAi rapidly applied in generating a gene knockdown organisms, in functional genomics studies, especially in medicine such as creating treatments against viral diseases (HIV, HBV, HCV...), cancer, etc. and in plant biotechnology. In order to master the RNAi technology our laboratory first tested it on tobacco plant for CMV&PVY resistance and then applied it on papaya for PRSV resistance. Following steps have been conducted: Isolation of genes of pathogenic viruses; Design of transgenic vectors carrying RNAi structure of viruses; Transformation of transgenic vectors carrying RNAi structure through bacteria *A. tumefaciens*; Screening of transgenic plants carrying RNAi structure and testing the virus resistance of transgenic plants. Double resistance to CMV and PVY in tobacco lines and stable resistance to PRSV in papaya have been successful created.

CRISPR/Cas systems, a site-directed genome-editing tool, administer adaptive immunity to all living organisms against viruses and plasmids by using CRISPR RNAs (crRNAs), which direct Cas endonuclease to cleave invading nucleic acids on the basis of sequence complementarity. Plant viruses can either be targeted directly by CRISPR for dsDNA/ssRNA breaks or essential host component genes can be targeted for mutations so that the virus can no longer infect. “Simultaneously induced mutations in *eIF4E* genes by CRISPR/Cas9 enhance PVY resistance in tobacco”.

SUSTAINABLE BIOREFINERY: HARNESSING AGROWASTE MATERIALS FOR ENZYMES AND VALUE-ADDED PRODUCTS

Suraini Abd-Aziz[1]*, Mohd Azwan Jenol[1], Mohamad Faizal Ibrahim[1], Lai-Yee Phang[1], Noorjahan Banu Alitheen[2], Shafinaz Abd Gani[3] & Mohd Azuraidi Osman[2]

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Abstract. A significant shift towards sustainable bio-based and renewable resources, while moving away from fossil-based alternatives, presents numerous advantages. Malaysia is fortunate to have abundant renewable energy sources, particularly biomass. The concept of a biorefinery involves the conversion of biomass into multiple valuable products through various bioprocess routes. The primary objective of a biorefinery is to maximize the utilization of biomass resources, minimize waste generation, and promote sustainability in the production of bio- based products. A bioprocess route refers to the specific conversion processes employed in a biorefinery to transform biomass into different products. It is crucial to note that the selection of agrowaste for a biorefinery depends on factors such as regional availability, cost- effectiveness, and the targeted end products for production. Our ongoing research aims to optimize conversion processes, improve product yields, and develop sustainable and economically viable biorefinery systems that utilize agrowaste materials. Additionally, we recognize the significance of considering the three pillars of sustainable development: economic, social, and environmental development. Therefore, our research group focuses on extracting or converting enzymes from agricultural wastes to produce value-added products. Through our research efforts, we strive to advance the field of biorefinery, foster technological innovation, and contribute to the transition towards a more sustainable and resource-efficient society. By combining scientific expertise, process optimization, and a comprehensive understanding of the environmental and social implications, we aim to drive the adoption of bio-based solutions and promote a greener and more sustainable future for Malaysia and beyond.

Keywords: Agrowaste, Green Feedstock, Enzymes, Value-Added Products, Biorefinery

THREE DECADES OF MOVEMENT ON PLANT BIOTECHNOLOGY APPLICATION

Prof. Dr. Kazuo Watanabe

Professor, Tsukuba Plant Innovation Research Center, University of Tsukuba, Japan

Adjunct professor, Section of Plant Breeding & Genetics, School of Integrative Plant Science, CALS, Cornell University, USA

Abstract. Genetic engineering has now five decades of the history with relevant product releases on biomedical, agriculture and food industries. Since early 1980's, emerging interest and application had been taken up with modern biotechnology to plants with the emphasis of the use of genetic engineering. Biotech crops have dominated over global markets nearly thirty years, and now the products and technology are essential for global food security. Development of such biotech crops is further fine-tuned with genomics and genome editing. Generally, wide applications have been made on food and agriculture areas over crops, fishery and livestock with streamlines of product candidates. Also reproducible energy sources and forestry areas are supported by modern biotechnology applications. For further materializing the technology applications, it is essential to contemplate on the public understanding and commercialization approaches. ELSI factors have been placed in parallel with such a commercial movement, and future outlook will be discussed over ELSI, climate change and sustainability.

ADVANCED PRODUCTION OF HIGH-VALUE PRODUCTS FROM RENEWABLE BIOMASS USING CUSTOMIZED CELL FACTORIES

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Abstract. Converting bioresources into high-value products is an important step in bioprocess to efficiently produce target chemicals from fermentable sugars. Designing microbes using systems metabolic engineering and protein engineering is a crucial biotechnology to produce valuable products. *Corynebacterium glutamicum* is one of the attractive microbial cell factories, because it can produce various amino acids and organic acids and is being used in the industry. Thus, customized designer cell factories were developed for large-scale and efficient production of high-value products from bulk to specialty chemicals from fermentable sugars or renewable biomass. Isopropanol is a bulk commodity chemical used in the chemical and medical industry. First, metabolic rewiring of isopropanol biosynthesis pathway and bypasses improved isopropanol production. Then, the development of a *Corynebacterium* whole cell biocatalyst by the surface display of cellulolytic and fungal-degrading enzyme complex allowed for the successful production of isopropanol from lignocellulosic biomass. Second, systems metabolic engineering of the heme biosynthesis pathway improved the biobased production of porphyrin derivatives, which are specialty chemicals applied in various fields. Customized metabolic redesign of two precursor routes, the core intermediate porphyrin pathway, and downstream heme pathway with transcriptional upregulation showed the enhanced production of various porphyrin derivatives such as Zn-porphyrin, heme, and biliverdin. Porphyrin extensively absorbs UV-visible light due to its unique structure. Hence, the possibility of bioderived porphyrin derivatives as a photoprotective or photocatalytic chemical was suggested in this study. The biorenewable sunscreen including Zn-porphyrins and lignins extracted from lignocellulosic biomass exhibited the UV-blocking effect on the broad-spectrum UV wavelength. A porphyrin-coating membrane showed the excellent antimicrobial activity under visible light. In conclusion, the customized design of microbial cell factories for the advanced production of high-value products could be an attractive strategy for their versatile application and industrialization.

Keywords: Microbial cell factory, isopropanol, porphyrin derivatives, photoactive application, renewable biomass utilization

YEAST SURFACE DISPLAY: POTENTIAL STRATEGY FOR CONTROLLING PATHOGENS IN AQUACULTURE

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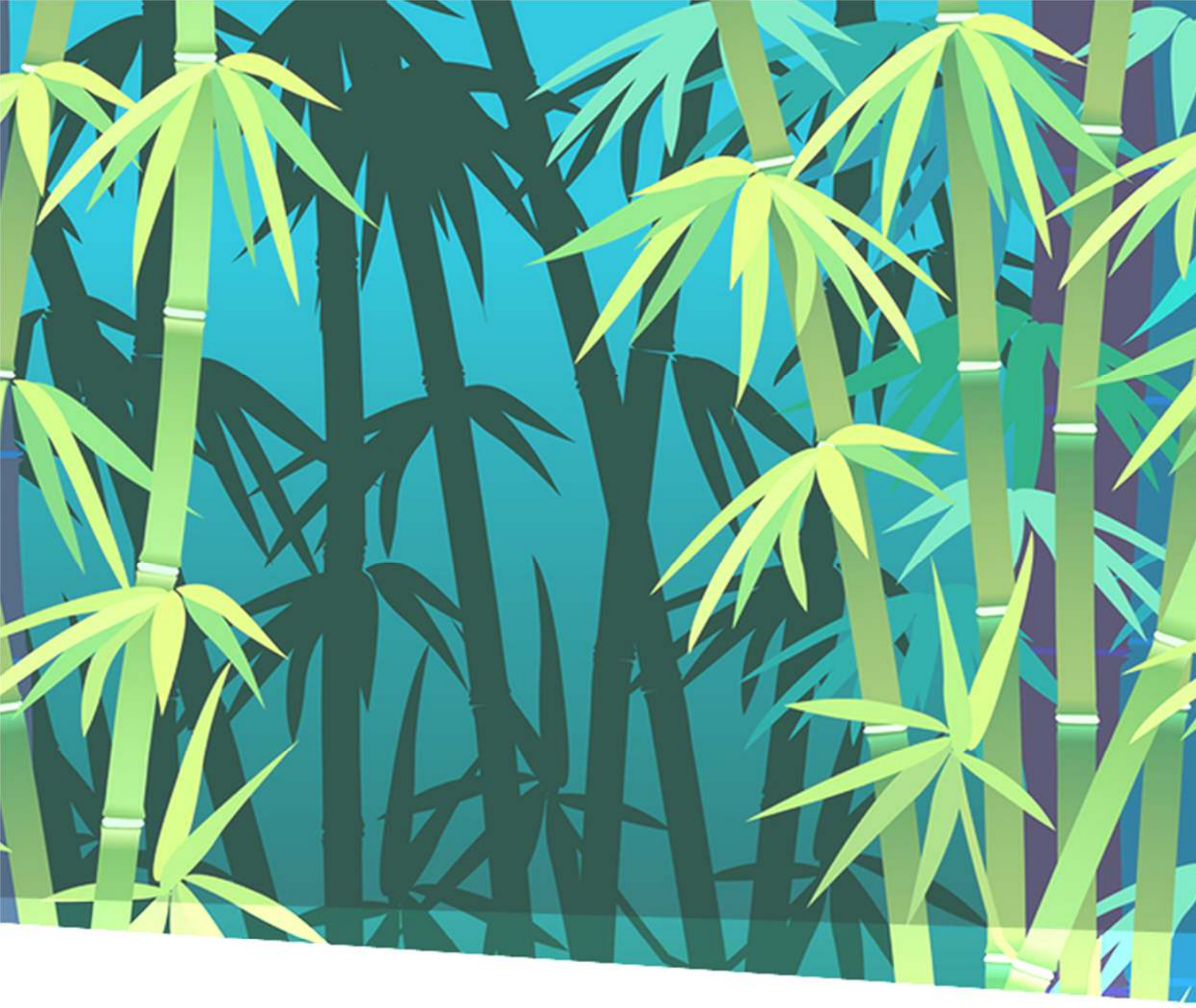
Abstract. Yeast surface display (YSD) has been widely employed for the heterologous expression of proteins with many potentials for biomedical and biotechnological applications. *Saccharomyces cerevisiae* and *Pichia pastoris* are robustness organisms which successfully accommodates a number of recombinant proteins displayed on their cell surfaces due to the rigid structure of cell wall. Being save vehicle of yeast cells, increasing numbers of investigation attempt to develop YSD as a live vaccine against pathogens in farm animals and human. White spot syndrome virus (WSSV) is one of the most devastating pathogen of penaeid shrimp that has been a significant impact on the shrimp aquaculture industry worldwide. So far, no effective treatments have been implemented. The prevention of infections in this shrimp is important to control the outbreaks of this pathogen. This presentation will go to the approach in protection of shrimp from WSSV infection using YSD of *Penaeus monodon* Rab7 (*Pm*Rab7) protein which was previously characterized as a binding partner of envelope protein VP28 of WSSV. Genetically engineered *P. pastoris* displayed biologically active *Pm*Rab7 in binding with partner protein VP28 and WSSV particles. Significant increase in shrimp survival was observed when shrimp fed with YSD-*Pm*Rab7 yeast cells supplemented feed prior to challenge with WSSV. Nested PCR assays and histopathological analysis confirmed significantly lower WSSV replication levels. The observation indicated potential for development of YSD-*Pm*Rab7 cells as an oral prophylactic against WSSV in shrimp. Since YSD platform has been applied for investigation of protein engineering and characterization without protein purification. The modification of YSD-*Pm*Rab7 to improve binding to VP28 and WSSV particles will be discussed.

Keywords: Yeast Surface Display, *Penaeus monodon* Rab7, *Pichia pastoris*, VP28, White Spot Syndrome Virus



1. Agricultural and Food Biotechnology

APPLICATION OF NANOPARTICLE IN PLANT CELL, TISSUE AND ORGAN CULTURE

Nhut Duong Tan[1,*] Khai Hoang Dac[1], Tung Hoang Thanh[1], Cuong Do Manh[1], Mai Nguyen Thi Nhu[1], Luan Vu Quoc[1], Nguyen Phan Le Ha[1], Bao Huynh Gia[1], Phong Truong Hoai[1], Diem Le Thi[1], Anh Truong Thi Lan[1], Hanh Nguyen Thi My[1], Bien Le The[1], Hiep Phan Phuoc Minh[1], Thuc Le Van[1], Ngan Ha Thi My[1], Hieu Tran[1], Mo Vu Thi[1], Huong Trinh Thi[1], Hien Vu Thi[1], Nghia Luong Van[1], Hien Do Thi[1], Thuy Nguyen Thi Thanh[1], Hoa Ho Cam Khanh[1], Khang Nguyen Nhat[1], Phuong Hoang Thi Nhu[2], Nam Nguyen Ba[2] , Vinh Bui Van The[3], Vinh Nguyen Quang[4], Dung Doan Manh[4], Hoang Cao Van[5], Phuong Truong Thi Bich[6], Buu Ngo Quoc[7], Chau Nguyen Hoai[7]

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Abstract. Nanotechnology is considered the industrial revolution, promoting development in all fields, especially biomedicine, energy, environment, agriculture, and information technology. Recently, nanomaterials affecting plant growth have attracted researchers around the world. Studies on the effects of metal nanoparticles on plants have been shown. Both the positive and negative effects of nanomaterials on plant growth depend on many factors, such as composition, structure, concentration of nanoparticles, and plant species. The research team of Prof. Dr. Duong Tan Nhut is the first group in the world to research and develop this problem for more than 10 years and has more than 50 publications in domestic and intentional journals (Q1), contributing to building an exciting new orientation in plant cell technology. Nanoparticles of metal, metalloid, and rare earth have been helpful in many plant micropropagation applications, acting as a disinfectant for explants, medium sterilization, and acting as plant growth regulators to improve the plant regeneration process, improving abnormal phenomena in micropropagation, thereby increasing the survival rate of plantlets under nursery conditions. Furthermore, adding nanoparticles, especially silver nanoparticles, help to study flowering *in vitro*. Interaction of metal nanoparticles with plants causes changes in the physiology and biochemistry of plants, such as endogenous hormone levels, ethylene gas accumulation, antioxidant enzymes, non-antioxidant non-enzymes, biosynthesis of secondary compounds, the ability to absorb nutrients from the culture medium, ... Some mechanisms of action of nano on the explants have also been clarified, thereby giving a better insight into the role of nano in plant cell technology. These pioneering researches contribute to the improvement of micropropagation of some valuable plant species such as *Panax vietnamensis* Ha *et* Grushv., *Panax vietnamensis* var. *langbianensis*, *Passiflora edulis*, *Gerbera* L., Fragaria × ananassa, *Limonium sinuatum* L., *Phyllanthus amarus*, *Begonia* × *tuberhybrida* Voss, *Actinidia chinensis*, *Dianthus caryophyllus* L., *Chrysanthemum morifolium*, *Saintpaulia ionantha* H. Wendl., creating new medium cultures in micropropagation and advanced plant cell technology studies. In this report, we will present the latest trends in the application of nanoparticles in plant cell, tissue, and organ culture.

Keywords: nanotechnology, plant cell, tissue and organ culture, micropropagation, growth and development

Acknowledgements: *This research was supported by Vietnam Academy of Science and Technology under grant number NCXS01.03/22-24.*

CREATING A NEW CLONE OF 'LỬA' ROSE (*ROSA HYBRIDA* L.) BY *IN VITRO* GAMMA IRRADIATION

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Abstract. *Rosa hybrida* cv. Lửa has been one of the most important roses for a long time in Sa Dec city Dong Thap province and being most often use for ornamental purposes. The study aimed to select new mutational clones of Lửa rose using tissue culture technique combining with *γ*-rays' treatments. *In vitro* single node cuttings (25 in vitro cuttings per treatment with four replications) were irradiated with different doses of γ-rays (0, 5, 10, 15, 20, 25 or 30 Gy) using a 60Co source (India) at Da Lat Nuclear Research Institute. The γ-irradiated explants were then cultured aseptically on Murashige and Skoog basal medium supplemented with 1.0 mg/L BAP to induce sprouting, shoot proliferation and acclimatization at Southern Horticultural Research Institute (SOFRI). The LD50 dose was determined to be 20-25 Gy treatments. Explants treated at all doses showed deleterious effects of ionizing radiation. Morphological abnormalities such as fused leaves, leaf albinism, leaves with lower levels of chlorophyll, variegated leaves, and stunted growth were observed at all doses. Two clones with altered or novel flower colors compared to the original flower color were isolated, such as Type 1 had orange-pink (Red 52C) and Type 2 had pink (Red 54B).

Keywords: gamma ray, irradiation dose, in vitro, mutation, Rosa hybrida.

JUTE ENDOPHYTES: A CLUSTER OF MICROBIAL SYMBIONTS WITH HUGE AGRICULTURE AND MEDICINAL POTENTIALS

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Abstract. A wealth of resources remaining untapped is the microbiome, the communities of microorganisms living together in a particular habitat. One such microbiome is the *endophytic population which* are often bacteria or fungi, that live within plants and do not apparently cause any harm to their hosts. Bioprospecting for microbial endophytes and their natural products can be related to the 'gold rush', of the early 19th century. Endophytes produce an abundance of bioactive compounds of potential use to modern medicine, agriculture, and industry. Jute plants known for their fiber are also rich sources of secondary metabolites. Jute leaves have been used in traditional medicine from time immemorial in countries where the plant is grown. Bioactive compounds from jute are known to be effective against tumors, diabetes, and cardiovascular diseases. While jute has been extensively explored for new chemical entities for therapeutic purposes, jute endophytic microbes which also constitute an important source for discovering new drugs remain uncharted. A few researches in this research area have found endophytes isolated from jute plants to harbor a plethora of bioactive compounds ranging from AMPs to anti-cancer drugs like the epothilones to biocontrol agents useful in agriculture and to industrially important enzymes, like the amylase, cellulase etc. Our work on jute endophytes is suggesting ways in which endophyte derived biologically active molecular entities and their analogs could lead to the development of new bioactive agents in medicine, agriculture and industry. Jute endophytes are providing multi-faceted examples of a wealthy array of finely evolved plant–microbe interactions that have immense opportunity to be exploited for the benefit of the society. We aimed to open new windows for potential application of endophytes in improving growth and cultivation of jute, phytoremediation, organic farming, bio-control, accelerated retting, etc. by identifying the fungal and bacterial endophytes of jute, determining their mutualistic relationship through biochemical, molecular biology, genomics and proteomics analyses, investigating potential industrial and medical applications through identifying important secondary metabolites and genes, assessing their roles as mediators of biotic and abiotic stress resistance and growth enhancers as this important crop is being pushed towards inhospitable terrains in our country to make space for the food crops.

Keywords: Jute, endophyte, secondary metabolite, biofertilizer, anticancer compound, antibiotic

AN UNEXPECTED PATTERN OF GENETIC DIVERSITY AND A HIGH LEVEL OF COMPLEXITY IN BOTH THE MORPHOLOGY AND THE PHYTOCHEMICAL COMPOSITION OF THE LEAVES OF *PANAX VIETNAMENSIS*

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Abstract. *Panax vietnamensis*, a perennial plant with significant medicinal value, is experiencing a decline in its population because of excessive harvesting activities in Vietnam. Sufficient data pertaining to the genetic, morphological, and phytochemical diversity of this significant species is imperative in devising an appropriate approach toward its preservation. Between 2017 and 2023, a total of over 450 samples of *Panax* in Vietnam underwent examination utilizing chloroplast species-specific SNP loci to establish molecular diversity patterns. Out of the given samples, approximately 200 samples were analyzed for their leaf morphology and/or subjected to thin-layer chromatography analysis. Molecular diversity patterns indicated that the *P. vietnamensis* populations have largely diverged, but the divergences were not correlated with either leaf morphology or phytochemistry. Clustering analyses revealed a highly complex spatial structure pattern associated with *P. vietnamensis* var. *fuscidiscus*. The findings of our study suggest that it is necessary to take into account over nine genetically distinct groups for the purposes of *P. vietnamensis* conservation, management, breeding programs, and pharmaceutical applications in Vietnam.

Keywords: Panax vietnamensis, molecular diversity patterns, morphological and phytochemical complexity

CAS9-SGRNA RIBONUCLEOPROTEIN MEDIATED GENOME ENGINEERING IN *LEUCONOSTOC* AND *BIFIDOBACTERIUM*

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Abstract. The CRISPR-Cas9 system is a powerful genome editing tool that has been widely used in various cell types. The delivery of Cas9 through ribonucleoprotein complexes (RNP), in which the Cas9 protein is preassembled with sgRNA, is a preferable method. However, this technique has not been widely applied to lactic acid bacteria (LAB) due to limitations such as their complex cell wall structure and lack of recombinase. In this study, we aimed to overcome these limitations and establish an efficient genome editing system in *Leuconostoc* and *Bifidobacterium* using Cas9-sgRNA ribonucleoprotein complexes. We targeted the dextransucrase gene (*dsr*) in *Leuconostoc* and the uracil phosphoribosyl transferase (*upp*) gene in *Bifidobacterium* as our genes of interest. By introducing the Cas9-sgRNA ribonucleoprotein complex, phosphothiolated DNA, and RecT recombinase into the cells via electroporation, we successfully obtained dextran-free *Leuconostoc* colonies with significantly improved mutation efficiency. Moreover, we achieved *Bifidobacterium* strains resistant to 5-fluorouracil (5-FU) with increased mutation efficiency. These findings highlight the potential of the Cas9-sgRNA RNP system as a powerful genome editing tool in LAB, enabling genetic engineering across different genera. The Cas9-sgRNA RNP system demonstrates its potential as a powerful genome editing tool in LAB, surpassing the genus barrier and providing a new strategy for developing LAB-based cell factories for bioresource production and therapeutic protein.

Keywords : CRISPR-Cas9, Ribonucleoprotein, Genome engineering, Lactic acid bacteria, *Leuconostoc*, *Bifidobacterium*

FROM MULTI-OMICS ANALYSIS TO DEVELOPMENT OF AGRICULTURAL DIGITAL TWIN

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Abstract. Technological innovation has been demanded to achieve a sustainable crop production fed the growing human population without generating environmental burdens. The achievement of sustainable agriculture by balancing food production and global environmental conservation is one of the challenges that we must urgently address, and one possible solution is tailor-made cultivation aimed at optimizing the various environments. Therefore, we have been digitizing agroecosystems through multi-omics analysis in agricultural fields and have initiated a national project to develop an "Agricultural Digital Twin" that uses these digital data to simulate agricultural ecosystems in cyberspace. This system is designed to predict crop yield, quality, and environmental impact based on weather forecasts during the harvest season and soil data, and to provide the best solution for the farming management practice. This system is expected to enable tailor-made production of crops with stable yield and quality for each site, and to realize efficient resource recycling as well as high profitability. Today I will introduce our multi-omics studies and share our progress in large scale soybean field trials dissected by multi-omics analysis and development of integrated model framework for the development of agricultural digital twin.

Keywords: Agroecosystem, digital twin, multi-omics, soybean

PLATELET-RICH PLASMA INDUCES THE PROLIFERATION AND CHONDROGENIC DIFFERENTIATION OF CANINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS *IN VITRO.*

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Abstract: Platelet–rich plasma (PRP) is a good application in regenerative medicine. In this study the effect of PRP on the proliferation and chondrogenic differentiation of canine bone marrow–derived mesenchymal stem cells (BMSCs). **Methods**: BMSCs are isolated from immature dogs. The colony-forming unit assay, flow cytometry, and multi-differentiation (osteogenesis, adipogenesis, and chondrogenesis) are used to define the stem cell characterizations. PRP is isolated from the peripheral blood of mature dogs. Then, the effect of PRP on the proliferation of BMSCs is measured by WST-1 assay. And the effect of PRP on the chondrogenic capacity is tested by glycosaminoglycan formation (Safranin-O stain). **Results:** BMSCs define colony forming in the basic growth medium, and express the stem cell markers (CD34neg, CD45neg, CD105pos, CD90pos). For multi-differentiation, it is shown the osteogenic, adipogenic, and chondrogenic ability. PRP is isolated and shows the induced proliferation, and chondrogenic differentiation of BMSCs. **Conclusions:** The results show that PRP can induce the proliferation and chondrogenic differentiation of BMSCs in a canine model.

Keywords: Bone marrow, mesenchymal stem cells, PRP, chondrogenesis

THE USE OF *BACILLUS* SPECIES AS BIOCONTROL AGENTS IN CROP FUNGAL DISEASE MANAGEMENT

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Abstract. *Bacillus* species are becoming popular tools for crop protection because of their effectiveness and sustainability, especially on fungal diseases. Using the in vitro antagonistic screening, we found an array of *Bacillus* species with high antifungal potential. The *Bacillus* K31 showed strongest in vitro inhibition against *Fusarium oxysporum* f. sp. *cubense* TR4 (*Foc* TR4) – causing Panama disease TR4 in banana and *Ganoderma boninense* – causing Basal Stem Rot (BSR) disease in oil palm. The average percent inhibition of mycelial growth on the 7th day of four *Foc* TR4 ecotypes and *G. boninense* were 58.66 ± 1.6 %, 58.84 ± 2.5 %, 59.67 ± 1.4 %, 59.77 ± 2.1 %, and 65.8 ± 3.6 %, respectively. Through sequencing and analysis of the *Bacillus* K31 complete genome, we found 42.85 % of its genome was annotated as metabolism genes. Among them, 5.64 % was related to the biosynthesis of secondary metabolites including several antifungal compounds (fengycin, difficidin, bacillibactin, bacilysin, surfactin, macrolactin, and bacillaene). The comparative complete genome analysis between the *Bacillus* K31 and others published *Bacillus* species (*B. amyloliquefaciens*, *B. velezenesis*, *B. subtilis*) indicated that the *Bacillus* K31 was the most unique (highest singleton, 646) and 51.1 % of them were annotated as hypothetical proteins. We suggest the *Bacillus* K31 is a potential biological control agent in controlling phytopathological fungi.

Keywords: Elaeis guineensis, Musa spp., decorative palm, fox tail palm, coconut.

THE EFFECTS OF SOLID-STATE FERMENTATION ON THE PRODUCTION OF L-CARNITINE IN AGRICULTURAL MATERIALS AND THEIR BIOCHEMICAL CHARACTERIZATION

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Abstract. L-carnitine, vital for transferring long-chain fatty acids to mitochondria by β-oxidation, is a quaternary amine synthesized from lysine and methionine. L-carnitine was commonly found in fish and meat but in low amounts in agricultural products. This study aims to enhance the L-carnitine content and biochemical properties of agricultural materials, including quinoa, sorghum, buckwheat, and wild turmeric, by solid-state fermentation using *R. oligosporus*. The results show that the L-carnitine in fermented agricultural materials, including quinoa, sorghum, and buckwheat, increased to 24.2-, 33.4-, and 4.0-fold. In the case of wild turmeric, L-carnitine was not detected in non-fermented, and it was detected as 242 μg/g after 7-day fermentation. The phytochemical compounds of fermented material were increased. The highest antioxidant activities of fermented sorghum and quinoa were obtained after 5-day fermentation, while it was obtained at 7-day for fermented wild turmeric. Furthermore, these fermented materials inhibited NO release in LPS-stimulated RAW264.7 cells. Based on these results, these fermented materials can be used as functional materials in the pharmaceutical and food industries.

Keywords: L-carnitine, solid-state fermentation, R. oligosporus, phenolic, antioxidants,

MICROWAVE-ASSISTED EXTRACTION (MAE) OPTIMIZATION, CHARACTERIZATION, AND ANTIDIABETIC AND CYTOTOXIC EVALUATION OF KEY PHENOLIC COMPOUNDS FROM PIGEON PEA (*CAJANUS CAJAN* L.)

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Abstract. The objective of this research is to propose and validate the antidiabetic and cytotoxic effects of phenolic compounds extracted from the leaves of the *Cajanus cajan* L. plant. The total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and antioxidant activities like 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) were optimized using response surface methodology over the course of 17 experimental runs using microwave-assisted extraction parameters such as temperature (50-70 °C), power (400-1000 W), and time (15-45 min). At 700 W of power, 60 °C of temperature, and 40 min of processing time, the optimal values of response variables were achieved. Eighteen bioactive phenolic compounds were found in the plant extract after it was purified, analysed by thin-layer chromatography, Fourier transform infrared (FTIR), and liquid chromatography-mass spectrometry (LC-MS). Fifteen out of eighteen bioactive phenolic compounds were found to have high binding affinities (in the range of -4.0 to -8.8 kcal/mol), improved pharmacokinetic characteristics, and a low to moderate toxicity after an in silico investigation. High levels of enzymatic inhibition activity were observed in vitro, with IC_{50} values of 46.49±2.2 and 59.51±3.57 µg/mL for α-amylase and α-glucosidase, respectively. Furthermore, cytotoxicity was low up to a concentration of 62.5 µg/mL as shown by the MTT assay and chorioallantoic membrane (CAM) test, *in vitro* and *ex vivo* respectively. This research will pave the way for scientists and food and drug manufacturers to create new antidiabetic food formulations and medications, helping diabetics better control their condition and enhance their health.

Keywords: *Diabetes mellitus, Cajanus cajan, Phytochemicals, Optimization, in silico, in vitro, ex vivo*

IMPROVING TRANSPLANT PRODUCTION BY IN VITRO CULTURE OF JATROPHA CURCAS L., A POTENTIAL BIOFUEL PLANT, WITH NATURAL- AND FORCED-VENTILATION PHOTOAUTOTROPHIC MICROPROPAGATION

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Abstract. The jatropha plant (*Jatropha curcas* L.) is a biofuel plant with great potential due to its fast growth and high oil yield from the seeds. However, finding better methods for the production of large number of uniform transplants from elite stock plants through *in vitro* culture is essential for the application of this plant in biofuel production. In this study, *in vitro* culture of jatropha shoots was conducted with a new method, photoautotrophic micropropagation, with both small-scale culture vessels using natural ventilation, and large-scale (7 liters) culture vessels using forced ventilation. Compared to the conventional photomixotrophic culture method, using sugar- and vitamin-free nutrient medium led to significantly greater results in most parameters such as increased plant fresh weight, leaf area, and shoot and root lengths. The effects were greater with a higher ventiration tate of the vessels. Photoautotrophically micropropagated plants also showed improved physiological characteristics, such as higher chlorophyll contents, increased net photosynthetic rates, higher stomatal densities, and reduced water loss rates through leaf transpiration, especially in forced ventilation treatment. When transplanted to the *ex vitro* condition, jatropha plants micropropagated with the forced-ventilation photoautotrophic method achieved a 100% survival rate, compared to only 55.6% in photomixotrophically micropropagated plants, as well as subsequent superior plant growth and performance. These results show the great potential of the photoautotrophic micropropagation method to be applied in the production of high-quality jatropha nursery plants for large-scale cultivation.

CHARACTERIZATION OF CELLULOSE ISOLATED FROM DENDROBIUM SONIA EARSKUL

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Abstract. Dendrobium is a most cultivateds orchid species in Thailand, Estimated at 80 % of orchid production or 84 thousand tons. Usually, stem of dendrobium is cut during quality control and the flower part is taking, leaving the stem... In this study, we aim to maximize the utilization of the leftover orchid stems, exploring it as a source of high-quality cellulose. We studied the effect of the pretreatment and bleaching processes, which is a critical part for cellulose production. Our results showed that the use of xylanase and laccase enzymes enhanced the whiteness index of the extracted cellulose, as high as 90.84%. Enzymes treated fibers were also observed to have 10.9% increase in crystallinity when compared to the native fibres. The surface of fibers obtained from synergistic enzyme pretreatment appeared torn. The chemical composition of the fibers was also analyzed, to determine the effect of the enzyme pretreatment on the fibers. Effect of the enzyme to showed production of environmentally friendly products.

Keywords: Orchid, Dendrobium Sonia Earsakul, Cellulose, Enzyme

ESTABLISHMENT OF MULTIPLEX PCR FOR QUICK IDENTIFICATION OF BBS9 MUTANTS IN COMMERCIAL PIG BREEDING LINES

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Abstract. Piglet lethality is one of the major concerns in pig breeding programs. Deletion of 212kb within the Bardet-Biedl Syndrome 9 (BBS9) gene has been linked to the reduction of the number of piglets born per litter. The carrier-by-carrier mating scheme of BBS9 gene could result in stillbirth individual piglets, which ultimately affect the reproductive performance of the sow. The aim of this study is to develop a simple, quick, and cost-efficient method that could be applied in BBS9 carriers screening program in developing countries with basic lab settings. 723 animals from Yorkshire, Duroc and Landrace purebred populations were analyzed. Among them, while the frequency of the BBS9 mutant allele was not identified in Duroc and Landrace, BBS9 carrier was detected at 7% in Yorkshire populations. The results from this study were subsequently validated by Sanger sequencing approach. In conclusion, our multiplex PCR method could be utilized in BBS9 screening test for suitable pig breeding program.

Keywords: Bardet-Biedl Syndrome 9, Multiplex PCR, piglet lethality, Yorkshire

FACILITY-BASED PLANT PRODUCTION SYSTEMS WITH MATERIAL CYCLING FOR A SUSTAINABLE FOOD PRODUCTION

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Abstract. In recent years, emission control, reuse, and recycling of wastes generated in large quantities have been important issues, especially in urban areas. High-quality plant production and thus quality food supplies will play an increasingly important role in human health and welfare. Developing low-cost technologies for controlling plant growth and food production efficiently is an important issue under consideration for environmental protection. To achieve these technologies, material cycling is necessary for sustainable food production. Therefore the development of efficient plant production systems with less environmental loads must be promoted. I consider that ecosystems, including human activities in urban areas, are semi-closed, and discuss the construction of plant production systems based on material circulation as well as the nutritional food supply within semi-closed ecosystems with plant production facilities such as plant factories. First, I introduce the concept of the circulation of materials in an entirely closed ecological life support system to enable human habitation in space. Next, as an application of resource recycling and energy conversion technology in agricultural production, I suggest a combined system with plant production and methane fermentation using organic waste from urban and agricultural areas. I also propose some items as examples of possibilities and perspectives of facility-based material circulation plant production systems with other organisms such as mushrooms, microalgae, and fish for a sustainable society.

Keywords: environmental protection, material recycling, methane fermentation, plant factory, sustainable society

PREBIOTIC POTENTIAL OF WATER-SOLUBLE NON-STARCH POLYSACCHARIDES FROM BARNYARD MILLET GRAIN

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Abstract. Increasing awareness of millet among the general public due to its high nutraceutical value is encouraging. The year 2023 being declared the 'International Year of Millet', draws attention to focus research endeavours to explore the potential of various millet polysaccharides with high nutritional values. High nutraceutical value and cost-effectiveness make Barnyard millet a potential candidate for extracting polysaccharides. Water-soluble non-starch polysaccharides were obtained from the residual part of Barnyard millet after ethanol extraction of low molecular weight sugar and treatment with water at a 1:2 sample solvent ratio for 2 hours at 40 °C. Its yield was found to be approximately 2-3% (w/w). On subjecting the dialyzed and lyophilised residue to gastric acidity, salivary and pancreatic amylases it was found to be non-digestible. Interestingly, in presence of various Lactobacilli strains, positive prebiotic scores of water-soluble non-starch polysaccharides indicated the prebiotic potential of barnyard millet.

Keywords: Prebiotic, Non-starch polysaccharides, Non-digestible, Nutraceutical.

EVALUATION OF PLANT GROWTH PROMOTING ACTIVITY AND MICROBIOME PROFILE OF PSEUDOMONAS LIBANENSIS IN MAIZE FOR SUSTAINABLE APPROACH IN THAILAND

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Abstract. Application of plant growth-promoting bacteria (PGPB) plays the important role to improve agricultural practice in the organic farming. Maize (*Zea mays L.*) has become the major grain crop in the world in terms of total production. This study demonstrated the effectiveness of *Pseudomonas libanensis,* isolated from coffee roots cultivated in Thailand, as PGPB that promote maize growth. The beneficial activity study showed that *P. libanensis* has a strong ability to fix-nitrogen, utilize urea, and solubilize potassium (K). However, the tested bacterial strain did not provide the ability to solubilize phosphorous (P). The optimal concentration of *P. libanensis* to promote maize growth was 10^6 CFU/ml. After four weeks of *in vivo* cultivation, *P. libanensis* showed remarkable effectiveness in improving maize growth. Significant difference ($p<0.05$) in maize height and biomass were observed in the treatments with *P. libanensis*, starting from two weeks of cultivation, when compared with the control group. The microbiome profiles were analysed and revealed that the application of *P. libanensis* alters the soil microbial communites. This study demonstrates the high effectiveness PGPB isolated from Thailand in improving maize productivity, even though the bacteria was originally isolated from the non-related plant. This suggests that this PGPB might also effective in promoting the growth of various economic plants in Thailand.

Keywords: PGPB, Pseudomonas libanensis, Maize growth, Organic farming

AMYLOSE CONTENT AND VARIATION IN GRANULE BOUND STARCH SYNTHASE 1 (*GBSS1*) GENE IN RICE MEGA VARIETIES AT THE MEKONG DELTA.

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Abstract. Rice (Oryza sativa L.) is one of the most essential crops, feeding more than half of the world's population, including more than 90% of Asians. Several essential characteristics, including amylose content, aroma, grain shape, and size, are involved in the quality and commercial value of rice grains. This study was conducted to investigate the variation in the GBSS1 gene that regulates amylose content in improved rice varieties growing in the Mekong Delta. The data on amylose content showed that rice varieties were classified into four groups: sticky, low, medium, and high amylose content. Furthermore, the association between genotype and amylose content revealed that varieties with low amylose content possessed a combination of SNP G on Exon 4 and SNP A on Exon 6. Medium and high amylose is a combination of SNP A on Exon 4 and SNP C on Exon 6. A 23 bp insertion on Exon 2 is specific for glutinous rice. The FAMD analysis model shows that the best traits that reflect the diversity in the population of the surveyed rice varieties are seed shape, grain size, and amylose content-related genotype. These results can be used in the research programs for rice improvement in the Mekong Delta.

Keywords: Amylose, GBSS1, quality, rice

INTERTWINED ROLES OF BIOTECHNOLOGY AND CLASSICAL PHYSIOLOGY STUDIES IN RARE PLANT CONSERVATION

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Abstract. Anthropogenic climate change and over-exploitation threaten many plant species. Different biotechnology solutions were developed at the Research Center for High-technology Applications in Agriculture (RCHAA) to protect valuable plant species better. We tested and applied new solutions, such as PPM™ mixture, to increase the sterilization efficiency for Orchidaceae species (*Dendrobium* and *Nervilia* spp). Another example, such as shoot forcing gel, comprising polyvinyl carboxy polymer capable and BA was developed and tested on five local *Dendrobium* species. Our program highlighted the role of plant physiology and propagation biology in rare species protection. The mass propagation procedure of endangered *Nervilia plicata*, which is physiologically complicated, was successfully developed for both conservation and medicinal plant production at a commercial scale. In addition, the seed physiology and germination model of the medicinal plant *Pyrenria jonqueriana* was successfully developed in our program. Our results not only allowed new populations of rare species to be reintroduced in their native environment but also allowed the industry to develop practical plant production procedures without threatening the valuable yet vulnerable ecosystem.

Keywords: Orchidaceae, Theaceae, Carbon neutralization, Conservation education

GENOTYPING OF DURIAN CULTIVARS FROM ELITE TREE ORCHARDS BY ISSR MARKERS: A CASE STUDY IN BEN TRE PROVINCE

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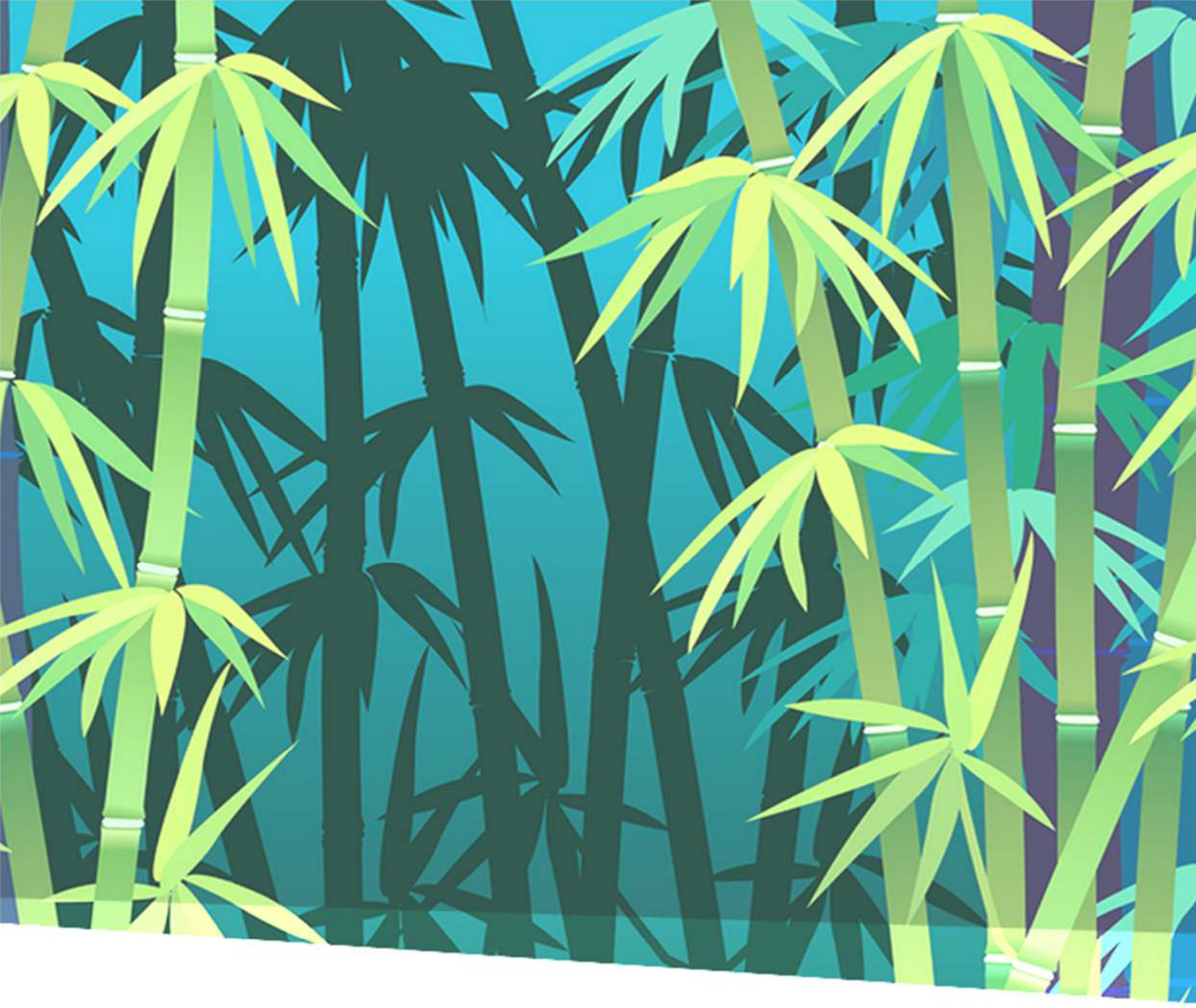
Abstract. Durians are valuable fruit in Southeast Asia with a distinct taste and aroma. Through various breeding methods, several indigenous cultivars have been developed, which have posed challenges in terms of identification. Thirty-one durian elite trees from four cultivars (Ri6, Monthong, Musang King, Chin Hoa, and Black Thorn), which were collected in Ben Tre province, were genotyped by 13 ISSR markers. Based on the amplicon profiles, 100 bands were amplified with 43 polymorphic bands, revealing the high genetic similarity among cultivars. From the dendrogram constructed by the UPGMA method, all individuals were classified into four clusters based on their cultivar. Ri6 and Monthong were in a monophyletic group with a closed genetic relationship. Furthermore, eight cultivar-specific markers, comprising 3 markers for Ri6 (UBC 825, UBC 809, UBC 890), 1 marker for Monthong (UBC 855), 2 markers for Musang King (UBC 829-11, UBC 840), and 2 markers for Chin Hoa (UBC 811, UBC 856) were explored. Such informative markers should be investigated to develop a molecular kit to validate durians accurately.

Keywords: Durian cultivars, genotyping, elite fruit trees, ISSR markers



2. Biopharmaceutical and Medical Biotechnology

NON-INVASIVE TRANSDERMAL DELIVERY OF PHARMACEUTICAL DRUGS ALTERNATIVE TO INJECTION

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Abstract

In the pharmaceutical industry, small molecule compounds have traditionally been primarily used as active pharmaceutical ingredients for therapeutic drugs. However, in recent times, the proportion of novel therapeutic approaches, collectively referred to as "new modalities," has been steadily increasing within the overall pharmaceutical sales landscape. This category includes biopharmaceuticals and gene therapeutics, such as antibodies, recombinant vaccines, and nucleic acid drugs. The reported share of these new modalities in total pharmaceutical sales reached as high as 37% by the year 2020, with expectations for continued growth in the future.

Biopharmaceuticals, composed of peptides and proteins, hold the promise of greater specificity when targeting molecules within the body compared to small molecule drugs. Nevertheless, the majority of these biopharmaceuticals require administration via injection due to their vulnerability to enzymatic digestion when taken orally. Conversely, attempts at transdermal administration—delivering substances through the skin—have encountered challenges, particularly for large molecules with molecular weights exceeding 500 Da. This is due to the formidable barrier presented by the stratum corneum, the outermost layer of the skin, which impedes passive diffusion.

Hence, this study introduces a novel transdermal penetration technique designed to surmount this challenge. Through the application of this method, the transdermal delivery of high molecular weight peptide, protein, and nucleic acid drugs has become feasible. For instance, a well-established peptide drug like insulin can now potentially be administered transdermally through a single formulated patch, offering an alternative to traditional injections. By extending this technique to vaccine antigens like those for influenza and malaria, non-invasive transdermal vaccine delivery becomes attainable.

Furthermore, as nucleic acid drugs gain prominence as an emerging therapeutic modality, the implementation of this technique facilitates the transdermal delivery of antisense oligodeoxynucleotides (ASOs). The ensuing discussion will delve into the impact of transdermal nucleic acid drug delivery on cancer suppression.

Keywords: Pharmaceutical Drugs, DDS, Transdermal Drug Delivery, Vaccine

THE ROLE OF WHOLE GENOME SEQUENCING FOR CONTROLLING TUBERCULOSIS

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Abstract. Tuberculosis (TB) is the leading cause of deaths among any infectious diseases, with more than 10 million new cases annually. Intensifying research and innovation have become an integral of the global End TB strategy. Whole genome sequencing (WGS) is a promising technology that can revolutionize the diagnosis and controlling the transmission of TB as well as open new opportunities for developing new treatment and vaccines. Diagnosis of drug resistance by WGS will provide results more rapidly, and probably more reproducible, than the standard phenotypic assays. Attempts to diagnose TB and drug resistance rapidly by directly metagenomic sequencing clinical samples has been explored widely. WGS-based molecular epidemiology allow identification of prison as a hot-spot for TB transmission in Thailand. We are developing a way to timely utilize WGS for controlling TB transmission in community settings. WGS-based genotyping has recognized a sublineage of *Mycobacterium tuberculosis*, L1.2.2, to be hypersusceptible to clarithromycin, opening an opportunity to repurpose the drug to TB treatment. The sublineage is common in Southeast Asia. Finally, the recent advance in the study of genome-to-genome interactions will allow a better design of tuberculosis vaccines. If successful, similar strategies can be applied for controlling other major infectious diseases, including the spreading of anti-microbial resistance.

Keywork: tuberculosis, whole genome sequencing, Southeast Asia, transmission, disease control

TARGETING METABOLIC FEEDBACK RESPONSE TO ENHANCE THE EFFICACY OF STATIN THERAPY

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Abstract. Neuroblastoma is one of the most common childhood extracranial solid malignancies and the 5-year survival of high-risk neuroblastoma patients remains less than 50%. Many treatments for neuroblastoma have been developed such as surgery, chemotherapy, radiation, stem cell therapy, imnunotherapy. Among them, targeting metabolic pathway also is a promising therapy to treat metabolic vulnerable neuroblastoma. Statins, a class of commonly prescribed medications for their cholesterol-lowering effect, have been reported to associate to reduction of cancer risk and to exert anti-tumor effect. However, statin treatment induces feedback activation of the mevalonate-cholesterol synthesis pathway, leading to statin resistance in some cancer cells. In this study, we found statin induced metabolic feedback response in some neuroblastoma cell lines, which was confirmed via GO analysis, mRNA and protein expression levels of some enzymes related in the mevalonate pathway. On the contrary, caffeine, a well-known antagonist of adenosine receptors, could enhance the efficacy of statin therapy in neuroblastoma. Mechanistically, caffeine blocks statin-induced activation of SREBP2 and downregulates the expression of enzymes in the mevalonate pathway, including HMGCS1, FDFT1, and ACSS2. Note that, istradefylline (KW6002), an adenosine receptor inhibitor, could imitate the synergistic effect of caffeine whereas adenosine supplement partly reversed the inhibitory effect of caffeine on statin-induced metabolic feedback activation. Our study uncovers a molecular link between adenosine receptor signaling and regulation of cholesterol metabolism and suggests a potential treatment for statin resistant neuroblastoma with combination of statins and adenosine receptor.

Keywords: neuroblastoma, statin therapy, caffeine, mevalonate pathway, adenosine receptor

AMYLOID-B ADSORPTION ON ENDOPLASMIC RETICULUM STRESS-MIMICKING MEMBRANES

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Abstract.

Endoplasmic reticulum (ER) stress resulting from abnormalities in protein folding is believed to be a major factor involved in the etiology of Alzheimer's disease (AD). To reduce ER stress, the unfolded protein response (UPR) is induced to cope with misfolded proteins by increasing protein folding ability, decreasing protein synthesis, and promoting ER-associated degradation. If ER stress cannot be alleviated, the UPR triggers cell death. Amyloid-β (Aβ) is prone to misfolding, and the accumulation of Aβ causes AD due to neuronal cell death induced by ER stress.

Two forms of hydrophobic vitamin E (VE), α-tocopherol (Toc) and α-tocotrienol (Toc3), have been proposed to be effective against Alzheimer's disease (AD), the etiology of which is thought to involve endoplasmic reticulum (ER) stress. However, previous studies reported conflicting effects of Toc and Toc3 on the risk of AD. We prepared liposomes mimicking the phase separation of the ER membrane (solid-ordered/liquid-disordered phase separation) and studied how VE can influence the interaction between amyloid-β (Aβ) and the ER membrane.

We found that Toc could inhibit the formation of the solid-ordered phase more significantly than Toc3. Furthermore, Aβ protofibril adsorption on ER stress-mimicking membranes was more strongly suppressed by Toc compared with Toc3.

Therefore, we concluded that VE can relieve ER stress by destabilizing the solid-ordered phase of the ER membrane and subsequently reducing the amount of Aβ adsorbed on the membrane. Moreover, Toc exerted a stronger effect than Toc3.

Keywords: Amyloid -β, Liposome, Vitamin E, Endoplasmic Reticulum (ER) Stress,

FUNCTIONAL AI SCREENING OF ACNE AND ITS PATHOGEN ISOLATES TO REVEAL DETERMINANTS OF SKIN LESION TYPES

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Abstract

Background: Skin disorders, particularly Acne, exhibit diverse clinical manifestations that arise from complex interactions between host genetics, environmental factors, and the microbiome. This study employs a multifaceted approach, combining Functional AI Screening with advanced molecular analyses, to uncover the determinants underlying distinct skin lesion types in Acne and their correlation with pathogen isolates.

Methods: Utilizing a variety of Machine Learning (ML) algorithms including linear, ensemble, and deep learning-based regression models, we integrated genetic, clinical, and environmental data to develop predictive models. Rigorous model validation was performed, and the domain for Acne lesion types was effectively determined. Additionally, pathogen isolates were examined, revealing a common thread—an identified linear plasmid—associated with inflammation.

Results *In vitro* and *in vivo* investigations demonstrated that the presence of the linear plasmid in Acne-causing pathogen isolates leads to a substantial upregulation of inflammatory genes in Normal Human Epidermal Keratinocytes (NHEKs) and distinct fibroblast subsets. This finding underscores the critical role of the plasmid in modulating inflammatory responses within key cell types. Contrary to common expectations, the behavioral variations among C. acnes strain-types were not elucidated by prevalent virulence determinants. Intriguingly, the susceptibility to disease could not be predicted solely based on individual colonization by a specific C. acnes sequence type. This suggests that distinct sequence types do not inherently dictate their inflammatory potential.

Conclusions: In conclusion, this study underscores the significance of Functional AI Screening in deciphering the complex interplay of factors driving diverse Acne lesion types. The correlation of a linear plasmid with inflammation and the interdependence of host-microbiome interactions redefine our understanding of Acne pathogenesis, paving the way for more nuanced and effective treatment strategies.

Keywords: Acne, inflammatory, machine learning, single cell RNA-Seq

ACCELERATED IDENTIFICATION OF RECCURRENT NEOANTIGENS FOR THE DEVELOPMENT OF OFF-THE SHELF CANCER VACCINES

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Abstract. Neoantigens, a type of tumor-specific antigens derived from non-synonymous mutations, have become attractive targets for cancer immunotherapy. Approaches targeting private neoantigens derived from mutations that are unique to individual patients' tumors are costly, labour-intensive and could lead treatment resistance due to antigen loss during clonal evolution. By contrast, vaccines targeting public neoantigens derived from reccurent mutations in cancer driver genes could be designed as "off-the-shelf" vaccines and would be broadly applicable to many cancer patients. However, this therapeutic approach relies on the accurate selection of highly reccurent mutations and identification of immunogenic neoantigens. Here, we developed a pipeline with both computational prediction tools and experimental validation assays, known as NEX-NEO to expedite the identification of public neoantigens in 100 patients with colorectal cancer (n=50) and lung cancer (n=50). By using NEX-NEO, we constructed an off-the-shelf neoantigen panel of 67 neoantigen candidates which cover 63% and 49% of colorectal and lung cancer patients, respectively. Furthermore, we developed a robust screening assay using K562 cells expressing HLA-A*11:01 as antigen presenting cells to validate their immunogenicity. Of the 47 candidates, we identified 23 (48%) immunogenic peptides which are capable of activating CD8 T cells to produce IFN-γ and Granzyme B in PBMC from 10 healthy donors. In conclusion, our study proposed a novel pipeline for the development of off-the-shelf neoantigen vaccines that could benefit a large proportion of cancer paptients.

Keywords: immunotherapy; public neoantigens; off-the-shelf vaccines, colorectal cancer, lung cancer

STEM CELLS AND FEMALE GERMLINE STEM CELLS: APPLICATION IN REPRODUCTIVE AND REGENERATIVE MEDICINE

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Abstract. Two major discoveries in the field of stem cells in the 21st century are the reprogramming of somatic cells into totipotent cells , induced pluripotent stem cells, and the discovery that there are female germ line stem cells in mammalian ovaries. By transferring the nucleus of a somatic cell into an enucleated egg cell, Dolly, the world's first mammal was cloned in 1995, which fundamentally changed the basic knowledge of cell biology. In 2006, Shinya Yamanaka made the groundbreaking discovery that adult somatic cells can be reprogrammed to become pluripotent cells by the introduction of four pluripotent genes into somatic cells. In 2009, Ji Wu and colleagues significantly reported that female germline stem cells collected from neonatal ovaries can develop into mature oocytes that can produce offspring after IVF. Those discoveries open up many application prospects in infertility treatment, transgenic animals, human recombinant proteins, human biological organs, pharmaceuticals, etc. In this talk, I will present advanced methods to improve the success rate of reprogramming a somatic cell into totipotent cells and pluripotent stem cells. I will present the latest methods to increase the success rate of animal cloning and the applications of somatic cell reprogramming in pharmaceutical production (Pharming), regenerative biomedicine, and human bio-organ. In addition, we will discuss the applications of female germline stem cells in assisted reproductive technology.

PHARMACOGENOMICS AND PRECISION MEDICINE: CLINICAL IMPLEMENTATION IN THAILAND

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Abstract. Thailand has advanced PGx research extensively over the last decades and is implementing such research outcome into the clinical practice, especially in eradicating severe cutaneous adverse drug reactions (SCARs). Thailand has become the focal point within the Southeast Asia to advance Pharmacogenomics (PGx) research for clinical implementation in this region. National PGx screening tests were commenced as part of the standard care in Thailand to prevent drug-induced severe cutaneous adverse drug reactions (SCARs), substantially. A multidisciplinary approach, where doctors, dentals, pharmacists, pathologists, medical technologists and medical informatics will work as a team, may help to adopt PGx successful in routine clinical care. Clinical decision support (CDS) system has been upgraded in recent years by insertion of PGx alerts into electronic health record (EHR) software in many developed countries especially in hospital settings where PGx information is routinely used in clinical practice. Approximately 19 PGx laboratories are currently functional in Thailand. Dr. Chonlaphat Sukasem has invented the PPM card, which is a pharmacogenomics identity card (PGx ID). This card may be available in smart mobile phone as app for easy application in clinical settings. In future, it is speculated that preemptive PGx testing through whole genome sequencing (WGS), whole exome sequencing (WES) will be available in patient care in Thailand. The PGx test information obtained from WGS/WES will then be incorporated into the PGx-ID for easy application into clinical settings or even direct consumer PGx testing may be available. The Genomics Thailand initiatives taken by the Thai government and other research institute/hospital that are driving Thailand to implement PGx into the routine clinical practice are explained in this presentation.

HOST-MICROBE INTERACTION: *PLASMODIUM VIVAX* TRYPTOPHAN-RICH ANTIGEN PVTRAG36.6 OF PVFAM-A FAMILY INTERACTS WITH RETICULOCYTE RECEPTOR CD71 FOR INVASION PROCESS OF MALARIA PARASITE

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Abstract. *Plasmodium vivax* is a very common human malaria parasite. It affects millions of people worldwide. Recent literature suggests that the parasite can cause complications in humans leading to deaths. The utmost important information available on the molecular mechanisms of host-parasite interaction, particularly the receptor-ligand interactions that takes place during red cell invasion by the merozoite, is very limited. Such molecular interactions during *P. vivax* infection need to be elucidated in order to understand the parasite biology and then to develop the newer therapeutic reagents to treat/control the disease. In this regard, only a very few host cell receptors and their corresponding parasite ligands are known for *P. vivax* as compared to *P. falciparum*, the parasite which cause cerebral malaria. The limited information available on the interaction mechanisms between host and parasite molecules during the red cell invasion is an impediment to the development of immuno-therapeutics against a most common human malaria parasite *Plasmodium vivax*. The genome of this parasite encodes for a large number of Tryptophan-rich proteins belonging to PvFam-a family. Several of these proteins bind to host erythrocytes utilizing various receptors. However, the identification of these receptors and the mechanisms of their interaction with the respective parasite ligand(s) requires further investigation. We describe here the identification of red cell receptor for one of the important *P. vivax* tryptophan-rich antigen called PvTRAg36.6. This parasite protein is known to be expressed and localized at the apical end of merozoites, has its sequences conserved in the parasite population, and produces cellular and humoral immune responses during *P. vivax* infection. Here, we show that PvTRAg36.6 binds to the human reticulocytes, and anti-PvTRAg36.6 antibodies were able to inhibit the parasite growth during the short-term *P. vivax* in-vitro culture. This protein was found to interact with the reticulocyte specific receptor, CD71, based on LC-MS analysis of proteins obtained during pull down assay. This receptor-ligand interaction was specific as confirmed by direct binding between PvTRAg36.6 and CD71 during solid-phase binding as well as by Surface Plasmon Resonance assays. Furthermore, the eukaryotic expression system; yeast-two-hybrid assay, and rosetting of erythrocytes around mammalian HEK cell expressing PvTRAg36.6 on its surface, also confirmed this receptor-ligand interaction. Dissection of PvTRAg36.6 molecule revealed that the CD71 binding activity was residing in its two different regions i.e. 119 KESSWYTWLKGTKK 132 and 228 YFSLWKDHRRKELD 241. This receptor - ligand interaction involved multiple amino acid residues from these two binding regions of PvTRAg36.6. In conclusion, the parasite ligand PvTRAg36.6 interacts with its reticulocyte receptor CD71 through its two peptide regions, and is involved in red cell invasion. These results may help in developing the immuno- therapeutics against this parasitic infection.

Keywords: protein-protein interaction, *Plasmodium*, malaria, tryptophan-rich antigen, host-pathogen interaction

OPTIMIZATION OF PLATE-BASED DIGITAL PCR ASSAYS TO SIMULTANEOUSLY QUANTITATE HEPATITIS B AND D VIRAL LOADS IN PATIENTS' SERUM

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Abstract. Hepatitis is an infectious disease caused by the hepatitis virus, affecting mainly the liver. Chronic viral hepatitis mostly progresses gradually. In the early stages, there are often no symptoms. However, if not detected early, the disease might have been passed into the stage of cirrhosis or even cancer. Many viruses cause hepatitis. Between them, co-infection or superinfection with HBV and HDV causes patients severe liver disease with rapid progression to cirrhosis and are at high risk of hepatocellular carcinoma. This study aims to optimize plate-based digital PCR assays that will accurately, specifically, and sensitively measure HBV and HDV viral loads in patients' serum simultaneously. Based on previously optimized real-time PCR assays' components and conditions for HBV and HDV quantification, digital PCR assays were developed. The technical sensitivity of the optimized digital PCR procedures was 1-5 copies/reaction for both HBV and HDV. Compared to real-time PCR and real-time RT-PCR assays, HBV and HDV quantitative digital PCR assays showed strong correlation values (R^2_{HBV} = 0.944 and R^2_{HDV} = 0.900). Substantial levels of agreement were indicated by Lin's Concordance Coefficient (CCC_{HBV} = 0.963 and CCC_{HDV} = 0.933). In addition, 2% (2/97) of the clinical samples had low HBV concentrations that the digital PCR was able to identify but not the real-time PCR. The HDV prevalence found in the study was 3% (3/97) by real-time PCR but 5.2% (5/97) by digital PCR. In summary, the optimized digital PCR assays successfully measured hepatitis B and hepatitis D viruses in serum samples with low viral loads that real-time PCR assays were unable to detect.

Keywords: digital PCR, hepatitis B virus, hepatitis D virus, microfluidic array plate, quantification, real-time PCR

CHARACTERIZATION OF PLASMA CELL-FREE RNA IN VIETNAMESE ALZHEIMER'S PATIENTS: A PILOT STUDY

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Asbtract. Introduction Circulating cell-free RNA (cf-RNA) is a potential hallmark for early diagnosis of Alzheimer's Disease (AD) as it represents the genetic expression level, giving insights into the pathological progress at the outset. Profiles of cf-RNA in Caucasian AD patients have been investigated thoroughly, yet there was limited report inspecting changes in the ASEAN groups. This study aimed to address such a gap, expecting to support the development of point-of-care AD diagnosis. **Methods** cf-RNA profiles were analyzed from 20 Vietnamese plasma samples (10 probable AD and 10 age-matched controls). RNA counts were subjected to differential expression (DE) analysis, followed by weighted gene correlation network analysis (WGCNA) to cluster genes (modules) that significantly co-expressed with one another. These modules were then correlated with clinical traits such as cognitive scores, ages, etc to identify pathologically relevant modules. The hub genes - potential drivers of each module - were spotted by cytoHubba via Maximal Clique Centrality (MCC) score ranking. **Results** 136 genes were identified as significant AD hallmarks ($p < 0.05$), with 52 downregulated and 84 upregulated in the AD cohort. 45.6% of these genes are highly expressed in the hippocampus, cerebellum, and cerebral cortex. Notably, all markers related to chronic inflammation were upregulated, and there was a significant shift in all apoptotic markers. Three out of five co-expressed modules were found to be significantly correlated with Alzheimer's status ($p < 0.05$; $R^2 > 0.5$). Functional enrichment analysis on these modules reveals an association with focal adhesion, nucleocytoplasmic transport, and cytoplasmic translation pathways, suggesting the potential participation of these pathways in AD pathology. 15 hub genes were found to be differentially expressed genes with the highest connectivity. Many of these hub genes are associated with inflammation, apoptosis, and signaling (FOXO1, ZFAND6, SASH1, TAOK3), indicating their potential role as key drivers in AD gene expression.

Keywords: *Alzheimer's disease, Molecular biomarkers, Cell-free RNA, inflammation, apoptosis.*

ANTICANCER BIOACTIVITY OF *E. MOLLIS* EXTRACTS TOWARDS SEVERAL HUMAN CANCER CELL LINES

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Abstract. *Elephantopus mollis*, a flowering tree belonging to the Asteraceae family, has been used as a traditional medicine for the treatments of diarrhoea, colic, wound, skin disease, etc. In this study, ethanol, ethyl acetate (EA), petrolium ether (PE) and water extracts of *E. mollis* were prepared and subjected to the quantitative analysis of their second metabolite contents, which revealed the presence of saponin, flavonoid, phenolic compounds, tannin, steroid, and terpenoid in the extracts. Especially, we found that the EA and PE extracts of this plant showed inhibitory effects on lung cancer A549, leukemia HL60, lymphoma Raji, and breast cancer MDA-MB-231 cell lines with IC_{50} < 20 µg/ml but did not have a significant effect on the normal fibroblast cells NIH-3T3. The treatments of A549 and HL60 cells with these extracts induced nuclei fragmentation, activated caspase 3, and caused the increase in the expression of some apoptosis related genes, indicating that EA and PE extracts induce apoptosis in these cancer cell lines. In case of MDA-MB-231 cells, PE extract was able to induce cellular senescence in MDA-MB-231 cancer cells, this assumption was based on characteristic morphological changes (flattened and enlarged cells with dim edge), the increase in β-galactosidase activity, which is an important hallmark of cellular senescence, and the upregulation of cell cycle inhibitory genes CDKN1A (p21) and CDKN1B (p27). Addionally, we also found that the EA extract inhibited the migration, a prerequisite step for metastasis, of MDA-MB-231 cells. These findings provided more scientific evidences for the use of *E. mollis* as a medicine in cancer treatment. However, the specific compound(s) which is(are) responsible for the anticancer activity of the extracts is(are) still unknown and thus, further study on the identification of bioactive compounds is now underway.

Keywords: Elephantopus mollis, anticancer activity, anti-migration, apoptosis, cellular senescence.

THE POTENTIAL FOR DIETARY FACTORS TO PREVENT OR TREAT OSTEOARTHRITIS

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Abstract.

Osteoarthritis is a degenerative disease of the joints and with more than 500 million patients worldwide, it is a major cause of disability. There is currently no pharmaceutical treatment that can slow the progression of osteoarthritis. Increasing age and obesity are the two major risk factors for disease, and with changes in population demographics, osteoarthritis will be an increasing health and socio-economic burden.

Diet is a key modifiable risk factor for the prevention of osteoarthritis. Population studies show that diet or dietary patterns associate with joint health, with a diet rich in fruit, vegetables and whole grains having a positive impact. However, there is a lack of supporting evidence for the effect of specific components of the diet and the molecular mechanisms by which these act.

Our work has focused on sulforaphane, a compound derived from the consumption of cruciferous vegetables, particularly broccoli. We have shown that sulforaphane inhibits surrogate markers of osteoarthritis in cells and tissue explants and reduces osteoarthritis in a murine model of disease. Experimental medicine showed that a high broccoli diet leads to sulforaphane and its metabolites being detectable in synovial fluid, altering the proteome. A proof-of-principle clinical trial in osteoarthritis shows that consumption of broccoli improves pain and physical function, though patient numbers were small.

This seminar will review research on dietary factors in osteoarthritis and the levels of evidence for their efficacy in prevention and treatment of disease. This will include potential mechanisms of action and differences to conventional pharmaceuticals. I will present detailed data from our own research on the role of sulforaphane in osteoarthritis. The issues that need to be addressed in research on dietary factors in osteoarthritis and the problems of undertaking clinical trials in this area will also be discussed, as well as a look to the future.

Keywords: Osteoarthritis, diet, nutrition, cartilage, bioactive

THERAPEUTIC EFFICACY OF MESENCHYMAL STEM CELL PRODUCTS BASED ON SCM TECHNOLOGY FOR INCURABLE DISEASE

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Abstract. SCM Lifescience, Inc. is a Biopharmaceutical company specializing in cell therapy using mesenchymal stem cells. The company is developing therapeutic products for rare and incurable diseases based on the "subfractionation culturing method (SCM)", a unique stem cell isolation method. Recently, two clinical studies were completed: one Phase 1/2a for acute pancreatitis, the other one Phase 1/2 for atopic dermatitis. Here, we want to share our clinical results, the company's technology, and some possibility for the commercialization of stem cell therapies.

THE VENOMOUS SNAKES AND RESEARCH PROGRAM TO PRODUCE ANTIVENOMS TO SAVE THE LIVES OF VICTIMS ARE BEING POISONED BY SNAKE VENOM IN VIETNAM

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Abstract. Vietnam (VN), an agricultural & tropical country. It is very favorable for the development of 9 poisonous snakes, harming up to 30,000 (thirty thousand) people per year with a very high mortality rate (19,5%). Because there is no antivenom (AV), *the most effective drug, widely distributed around the world since many years (WHO).* In 1895, Dr. A. Calmette, the first scientist in the World invented AV right at the Pasteur Institute in Saigon. Yet, more than 100 years later, there is still no AV in VN. *The aim of this research program is manufacturing specific AV effects with the venom of each species of venomous snakes circulating, and its clinical application to save the lives of many thousands venomous snake victims in VN. Method:* Survey and selection of dangerous snakes in VN (Med of Aberdeen U). Antigen production, equine immunology, plasma extraction, AV purification, quality control (Med: Liverpool U). AVs clinical application of first aid for snake envenoming patients under the guidance of Prof. D.Warrell. Oxford U. *Result: Vietnam Institute on Toxicology (VIT)* Has successfully made most of the specific AVs to each major venomous snake species: *Naja kaouthia, Calloselasma rhodostoma (Patents have been granted, 2003).* The Avs (*Bungarus candidus, King Cobra, Naja siamensis*) have met the standard of pharmacopoeia VN (III). The National Institue for Control of Vaccine & Biologicals, MOH, VN has issued the Certificate of inspection results. Over 3,000 (three thousand) of very severe envenoming victims at the hospitals across the country have been saved safety and effectively by VIT's AV. However, snakebite is a neglected tropical disease. Urgently requesting the attention of the State, Charities and Benefactors to support the development of AVs production in time to save the lives of thousands of venomous snake victims each year in VN.

Keyword: HTKNR

MANUFACTURING CHALLENGES FOR BIOLOGICS PRODUCTION IN JAPAN

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Abstract Biologics include a wide range of products such as vaccines, blood and blood components, antibodies, gene therapy vector, cellular medicine, tissues, and recombinant therapeutic proteins. Biologics can be composed of sugars, proteins, nucleic acids, or complex combinations of these substances, or may be living entities such as cells and tissues. Mammalian cell lines are important host cells for the industrial production of therapeutic proteins owing to their capacity for correct folding, assembly, and post-translational modification. With the increase of therapeutic antibodies, bio-similar, bio-better, and/or artificial therapeutic antibody formats, the Chinese hamster ovary (CHO) cell is the most dependable host cell for the industrial production of therapeutic proteins. CHO cell was established from a Chinese hamster (*Cricetulus griseus*) in 1957 by Puck and cultivated in vitro conditions for more than 50 years. In this presentation, I focused on the recent progress of CHO cell-based platform cell and cell culture engineering for biopharmaceutical industries and introduce the activities of the Manufacturing Technology Association of Biologics (MAB) (non-profit mutual benefit organization, including 36-Companies, 5-Universities, 2-National Research and Development Agencies, 3-Organizations) in Japan.

Keywords: biologics, recombinant protein, therapeutic antibody, Chinese hamster ovary cell, cell engineering, cell culture engineering

BIOLOGICAL ACTIVITY EVALUATION OF PLANT EXTRACTS IN INDIGENIOUS MEDICINE AGAINST INFLAMMATION AND OSTEOARTHRITIS

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Abstract. Traditional herbal medicine has been playing an essential role in healthcare globally, and especially in a country that is rich in natural herb such as Vietnam. There are several remedies that use different kinds of plants in treatment against inflammation, osteoarthritis, cancer, etc. To provide a scientific basis for medicinal applications in the treatment of diseases related to inflammation and bacteria, medicinal plants from the southern part of Vietnam were collected and evaluated for their antioxidant, antibacterial, and anti-inflammatory activities. Our results demonstrated that most of the plant extracts tested showed low or average anti-bacterial activity (MIC ranging from 250-500 µg/mL against *E. coli*, *P. aeruginosa*, *A. baumanni,* and *C. albicans*) with dichloromethane and methanol extracts of *Caesalpinia sappan* (TMA and TMB) having the best potential against biofilm development on *S. aureus* (reducing more than 50% of biofilm formation compared with negative control). On the other hand, potential plants were also evaluated for their anti-inflammatory activity and the capability to reduce osteoarthritis. Methanol extract of *Dracaenae* cambodianae Pierre ex Gagnep (HGB) and dichloromethane extract of *Acanthopanax aculeatus* Seem (NGBTA) had inhibitory effect on the expression of pro-inflammatory iNOS, IL-1β, IL-6 and TNF-α genes (HGB reduced expression of iNOS, IL-1 β, IL-6 by 3.4, 1.4 and 1.9 times on RAW264.7 cell model compared with untreated cells induced by LPS; while NGBTA downregulated expression of iNOS, IL-1 β, IL-6 and TNF-α by 5.5, 1.1, 2.3 and 3.3 times, respectively). Moreover, these extracts also presented strong osteoarthritis inhibition ability on SW1353 cells induced by IL-1β. Through these results, potential plant extracts are being further investigated for their effects on mouse model of osteoarthritis as well as being studied to develop a product to treat atopic dermatitis.

Keywords: anti-bacterial, anti-inflammatory, osteoarthritis, plant extract.

CONSTRUCTION OF AN ALL-IN-ONE PRIME EDITING BACULOVIRUS BY USING A NOVEL CRISPR-ASSOCIATED TRANSPOSON FOR HUMAN GENOME EDITING

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Abstract. The Prime Editing (PE) system is a novel genome editing tool that can precisely generate point mutations, insertions, deletions and their combinations in the human genome without causing DNA double-strand breaks. However, one limitation is that the size of the PE system is too large (>10 kb) for efficient delivery into human cells. To deliver large PE systems into target cells, we explored the potential of using baculovirus, a promising viral vector. The bac-to-bac system is commonly used to construct recombinant baculovirus. However, bac-to-bac only allows the cloning of transgene into a pre-designed target site on the baculovirus genome. Here, we have developed a novel CRISPR-associated transposon called SHOT. SHOT allows RNA-guided DNA insertion up to 14 kb into the target site and works with bac-to-bac. Finally, we constructed an all-in-one PE baculovirus by using SHOT and bac-to-bac. This all-in-one PE virus showed remarkable efficacy, inducing 69.7% CTT insertions, 30.2% T deletions, and 52.7% T to G base editing on the *hek3* gene in Hek293T cells. We confirmed that using the all-in-one viral vectors has better editing efficiency than using plasmids as vectors. Ultimately, this study significantly enriches the toolkit for baculovirus construction and promises to advance human genome editing research.

Keywords: Prime editing, baculovirus, gene delivery, CRISPR, Transposon

PRIMARY EVALUATION OF *BOESENBERGIA PANDURATA* RHIZOME ROOT EXTRACT ON SOME SENESCENT CHARACTERISTICS OF AGED HUMAN FIBROBLAST

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Abstract.

Today, aging is one of the concerns of society due to the increasing demand for beauty. Using ingredients extracted from plants that affect aging is an outstanding research direction. *Boesenbergia pandurata* (Roxb.) Schltr or *Boesenbergia rotunda* (L.) Mansf is a perennial herb with great potential in research into its effects on aging.

In this study, *Boesenbergia pandurata* were soaked in ethanol for 72 h and freeze-dried to obtain the extract of *Boesenbergia pandurata*. Then, the effect of the treated extract on senescent fibroblasts was induced by etoposide to evaluate some characteristics of senescent cells such as cell size, expression of SA-ß-galactosidase enzyme, the expression of aging-related genes such as *p16, p21, p53* and finally, the characterization of fibroblasts through the CD90 marker. In addition, we determined the antioxidant potential of *Boesenbergia pandurata* extract through DPPH assay to initially determine the mechanism of the senescent process of this extract. The results showed that senescent fibroblasts were treated with finger root extract at a concentration of 15 µg/ml, some senescent fibroblast characteristics were improved, such as cell size reduction (decreased 27%), decreased expression of SA-β-Galactosidase enzyme (decreased by 1,2 times), reduced gene expression of *p16, p21, p53* and retained the characteristics of fibroblasts through the positive rate for marker CD90. Besides, *Boesenbergia pandurata* extract had antioxidant activity through DPPH free radical scavenging experiment with EC50 of 337,6 µg/ml ± 28,4 µg/ml. From the above results, we have demonstrated an improvement in some characters of the senescent cellular of *Boesenbergia pandurata* extract at a concentration of 15 µg/ml.

Keywords: cellular senescence, human fibroblast, Boesenbergia rotunda, finger root

POTENTIAL APPLICATIONS OF THE AIR-DRYABLE QPCR MIX IN THE PRODUCTION OF REAL-TIME PCR DIAGNOSTIC KITS

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Abstract. qPCR mix is a mixture consisting of important components that determine the efficiency of Real-time PCR reaction. The appearance of lyophilized qPCR mix has helped to optimise storage conditions and transportation costs. Later, the appearance of the Air-dryable qPCR Mix also simplifies the production process compared to freeze-drying. In this study, we evaluated the potential application of Air-dryable qPCR Mix in kit production against HBV targets (Air-dryable HBV qPCR Mix). The application efficiency of the dried mix was evaluated through analytical performance (Linear Range, LOD) and stability after 12 months of storage at different temperatures. The results show that Air-dryable HBV qPCR Mix has the same or better analytical performance than liquid HBV qPCR mix with a linear range of 5-10^9 copies/µL, PCR amplification efficiency (E%) is 100.22%, R^2=0.9991 and LOD_{95} is 44 copies/reaction. Air-dryable HBV qPCR Mix can be stored stably at -20^{o}C and cool temperature (2-8^{o}C). In addition, the product remains stable during transport at ambient temperature for 7 days. The above results show the potential application of Air-dryable qPCR Mix in the production of Real-time PCR kits to solve problems in terms of production, storage, and transportation of biological products.

Keywords: *Air-dryable HBV qPCR Mix*, *qPCR mix, Real-time PCR.*

ELECTRON BEAM IRRADIATED – GUM ARABIC STABILIZED SELENIUM NANOPARTICLES INDUCES APOPTOSIS IN AGS GASTRIC CANCER CELLS VIA CONTROLLING THIOREDOXIN AND GLUTAREDOXIN EXPRESSION

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Abstract. Gastric cancer (GC) is the fifth most common malignancy worldwide and has a poor prognosis with a 5-year survival rate of 36%. The chemotherapy for gastric cancer is still facing to real trouble of drug resistance and adverse side effects. Various types of selenium nanoparticles (SeNPs) were studied and suggested to be potential in cancer treatment due to their selective cytotoxicity. However, the effect of selenium nanoparticles, particularly, electron beam irradiated – gum arabic stabilized SeNPs (EB-GA SeNPs), on AGS gastric cancer cells is still not uncovered. In this study, we present our data which showed the high potential in applying EB-GA SeNPs for gastric cancer treatment. EB-GA SeNPs showed significant toxicity on AGS cells with IC_{50} as 4.3 μg/mL and selectivity index (SI) as 5.2 in comparison to BJ-5ta normal cells. Under the effect of EB-GA SeNPs, AGS cells changed its features such as nuclei fragmentation, chromatin condensation and caspase-3 activation. Based on fluorescence staining method, we determined that EB-GA SeNPs increased reactive oxygen species (ROS) levels in AGS cells. Consequently, by RT-qPCR we found the transcriptional increasing of either thioredoxin, glutaredoxin related genes such as *Trx, TrxR, Grx-GR* genes or *cJUN*, *NFκB* genes. The results revealed that EB-GA SeNPs triggered higher expression of thioredoxin, glutaredoxin system and by which led to the accumulation of ROS in AGS cancer cells. The cellular oxidative stress induced by EB-GA SeNPs pulled along the significant upregulation of JNK/cJUN and NFκB pathways and resulted as apoptosis activation.

Keywords: Selenium nanoparticles, gum arabic, AGS gastric cancer cells, apoptosis, thioredoxin system, glutaredoxin system.

A SINGLE CELL TRANSCRIPTOME COMPARISON BETWEEN COMMON CHO CELL LINES AND A NOVEL CELL LINE DERIVED FROM LUNG TISSUE OF CHINESE HAMSTERS (CHL-YN CELLS)

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Abstract. Chinese hamster ovary (CHO) cells play an important role as a heterologous host in the commercial-scale production of protein pharmaceuticals. The adaptability of CHO cells to high cell density processes has ensured their successful application for this role. Cell culture times are generally long (e.g., 12 days for fed-batch cultures), and advances have further extended culture times. For example, perfusion or continuous manufacturing processes (e.g., 60 days) is widely recognized as the next generation biomanufacturing platform to achieve superior product quality and reduce manufacturing costs. A potential problem with the use of long-term culture to produce therapeutic proteins is the increase in cellular heterogeneity over time. Deep characterization of heterogeneity within cell line cultures, such as single-cell sequencing, would allow studying cooperative and competitive interactions between cell populations and the mechanisms underlying relevant cellular functions. In this study, we performed single-cell transcriptome sequencing (scRNA-Seq) of serum-free suspension-adapted CHO-K1 and CHO-S cells and a novel cell line derived from lung tissue of Chinese hamsters and adapted to serum-free medium (Chinese hamster lung (CHL)-YN cells). The degree of cellular heterogeneity was determined based on gene expression analysis and mitochondrial genome sequencing. According to expression analysis, CHL-YN and common cell lines (CHO-K1 and CHO-S) had different gene expression profiles at the single-cell level. In addition, for each cell line, the clonality of the cells based on the mitochondrial genome sequence and the expression pattern of endogenous viruses were measured.

Keywords Chinese hamster ovary (CHO) cells, *single cell transcriptome sequencing, heterogeneity, mitochondrial genome, clonality*

DATABASE CHEMISTRY IN GENOMICS-BASED SAFETYAND QUALITY EVALUATION STUDY OF BIOLOGICS

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Abstract. ICH Q5A (R2) is in progress. In the text, the Next Generation Sequencing (NGS) Technology is applicable for broad and specific virus detection. For the virus safety study using NGS, the reference genome of the host cell is necessary. Any technologies depending on some genome would have qualification to be called genomics; the applicability of genomics for biologics production is not restricted to viral sequences monitoring. The genomics would be potentially able to brush up safety and quality evaluation studies of biotechnology products; viral safety (using NGS), host cell protein control (using LC-MS/MS), products heterogeneity control (using RNAseq), cellular heterogeneity control (using scRNAseq) and process reproducibility monitoring (using NGS and/or LC-MS/MS). These genome-scale measuring methods must depend on databases: genome sequences, genome annotations, transcriptome sequences, protein sequences, ion liberality. The optimization of databases is depending on the purpose of each measurement, simple benchmarking would not work. Here, we assembled several CHO genomes using Hi-Fi reads and performed Hi-C, RNAseq, sc RNAseq, BS-seq, IDA-MS, SWATH-MS, virus spike-in test and observed using transmission electron microscopy. We managed the databases to increase true positives and decrease false negatives. We also developed novel database combination methods which decrease false negatives.

PROMISING POTENTIAL OF A NOVEL ANTI-CANCER DRUG FROM SNAKE VENOM IN VIETNAM

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Abstract. Vietnam (VN) is a tropical and agricultural country. It is very favorable for the development of 9 poisonous snakes. In this, the *Trimeresurus mucrosquamatus (TM)* is the one of the most dangerous snakes of *Viperidae* with the very severe coagulopathy and the mortality rate is 20%. Derived from clinical practice, the idea of researching and manufacturing anti-cancer biological products from the *TM* venom in VN was risen. The aim of the study was purification of *disintegrin* from *TM* venom (*TMd*.VN), determination of its molecular weight (MW), structure, and anti-cancer activity. The collection and lyophilization of *TM* venom in VN and its protein concentration was determined by Bicinchoninic Acid (BCA) Protein Assay. High Pressure Liquid Chromatography (HPLC), Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE), Mass spectrometry (MS) analysis and sequencing were used for purification and determination of MW and structure of *disintegrin* from *TM* venom (*TMd.*VN). Standard cell biological methods were employed to characterize the *TMd*.VN's abilities of anticancer activities included the adhesion on cancer cell surfaces, inhibition of platelet aggregation, migration, invasion and anti-angiogenesis of cancer cells (*in vitro*). *Results:* The (*TMd*.VN) appeared at the Peak N^{O}:7 of HPLC. It was also indicated a single band on SDS-PAGE gel. Its MW is 7.30 kDa with the structure and sequence are a monomer containing 68 amino acids, an RGD motif (position 49-51) and 5 disulfide bonds. The anti-cancer activity of the (*TMd*.VN) was very strong and safe. It is a reliable scientific foundation and a potential agent to meet the requirements of clinical anti-cancer drugs.

Keywords: TMd.VN anti-cancer disintegrin.

INTEGRATED DNA AND RNA SEQUENCING IMPROVES NEOANTIGEN DETECTION IN COLORECTAL CANCER PATIENTS

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Abstract. Studies have demonstrated that tumor-specific antigens, known as neoantigens, originating from non-synonymous mutations within tumor DNA, are promising targets for precision immunotherapies which can improve the life expectancy of cancer patients. These therapeutic approaches heavily rely on accurate identification of cancer mutations using DNA sequencing (DNAseq) data. However, current workflows tend to provide a large number of neoantigen candidates, of which only a limited number elicit efficient and immunogenic T-cell responses suitable for downstream clinical evaluation. To overcome this limitation and increase the number of high-quality immunogenic neoantigens, we propose integrating RNA sequencing (RNAseq) data into the mutation identification step in the neoantigen prediction workflow. In this study, we characterize the mutation profiles identified from DNAseq and/or RNAseq data in tumor tissues of 25 patients with colorectal cancer (CRC). We detected only 22.4% of variants shared between the two methods. In contrast, RNAseq-derived variants displayed unique features of affinity and immunogenicity. By performing ELISpot assay, we further showed that neoantigen candidates identified by RNAseq data significantly increased the number of highly immunogenic neoantigens that would otherwise be overlooked if relying solely on DNAseq data. In conclusion, this integrative approach holds great potential for improving the selection of neoantigens for personalized cancer immunotherapy, ultimately leading to enhanced treatment outcomes and improved survival rates for cancer patients.

Keywords: immunotherapy; somatic mutation; neoantigen; prediction.

EXPLORING PHYTOCHEMICAL DIVERSITY OF VIETNAMESE SMILAX GLABRA ROXB EXTRACT AND ITS ANTIOXIDANT EFFECTS IN ZEBRAFISH

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Abstract. This research explores the phytochemical constituents and antioxidative capabilities of an ethanol extract obtained from *Smilax glabra* Roxb (SGB). Comprehensive analysis revealed a diverse spectrum of bioactive compounds, including steroids, phenolics, flavonoids, terpenoids, aldehydes, and alkaloids. Notably, the identified compounds encompassed 11-O-p-Coumarylnepeticin, Bufotalinin, 5-Dehydrokarounidiol, Bufalin, and 5-O-Caffeoyl quinic acid butyl ester, each exhibiting distinct biological activities. To evaluate its antioxidative potential, zebrafish larvae were subjected to arsenic ($NaAsO_2$) and hydrogen peroxide (H_2O_2) exposure. The extract exhibited a remarkable capacity to enhance survival rates, indicating its efficacy in mitigating oxidative stress. Molecular investigation centered on the gene expression profiles of glutathione S-transferase pi 1 (*gstp1*) and peroxiredoxin 1 (*prdx1*), revealing intriguing antioxidative mechanisms that may extend beyond the classical Keap1/Nrf2 pathway. These findings unveil the multifaceted bioactivity of SGB extract, underscoring its promise as a therapeutic candidate for conditions linked to oxidative stress. This study not only advances insights into its mechanisms but also establishes a foundation for future research endeavors aimed at harnessing its therapeutic potential.

Keywords: phytochemical, antioxidant, *Smilax glabra* Roxb, Keap1/Nrf2, oxidative stress

POTENTIAL OF *BACILLUS SUBTILIS* ENDOSPORE IN ORAL VACCINE DEVELOPMENT AGAINST *STAPHYLOCOCCUS AUREUS*

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Abstract. *Bacillus subtilis* spores are proven to be safe for oral usage. They can be engineered to display heterologous antigens and applied in vaccine development. The spores themselves also have adjuvant properties. *S. aureus* is a global burden that causes various infectious diseases in human and animals. Suitable strategies with the One Health approach are required to control the transmission of this pathogen. Therefore, we aim to study the potential of *B. subtilis* spore as a vector for an oral vaccine against *S. aureus*, which can be further advanced for both human and livestock applications. In this research, we display antigens of *S. aureus* that are involved in iron acquisition and host immune resistance, including alpha-toxin and iron-regulated surface protein, on the *B. subtilis* spore surface and deliver them into the mice model. The target protein was translationally fused with the anchor protein CotB for surface display. Recombinant vectors were cloned in *E. coli* and transferred into *B. subtilis*. PCR was the method for checking the integration of the interested gene into bacterial chromosomes via a double cross-over event. *B. subtilis* sporulation was stimulated in DSM medium and observed under the microscope. The surface display of fusion protein was confirmed by sporeELISA. Next, three doses of *B. subtilis* spores were orally administrated to Swiss mice. The level of IgG in serum and IgA in feces were analyzed by ELISA. The significant increase in IgG and IgA levels indicated that antigens had been delivered into the mice gut by *B. subtilis* spore vector and triggered specific antibody production. The overall data provided an evaluation of *B. subtilis* spore displaying *S. aureus* antigens on the surface for oral vaccine development.

Keywords*: Bacillus subtilis, spore, Staphylococcus aureus, oral vaccine, alpha toxin, iron-regulated surface protein*

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APPLICATION OF LED IRRADIATION ON GROWTH OF *PANAX VIETNAMENSIS* IN *IN VITRO* CULTURE

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Abstract: *Panax vietnamensis Ha et Grushv*., a Vietnamese ginseng generally distributed in the Ngoc Linh mountain areas, is one of the most precious ginseng with a high saponin content. To produce large-scale plantlets of Vietnamese ginseng, plant cell and tissue culture methods have been applied. In which, lighting plays an essential role in controlling plant morphogenesis and metabolism in micropropagation. Light—emitting diodes (LEDs) have recently emerged as an alternative for fluorescent lamps (FLs) in *in vitro* micropropagation. LED emits at wavelengths suiting with plant photoreceptors and may provide more production, plant morphology, and metabolism. However, there has been little discussion about using LED *in vitro* cultures of *Panax vietnamensis Ha et Grushv*. This study examines the influence of various LED systems on hairy root growth, buds, and plantlets development of Panax vietnamensis. The hairy roots, cluster of buds, and plantlets were exposed to 7 different lighting conditions: 5 LED systems consisting of a mixture of 460 nm and 640 nm LEDs with different ratios, an FLs lamp, and a control treatment. The results present that the most suitable lighting system for the rapid multiplication of buds and plantlet development of P. vietnamensis is the LED system with the ratio of 60% red and 40% blue, and the fresh weight and morphogenesis of Panax vietnamensis hairy roots is the blue LED treatment correspondingly.

Keywords: Panax vietnamensis Ha et Grushv., LED, in vitro micropropagation, biomass.

QUALITY AND PRECLINICAL EVALUATION OF DIPHTHERIA – TETANUS - ACELLULAR PERTUSSIS TRIVALENT VACCINE (DTAP) DEVELOPED BY THE INSTITUTE OF VACCINES AND MEDICAL BIOLOGICALS (IVAC)

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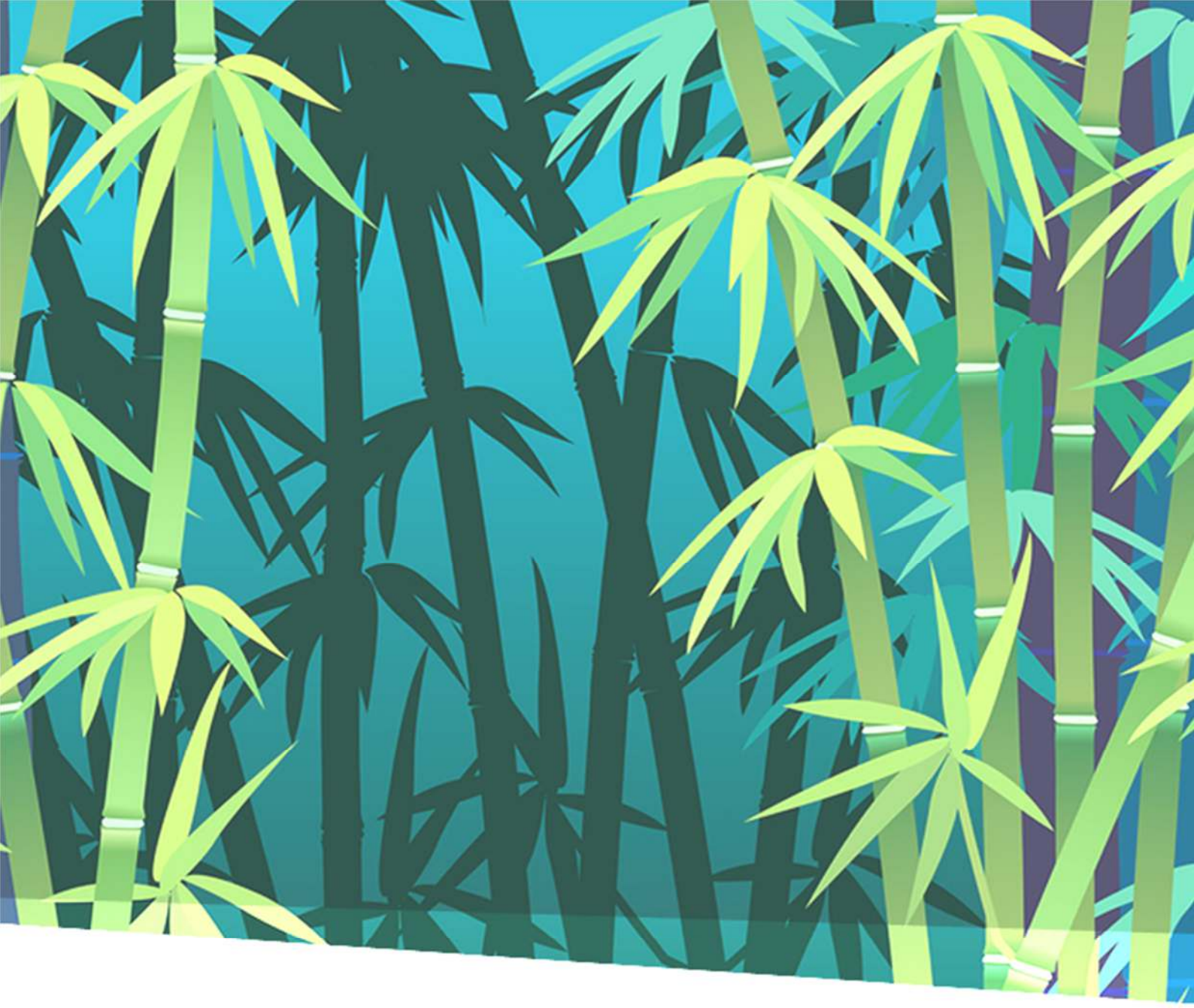
Abstract. Diphtheria - Tetanus - Acellular Pertussis (DTaP) vaccine was developed with a single dose of Diphtheria (20 Lf), Tetanus (10 Lf), PT (5 µg), FHA (10 µg) and aluminum hydroxide (0.9 mg). The DTaP vaccine has been qualitatively and preclinically evaluated according to World Health Organization (WHO) guidelines. The results showed that the DTaP vaccine meets the requirements of sterility, safety, potency and physico-chemical according to WHO recommended standards. The potency of DTaP vaccine was still stability after 4 weeks at 37^{o}C, 3 months at 25°C and 24 months at storage conditions (2-8^{o}C). Evaluation of repeated dose toxicity in rabbits showed that 100% of rabbits were alive and developing normally, feed consumption and average weight gain of a rabbit were within normal physiological limits and there was no difference between the group of rabbits receiving the vaccine and placebo. DTaP vaccine is safe, well tolerated by intramuscular injection and has a low rate of post-injection local reaction (10%) that completely disappears 48 hours after injection. Laboratory evaluations (hematology, selective blood biochemistry) and histopathologic examination were not significantly different between the two groups of rabbits vaccinated and placebo. In terms of immunogenicity, all vaccinated rabbits had an IgG antibody response with an increase of GMTR ≥ 4 times for all antigenic components. Preclinical and qualitative evaluation records show that the DTaP vaccine produced by IVAC is sufficient for clinical trials in human.

Keywords: DTaP vaccine, preclinical, toxicity, IVAC



3. Tissue Engineering and Biomaterials

INNOVATIVE ADHESIVE PLATFORM BIOMATERIAL FOR EFFECTIVE BONE REGENERATION

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Abstract. Marine mussel adhesion is known to be mediated by adhesive proteins, which are secreted through the mussel byssus and have great potential as biologically and environmentally friendly adhesive biomaterials due to their biocompatibility and biodegradability. In addition, mussel adhesive proteins have strong adhesion ability even on wet and underwater surfaces due to their unique amino acid arrangements and composition. However, research using the natural amino acid composition has been limited due to extreme difficulties in obtaining sufficient quantities of mussel adhesive proteins for practical applications and commercialization. Previously, we successfully produced redesigned mussel adhesive protein using a bacterial expression system and this bioengineered mussel adhesive protein showed significant adhesion ability and biocompatibility. In this talk, I will present our research team's efforts in developing and evaluating mussel adhesive protein as an innovative adhesive biomaterial in diverse tissue engineering applications, especially in bone regeneration.

Keywords: Adhesive biomaterial, Mussel adhesive protein, Bone regeneration, Bone grafting binder, Tissue engineering

AN ALIGNED POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) SCAFFOLD FIXED WITH FIBRONECTIN TO ENHANCE THE ATTACHMENT AND GROWTH OF HUMAN ENDOTHELIAL PROGENITOR CELLS FOR VASCULAR TISSUE ENGINEERING

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Abstract. Repair and regeneration of vascular tissue is a crucial current research focus in the fields of biomedical engineering and regenerative medicine. Numerous studies revealed that cells are required to grow on an appropriate extracellular matrix to maintain or enhance functionality. In the present study, various surface modification methods were evaluated to fix fibronectin on the surface of a bio-based and aligned poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) film for vascular tissue engineering. After chemical modification, the properties of the fibronectin-fixed PHBV films were examined and compared with the original films, including -NH2 group expression, contact angle, mechanical properties, and fibronectin binding amount. Then, cytotoxicity and biocompatibility were measured by culture with L929 cells and endothelial progenitor cells (EPCs) of the fibronectinfixed PHBV films. In addition, cell morphology, cell growth kinetics, acetylated low-density lipoprotein uptake ability, lectin binding ability and specific gene expressions of cultured EPCs on fibronectin-fixed PHBV films were also analyzed. Taken together, our data demonstrated that the surface of the aligned PHBV films could be successfully modified to immobilize fibronectin. Importantly, EPCs cultured on the fibronectin-fixed PHBV films showed excellent cell biocompatibility, a rapid proliferation rate, an aligned growth direction and correct cell functions. We believed that fibronectin-fixed PHBV films can serve as a potential scaffold for vascular tissue engineering.

Keywords: endothelial progenitor cell, fibronectin, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), surface modification, vascular tissue engineering

INJECTABLE THERMOGEL INCORPORATING REACTIVE OXYGEN SPECIES SCAVENGER AND NITRIC OXIDE DONOR TO ACCELERATES THE HEALING OF DIABETIC WOUND

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Abstract. The healing of diabetic wounds is challenging due to a redox imbalance. In this study, a thermogelling system AR-ACP hydrogel, with encapsulated biosafe nitric oxide (NO) donor L-arginine, and resveratrol as ROS scavenger, is established for sustainable wound therapy in diabetic condition. The innovated AR-ACP hydrogel dressing were liquid in cold condition and undergo a sol-to-gel transition when exposed to body temperature, allowing the hydrogel to be covered wounds with various size or shape. In addition, the combination of L-arginine and resveratrol performed prominent effect in anti-oxidative activity. The elimination of superoxide anion synthesizing from the activation of immunity cells due to resveratrol prolonged the stability of NO-proangiogenic factors generated from L-arginine. AR-ACP hydrogel also exhibited significantly better protection for cells against external oxidative stress as compared to single loading hydrogel. Furthermore, the dual hydrogel system endowed their outstanding features such as hemocompatibility, non-skin irradiation as well as antibacterial activity. In the in vivo diabetic mice model, AR-ACP hydrogel reveals significant wound closure as compared to saline or native ACP hydrogel treated group. At 18 days, AR-ACP hydrogel promote epidermal regeneration with comparable to undamaged skin. In addition, the synergy between L-arginine and resveratrol in ACP hydrogel facilitated the neovascularization at the early stage, resulting in the higher balance in cellularity growth and collagen deposition in the dermal layer than control groups. Taken together, our findings demonstrate that the use of customized ACP-based hydrogel, without the additional L-arginine and resveratrol, resulted in significant skin regeneration in condition of diabetic. This highlights the potential of such AR-ACP hydrogel formulations as innovative treatments for chronic wounds.

Keywords: Thermoresponsive hydrogel, nitric oxide, oxidative stress, diabetic wound

NEW GENERATION MAGNESIUM BASED BIODEGRADABLE IMPLANTS FOR ORTHOPEDIC APPLICATIONS

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Abstract. Magnesium-based biomaterials have been in extensive research for orthopedic applications for decades due to their optimal mechanical features and osteopromotive nature; nevertheless, rapid degradation restricts their clinical applicability. In this study, pristine magnesium was purified (P-Mg) using a melt self-purification approach and reinforced using indigenously synthesized nanohydroxyapatite (HAP, 0.6 wt.%) and strontium substituted nanohydroxyapatite (SrHAP, 0.6 wt.%) using a low-cost stir assisted squeeze casting method to control their degradation rate. Using electron back-scattered diffraction (EBSD) and X-ray diffraction (XRD) examinations, all casted materials were carefully evaluated for microstructure and phase analysis. Mechanical characteristics, in vitro degradation, and in vitro biocompatibility with murine pre-osteoblasts were also tested on the fabricated alloys. For in vivo examination of bone formation, osteointegration, and degradation rate, the magnesium-based alloys were fabricated as small cylindrical pins with a diameter of 2.7 mm and a height of 2 mm. The pins were implanted in a critical-sized defect in a rat femur shaft (2.7 mm diameter and 2 mm depth) for 8 weeks and evaluated by micro-CT and histological evaluation for bone growth and osteointegration. When compared to P-Mg and P-MgHAP, micro-CT and histological analyses revealed that the P- MgSrHAP group had the highest bone formation towards the periphery of the implant and hence maximum osteointegration. When the removed pins from the bone defect were analyzed using GIXRD, they displayed hydroxyapatite peaks that were consistent with bio-integration. For P- Mg, P-MgHAP, and P-MgSrHAP 8 weeks after implantation, in vivo degradation rates derived from micro-CT were around 0.6 mm/year, 0.5 mm/year, and 0.1 mm/year, respectively. Finally, P-MgSrHAP possesses the requisite degradation rate and sufficient mechanical and biological properties, indicating that it can be used in the development/fabrication of biodegradable bioactive orthopedic implants.

Keywords: Magnesium purification, Reinforcement, Degradation rate, Nanocement, Bone regeneration

SPLIT DCAS12A ACTIVATOR FOR LNCRNA H19 ACTIVATION TO INDUCE BMSC CHONDROGENESIS AND PROMOTE CALVARIAL BONE HEALING

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Abstract. Healing of large calvarial bone defects in adults remains to be a challenging task. Although implantation of bone marrow-derived mesenchymal stem cells (BMSC) or adipose-derived stem cells (ASC) - based therapy is still far from perfect, thus requiring new methods to stimulate cell differentiation so as to further augment calvarial bone healing. In recent years, H19 was exposed to enhance chondrogenic differentiation, but its function in mesenchymal stem cells and bone regeneration *via* the endochondral ossification pathway has yet to be explored. In this study, we verify whether using a Split dLbCas12a platform is able to activate H19 expression in BMSC or ASC and promote calvarial bone formation. We constructed the Split dLbCas12a system where dCas12a is expressed as N domain and C domains, each of which is fused with two transcription activators consisting of p65 and HSF1 (p65-HSF1). After expression, the split N and C domains spontaneously assemble at the split position to become Split dLbCas12a activator with 4 synthetic transcription activators to activate H19 expression. We further packaged the entire split dCas12a activator system (13.2 kb) into a hybrid baculovirus vector to enhance and prolong H19 activation. As the result, we demonstrated that the split dLbCas12a system activated H19 expression with higher efficiency than the full-length dLbCas12a in rat ASC and BMSC. Furthermore, we showed that the Split dLbCas12a-mediated H19 activation stimulated rat BMSC chondrogenesis and inhibited adipogenesis. Consequently, the engineered BMSC promoted in vitro cartilage formation and augmented calvarial bone healing in rats. Thus, our results demonstrated that Split dCas12a-mediated H19 activation drives rat BMSC towards chondrogenesis lineage and offers a potential therapeutic target to regenerative medicine for bone regeneration.

Keywords: Bone healing, CRISPRa, Split dCas12a, H19, lncRNA

FABRICATION OF PCL/GELATIN NANOFIBER INCORPORATING STARFISH POLYDEOXYRIBONUCLEOTIDES FOR WOUND HEALING APPLICATIONS

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Abstract. Conventional wound dressings have several shortcomings because they are unspecific and partially effective for wound healing. To enhance the therapeutic efficiency of wound dressings, we extracted polydeoxyribonucleotides (PDRN) from *Patiria pectinifera*, and characterized using absorbance-based analysis, chemical composition analysis, and electrophoresis. We evaluated their cytotoxicity and wound healing activity on human dermal fibroblast (HDF) and human keratinocytes (HaCaT). Next, we used electrospinning to fabricate polycaprolactone (PCL)/gelatin (Gel)/PDRN nanofibers for wound dressing application. The average fiber diameters of PCL (P), PCL/Gel (PG), and PCL/Gel/PDRN (PGP) nanofibers were 582.88 ± 202.65, 435.65 ± 149.87, and 334.63 ± 98.09 nm, respectively. The biocompatibility of the nanofibers was assessed using MTT assay and FDA/PI staining on HDF and HaCaT, no cytotoxicity was observed. In vivo experiments with full-thickness skin defect mouse models confirmed that the PGP nanofiber accelerated the initial wound healing process, as shown by wound closure analysis and histological analysis. Our results suggest that the PGP nanofiber has potential as a biomaterial for wound dressing applications and skin tissue engineering.

Keywords: Polydeoxyribonucleotides, *Patiria pectinifera*, Nanofiber, Wound healing

INTELLIGENT CRISPR DESIGN FOR TISSUE REGENERATION

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Abstract. Gene editing by CRISPR and gene regulation by microRNA or CRISPR activation have dramatically changed the way to manipulate cellular gene expression and cell fate. In recent years, various gene editing and gene manipulation technologies have been applied to control stem cell differentiation to enhance tissue regeneration. This presentation will focus on how to develop CRISPR, CRISPR activation (CRISPRa), CRISPR inhibition (CRISPRi) as well as bi-directional CRISPR-AI gene regulation technologies to control cell differentiation and bone regeneration.

Keywords: CRISPR, gene activation, gene inhibition, tissue regeneration, bi-directional gene regulation, stem cells

TAILORING FUNCTIONAL NANO-BIOMATERIALS FOR EFFICIENT GENE THERAPY AND TISSUE REGENERATION

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Abstract. In numerous preclinical research, remarkable therapeutic results have been documented using gene therapy approaches for the regeneration of bone, cartilage, and nerve tissue. In particular, numerous gene delivery vectors, including as nanoparticles and recombinant viruses, have been developed for the purpose of central nervous system (CNS) gene therapy and tissue engineering. However, their clinical applicability has been constrained by the inadequate delivery of genes to CNS target cells and tissues, mainly caused by the poor penetration of vectors through blood-brain barrier (BBB). Among these vectors, adeno-associated virus (AAV) is a preferred gene delivery vector owing to its safety profile, long-lasting expression in non-dividing cells, and relatively low immunogenicity compared with other viral vectors. However, the gene transfer efficacy of AAV to CNS tissues after intravenous injection is at least an order magnitude lower than to liver, which poses a significant challenge in CNS gene therapy. Therefore, we aimed to develop novel AAV capsid variants with greater specificity to desired CNS tissues to enhance the gene transfer efficacy and minimize the unwanted transduction in other tissues including liver.

Keywords: Adeno-associated virus (AAV), CNS gene therapy, BBB, nerve regeration

BIO-FABRICATION OF HUMAN SKIN EQUIVALENTS CONTAINING IMMUNE CELLS AS A MODEL TO STUDY CUTANEOUS RESPONSE TO STIMULI AND MICROBES

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Abstract. The human skin equivalent (HSE) models are useful for studying skin biology and pharmaceutical testing for replacement of lab animals. However, the physiologically complex models such as the involvement of skin resident immune cells are still poorly developed and characterized. In this study, we fabricated an *in vitro* HSE incorporating monocyte/macrophages, THP-1, as skin resident immune cells to investigate responses of HSE and dermal macrophages to UVA irradiation. The fabrication involved the use of fibrin and plasma treatment of culture surface. The tissue histology of HSE model showed patterns closely similar to those of the native human skin. The expression of dermal and epidermal markers such as cytokeratin and tight junctions in epidermis were clearly detected that indicated proper cellular differentiation. Furthermore, the developed HSE was used to study cellular responses to UVA exposure in the presence or absence of applied a natural sunscreen compound, mycosporine-2-glycine (M2G). Upon UVA irradiation, the deformation of HSE layers, and abnormal decreased expression of dermal and epidermal markers were detected. In addition, the genotoxic stress and DNA damage signal were observed. Currently, the responses of dermal macrophages integrated in the HSE is under investigation to assess the immunophenotypes by gene expression profiles after UV exposure with or without M2G. In addition, the HSE is used to study skin-microbe interactions that reflects human skin conditions.

Keywords: Human skin equivalent; Immune cells; UV response; inflammation

PHENOTYPIC SCREENING OF BIOACTIVE COMPOUNDS BY USING A SYSTEM FOR MEASURING CONTRACTILE FORCE OF 96-WELL SCALE HUMAN SKELETAL MUSCLE TISSUE MODELS

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Abstract. Loss of skeletal muscle mass and strength causes systematic effects and worsens the quality of life. In the super aged society, there is a growing need for the development of bioactive compounds against it. Tissue-engineered skeletal muscle has the potential to be used as a tool for screening bioactive compounds and its contractile force is considered a useful index for assessing the properties of tissues. In this study, we developed a 96-well format system for measuring the contractile force of tissue-engineered human skeletal muscles and demonstrated the screening of bioactive compounds increasing contractile force of the skeletal muscle tissues [Yamamoto et al., Biotechnol Bioeng. 2022;119:2196-2205]. To reduce the variability in the contractile forces of each tissue as measured using the 96-well format system, we developed a process using centrifugation. We constructed human skeletal muscle tissue using human immortalized skeletal muscle cells, Hu5/KD3, in the 96-well system and induced skeletal muscle atrophy by treating the tissues with dexamethasone. Eight peptides consisting of less than 15-mer natural amino acids were selected from the literature and used for the screening of peptides for preventing the decrease of the contractile force in human atrophic muscle tissues. As results, one peptide, QIGFIW, showing preventive activity was selected as a seed sequence, and the peptide with the highest preventive activity, QIGFIQ, was obtained by amino acid substitution. These results suggest that the developed phenotypic screening system represents a practical and powerful tool for the development of drugs/functional food ingredients against loss of skeletal muscle mass and strength.

Keywords: microdevice, organ-on-a-chip, tissue engineering, skeletal muscle cells

FUNCTIONALIZED SCAFFOLDS FOR CHRONIC AND INFECTIOUS WOUNDS

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Abstract. Chronic wounds emerge due to delayed healing, underlying health issues, bacterial presence, and other factors, all of which are connected to challenging characteristics. These characteristics primarily involve persistent inflammation, oxidative stress, infections, and hypoxic conditions at the wound location. In the present study, a versatile bilayer cryogel is developed using chitosan-gelatin as the foundational layer, which accommodates bioactive components. These components consist of nitric oxide nanoparticles (as a signaling molecule), cerium oxide microparticles (as antioxidants), and calcium peroxide microparticles (for oxygen release). The cryogelation technique is employed for fabrication, and an additional top layer embedded with iodine serves as an antibacterial element. The engineered scaffolds exhibit notable attributes such as a high-water absorption capacity (around 93%-98%), substantial swelling ratio (approximately 18.3–22.5), and over 90% degradation within 15 days. Release kinetics reveal gradual release percentages for iodine, oxygen, and nitric oxide (approximately 80% in 3 days, 78% in 10 days, and 94% in 10 days, respectively). *In vitro* assessments demonstrate the scaffold's excellent biocompatibility, cell proliferation, robust antioxidant capabilities, and cell migration potential. Furthermore, *in vivo* investigations underscore the bilayer scaffold's ability to significantly accelerate wound closure (around 99.6% within 21 days) and regeneration, surpassing outcomes observed in control groups. Histological analysis illustrates well-organized skin structure in healed wounds treated with the bilayer scaffold, exhibiting granulation tissue, blood vessels, and hair follicles. Results from scaffold-treated ischemic wounds indicate not only the prevention of necrosis but also the preservation of the native skin architecture through the scaffold's nitric oxide and oxygen release properties. In conclusion, the combined approach applied to chronic wounds proves to be effective and introduces novel avenues for advanced strategies in managing complex wound conditions.

Keywords: *nitric oxide, antioxidant, chronic wounds, ischemic wounds, cryogels*

EXTRACELLULAR MATRIX MEMBRANE UTILIZING ONE-DAY-OLD PORCINE CARTILAGE-DERIVED STEM CELLS FOR CARTILAGE REPAIR

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Abstract: The most common complication following Autologous Chondrocyte Implantation (ACI) was periosteal hypertrophy and delamination, which can lead to failure. However, the study on extracellular matrix (ECM) membrane and its application to replace periosteal flap applied in Autologous Chondrocyte Implantation has not been widely developed. Thus, this study aimed to develop the ECM membrane fabricated by stem cells derived from one-day-old porcine cartilage for application in treating knee cartilage injuries. **Methods:** Cells were isolated from one-day-old porcine cartilage tissue, cultured, and characterized their biological properties. Additionally, ECM membranes were fabricated based on cell sheet technology. Then, assessing this membrane for cellular toxicity according to ISO 10993-5 standard and examining rabbit chondrocyte proliferation. Finally, this membrane covered the *in vitro* porcine partial-thickness chondral defect following the ACI. **Results:** One-day-old porcine cartilage-derived cells expressed with stem cell markers. They had a population doubling time of approximately 1 to 2 days, maintained their polygonal morphology over 18 passages, and possess the potential for multilineage differentiation. The ECM membrane was not toxic to rabbit chondrocytes and the proliferation of rabbit chondrocytes in the ECM membrane extract medium increased. The histological assessment indicates the presence of repair tissue at the defect and surrounding the native tissue was also conserved. **Conclusions**: One-day-old porcine cartilage-derived cells have stem cell properties to some extent and offer potential as a cell source for extracellular matrix membrane fabrication. Furthermore, the ECM membrane holds promise for preclinical investigations owing to its biocompatibility and ability to enhance the rabbit chondrocytes proliferation, while also facilitating tissue regeneration in ACI.

Keywords: Cartilage Repair, Extracellular Matrix Membrane, One-Day-Old Porcine, Cartilage-Derived Stem Cells.

BIOENGINEERED APPROACHES IN THE INTERVENTION OF CARDIOVASCULAR DISEASES

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Abstract: Cardiovascular diseases (CVDs) are the leading cause of mortality globally. Therefore, we have developed different therapeutic and regenerative approaches to combat the key pathological conditions leading to heart failure – thrombosis, atherosclerosis, and myocardial infarction (MI). To treat thrombosis, we isolated clot-busting exosomes from HT1080 fibrosarcoma cell line and encapsulated them in a thermo-responsive polymer poly(*N*-vinyl caprolactam) to ensure sustained release pattern. As a proof-of-concept, this combinatorial therapy was applied to rat carotid artery localized thrombus model, and we visualized successful clot dissolution after 5 days by ultrasound imaging. We further fabricated a small diameter trilayered conduit as a vascular graft to treat atherosclerosis. A blend of slow-degrading polycaprolactone and fast-degrading polyglycolic acid was used to design the backbone, which was coated by gallic acid containing antioxidant polyurethane. The material was found to be antioxidant, biocompatible, and hemocompatible. As a proof-of-concept, the scaffold was utilized as a rat femoral artery vascular graft to assess its patency. After 35 days, we visualized blood flow by ultrasound imaging, whereas cell infiltration and matrix deposition were confirmed by immunohistochemical analysis. Lastly, we treated MI using cardiac patches composed of ascorbic acid and gallic acid-modified polyurethane. Immunostaining using cardiac tissue maturation markers revealed a functional tissue after culturing neonatal cardiomyocytes on the scaffold. Exosomes from adipose tissue-derived stem cells were added to the scaffold for therapeutic use. Echocardiography showed improved cardiac heart function after 8 weeks in a rat MI model. Immunohistochemistry showed enhanced cell proliferation, angiogenesis, and cardiac tissue function. Since scaffold implantation requires open-heart surgery, minimally invasive therapeutic formulation delivery has better translational potential. Hence, an injectable and adhesive chitosan-guargum-borax hydrogel was synthesized which can form a patch-like structure after injecting into pericardial cavity. The fabricated hydrogel displayed excellent biocompatibility and sustained release behaviour of exosomes. Overall, we believe our therapeutic and regenerative approaches are clinically relevant and can aid in treatment of CVDs.

Keywords: thrombosis, atherosclerosis, myocardial infarction, cardiac tissue engineering, regenerative medicine

STUDY ON THE HEMOCOMPATIBILITY AND ENDOTHELIALIZATION OF BOVINE PERICARDIAL SCAFFOLD FOR CARDIOVASCULAR PATCH APPLICATION

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Abstract. The bovine pericardium has been introduced to tissue engineering as a highly versatile material. Bovine pericardial scaffolds (BPSs) have been fabricated and presented good mechanical properties and biocompatibility. In the cardiovascular field, thrombogenicity is the main obstacle limiting the effectiveness of BPSs due to thrombus formation. Heparinization is a potential approach to improve the hemocompatibility of engineered constructs derived from decellularized tissues. BPSs were successfully heparinized using heparin and DHI which were alternatively deposited on the surface of BPSc microfibers via the desired number of Layer-by-Layer cycles. The heparinized membranes should exhibit the resistance to blood clotting upon blood-contacting which is a critical challenge for tissue-derived biomaterial when directly contacting the bloodstream. In addition to surface modification with heparin, endothelialization of the grafted material is suggested to improve long-term clinical efficacy. This study would evaluate the hemocompatibility and the ability to endothelialize in vitro of heparinized bovine pericardial scaffolds. The bovine pericardial scaffolds were fabricated and heparinized using a layer-by-layer assembly technique. The heparinized scaffolds were characterized blood compatibility, including platelet adhesion and anti-thrombus for 30 days. The in-vitro endothelialization was determined via human endothelial cell attachment and proliferation on the scaffold within 7 days.

CRYOPROTECTANT-FREE CRYOPRESERVATION AND A NEW AGE OF MESENCHYMAL STEM CELL DRUGS

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Abstract..Introduction: Mesenchymal stem cell (MSC) therapy is a promising therapy for various diseases, especially inflammation-related diseases and degenerative diseases. However, the greatest issue with this therapy is the high cost of the treatment procedure. The scale-up of MSC manufacturing in combination with allogenic transplantation can significantly reduce the cost of this treatment. This study aimed to provide a new strategy for cryopreservation and usage of allogenic MSCs that can decrease the cost and make this therapy easier to use at hospitals. **Methods**: MSCs were manufactured in Quantum Cell Expansion and then cryopreserved in cryoprotectant-free cryopreservation (CFC) medium. The viability of MSCs was evaluated at 3, 6, 9, and 12 months at -86 °C and -196 °C. The toxicity of the CFC medium was carefully evaluated in vitro and in vivo. Freshly thawed MSCs were infused into mice to check their toxicity and treatment efficacy in some diseases. **Results**: The results showed that the CFC medium can maintain the cell viability of more than 90% viable cells at both -86 °C and -196 °C after 12 months. CFC medium is not toxic in vivo. Freshly thawed MSCs can have therapeutic effects in some disease murine models. **Conclusion**: Cryoprotectant-free cryopreservation medium can be used to cryopreserve MSCs and produce units of MSCs as drugs. Freshly thawed MSCs are safe and have therapeutic effects in mice.

Keywords: Stem cell cryopreservation, DMSO-free cryopreservation, Cryoprotectant-free cryopreservation medium, Stem cell drugs

COMBINED LOW ENERGY SHOCK WAVE WITH MELATONINTHERAPY ENHANCES PODOCYTE REGENERATION AND AMELIORATES KIDNEY FUNCTION IN DIABETIC NEPHROPATHY RAT MODEL

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Abstract. (1) Background: Diabetic nephropathy (DN) is a common complication of diabetes. Current therapy for DN does not promotion of podocyte regeneration. Therefore, we investigated the therapeutic effect of low-energy Shock wave (SW) combined with melatonin (Mel) therapy on a DN rat model. **(2) Methods**: The DN rats were treated with Mel (5 mg/kg) twice a week for 6 weeks and SW treatment once a week (0.13 mJ/mm^2) for 6 weeks. We assessed urine microalbumin, albumin to creatinine ratio (ACR), glomerular hypertrophy, glomerular fibrosis, podocyte markers (Wilm's tumor protein-1, synaptopodin and nephrin), cell proliferation, cell survival, cell apoptosis, renal inflammation and renal oxidative stress. **(3) Results:** The SW combined Mel therapy regimen significantly reduced urine microalbumin excretion, ACR, glomerular hypertrophy, and glomerular fibrosis. Moreover, the SW combined Mel therapy regimen significantly increased podocyte number in the SW combined Mel group. This is likely primarily because SW combined Mel therapy significantly reduced renal inflammation, renal oxidative stress, and cell apoptosis, and also significantly increased cell proliferation, cell survival, and nephrin level. **(4) Conclusions**: SW combined Mel therapy enhances podocyte regeneration and ameliorates kidney function in a DN rat model. SW combined Mel therapy may serve as a novel noninvasive and effective treatment of DN.

Keywords: low energy Shock wave, melatonin, podocyte regeneration; diabetic nephropathy

THE EFFICACY OF TRANSPLANTING HUMAN UMBILICAL CORD MESENCHYMAL STEM CELL SHEETS IN THE TREATMENT OF MYOCARDIAL INFARCTION IN MICE

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Abstract. The transplantation of mesenchymal stem cell (MSC) sheets derived from human umbilical cords (hUCs) was investigated in this study as a potential application in treating myocardial infarction (MI). Two groups of hUC-MSC sheets were formed by populating LunaGel^{TM}, which are 3D scaffolds of photo-crosslinkable gelatin-based hydrogel with two different cell densities. An MI model was created by ligating the left anterior descending coronary artery of healthy BALB/c mice. After two weeks, the cell sheets were applied directly to the MI area and the efficacy of the treatment was evaluated over the next two weeks by monitoring the mice's weight, evaluating the left ventricle ejection fraction, and assessing the histology of the heart tissue at the end of the experiment. Higher cell density showed significantly greater efficiency in MI mice treatment in terms of weight gain and the recovery of ejection fraction. The heart tissue of the groups receiving cell sheets showed human-CD44-positive staining and reduced fibrosis and apoptosis. In conclusion, the hUC-MSC sheets ameliorated heart MI injury in mice and the efficacy of the cell sheets improved as the number of cells increased.

Keywords: myocardial infarction; mesenchymal stem cell; umbilical cord stem cell sheet; regenerative medicine

ANTIOXIDANT AND ANTIBACTERIAL OXIDIZED ALGINATE/GELATIN/ISHOPHLOROGLUCIN A HYDROGEL FOR ENHANCED SKIN WOUND HEALING

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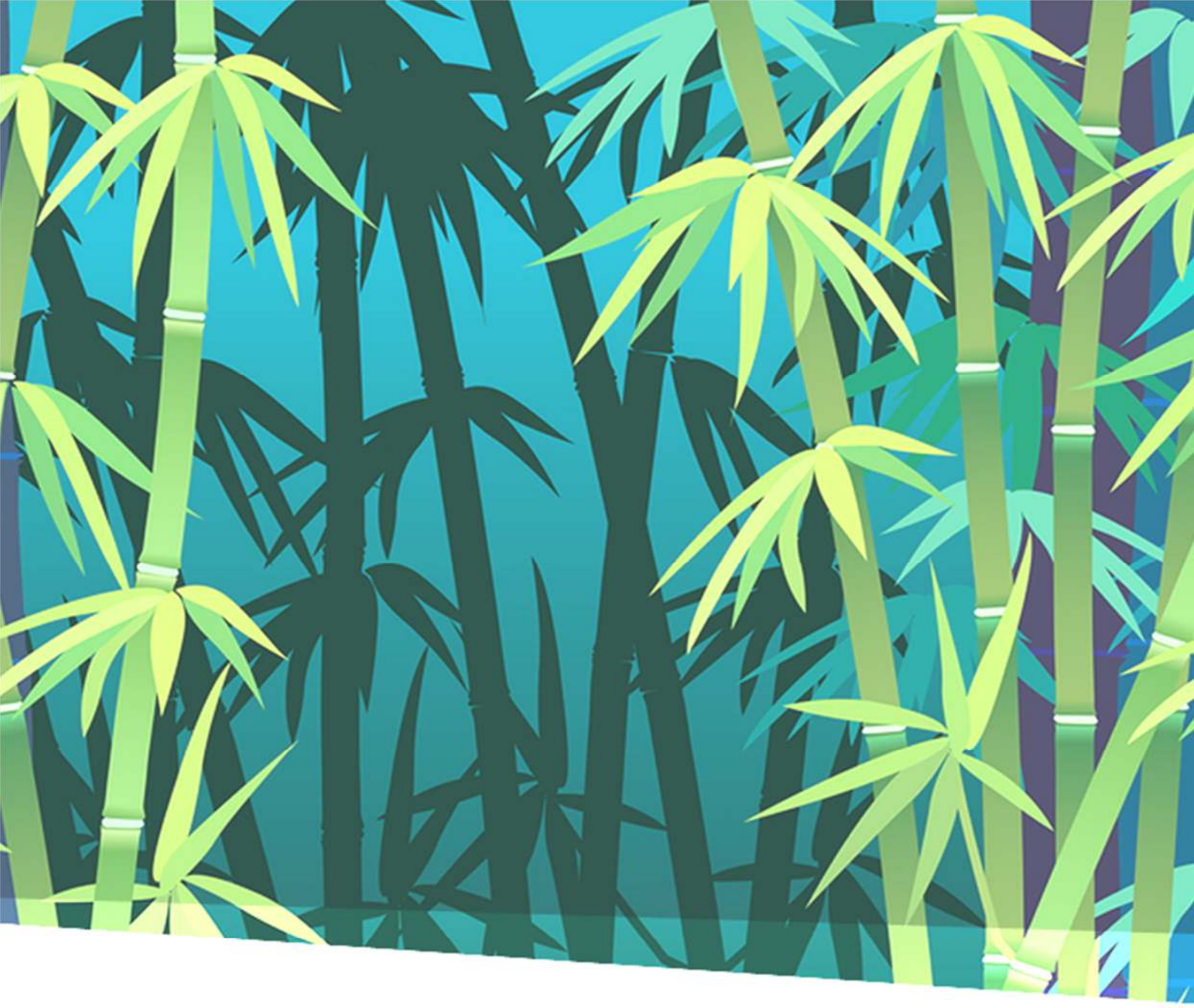
Abstract. This study investigated the potential applicability of wound dressing hydrogels for tissue engineering, focusing on their ability to deliver pharmacological agents and absorb exudates. Specifically, we explored the use of polyphenols, as they have shown promise as bioactive and cross-linking agents in hydrogel fabrication. Ishophloroglucin A (IPA), a polyphenol not previously utilized in tissue engineering, was incorporated as both a drug and cross-linking agent within the hydrogel. We integrated the extracted IPA, obtained through the utilization of separation and purification techniques such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) into oxidized alginate (OA) and gelatin (GEL) hydrogels. Our findings revealed that the mechanical properties, thermal stability, swelling, and degradation of the multifunctional hydrogel can be modulated via intermolecular interactions between the natural polymer and IPA. Moreover, the controlled release of IPA endows the hydrogel with antioxidant and antimicrobial characteristics. Overall, the wound healing efficacy, based on intermolecular interactions and drug potency, has been substantiated through accelerated wound closure and collagen deposition in an ICR mouse full-thickness wound model. These results suggest that incorporating IPA into natural polymers as both a drug and cross-linking agent has significant implications for tissue engineering applications.

Keywords: Ishophloroglucin A (IPA), Oxidized alginate, Gelatin, Non-covalent interaction, Wound healing



4. Bioenergy and Biorefinery

INNOVATIVE APPROACHES TO CREATING SMART CELLS FOR BIOREFINERY

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Abstract. We have developed methods and programs to design new artificial metabolic pathways and artificial enzymes, methods to create diverse gene switches that artificially control the On-Off of gene expression, automated technologies for gene transfer to microorganisms and screening and so on. We have also developed a number of basic technologies, such as the base editor system, which links base-editing enzymes such as deaminase with dCas9, genome synthesis technology that enables the synthesis of long DNA strands of more than 100,000 bases using *Bacillus subtilis*, and automated systems for high-throughput analysis of metabolites and other substances. Furthermore, by integrating the results of these fundamental researches, we constructed the biofoundry. In the biofoundry, the metabolic pathways and regulatory systems of microorganisms are designed in "Design: D", the designed microbial cells are generated in parallel and rapidly using robotics in "Build: B", and the constructed microorganisms are evaluated in high-throughput using automated equipment in "Test: T". The obtained data is then subjected to machine learning, etc., to extract rules, "Learn: L" and the design is further improved. By rapidly rotating this DBTL cycle, robust cells "smart cells" that produce high levels of target substances can be quickly constructed. Furthermore, by accumulating the data obtained in this DBTL cycle in the system, a database and knowledge base can be constructed to further shorten the development time. In other words, it is a fusion technology of biotechnology, digital technology, robotics technology and process technology, and this area has recently come to be known as “Engineering Biology”. Today, biofoundry technology is a technology platform that will drive the bioeconomy, and start-up companies are emerging to develop biofoundry businesses. We have been working on the realization of bio-manufacturing for biorefinery through the rapid development of smart cells by utilizing the biofoundry.

Keywords Biofoundry, Bioproduction, DBTL cycle, Engineering Biology, Biorefinery

BIOPROCESSING STRATEGIES TO IMPROVE BIOFUEL PRODUCTION FROM BIOMASS

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Abstract. The global crisis stemming from fossil fuel depletion, environmental pollution, and its significant impact on climate change have spurred a crucial shift towards renewable energy sources, particularly the exploration of biobased fuels. Among the abundant renewable resources, agricultural biomass has emerged as a prominent contender, boasting a high composition of polysaccharides, such as cellulose and starch, rendering it an ideal candidate for replacing our reliance on diminishing fossil fuels. Biobutanol, a 4-carbon alcohol, has garnered attention as a potential alternative to gasoline, with its production facilitated through acetone-butanol-ethanol (ABE) fermentation primarily orchestrated by anaerobic bacteria, prominently *Clostridium* sp. Despite the feasibility of producing biobutanol from agricultural biomass, its current yield remains limited, impeding widespread industrial production and posing a challenge in competing with fossil fuel prices effectively. Addressing this constraint, researchers have been diligently exploring various strategies to enhance biobutanol production, intensifying efforts to increase yield and reduce processing costs. One promising advancement is the adoption of simultaneous saccharification and fermentation (SSF), which ingeniously combines saccharification and fermentation in a single process, leading to heightened biobutanol yield and streamlining processing steps concurrently. Moreover, to further augment production, the introduction of sequential substrate and enzyme feeding has demonstrated notable results, effectively bolstering sugar concentration up to 120 g/L while significantly reducing enzyme loading, particularly cellulase usage by 50% and amylase usage by 80%. This breakthrough not only promises elevated cost efficiency but also aids in overcoming a major hurdle in biobutanol production. In a noteworthy revelation, the ABE fermentation process exhibits an additional beneficial output by generating biohydrogen during its initial stages. Recovering and harnessing this biohydrogen not only enhances the overall production process's sustainability but also presents an opportunity to diversify revenue streams and bolster the economic viability of biobutanol production. In summation, harnessing renewable biomass for biobutanol production, coupled with these innovative bioprocessing approaches, holds tremendous potential in securing a greener and more sustainable energy future, driving us closer to a harmonious coexistence with our environment.

Keywords: Bioprocessing; Biobutanol; Biohydrogen; ABE Fermentation; Agricultural Biomass

THE SUSTAINABLE BIOREFINERY PLATFORM OF VALUE-ADDED BIOMOLECULES FROM BREWING WASTES WITHIN SUPPORTING CIRCULAR ECONOMY

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Abstract. Craft beers have been steadily gaining market share, challenging both national and international beer breweries. However, the comprehensive utilization of lignocellulosic biomass and waste generated from brewing processes remains a significant challenge. In this study, we have developed integrated fermentation technologies to produce various valuable products, including pigments, animal feed, gamma-aminobutyric acid, quantum dots, and recycled coasters. This approach enables the complete utilization of brewery spent grain (BSG), including the spent solid residue and liquid waste. The results demonstrate that these sustainable processes, focused on waste reduction and resource utilization, provide cost-effective feedstocks for the production of high-value biochemicals. As a consequence, this integrated technology not only enhances carbon footprint efficiency but also reduces waste disposal while increasing economic revenue for the brewing industry. By implementing these innovative technologies, the brewery industry can achieve a more sustainable and circular approach to waste management. The conversion of lignocellulosic biomass and brewing waste into valuable products contributes to a more efficient use of resources and reduces the environmental impact associated with waste disposal. Furthermore, the economic benefits derived from the production of high-value biochemicals enhance the overall profitability of the brewing industry. Overall, the integrated fermentation technologies proposed in this study offer a promising solution to address the challenge of waste utilization in the craft beer industry. It paves the way for a more sustainable future by transforming waste into valuable resources and driving the industry towards a circular economy model.

Keywords: biorefinery, lignocellulosic biomass, brewery spent grain, gamma-aminobutyric acid, quantum dot

INSECT BIOREFINERY: AN ECO-MIMIC PATHWAY TOWARDS NET ZERO EMISSIONS

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Abstract. The global commitment to Net Zero emissions necessitates innovative pathways to reduce carbon footprints, and insect biorefinery stands as a promising avenue. This talk explores the confluence of insect biorefinery with biotechnology, elucidating a comprehensive, sustainable approach that contributes to Net Zero targets. Insect biorefinery, leveraging biotechnological advancements, facilitates the transformation of insect biomass into a plethora of valuable products such as proteins, lipids, chitin, and biofuels. Through cutting-edge bioprocessing techniques and genetic engineering, insect biomass is valorized, resulting in minimal waste and efficient resource utilization. Insect farming, as the foundation of biorefinery, presents a low-carbon alternative to traditional animal farming. It offers a sustainable source of nutrition for both humans and animals while reducing the demand for land, water, and energy. The integration of biological pretreatment methods and the utilization of waste feedstock further enhance sustainability. Additionally, the development of novel products like insect-derived bio-lubricants and nanocarriers highlights the multifaceted applications of insect biorefinery. These innovations not only contribute to the circular economy but also underscore the potential for carbon reduction. This presentation offers a panoramic view of the insect biorefinery landscape, from farming to end products, emphasizing its alignment with modern biotechnological advancements. It serves as a rallying call for researchers, policymakers, and industry stakeholders to explore this promising frontier, leveraging the synergies between insect biorefinery and biotechnology in our shared pursuit of a sustainable, Net Zero future.

Keywords: Net Zero, insect biorefinery, biomass, agroindustrial wastes, circular economy

EVALUATION OF THE CO-FERMENTATION POTENTIAL OF *GLUCONOBACTER OXYDANS* FOR SIMULTANEOUS UTILIZATION OF REDUCING SUGAR TO GENERATE GLUCONIC AND XYLONIC ACID

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Abstract. Gluconic and xylonic acids are considered pivotal building block chemicals and are listed among the top 30 compounds with remarkable potential. Following glucose, xylose stands as the most abundant sugar in lignocellulosic biomass. The effective utilization of these compounds holds the key to unlocking the full potential of lignocellulosic materials. While glucose is extensively utilized, xylose, classified as a bioinert chemical, finds limited microbial application. However, *Gluconobacter oxydans* exhibits the unique capability to concomitantly ferment glucose and xylose, yielding gluconic and xylonic acids simultaneously. The study demonstrated the capacity of *G. oxydans* to ferment xylose, even when co-cultured with 50 g/L of xylose in media containing glucose levels ranging from 25 to 75 g/L. Remarkably, the presence of xylose not only failed to hinder bacterial growth but also appeared to enhance *G. oxydans* proliferation. Despite variations in glucose concentrations, the presence of xylose exhibited negligible impact on growth. The dry weight growth of *G. oxydans* reached 1.9 kg/m^3 within 120 hours, characterized by rapid initial growth within the first 48 hours, followed by a deceleration. However, a limitation of *G. oxydans* lies in its metabolic pathway's potential consumption of gluconic acid. Analogous to prior studies focusing on *G. oxydans* growth in glucose media, our investigation revealed an initial peak followed by a decline in gluconic acid concentrations over extended fermentation. Co-fermentation of glucose and xylose led to the accumulation of xylonic acid at 71.5 ± 13.8 g/L, concomitant with inhibited glucose concentration. Notably, the presence of higher glucose concentrations led to increased gluconic acid production, reaching 110±0.4 g/L within 120 hours at 75 g/L glucose levels. Intriguingly, the bacterium ceased gluconic acid production at 25 g/L glucose concentrations, generating xylonic acid instead. This phenomenon underscores *G. oxydans*' potential in utilizing glucose and xylose to generate gluconic and xylonic acids, thus showcasing its metabolic versatility.

Keywords: co-fermentation, gluconic acid, Gluconobacter oxydans, simultaneous utilization of reducing sugar, xylonic acid

MATHEMATICAL MODELS ON THE *IN-SITU* TRANSESTERIFICATION OF WET MAHOGANY (*SWIETENIA MACROPHYLLA*) SEED LIPID USING AZEOTROPIC ETHANOL AND SUPERCRITICAL CARBON DIOXIDE

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Abstract. To produce biodiesel competitive with petroleum diesel, the cost of manufacturing must be reduced. This problem can be overcome using the novel method employed in this study. The research entails mathematical modelling of a simultaneous extraction-transesterification method of Mahogany (*Swietenia macrophylla*) seed lipid that makes use of the synergy of in- situ catalyst production, carbonic acid, and the carbon dioxide-expanded ethanol-water system. This approach reduces manufacturing costs by eliminating the energy-intensive process of drying seeds and replacing it with a cheap and ecologically friendly solvent, azeotropic ethanol, and the recoverable co-solvent, carbon dioxide. Despite initial experimental data indicating low conversion, it is critical to understand how to enhance conversion and yield using a mathematical model. The model and simulations may give insight into the many mechanisms behind the synergistic effect of the CO_2-ethanol-water system on the reactive extraction of wet seed lipids, perhaps leading to process improvement. This research develops complicated mathematical models that might be beneficial in the design of process control systems. This study validates the unsteady-state and steady-state mathematical models for the simultaneous extraction and transesterification process on a heterogeneous solid (seed matrix) - liquid (CO_2-water-ethanol combination) medium. It then goes over the model extensions that are needed to create a predictive model for in situ biodiesel processes. Matlab is used to calculate kinetic rate equations and mass transfer equations of chemical species involved in heterogeneous transesterification, respectively. AspenOne is used to investigate the model's effects, including the effect of temperature and other factors, and then simulate the performance of large SCF reactors at various reactor modes, resulting in cost and time savings. This present work is innovative, with substantial scientific and industrial promise.

Keywords: Mahogany, transesterification, modelling, simulation, supercritical fluid

THE DEVELOPMENT OF GREEN PROCESS OF FURFURAL PRODUCTION FROM OIL PALM SOLID WASTE

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Abstract. Most of the biomass produced in Indonesia is oil palm empty fruit bunches (OPEFB). One of the uses of OPEFB is the conversion into Furfural, which can then be synthesized into various derivative compounds with high added value. This study describes the production of Furfural carried out at the Research Center for Biomass Valorization UI using acid hydrolysis and greener methods. This review describes the chemical and biological routes for furfural production. A lab-scale study examined the production of Furfural from POEFBs, which were pretreated by soaking in aqueous ammonia (SAA). The highest furfural yield was 27.94 mol%, which was obtained at a reaction temperature of 170°C after 20 min in an acidic solution with a concentration of 0.5 M. Subsequent experiments used DES [ChCl][AlCl3.6H2O] as a solvent, and MIBK nonpolar solvent to avoid using acids that are not environmentally friendly. The yield of Furfural from OPEFB given pre-treatment under optimum conditions was 34.27 % mol (7.05 % by mass). This research resulted in relatively low processing conditions with high yields so that it can be applied on an industrial scale). The presentation will also discuss other efforts towards a greener process of furfural production.

Keywords: acidic hydrolysis, biphasic system, deep eutectic solvent, Furfural, palm oil empty fruit bunch

UPCYCLING SOYBEAN PULP FOR SUSTAINABLE PROTEIN BIOMANUFACTURING *VIA* A ONE-POT THERMOPHILIC PROTEASE CASCADE

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Abstract. Amino acids are essential components of culture media for fermentative protein production as *de novo* amino acid synthesis is highly energy-consuming for the host. Soybean pulp (*i.e.*, okara) is a low-value byproduct from soybean processing; however, okara is rich in insoluble proteins. Therefore, okara could be a sustainable source of amino acids for fermentative protein production. Conventional industrial methods for amino acid harvesting employ high-temperature acidic proteolysis to hydrolyze protein sources. However, these conditions are harsh and the process is time-consuming. To increase throughput and yield, we developed a one-pot, two-protease cascade capable of complete okara proteolysis into oligopeptides and individual amino acids in 3 hours. Interestingly, we observed an unprecedented synergy between the thermophilic endopeptidase (alcalase) and hyperthermophilic exopeptidase (TETamp), which allows the two-protease cascade to function optimally at 60°C and pH 7.5. Unlike the conventional method, the enzymatic process preserves tryptophan and asparagine, resulting in an almost complete recovery of total amino acid equivalent from okara. Furthermore, both *E. coli* and *Bacillus megaterium* cultures cultivated in the enzymatic okara hydrolysates demonstrated comparable GFP yields compared to those cultivated in LB medium, respectively. We also used the enzymatic okara hydrolysates for fermentative production of the two proteases used in the enzymatic proteolysis. The cell-lytic activity of alcalase even allows okara proteolysis directly using protease-expressing *B. megaterium* whole-cell biocatalyst, bypassing the costly protease purification step. In conclusion, this study represents a renovated circular bioeconomy model that converts abundant and low-value agro-waste into sustainable feedstocks of the biotechnological industry.

Keywords: Circular bioeconomy, fermentative protein production, thermophilic protease, one-pot enzyme cascade, agro-waste biorefinery, resource recovery.

CASCADE UTILIZATION OF INEDIBLE SEEDS OIL FOR RENEWABLE ENERGY AND CHEMICALS

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Abstract. Our processes for the cascade utilization of rubber seeds, which are now thrown away as waste, for the production of high quality (98%<) but cheaper than petro-diesel fuel, and other chemicals such as cosmetic oil with high concentration of ω3 and ω6, super vitamin E, and metal soap, and high-quality glycerin. Production of Metal Soap, which is widely used for the plasticizer, from free fatty acid in waste oil.

(1) Co-solvent method for the production of high quality Bio-diesel Fuel (BDF) (98% FAME) with low energy consumption and low waste emission.

(2) Cascade Utilization of inedible oil seeds, such as Para rubber seeds (*Hevea brasilensis*), *Jatropha curcas* seeds, *Callophyllum inophyllum* seeds, *Pongamia pinatta* seeds, and *Hibiscus sabdariffa* for the production of renewable energy and biochemicals.

(3) Utilization of glycerin, which is by-product of BDF production process,

A: For the hydrogen generation from water with TiO2 photo-catalyst by solar energy.

B: For the fuel of glycerin fuel cell.

C: Utilization of raw materials for the production of super water absorbent polymer.

(4) Extraction of sucrose by water and Vitamin E and other medicinal compounds by alcohol.

(5) Utilization of oil seed's shell and solid waste from oil expression for the coal-mixed burning of biomass for electric generation.

Keywords: rubber seeds oil, high quality BDF, Vitamin E, Metal soap, glycerin

SUSTAINABLE STRATEGY OF ALGAL BIOMASS PRODUCTION USING NANO UREA AND SUBSEQUENT RAPID BIOMASS HARVESTING USING NOVEL BIOFLOCCULANT

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Abstract. Algal biomass is considered a promising feedstock for bioenergy generation. Obtaining the highest energy yield from microalgae necessitates improving their biomass, carbohydrate, and lipid content. The present study aimed to investigate the effect of nano urea (NU) on biomass concentration and the biochemical composition of microalgae. The results revealed that the biomass concentration was improved by 47.67% and 16.24% using NU-supplemented Bold's Basal Medium (BBM) over BBM and urea-supplemented BBM medium, respectively. Similarly, carbohydrate, lipid, and total chlorophyll content also increased in the presence of nano urea. Moreover, this study also showed harvesting microalgae by flocculation using a chemically modified eco-friendly novel biopolymer. A cationic polymer was used as a flocculant to harvest microalgal cells from the liquid cultures. The multiple process parameters viz. B/P ratio, $FeCl_3$ solution, and pH were optimized together using Taguchi design in order to determine the maximum flocculation efficiency. Under optimized conditions, the combination of biopolymer and $FeCl_3$showed significant results with a flocculation efficiency of 98.87%. The present study suggests that the use of nano urea at a minimal dosage could be a suitable strategy to improve the quality of microalgal biomass. Additionally, harvesting of algal cells with the novel green flocculant could be applied to reduce reliance on hazardous inorganic flocculants

Keywords: *Nano urea, BBM, biomass, Guar gum, L-arginine, flocculant, Microalgae, Cationic polymer*

UTILIZATION OF RECYCLED YEAST CELLS FOR VERY HIGH GRAVITY BIOETHANOL PRODUCTION USING SAGO HAMPAS HYDROLYSATE AS FERMENTATION MEDIUM

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Abstract. The osmotic pressure in very high gravity (VHG) bioethanol fermentation has led to defective yeast metabolism, cell lysis and mortality, which ultimately caused incomplete fermentation. Fed-batch mode was demonstrated to minimise the negative effects of the VHG condition. An ample amount of active yeast cells was produced from VHG fed-batch bioethanol fermentation using sago hampas hydrolysate (SHH) supplemented with yeast extract and peptone. The cells were recycled to start a new cycle of bioethanol fermentation, which reduced the time and operational cost of fermentation. This study explores the effect of utilising recycled yeast in repeated batch VHG bioethanol fermentation using SHH as the feedstock. Yeast was allowed to naturally sediment at the bottom of the fermenter for 6 h. After sedimentation, the concentration of viable cells recorded was 1.21 x10^6 ± 0.135 cells/mL. The results of the repeated batch fermentation using recycled cells had attained similar ethanol fermentability ($Y_{E/s}$: 0.505 ± 0.02, P_E: 126.20 ± 3.00 g/L) to the fed-batch experiment. Meanwhile, the results showed a 1.2-fold increase in Y_E and a 40% enhancement in P_E compared to a single batch fermentation. As a whole, the findings are crucial in developing a cost-effective VHG fermentation system using sago-based feedstock.

Keywords: bioethanol, recycled yeast cells, sago *hampas*, very high gravity fermentation

ENHANCING CELLULOSE EXTRACTION FROM SUGARCANE BAGASSE THROUGH *LACTOBACILLUS* PRETREATMENT

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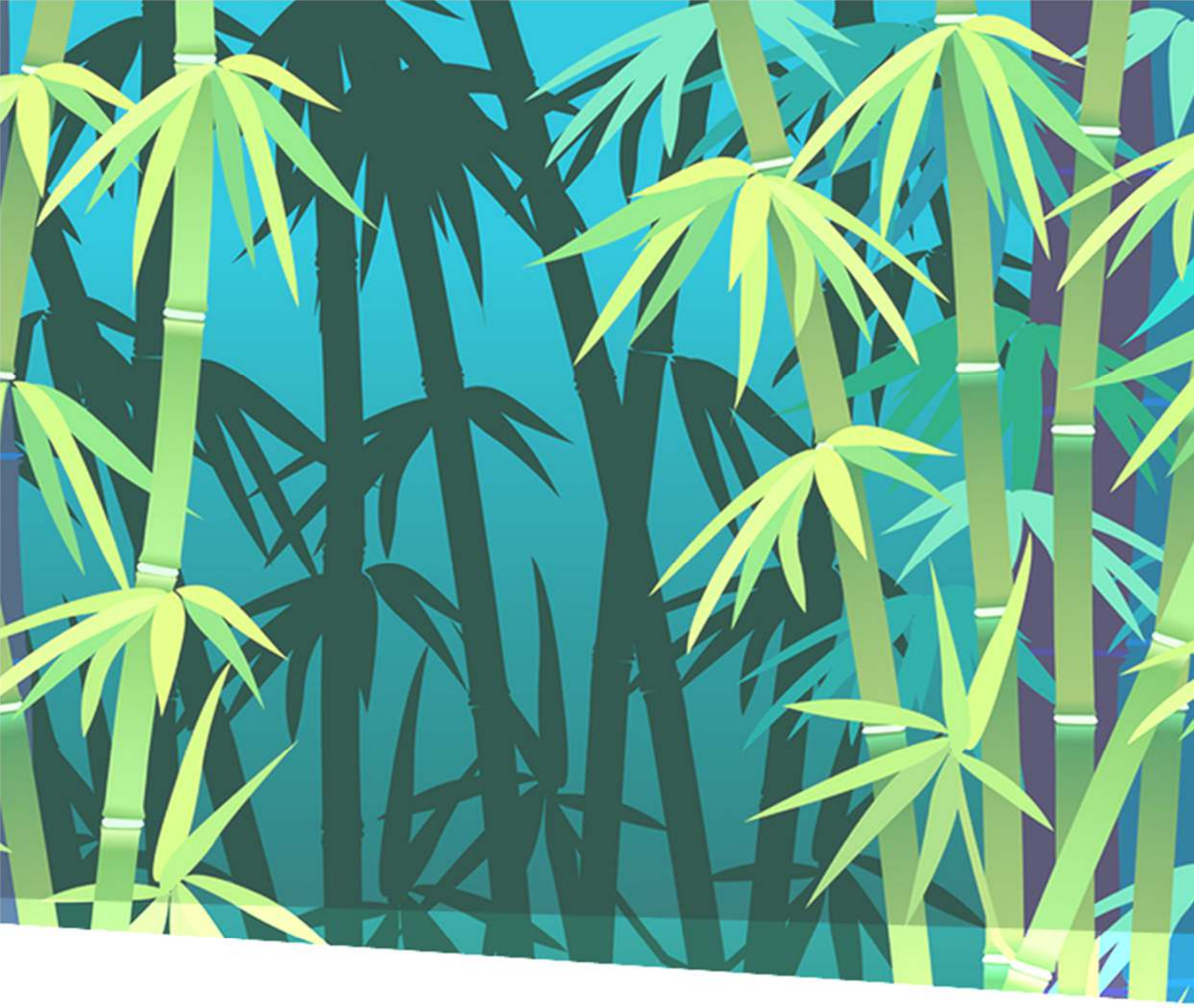
Abstract. Sugarcane bagasse, a byproduct of the sugar industry, has long been recognized as a promising source of cellulose, a valuable biopolymer with numerous industrial applications. This study investigates the efficacy of Lactobacillus pretreatment for enhancing cellulose extraction from sugarcane bagasse (SCB). Lactobacillus, known for its cellulolytic potential and eco-friendly nature, it is utilized as a biological pretreatment agent. The pretreatment process involves the cultivation of Lactobacillus strains on sugarcane bagasse under controlled conditions, optimizing factors such as temperature, pH, and incubation time. The treated SCB is subsequently subjected to a cellulose extraction process. The extracted cellulose is characterized by various analytical techniques, including SEM, FTIR, and XRD, to assess its purity and crystallinity. The SCB-P fiber showed an increase in cellulose content and crystallinity index, while lignin content decrease with reduced bleaching time. The FTIR analysis also confirmed the elimination of lignin. Scanning electron microscope revealed the opening of the fibers structure, and increased internal surface area. Preliminary results indicated that Lactobacillus pretreatment significantly improves cellulose extraction yields from sugarcane bagasse compared to conventional methods. This approach demonstrates the potential of microbial pretreatment as a sustainable and cost-effective means of harnessing cellulose from agricultural residues. Furthermore, it contributes to the valorization of sugarcane bagasse, reducing waste and promoting a circular economy within the sugar industry.

Keywords: Lignocellulose, Sugarcane bagasse, Cellulose, Biological pretreatment



5. Bioprocess and Bioseparation Engineering

PERSPECTIVES ON BIOLOGIC PRODUCT MANUFACTURING AND BIOPROCESSING IN ASIA

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Abstract. The biologics industry produces many materials of importance to agriculture, food, medicine, energy and the environment throughout the world. As is true everywhere, in our area, development of biological processes focuses on products of local interest to the people of Southeast Asia and the Asia Pacific region. While there is a wide variety of products under development, in this presentation we shall focus on those areas that have been highlighted by regional companies, researchers, and governments. These include therapeutics and diagnostics directed toward locally occurring diseases; food and agriculture issues related to marketed plants and animals that are economically important in our region; and energy and environmental projects that have potentially significant impact on our well-being. Various research programs, government supported activities, and commercial enterprises are reviewed to help us understand what technologies are in development and what the future may look like as new processes are brought to commercial fruition.

Keywords: Bioprocessing, APAC, healthcare, agriculture, environment

PURIFICATION OF VIRUSES AND PLASMID DNA ON MONOLITHS

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Abstract. Monoliths are rather novel chromatographic stationary phases demonstrated to be especially powerful for isolation and analytics for large macromolecules and biologic nano-assemblies. In particular, they were demonstrated to efficient for purification of plasmid DNA and viruses, which required quantities recently increased substantially due to a progress in gene therapy as well as COVID pandemics.

Monoliths have a particular structure. Being a single piece of porous material in which the entire porosity is open and consists of interconnected channels, allowing convection transport over the entire matrix. Because of that, slow diffusivity of these large macromolecules and biologic nano-assemblies to be isolated in not any longer a bottleneck, as they are transported by the stream of a mobile phase, allowing fast exchange between mobile and stationary phase. As a consequence, separation resolution as well as dynamic binding capacity are flow unaffected. Much higher open porosity in comparison to particulate chromatographic supports results in low pressure drop even at elevated flow-rates. Besides, their pore structure can differ not only in pore size and porosity but also in microstructure topology affecting separation.

Plasmids and viruses differ significantly from smaller molecules by their strength of interaction, mechanical stability, and molecule symmetry. All these features have to be considered when high quantities are to be isolated.

In this work we will present monolith properties and effect of their structure on pressure drop and separation performance. Effect of ligand density on recovery and dynamic binding capacity will be discussed together with technique for non-invasive determination of ligand amount. Several examples will be presented.

Keywords: plasmid DNA, viruses, monoliths, pressure drop, non-invasive characterization

PROCESS MODELLING OF CHROMATOGRAPHY OF BIONANOPARTICLES

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Abstract. Various types of bionanoparticles (BNPs) such as viruses, virus-like particles, exosomes, RNAs and DNAs are important as drugs, vaccines or vectors for gene and cell therapy. Although purification of BNPs by chromatography is needed, it is not easy to build the efficient chromatography process due to the large size of BNPs, which lowers the mass transfer rate and the binding capacity significantly compared with proteins. In this presentation, modelling of ion-exchange chromatography (IEC) of BNPs with monolithic supports and porous particle packed beds will be presented in comparison with IEC of proteins. The model separation system is the separation of empty (E) and full (F) particles of adeno-associated virus (AAV) by IEC. Linear gradient elution (LGE) experiments at various gradient slopes and flow-rates were carried out. Parameters needed for the model simulation were obtained from LGE data by the Yamamoto method. The numerical simulations by the mechanistic models were carried out in order to understand the EF particle separation mechanism, and to optimize the separation process

Keywords: chromatography, bionanoparticles, simulation, process modeling

DEVELOPMENT OF RABBIT SINGLE-CHAIN FV-BASED AFFINITY CHROMATOGRAPHY FOR ON-DEMAND PURIFICATION OF BIOPHARMACEUTICALS

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Abstract. We developed affinity chromatographic resins that immobilized rabbit single-chain Fv antibodies (scFvs). By biopanning using antigen-coupled multilamellar vesicles (Ag-MLVs), a variety of original scFv clones that specifically bind to target protein interests were isolated and identified. Apparent dissociation rate constants, $_{app}k_{off}$, of several different candidates were less than 10^{-3} s^{-1} and their dissociation constants, K_Ds, were ranged from 5.56×10^{-10} to 4.04×10^{-8} M. Consequently, the clones, some clones were further investigated for use in affinity purification of human IgG. Two of them maintained more than 40% of antigen-binding activities on the surface of affinity resins. Especially, another had a relatively high alkaline resistance. The direct separation of human IgG from 10% FBS-D-MEM by use of the column with R1-27 achieved 97.2% purity, while the column with R3-26 showed almost 100% recovery. The affinity resins at the densities between 4.32 and 15.19 mg-scFv/cm^3 exhibited maximum binding amount of human IgG, while the highest ligand utilization was achieved by use of the resin at approximately 9 mg-scFv/cm^3. Therefore, the strategies to develop affinity ligands will be beneficial for development of on-demand affinity columns with higher affinity/selectivity, chemical resistance, while optimization of pore size and pore volume for scFv-coupled resins will further improve the EBC.

Keywords: rabbit scFvs, affinity ligand, affinity chromatography, biopharmaceuticals

VIRUS BREAKTHROUGH BEHAVIOR DURING VIRUS FILTRATION: EFFECTS OF VIRUS/PROTEIN CONCENTRATION AND OPERATING CONDITIONS

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Abstract. Virus filtration in the biopharmaceutical downstream process is a critical step to ensure viral safety with removal efficiency greater than 99.99%. Despite its robust performance, it has been reported that viruses can escape through the membrane under certain conditions. The objective of this study was to examine the inevitability of virus breakthrough using a commercial virus filter with PP7 bacteriophage under harsh conditions. To determine the effect of virus titer on phage breakthrough, virus challenge per unit was measured at which phage escaped for the first time. The effect of protein was also examined by varying the concentrations of protein in the feed solution where phage and protein coexist. In addition, phage breakthrough due to the diffusion effect occurring in post-buffer flush and low flow operations was observed, which has recently been reported in the research. The results show robust virus removal performance (LRV >6.8) up to 10^9 PFU/mL for 200 L/m^2, with the first phage breakthrough observation over 10^{12} PFU/m^2 of phage challenge in the membrane. As the protein concentration increased in the coexistence condition, a greater number of phages passed through the membrane. During the post-buffer flush and the operation at low filtrate flux, diffusion plays an important role in the escape of phages, which were detected as high as 10^{12} PFU/m^2, but as low as 10^9 PFU/m^2. These results suggest that the virus-retentive membrane may become susceptible to undesired virus breakthrough when exposed to phage challenges of >10^{12} PFU/mL, providing important key factors to ensure viral safety during the downstream process of biopharmaceutical production.

Keywords: Virus filtration, virus breakthrough, biopharmaceutical downstream process, phage challenge, low flux operation

VALORIZATION OF RICE BRAN AS AN ALTERNATIVE SOURCE FOR PRODUCING BIOACTIVE PHENOLIC COMPOUNDS

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Abstract. Rice bran obtained from a rice milling process contains polyphenolic compounds such as ferulic acid bound with the plant fibre matrix which can be degraded using fermentation This research was conducted to determine the optimum time of rice bran fermentation using *Aspergillus niger* to increase extract yield and bioactivity against various solvents. The results showed that the fermentation process of rice bran with *Aspergillus niger* reduced the content of hemicellulose, cellulose, and lignin each by 22.36%, 42.47%, and 22.96% after seven days. Solid substrate fermentation using *Aspergillus niger* can increase the yield of polyphenolic compounds by 32.8% after seven days of fermentation. Extraction of phenolic compounds was carried out for 180 minutes by maceration method using different solvents particularly methanol 80% (40°C), ethanol 80% (40°C), and acetone 40% (60°C). Solid substrate fermentation using *Aspergillus niger* increased the yield of phenolic compounds by 32.8% on the 7th day using methanol, 81.1% on the 3rd day using ethanol, and 64.4% on the 5th day using acetone as compared to the results without fermentation. The optimum total phenolic content using methanol solvent was obtained on the 7th day of 6.18 mg GAE/g rice bran, 10.93 mg GAE/g rice bran on the 3rd day using ethanol solvent and 59.61 mg GAE/g rice bran on 5th day using acetone solvent. The highest ferulic acid content was obtained on the 3rd day of fermentation with ferulic acid content in methanol, ethanol, and acetone solvents, respectively 0.130; 0.157; and 0.115 mg FA/g rice bran when compared to the results without fermentation. Determination of antioxidant activity of the phenolic extracts showed that the optimum IC_{50} value was obtained on the 7th day of fermentation using methanol solvent (150.25 ppm), 103.14 ppm on the 3rd day using ethanol solvent and 277.59 ppm on the 5th day for acetone solvent.

Keywords: Rice bran, *Aspergillus niger*, Solid state fermentation, Ferulic acid

EFFICIENT PURIFICATION METHODS FOR PHYCOCYANIN USING MEMBRANE TECHNOLOGIES

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Abstract. Phycobiliproteins (e.g., phycocyanin, phycoerythrin, and allophycocyanin) are main light-harvesting pigment antenna proteins. Phycocyanin (PC) is water-soluble, fluorescent, and utilized in various applications such as food, cosmetics, and pharmaceuticals due to its antioxidant, anti-inflammatory, and other bioactivities. The process mainly involves extraction, precipitation, dialysis, chromatography, and lyophilization . To improve the purity of phycocyanin, several purification steps are required, which can be time-consuming and costly. Dialysis is commonly used for desalting, but it is only suitable for small-scale applications and requires a large amount of buffer relative to the sample. Packed bed chromatography is employed to obtain high purity (>3.0) phycocyanin, but it can reduce the yield and hinder large-scale processes. Replacing the conventional process with membrane technologies would simplify the purification process and reduce the cost, providing an alternative for scale-up. The objective of this study is to apply a process suitable for the large-scale processes of phycocyanin by replacing dialysis with diafiltration (DF) and packed bed chromatography with membrane chromatography (MC). Phycocyanin was extracted from *Spirulina maxima*, followed by precipitation using ammonium sulfate. Subsequently, DF and MC were performed in comparison with dialysis and packed bed chromatography. The purity and yield of PC were measured using a UV-vis spectrophotometer. DF reduced buffer consumption by 67-fold and shortened the process time by over 3-fold, with an 18-fold increase in throughput. MC also reduced the process time by at least 5-fold and costs by consuming less elution buffer although the binding capacity of MC is about 3-fold lower than that of packed bed chromatography. These results suggests that membrane technologies can be a promising alternative for large-scale processes of phycocyanin.

Keywords: Phycocyanin, membrane chromatography, large-scale purification, diafiltration (DF), optimized purification

RECOMBINANT HPV58 L1 CAPSID PROTEIN PRODUCTION IN *HANSENULA POLYMORPHA*

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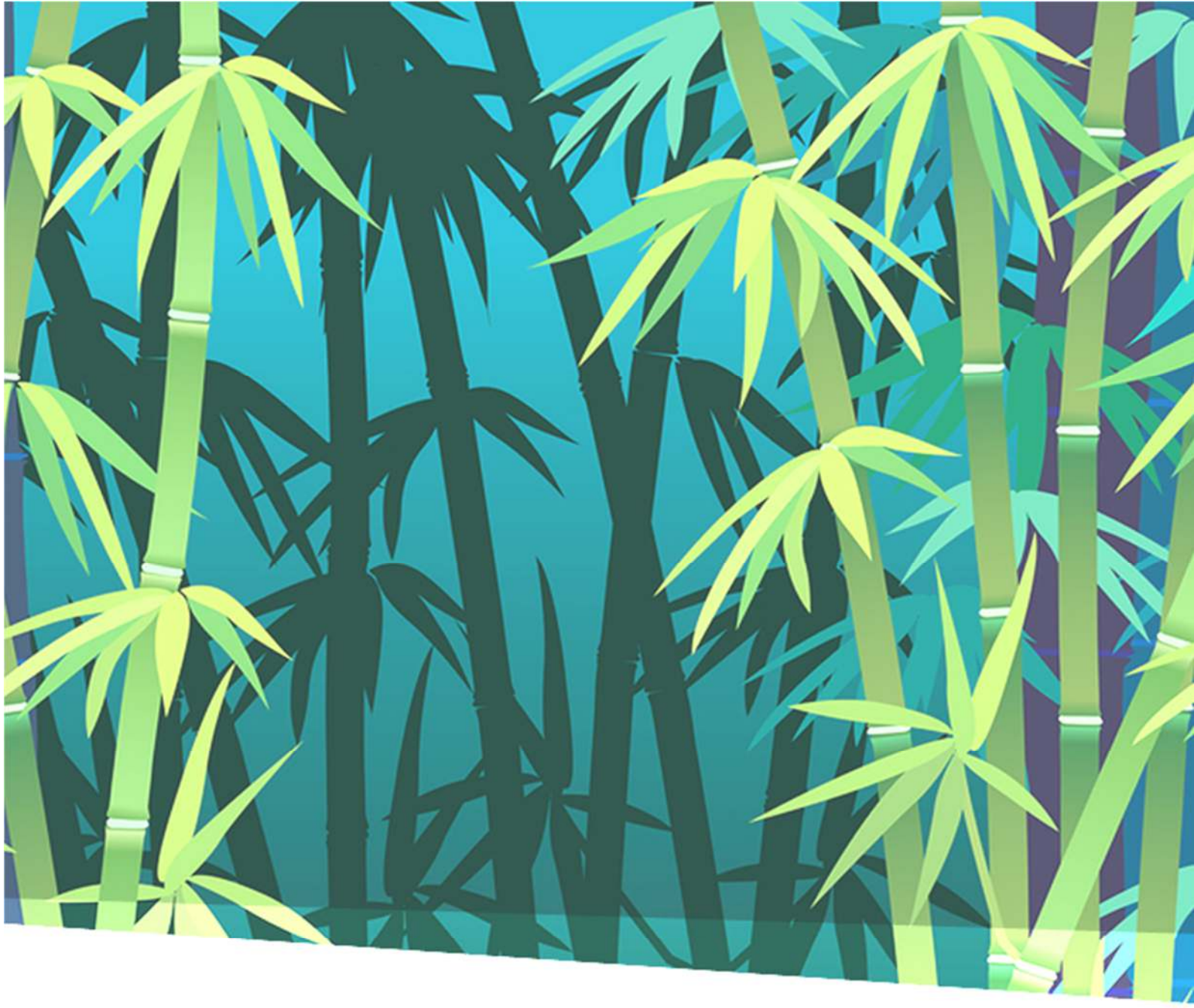
Abstract. HPV (Human Papilloma Virus) vaccine, derived from virus-like particles (VLPs) of a recombinant L1 capsid protein, is widely accepted as an efficient preventive measure against cervical cancer, the 4th most common cancer in women worldwide. In this study, the methylotrophic yeast *Hansenula polymorpha* was employed for production of recombinant L1 protein from HPV58, the 3rd most prevalent HPV subtype among Thai women. As the cultivation of each recombinant strain must be optimized specifically, this study aimed to optimize the cultivation medium and conditions to maximize the recombinant L1 protein yield from *H. polymorpha* HPV58, a strain previously constructed by our team. Various factors including types of iron supplement, methanol concentration for induction as well as supplementation of carbon (i.e., maltose) and complex nitrogen sources were investigated in detail using SYN6 (a defined medium previously developed for cultivation of *H. polymorpha*) as base medium. Finally, the optimized medium and condition were evaluated at a bioreactor-scale with a working volume of 2 L. In summary, ammonium iron (II) sulfate hexahydrate was selected as a suitable iron supplement based on its performance and cost-effectiveness. Methanol induction at 1%v/v was found to be the most optimal for expression of HPV58 L1 protein. Supplementation of a non-animal derived complex nitrogen source into SYN6 enhanced growth and production of L1 protein significantly. With a suitable control of pH and aeration in the bioreactor, L1 protein yield (mg/L-culture) from SYN6 medium increased by 2.5 folds compared to the flask-scale cultivation. Production of L1 protein from the optimized medium with supplementation of a complex nitrogen source at a bioreactor-scale enhanced the L1 protein yield even further (1.9-fold).

Keywords: human papilloma virus, HPV vaccine, L1 capsid protein, *Hansenula polymorpha*



6. Environmental Microbiology and Biotechnology

BACTERIA FEEDING ON ANTIBIOTICS – EATING THE POISONOUS

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University of Applied Sciences and Arts Northwestern Switzerland

Abstract.

Wastewater treatment plants (WWTP) are proposed to be a hub for the emergence of resistant bacterial strains and one of the major sources for the input of bactericidal micropollutants into the environment. Among these substances, sulphonamide antibiotics are the second most used antibiotics worldwide in human and in veterinary medicine with a release of ~20,000 tons year-1. The photo- and thermally stable sulfamethoxazole (SMX) as one representative of this chemical group, is often detected in significant concentrations reaching several µg/L. The biodegradation pathways are poorly understood, and several studies report insufficient sulphonamide removals by conventional sewage treatment.

We report here on the isolation of bacterial strains, which are not only resistant to the sulphonamide antibiotics, but also degrade and mineralize them. One of these isolates, namely *Microbacterium* sp. strain BR1 is able to feed on SMX as sole carbon and energy source. In this bacterium, the degradation of SMX and structurally related compounds is initiated by an *ipso*-substitution, catalyzed by a flavin-dependent monooxygenase acting in concert with a FMN reductase. The resulting *p*-aminophenol enters the central metabolism through a second monooxygenase activity, which leads to products amenable to ring opening. The cluster of genes involved in this degradation process was identified and each of these three enzymes could be heterologously expressed in *E. coli*. The presence of this gene cluster might represent an additional, yet unknown resistance mechanism for bacteria against sulfonamides. Even though the classic *sul1* gene is present as well in *Microbacterium* sp. strain BR1, its additional capacity to feed on SMX might represent a superior mechanism conferring to the bacterium clear advantages over a modified protein target especially in nutrient limited environments but also in case of human infection. Finally, we discuss the relevance of these findings addressing a series of questions arising from this research. Is the biodegradation of sulphonamides by *Microbacterium* BR1 a single case? Can the catabolism of sulphonamides be considered as a novel resistance to sulphonamides? What are the reciprocal effects of sadA and sul1? What is the significance of ipso-substitution during wastewater treatment? What if bacteria like *Microbacterium* infects human? Can bacteria feed on other antibiotic families? Do catabolic genes involved in the biodegradation of a given antibiotic impact the propagation of genes determining the resistance to this antibiotic and reciprocally?

INTRODUCTION AND UTILIZATION OF A GENE TARGETING SYSTEM IN A BASIDIOMYCETE *PLEUROTUS OSTREATUS* USING CRISPR/CAS9 GENOME EDITING TECHNOLOGY

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Abstract. The population of the planet has been growing recently. Global food demand, medical needs, and severe pollution are three possible outcomes. The oyster mushroom (*Pleurotus ostreatus*) has been used as a remedy for these issues. The oyster mushroom is now recognized as one of the most economically important species of cultivated edible mushrooms, with certain beneficial compounds and the ability to break down lignocellulosic wastes. Establishing CRISPR/Cas9-assisted genome editing in *P. ostreatus* is the goal of this research. We exhibited effective gene replacement and mutation via NHEJ and HDR using plasmid-based CRISPR/Cas9. After then, this method was used to clarify how *pcc1* and *clp1* affected the sexual development of *P. ostreatus*. Additionally, the split-marker strategy combined with Cas9 RNP can be used to address the drawbacks of the NHEJ-deficient strain. This study is the first to use CRISPR/Cas9 to modify the genome of the edible mushroom *P. ostreatus*, and it is the first step in the molecular breeding of non-GM edible mushrooms.

Keywords: oyster mushroom, CRISPR/Cas9, Cas9 RNP, gene targeting, homologous recombination

NITROGEN AND CARBON CO-REMOVAL FROM WASTEWATER WITH LOW CARBON TO NITROGEN RATIOS UNDER FEAMMOX-COUPLED-WITH-HETEROTROPHY CONDITIONS

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Abstract. Removal of nitrogen and carbon from wastewater is challenging for currently available technologies. Herein, effective treatment for synthetic wastewater (SW) and real anaerobic digester (AD) effluents was achieved via the feammox process by using a Multistage Feammox Bioreactor (MSFB). For the synthetic wastewater, batch incubations testing influents with different NH_4^+ and COD concentrations revealed that the [COD]/[NH_4^+] ratio of 1.4 (equivalent to a C/N ratio of 1.8) and the influent redox potential ranging from − 20 to + 20 mV led to the highest removal efficiencies, i.e. 98.3% for NH_4^+ and 58.8% for COD. N_2 was detected as the only product of NH_4^+ conversion, whereas NO_2^- and NO_3^- were not detected. While operating continuously with SW influent having a C/N ratio of 1.8, the system efficiently removed NH_4^+ (> 91%) and COD (> 54%) within 6 days of retention time. For the AD effluents, the C/N ratio was required to be 2.5 for the best performance. At a 6-day retention time, the MSFB reached removal efficiencies for NH_4^+ and COD at 99% and 97%, respectively, with a thorough conversion of NH_4^+ to N_2. Accordingly, the MSFB achieved removal rates for N and C of 14 and 34 mg L^{-1} d^{-1}, respectively. The C/N ratio of 2.5 is regarded to be the critical point above which the feammox is shifted to conventional iron reduction with organic carbon. Iron-reducing bacteria of the γ-Proteobacteria (*Pseudomonas* and *Acinetobacter*), and δ- Proteobacteria (*Geobacter*) were detected dominant in the MSFB and were supposed to drive the feammox process.

Keywords: Multistage Feammox Bioreactor (MSFB), Iron-reducing bacteria, *Pseudomonas*, *Geobacter*.

PROMISCUOUS METALLOHYDROLASE FROM A LOCALLY ISOLATED ORGANOPHOSPHATE DEGRADING *BACILLUS* SP. S3WAHI

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Abstract. Agricultural sector has emerged as a dynamic economic sector, contributing to overall economic growth worldwide. However, the growth of agriculture economy has caused the contamination of organophosphate (OP) compounds to rise in the environment and living organism. Hence, microbial organophosphate-degrading enzymes such as OP hydrolases, have been employed as environmentally friendly and effective agents to act as a bioscavenger in decontaminating OP compounds. This project utilized approaches to highlight the catalytic landscape of a locally isolated organophosphate degrading *Bacillus* sp. S3wahi and its structure-function relationship that contributes to the promiscuity. Genome sequence survey of *Bacillus* sp. S3wahi reveals a novel MBL enzyme, S3Wahi metallohydrolase, sharing a low sequence identity with MBL family protein. Steady state kinetic study demonstrated S3Wahi metallohydolase has the best binding affinity (K_M) and catalytic efficiency (K_{cat}/K_M), 8.09 μM and 1.242 E^{-08} $s^{-1}\mu M^{-1}$, respectively, towards organophosphate compound, paraoxon. The predicted 3D structure showed that S3Wahi metallohdyrolase appears to have a conserved sandwich αβ/βα metallo-β-lactamase-fold (MBL-fold) domain and the zinc bimetal nuclear at its catalytic site. S3Wahi metallohdyrolase showed catalytic promiscuity of distant family members and broad susceptibility of substrate. S3Wahi Metallohydrolase activity can be cofactor-dependently directed from β-lactamase to lipolytic activity, phosphotriesterase or glyoxalase II. The *in silico* molecular docking study also showed that S3Wahi metallohydrolase has a broad affinity towards various substrate compounds such as antibiotics, organophosphate compounds and oligopeptides, which are compatible to its catalytic metal binding site. By identifying S3Wahi metallohydrolase's active site architecture, substrate and cofactor ambiguity related to promiscuity, it demonstrate the multifunctionality of S3Wahi metallohydrolase. Thus, highlighting the unique features of MBL superfamily in exploring a novel organophosphate degrading metallohydrolase with improved characteristics to be used in bioremediation application.

Keywords: Metallo-β-lactamases, *Bacillus sp.*, Enzyme promiscuity, Zinc binding; Structure

IMMOBILIZATION OF A MULTIDOMAIN CARBOXYLIC ACID REDUCTASE FROM *MYCOBACTERIUM PHLEI* AS A STRATEGY TO IMPROVE ITS INDUSTRIAL RELEVANCE PROPERTIES

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Abstract. Many works are currently focusing on researching potentially robust aldehyde- producing enzymes. Carboxylic acid reductase (CAR) is a large multidomain oxidoreductase that can catalyze the one-step reduction of carboxylic acid to aldehyde. There is growing interest in the industrial application of this enzyme, for the synthesis of aldehyde as end-products especially in the flavor and fragrance industries, and aldehyde intermediates in enzymatic cascade reactions. Immobilization is one of the strategies to improve the stability and reusability of the enzyme. So far, the studies on CAR immobilization are still limited. Hence, this study aims to immobilize a CAR from moderate thermophile *Mycobacterium phlei* (*Mp*CAR), to investigate its potential as a biocatalyst for green industries. This work described the first immobilization of a bacterial CAR onto ionic polymer support, Seplite LX120 by a simple adsorption method. The optimal activity of immobilized *Mp*CAR was observed at 60 °C and pH 9. It was stable over a broad range of temperatures (10 to 100 °C) and pHs (pH 4 to 11), with >50% activity retained. The enzyme could be stored for up to 6 weeks at 4 °C and 3 weeks at 25 °C. The immobilized *Mp*CAR exhibited good reusability, up to 10 assay cycles. The thermostability of immobilized *Mp*CAR was also proven as 2.6 mM of benzaldehyde product was obtained, up to at 60 °C. The successfully immobilized *Mp*CAR demonstrated enhanced biocatalytic properties which potentially could increase its industrial utility.

Keywords: carboxylic acid reductase, *Mycobacterium phlei*, immobilization, adsorption, stability

BIOREMEDIATION OF DICLOFENAC AND IBUPROFEN USING IMMOBILIZED CALCIUM ALGINATE BEADS CONTAINING BACTERIAL CONSORTIUM

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Abstract. Diclofenac and ibuprofen are among the most commonly used NSAIDs, and are present ubiquitously in the aquatic environments around the world because of their biorefractory and persistent nature. The presence of these pharmaceuticals in plants as well as in bile of wild fishes demonstrate their penetration in our food webs. Their toxic effects are well reported in several model organisms. The aim of this study was to remove these target NSAIDs using an immobilized bacterial mixed culture. A bacterial consortium capable of degrading diclofenac and ibuprofen was isolated from contaminated hospital soils. This consortium was encapsulated in calcium alginate beads and used for bioremoval. The metagenomic analysis of the bacterial culture yielded the members of families of Alcaligenaceae, Bacillaceae, Clostridiaceae, Enterococcaceae, Paenibacillaceae, Pseudomonadaceae, Sphingobacteriaceae and Xanthomonadaceae. However, the highest composition of the culture included members from Pseudomonadaceae, Bacillaceae and Clostridiaceae. Above 80% and 70% removal were observed for all the test assays containing 5 mg/L diclofenac and 6 mg/L ibuprofen respectively in a period of 10 days. The effect of the number of beads on the degradation of diclofenac and ibuprofen was also analysed using HPLC. The highest removal was observed for the test assays with 20 beads where 84.49% and 81.1% removal was attained for diclofenac and ibuprofen respectively. The repeatability of these beads was also tested for determining their recycling capacity. Since immobilization offers protection from various shear forces and environmental stresses by providing stability to microbial cells, this method could prove to be efficient for the bioremoval of pharmaceuticals from wastewater treatment plants and aquatic ecosystems.

Keywords: Bacterial cell immobilization, diclofenac, ibuprofen, bioremediation, metagenomic analysis

DETERMINED MICROBIAL FLORA OF PROTEOLYSIS IN THE INITIAL STAGE OF FISH SAUCE FERMENTATION BY METAGENOMIC SEQUENCING

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Abstract. Nước mắm is a Vietnam traditional fish sauce made by fermenting salted anchovies. Fish sauce fermentation is a complicated process of 6 –12 months involving many metabolic processes. Protein metabolism plays a vital role with support from the microbiome in the initial stage of fish sauce fermentation. The present study aimed to reveal the whole microbial community structure in the initial stage of fish sauce fermentation, along with the prediction of functional microbial profiles by metagenome sequencing. We showed the presence of 22 phyla, 35 classes, 83 orders, 163 families, 349 genera, and 637 species of bacteria in the fish sauce. Bacillota and Pseudomonadota played a leading role in the initial stage of fish sauce fermentation, and their average relative abundance was greater than 50%. At the genus level, *Staphylococcus*, *Psychrobacter*, *Vibrio*, *Pseudoalteromonas*, *Streptomyces*, *Tetragenococcus*, *Halanaerobium*, *Macrococcus*, *Pseudomonas* were dominant bacteria genera in all three samples belonging to Bacillota and Pseudomonadota. Functional annotation of gene based on KEGG database in all three samples were related to metabolism with 57%. In addition, it revealed 15 pathways associated with amino acid metabolism and many enzymes with proteolytic activity related to species *Pseudoalteromonas* sp., *Halanaerobium praevalens*, *Macrococcus caseolyticus*, *Staphylococcus saprophyticus*, *Staphylococcus arlettae*, *Vibrio diabolicus*, *Vibrio alginolyticus*, *Psychrobacter* sp., *Tetragenococcus halophilus*, *Streptococcus pantholopis*. The results of this study provide important information for understanding the microbial community structure and functions and the effect of microbiome in fish sauce fermentation.

Keywords: Nước mắm, metagenomic sequencing, protein metabolism, microbial community.

AUXILIARY ACTIVITIES FOR LIGNOCELLULOSE DEGRADATION FROM BACTERIA: A QUESTION OF LOCATION

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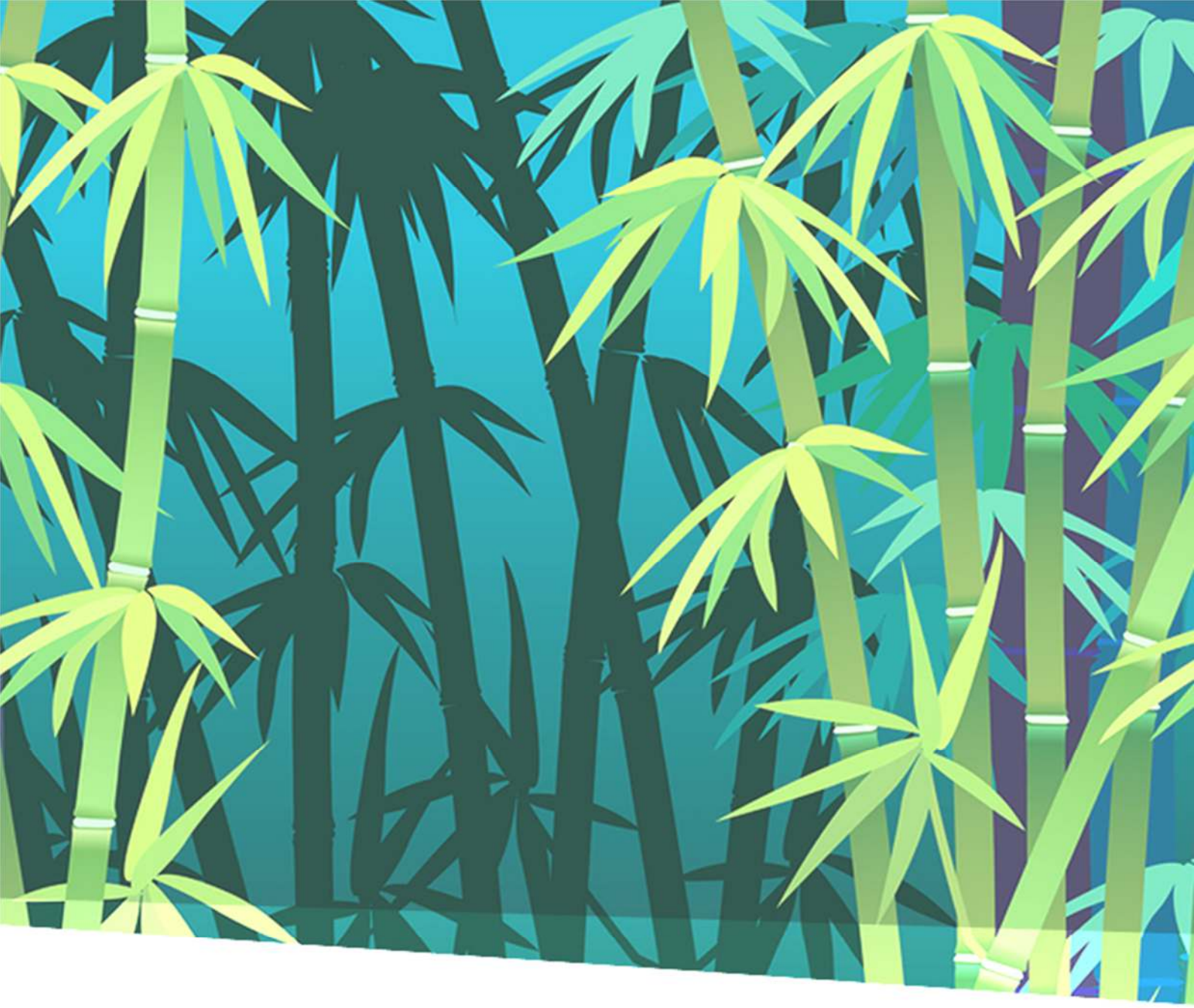
Abstract. Degradation of lignocellulose is best described in fungi, which degrade lignin through a cocktail of laccases and various peroxidases as well as auxiliary enzymes such as peroxide-generating oxidases. These enzymes can belong to the Glucose-Methanol-Choline (GMC) oxidoreductase family of flavoenzymes (Auxiliary Activities Family 3), such as aryl alcohol oxidases or pyranose oxidases, and may also be involved in the oxidative depolymerization of cellulose and hemicellulose through lytic polysaccharide monoxygenases (LPMOs), for which hydrogen peroxide is an essential co-substrate. Aryl alcohol oxidases and pyranose oxidases also have significant dehydrogenase activities using (substituted) quinones as electron acceptors, which may play a role in lignin depolymerization, as combining fungal laccase or peroxidase with pyranose oxidase increased the degree of depolymerization markedly *in vitro*. A potential for lignin depolymerization and modification in bacteria using similar enzymatic activities is increasingly recognized, however, only few reports on enzymes that can be considered Auxiliary Activities exist. The sequence of POx from *Kitasatospora aureofaciens* (*Ka*POx) is closely related to fungal POx and shows oxidase activity suitable for peroxide provision as well as dehydrogenase activity to prevent re-polymerization of degradation products and to protect the cells from damage by those radicals. Both roles are only plausible if POx is located extracellularly, otherwise hydrogen peroxide (for peroxidase activation) would have to be transported out of the cells, and free radicals from lignin depolymerization would have to be imported into the cells. We investigated the subcellular location of *Ka*POx by heterologous expression using Gram-positive bacterial expression systems and included additional sequences upstream of the annotated ATG that may constitute a functional signal peptide. We also investigated the occurrence of such potentially secretory Auxiliary Activities in Actinomycetes and related bacteria, and discuss the implications for further research.

Keywords: flavoprotein oxidoreductases, auxiliary activities, lignin depolymerization, Actinomycetes



7. Drugs Delivery and Development

SELF-ASSEMBLED POLY(AMINO ACID)-BASED NANO-DRUG FOR BIOMEDICAL APPLICATIONS

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Abstract. Amino acids have attracted considerable attention in drug development because they play important roles in many physiological and pathological processes. In the past several decades, various amino acid supplementations have been reported to have potential therapeutic efficacy for the treatment of many disorders in clinical trials. However, their effectiveness is controversially reported, which may be explained by poor pharmacokinetic properties of such low-molecular-weight (LMW) agents. The nanoparticle-based drug delivery systems (nano-DDS) have attracted considerable attention for the last several decades as an innovative medical technology, since these systems alter the biodistribution of the therapeutic agent with the controlled release manner at the target tissues, resulting in a significant therapeutic drug effect. Merely simple encapsulation of the conventional LMW amino acid in nano-DDS can not be stable enough and cannot control the release properly. In this presentation, we have discussed the current therapeutic applications of certain amino acids, and have introduced our approach of using amino acid-based self-assembled nanostructures as novel therapeutic agents.[1] Using animal disease models, poly(ornithine)-based nanoparticle was designed for treatment of hyperammonia in liver acute injury,[2] poly(dopamin)-based nanoparticle was used in treating the Parkinson's disease,[3] and poly(arginine)-based nanogel was used in treatment of myocardial infarction.[4] By covalently introducing a substrate into the nanosystem, amino acid-based self-assembled macromolecules could overcome the aforementioned drawbacks by improving the pharmacokinetic profile and accumulation of specific molecules at target sites to enhance the therapeutic effect. As the results, the therapeutic efficacies have been significantly improved, which is anticipated to open the future development of an emerging and novel concept of self-assembled macromolecule-based therapeutic approach.

Keywords: self-assemly, nanomedice, poly(aminoacid), drug delivery, inflammation, cancer, biopolymers.

Investigation into Antiviral Drug Candidates: Unveiling Targets and Unraveling Their Underlying Mechanisms

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Abstract. Our laboratory has dedicated its research endeavors to developing antiviral agents for combatting enteroviruses and influenza viruses. We have explored a wide range of sources, including natural products and synthetic compound libraries. Employing a cell-based antiviral assay, we successfully pinpointed potential inhibitors and further substantiated their efficacy using in vivo mouse models. Our findings reveal that specific compounds, notably rosmarinic acid and 3,4-dicaffeoylquinic acid derived from medicinal plants, exhibit the potential to disrupt the initial stages of enterovirus A71 infection by directly targeting viral particles. This interference disrupts virus-receptor interactions effectively. Subsequent sequencing analysis of plaque-purified drug-resistant viruses unveiled mutations in the five-fold axis of the structural protein VP1. These mutations involve positively charged amino acids known to be involved in virus-heparan sulfate (a host receptor) interactions through electrostatic attraction. Recently, our research has led to the discovery of a novel inhibitor with precise targeting capabilities toward the viral 2B protein of enterovirus D68 which acts as a viroporin, exerting influence over calcium flux within host cells. Our investigation has shed light on the pivotal role played by 2B in regulating intracellular calcium levels for viral replication. Through the identification of these antiviral inhibitors and our comprehensive exploration of their mechanisms of action, we have garnered valuable insights into previously undisclosed molecular functions of viral proteins within the context of viral entry and replication.

Keywords: drug development, enteroviruses, influenza virus, mechanism of action, viral replication

Bioassay accompanied machine learning as an efficient approach for identifying natural compounds towards regulating Nrf2 for cancer treatment

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Abstract

Nuclear factor erythroid-2 p45-related factor 2 (Nrf2) is the transcription factor known for cellular stress adaption. Inhibition of Keap1/Nrf2 is a promising approach for activating Nrf2. However, the elevated levels of Nrf2 in hepatocellular carcinoma cells induce cancer invasion and inhibit apoptosis. Medicinal plants are a diverse source for metabolites with various pharmacological activities. However, the isolation of active natural compounds targeting Nrf2 are time-consuming and require considerable effort. Bioassay accompanied by machine learning guided identification of natural compounds will be the promising approach to seek novel natural compounds for Nrf2 activation and inhibition.

In this study, we screened the effects of 52 methanol extracts from different parts of 24 medicinal plants to evaluate the ability to regulate Nrf2 at the crude extract concentration of 100 µg/mL. The study has successfully built the screening model for Nrf2 expression based on luciferase fluorescence assay of Huh7 cells transfected with the Nrf2 gene. The extracts of *Helicteres hirsuta* leaves, Curcuma zedoaria roots, *Cleome rutidosperma* stems and leaves, *Dicliptera chinensis* leaves, *Zingiber zerumbet* roots exhibited notable inhibition of Nrf2 expression levels, ranging from 1.91% to 49.27%. Additionally, the results revealed that *Phyllanthus amarus* leaves, *Piper sarmentosum* root and *Oroxylum indicum* stem extract exhibited an approximately 500% enhancement in Nrf2 activity within HaCaT cells.

We utilized machine learning algorithm-based model for discovering potential compounds on inhibiting Keap1/Nrf2. The Support Vector Machine algorithm achieved the highest accuracy of 90% after hyperparameter tuning in discovering potential compounds. Different natural compounds from the *Piper sarmentosum, Oroxylum indicum*, and *Phyllanthus amarus* are selected as a test dataset. Among them, top ten compounds exhibit activity against Keap1/Nrf2. The inhibition mechanisms of these natural compounds are analyzed using molecular docking simulation.

This study shows that bioassay accompanied by machine learning likely screen bioactive compounds targeting Nrf2 and offer the basis for further research into substances derived from Vietnamese medicinal plants.

Keywords : Nrf2, medicinal plant, machine learning, Huh7, Support Vector Machine algorithm, molecular docking

DEVELOPMENT OF POLYMERIC HYDROGELS FOR BIO-RELATED ENERGY STORAGE

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Abstract. The drug delivery system is a helpful tool for increasing therapy effectiveness and reducing the negative effects of the active ingredient. Paracetamol is often prescribed several times per day, which can make it difficult for some patients to adhere to it. The drug delivery system may be able to better regulate the release of paracetamol. By continuing to use the medicine at therapeutic doses, the risk of an overdose can be reduced. With less usage, the possibility of medication errors or missed dosages can also be reduced. Paracetamol release may be better controlled by the drug delivery system, leading to more constant and reliable medication levels in the body. The danger of an overdose can be decreased by maintaining therapeutic doses of the drug in this way. The goal of this study is to create a polysuccinimide-based paracetamol drug delivery system. In vitro experiments, the polysuccinimide-based hydrogels strengthened the mechanical properties, prolonged the drug release duration, and completely dissolved and removed the hydrogel from the body for 1 day. The results showed that this hydrogel has intriguing potential applications for promoting the rapid removal of extra hydrogel from the body and the long-term retention of paracetamol's analgesic action.

Keywords: alginate, drug delivery, hydrogel, paracetamol

ANTIFUNGAL FACIAL SOAP AND SHAMPOO FORMULATION FOR THE TREATMENT OF *MALASSEZIA FOLLICULITIS* AND *MALASSEZIA GLOBOSA* FROM *TETRAGONULA SAPIENS* AND *HETEROTRIGONA ITAMA* BEE PROPOLIS

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Abstract. Excessive inflammation causes inflammation on the surface of the skin that looks like pimples, is a disease caused by the population the fungus *Malassezia folliculitis*. Seborrheic dermatitis or dandruff is inflammation of the scalp, one of which is caused by the fungus *Malassezia globosa*. Antifungal drugs such as ketoconazole are a class of hard drugs, so they cannot be used freely. Indonesia's various types of propolis encourage innovation to utilize this natural resource as an active ingredient to create natural antifungal care products. The objective of the study was to formulate antifungal facial soap and shampoo for the treatment of *Malassezia folliculitis* and *Malassezia globosa* from Indonesian bee propolis. The resulting propolis extract was tested to determine antifungal activity, the value of total polyphenol and flavonoids by using spectrophotometry, and the compounds contained by using LC MS/MS. The antifungal activity results showed that the facial soap with Belitung propolis extract performed better than Sulawesi propolis. Through the broth microdilution method, Belitung propolis extract was found to have better inhibition against wild-type *Malassezia globosa* than Sulawesi propolis extract at an extract concentration of 64 µg/mL. The test results showed that the highest total polyphenol content was owned by Sulawesi propolis at 498.38 ± 1.29 mgGAE/g extract and the highest total flavonoid content was owned by Belitung propolis at 204.91 ± 0.47 mg QE/g extract. The content of propolis active chemical compounds can be determined by the LC-MS/MS method in which 31 active compounds and 2 marker compounds were obtained, namely Leptomycin A and Mangostin.

Keyword: Anti-fungal agents, Belitung Propolis, Sulawesi Propolis, Malassezia, natural face wash, antidandruff shampoo.

BACULOVIRUS-GENERATED PEG10 PVNPS PACKAGE THERAPEUTIC MRNA FOR ANTITUMOR IMMUNITY

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Abstract. Pseudovirus-like nanoparticles (PVNPs) are non-infectious viral particles that can serve as gene delivery platform. Recent research found that PEG10, a mammalian endogenous protein, can recognize specific packaging signals and encapsulate mRNA to form PVNPs. This novel encapsidation platform was capable of delivering mRNA cargo efficiently and reducing immunogenicity, which made it a promising tool for mRNA gene therapy. In this study, production of PEG10 PVNPs was attained by a baculovirus-mammalian cell expression-system, with a titer of 1×10^7 transducing unit (TU)/ml. PEG10 PVNPs could transfect mRNA into a myriad of cancer cell lines within 2 days and especially effective to CT26 which achieved 60% mRNA delivery efficiency.

Upon confirming the efficient transfection capability of PEG10 PVNPs, we proceeded to select OX40L and IL-12 as a notable therapeutic dual for mRNA cargo, aimed at bolstering immune stimulation. Analysis of OX40L/IL-12 expression profiles revealed that concentrations of OX40L and IL-12 reached 1 ng/ml and 10 ng/ml respectively, within the supernatant. In an in-vitro study involving co-culture of splenocytes and transfected CT26 cells, a remarkable six- to eightfold increase in T-cell expansion was observed compared to the mock group. Additionally, the ratio of $CD4^+$ T cells uplifted from 20% to 30% post PEG10 PVNP treatment, indicating an augmentation of Th differentiation and proliferation. The combined effect of OX40L and IL-12 elicited a potent therapeutic effect against tumor invasion and recurrence. Our next steps involve utilizing PEG10 PVNPs to encapsidate the OX40L/IL-12 mRNA cargo and applying this approach to cancer models for further exploration.

DEVELOPMENT OF SELF-AMPLIFYING (SARNA) SYSTEM FOR COMBINATION CANCER THERAPY

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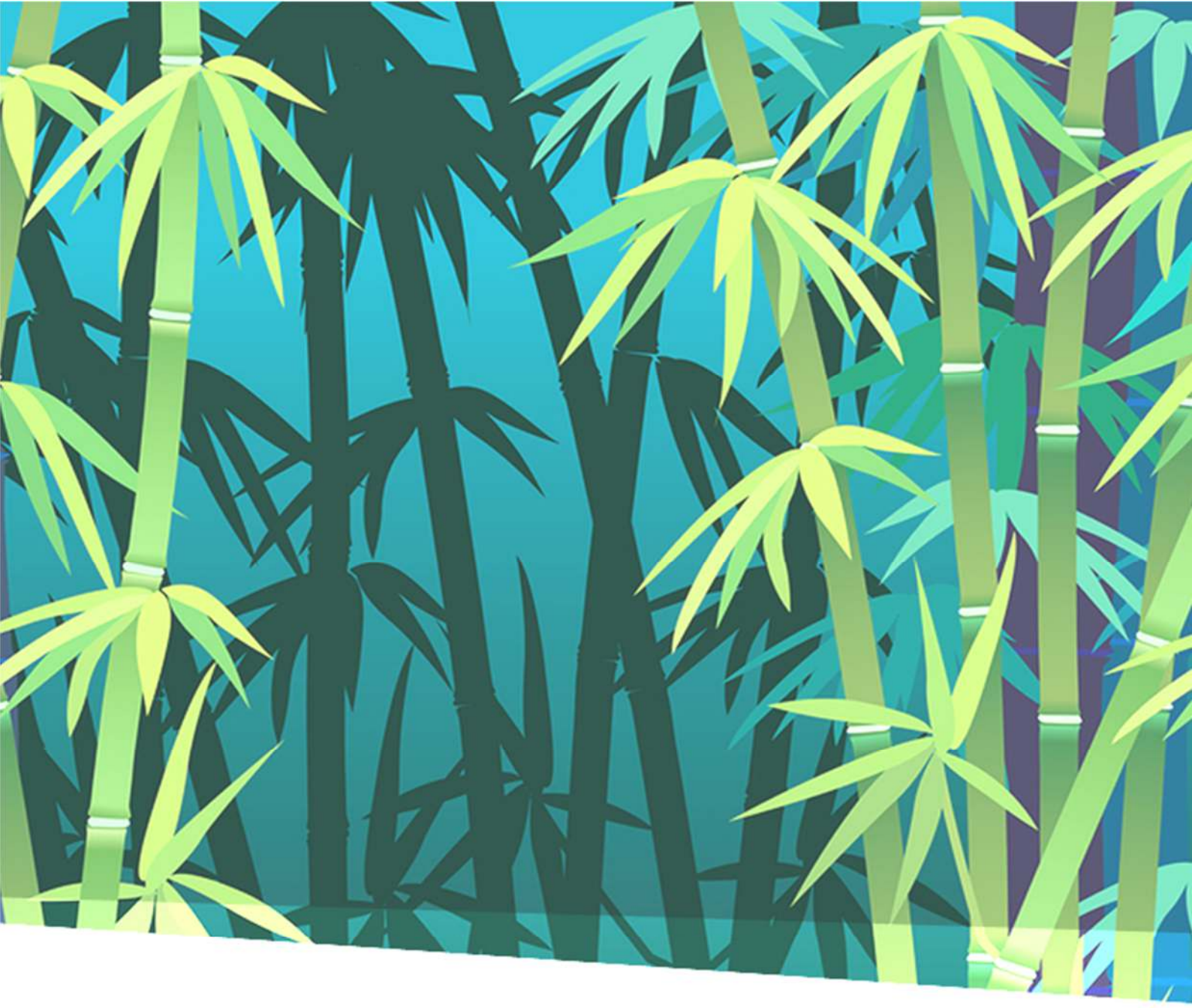
Abstract. Antitumor immune responses induced by chemotherapy have remained challenging of not being very effective due to the complexity of inducing all events relating to cancer cell killing and remodeling of the tumor microenvironment. Taking advantage of self-amplifying RNA (saRNA) over the conventional mRNA for exponential protein expression, we herein developed novel saRNA-encapsulated in TT3-lipid nanoparticles (saRNA-TT3-LNP) and combined it with a chemotherapy drug to induce and self-sustain antitumor immunity more effectively. We first verified the prolonged expression of our saRNA-TT3-LNP by transfecting into different cancer cell lines using saRNA-encoded ZsGreen protein. The results showed that the expression level of fluorescence could sustain up to 28 days in CT26 cell line. Next, saRNA payload was replaced by two effective antitumoral cytokines, OX40L and IL12, linked through P2A peptide sequence, and this saRNA system was called saOX12-LNP. The gene expression of OX40L and IL12 could sustain for up to 21 days in CT26 cell line when measured by qRT-PCR and ELISA. Then, the antitumor effect of saOX12-LNP was confirmed in the tumor-bearing mice model. After establishing tumors, mice were divided into several treated groups such as PBS, Oxaliplatin (OXA), saOX12-LNP, and the combined treatment between Oxaliplatin and saOX12-LNP. A single injection of saOX12-LNP after injection of OXA in the combined treatment outperformed the OXA only in eradicating the tumor with long-term survival of up to 17 days. The analysis of tumors after receiving treatments disclosed vital role of saOX12-LNP in enhancing T cell activation cascade after the priming of OXA in the combined treatment. In conclusion, we demonstrated a potential strategy that effectively inflamed the tumor microenvironment through immune responses and overcome the limitation of using chemotherapy drugs as monotherapy, offering a promising platform for combination cancer treatment.

Keywords: antitumor immunity, self-amplifying RNA, T-cell activation, combination cancer treatment



8. Applied Microbiology

ADVANCE RESEARCH ON MICROBIAL BIOTECHNOLOGY FOR ORGANIC AGRICULTURE TOWARD SUSTAINABLE DEVELOPMENT GOALS (SDGS)"

Kasem Soytong

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Abstract. ***Sustainable development goals (SDGs)*** is one of the target to promote food safety and security to serve human being for good health and long life. Organic agricultural production is needed the advances research om scientific basis to investigate agricultural inputs such as different formulations of biofertilizers, biofungicides, bioinsecticide, bionematicide, bioherbicide, and natural products from plants and potent microorganism , bioremediation and soil revitalizers etc. to be completely used instead of toxic agrochemicals. In accordance with the policy of sustainable development goals (SDGs), the Association of Agricultural Technology in Southeast Asia ***(AATSEA)*** has decided to promote the adoption of practical approaches to organic agriculture for the farmers. This is to provide the consumers consume safety food for good health and protect our surrounding environment. With this, ***Earthsafe foundation and AATSEA*** make a criteria for organic agriculture in practices as follows: ***Non-agrochemical production (NAP)*** which is defined as the growers must suddenly stop using synthetic agrochemicals (chemical fertilizers, chemical pesticides etc. for crop and animal production. The aim is to revitalize the surrounding agroecosystem and environment, improve soil biodiversity, soil fertility with high organic matter, and proper soil pH for plant growth. NAP farms are recommended to get rid of toxic chemical residues in the soil, water, and agricultural products in transition period. The growers combine conventional methods to maintain and improve soil fertility, biological activities, biodiversity, soil revitalization and remediation with beneficial microorganisms, apply biological products and natural products as agricultural inputs for their production to maintain the quantity and quality of agricultural products taking into account food security and safety. NAP is organic agricultural production *in transition period* until non-detected toxic agrochemicals in agricultural products. It is a crucial period of changing from the conventional and chemical agriculture to organic agriculture. ***Organic agriculture (OA*****)** is defined as a production system that relies on the natural ecosystem where negative environmental and social impacts which must be stopped the use of synthetic agrochemical inputs, such as synthetic fertilizers and pesticides, veterinary drugs, and genetically modified seeds/organisms. Synthetic chemicals are replaced by organic innovative products, natural products, beneficial microorganisms, biological products, natural substances, and management practices that maintain and increase long-term soil fertility. Organic agriculture promotes and enhances agroecosystem, biodiversity, biological cycles, soil fertility, and activities. Organic agriculture products do not contain toxic synthetic agrochemical residues and are thus safety food. Organic agricultural production includes crop and animal production, organic seed production, organic animal feed, organic food processing and so on. Organic certification is urgently needed to promote from farms to table as the market demand and to guarantee for reliable of the consumers.

STUDY ON DNA VACCINE AGAINST *NOCARDIA SERIOLAE* INFECTION IN ORANGE-SPOTTED GROUPER *EPINEPHELUS COIOIDES*

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Abstract. Nocardiosis in aquatic animals caused by Nocardia seriolae is a frequently occurring serious infection that has recently spread to many countries. In this study, DNA vaccines containing potential bacterial antigens predicted using the reverse vaccinology approach were developed and evaluated in orange-spotted groupers. In silico analysis indicated that proteins including cholesterol oxidase, LD-transpeptidase, and glycosyl hydroxylase have high immunogenicity and are potential vaccine candidates. In vitro assays revealed the mature and biological configurations of these proteins. Importantly, when compared to a control PBS injection, N. seriolae DNA-based vaccines showed significantly higher expression of IL1β, IL17, and IFNγ at 1 or 2 days, in line with higher serum antibody production and expression of other cellular immune-related genes, such as MHCI, CD4, and CD8, at 7 days post-immunization. Remarkably, enhanced immune responses and strong protective efficacy against a highly virulent strain of N. seriolae were recorded in DNA vaccine-cholesterol oxidase (pcD::Cho) injected fish, with a relative survival rate of 73.3%. Our results demonstrate that the reverse vaccinology approach is a valid strategy for screening vaccine candidates and pcD::Cho is a promising candidate that can boost both innate and adaptive immune responses and confer considerable protection against N. seriolae infection.

Keywords: Nocardia seriolae, reverse vaccinology, Vaxign-ML, in silico, DNA vaccine, orange-spotted grouper, cholesterol oxidase, LD-transpeptidase LppS, glycosyl hydroxylase, pcDNA3.1(+)

LEVERAGING YEAST FLOCCULATION FOR ROBUST LACTIC ACID FERMENTATION

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Abstract. Yeast-based lactic acid fermentation offers more robust production and simpler nutrient requirements compared to the process facilitated by bacteria. Numerous studies have aimed to amplify lactic acid accumulation in yeast through pathway engineering and optimization of process parameters. This study takes a different approach by leveraging the unique trait of yeast flocculation to enhance lactate production. Flocculating and non-flocculating *Saccharomyces cerevisiae* strains, each incorporating an exogenous lactate dehydrogenase gene, exhibited a distinctive profile of fermentation products, with the former being more stable when cultivated in different cell densities and in the presence of chemical inhibitors commonly generated from lignocellulose pretreatment. The non-flocculant yeast demonstrated significant alterations in the expression levels of genes related to glycolysis and energy metabolism as cell density was increased and chemical inhibitors were introduced. Additionally, the flocculating strain had lower expression levels of stress response genes, indicating the role of flocculation in protecting cells from acid stress. Key differences in flocculation behaviors between strains could be attributed to the variations in the amino acid sequences within the tandem repeat regions of the Flo5 protein. Notably, flocculation allows for immediate cell separation from the cultivation medium, thereby simplifying downstream processing and reducing overall production costs. This study highlights the potential of flocculation in bolstering lactic acid production in yeast, offering valuable insights into the associated metabolic mechanisms, and revealing promising gene targets for strain enhancement.

Keywords: yeast flocculation, *Saccharomyces cerevisiae*, lactic acid, cell density, chemical inhibitors

CHARACTERIZATION OF TWO *ESCHERICHIA* PHAGES AGAINST DRUG-RESISTANT HYBRID IPEC/EXPEC *E. COLI* ISOLATED FROM PIGS

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Abstract Pathogenic *Escherichia coli* is widely known as a serious issue for swine production, especially those that cause severe symptoms and death in piglets. Moreover, the emergence of drug-resistant pathogens has become the most serious obstacle to treating infected pigs ~~by~~ with antibiotics. Bacteriophages are viruses that naturally infect and lyse target bacteria, thus providing a promising alternative for combating drug-resistant bacteria. In this work, two *Escherichia* phages, vB_Eco-RN855i1 and vB_Eco-RN861i3, were isolated using pathogenic *E. coli* strains isolated from diarrheal pigs as hosts. Their bacterial hosts resisted 2–5 antibiotics and carried virulence genes of both intestinal (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC). Host ranges, efficiency of plating, and thermal and pH stability of both phages were characterized. The host range results revealed that vB_Eco-RN855i1 and vB_Eco-RN861i3 infected and lysed 12 and 6 of 23 tested *E. coli* isolates, respectively. vB_Eco-RN855i1 effectively produced clear plaques at 15–37°C, while 30–42°C was the optimal range for vB_Eco-RN861i3. vB_Eco-RN855i1 was stable at 4–45°C for 24 h, whereas vB_Eco-RN861i3 showed a slight reduction at 45°C. The pH stability of the phages in simulated gastric fluid showed that vB_Eco-RN855i1 was sensitive to a pH range of 2.5–4.0. In contrast, the vB_Eco-RN861i3 titer decreased gradually from pH 4.0 to 3.0 and became inactive at pH 2.5. According to the results, this suggested that vB_Eco-RN855i1 and vB_Eco-RN861i3 had ability to eliminate pathogenic *E. coli* at a wide range of temperatures, including pig body temperature. Furthermore, they could be stored in room and housing temperatures. Nevertheless, acidic sensitivity profiles should be taken into account for phage therapy by oral administration.

Keywords: Bacteriophage, Drug-resistant bacteria, E. coli, Pig

MICROBIAL DEGRADATION OF NITRILE RUBBER

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Abstract. In production of nitrile rubber (NBR), its approximately 50 wt% is discarded as mill ends and most of them is incinerated as well as used NBR. Thus, it is required to develop an effective recycle method. Our goal is development of bio-recycle method of NBR. We have isolated an NBR-degrading bacterium from activated sludge of wastewater treatment of a chemical synthesis plant. An NBR-degrading actinomycete, *Gordonia* sp. strain J1A, degraded 6.5-11 wt% of used NBR in one week cultivation at 37ºC. Strain J1A produced an NBR- degrading enzyme to the cell surface. The enzyme was partially purified, and the purified enzyme sample degraded 1.5 wt% in 24 h at 37ºC and pH 8. The nitrile unit contents of NBR were reduced from 39 wt% to 34 wt% by the enzyme reaction. Moreover, an aromatic compound with nitrile group was detected in the gas phase after the enzyme degradation by GC-MS analysis. In the aqueous phase, MALDI-TOF-MS analysis suggested the products of NBR oligomers containing one or two oxygen atoms. In addition, carbon black powder and white precipitates occurred in the reaction mixture. IR analysis suggested that the white precipitates were contained carbonyl group and C=C bond. A whole genome analysis showed that strain J1A possessed 59 oxygenases and 39 oxidases genes as well as oxidoreductases, esterases, and disulfide bond-degrading enzymes. We concluded that strain J1A assimilates NBR oxidatively and can also degrade plasticizers and sulfur cross-linking (-S-S-) bond. Furthermore, RNA-Seq analysis suggested that three candidates such as oxidase and oxygenase may be involved in NBR degradation.

Keywords: nitrile rubber, biodegradation, nitrile rubber-degrading enzyme, *Gordonia* sp.

DIVERSITY AND BIOLOGICAL CHARACTERISTICS OF ICE-BINDING PROTEINS FROM COLD-ADAPTED MICROORGANISMS

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Abstract. Many living organisms have various biochemical and ecological strategies to protect themselves from intracellular freezing. Ice-binding proteins (IBPs) have the unique ability to attach to hexagonal ice crystals to inhibit their growth, resulting in depression of the freezing point of water and leading to protection of cells from freezing injury. Such depression of the freezing point is 500 times greater than that of colligative salts on a molar basis and occurs non-colligatively because of IBP-induced thermal hysteresis: a disparity between the melting and freezing points of the solution. IBPs also affect the morphology of ice crystals, creating bipyramidal ice crystals. We found extracellular IBPs (ca. 25 kDa) from several taxa of fungi, and their genes were completely different from IBPs from animals and higher plants. Fungal IBPs were also found from prokaryotes (archaeum and bacteria) and eukaryotes without fungi. These findings were suggested that microorganisms acquired fungal IBP genes moved though the horizontal gene transfer. However, intracellular and/or various molecular masses of IBPs of microorganisms were also reported. In my presentation, I will overview the diversity and biological characteristics of IBPs from cold-adapted microorganisms.

Keywords: AFP, *Antarctomyces*, antifreeze proteins, snow mold, *Typhula*

DEVELOPMENT OF A GENOME-EDITING CRISPR/CAS9 SYSTEM IN *SACCHAROMYCES CEREVISIAE*

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Abstract. CRISPR/Cas9 is a revolutionary gene-editing technology that enables precise modifications to the DNA sequence in living cells. In this study, we developed a CRISPR/Cas9 system for genome editing in *S. cerevisiae* to develop new yeast strains for industrial applications without marker detection. CRISPR/Cas9 system was generated by constructing a recombinant vector carrying genes encoding for Cas9 protein and gRNA. To examine the operation of this system, two donor cassette genes HA::*zeo*^{R} và HA::*gfp* were designed and obtained for the yeast genome editing. The efficiency of cassette HA::*zeo*^{R} and HA::*gfp* integration was 90.0% and 40-70%, respectively. The antibiotic resistance gene (G418) contained in the CRISPR/Cas9 vector system was eliminated from the engineered yeast strains by culturing in a non-selective medium. Two genome-edited yeast strains showed the ability to express the corresponding heterologous proteins. Thus, the CRISPR/Cas9 vector system could be employed for the development of new yeast strains with markerless integration of expression cassettes in our further studies.

Keywords: CRISPR/Cas9, markerless integration, genome-editing, *Saccharomyces cerevisiae*

SILK WORM (*BOMBYX MORI)* GUT SYMBIONT *KOCURIA MASSILIENSIS* - A POTENTIAL PROBIOTIC FOR SERICULTURE

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Abstract. *Bombyx mori,* the domesticated silkworm, is of great importance as a silk producer and as powerful experimental model for basic and applied research. Silkworm is also associated with a large consortium of symbiotic microbes. These beneficial gut microbiome plays essential roles in nutrient acquisition, digestion, absorption, growth, development, immunity response and environmental adaptation. In the present study, we comprehensively characterized the gut microbiota of the domesticated silkworm. We isolated 89 bacterial isolates from silkworm gut and evaluated their potential use in sericulture. 10 isolates were screened down based on their ability to produce digestive enzymes such as cellulase, amylase, lipase and protease. Among them 5 isolates showed hemolysis activity and 2 isolates found to be silkworm pathogens hence omitted from further study. pH tolerance study reveals that SP28 isolate have highest tolerance across 3-11 pH and was selected for further studies. SP28 showed antibacterial activity against silk worm pathogens *Staphylococcus aureus, P. vulgaris* and *P. mirabilis*. It also exhibited cell adhesion properties such as auto aggression, co aggression and hydrophobicity. Genomic identification revealed that SP28 show 98.51% similarity towards *Kocuria massiliensis* & as per our knowledge it is the 1st report to identify this bacterium in the gut flora of silk worm. *In vivo* probiotic effect of *Kocuria massiliensis* was studied by supplementing to silkworm along with mulberry leaves. The results indicated that probiotic treatment, there is a profound increase in larval growth and cocoon characters than the control with enhanced immunity as well as quality & quantity of silk production.

Keywords: *Bombyx mori,* gut bacteria, *Kocuria massiliensis,* probiotic, sericulture.

ISOLATION, CHARACTERISTICS OF BACTERIOPHAGE L522 AND ITS EFFICACY AGAINST BACTERIAL LEAF BLIGHT OF RICE

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Abstract. Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is considered as a threat to rice cultivation worldwide, especially in tropical Asian countries. The improper usage of chemical pesticides has prompted increasing concern about the environmental and human health impacts, along with the evolution of resistant pathogens. Bacteriophage biocontrol is a promising strategy which is more environmental-friendly than antibiotics. In this study, a phage named L522 was isolated from a rice field in the Mekong Delta Vietnam. Burst size, latent period, morphology, and whole genome sequence of the phage were analyzed. The stability of phage L522 under various environmental factors was investigated. Phage L522 showed high tolerance over a wide range of temperatures (4, 20, 30, 37 and 50 ºC) and pH (4 – 11) and was also less affected by UV-A and UV-B light. In the *in vitro* test, the inhibition time of the phage on the growth of *Xoo* in tryptone soya broth was about 45 h. Moreover, the efficacy of phage treatment was equivalent to that of a popular commercial pesticide (Starner 20WP) in the *in vivo* and field trial on rice plants. This study indicated that phage L522 should be a suitable candidate to control BLB in rice farming.

Keywords: bacteriophage, rice, bacterial leaf blight, biocontrol

LACTOBACILLUS ISOLATES IMPROVE LEARNING AND SPATIAL MEMORY IN WISTAR RAT

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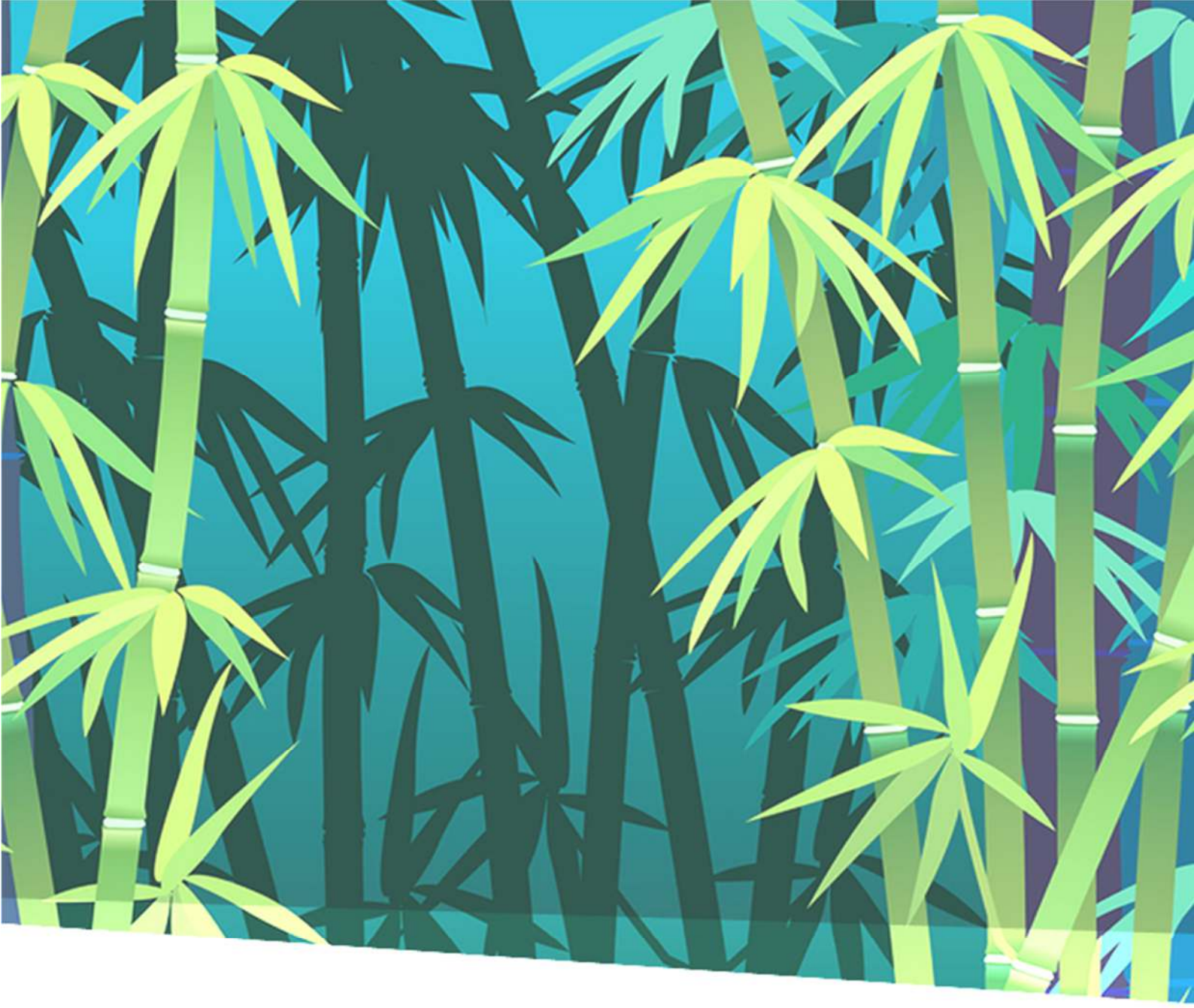
Abstract. *Lactobacillus* is a genus of bacteria that can be found in the gut microbiota and has a beneficial impact on various aspects of human health, including cognitive functions. These include improved memory and learning capabilities in mice/rat models and reduced cognitive decline in human subjects, especially in older adults. *Lactobacillus* can also be found in fermented food, with emphasize on Indonesian fermented food, such as *oncom* (fermented peanut press cake) and *terasi* (fermented shrimp paste). Three *Lactobacillus* isolates isolated from *oncom* and *terasi* have been investigated for containing the glutamate decarboxylase gene. The presence of this gene might indicate the ability of bacteria to influence brain function. This study aims to evaluate the ability of three *Lactobacillus* isolates from Indonesian fermented foods to improve learning and spatial memory in Wistar rats. Three *Lactobacillus* isolates (BoO, T1, and CH105F) were used in this study. The Morris Water Maze (MWM) test was performed to examine the learning and spatial memory of Wistar Rat. The results of the Morris Water Maze (MWM) test showed that supplementation of *Lactobacillus* isolates to BoO, T1, and CH105F groups was able to improve spatial memory and learning ability in the rats as indicated by the decrease of latency time, explored area, speed, dan shorten distance compared to control. Based on the results of this study, these three isolates were able to be developed as psychobiotics.

Keywords: Lactobacillus, learning, spatial memory, psychobiotics



9. Biocatalysis and Protein Engineering

COMPUTATIONAL AND EXPERIMENTAL STRUCTURE-FUNCTION INVESTIGATION OF MULTI-DOMAIN CARBOXYLIC ACID REDUCTASE FOR ENHANCED ALDEHYDE PRODUCTION

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Abstract. Currently, significant progress has been made in the exploration of highly efficient aldehyde-producing enzymes. This research presents a comprehensive study on the multi-domain carboxylic acid reductase (CAR) enzyme from *Mycobacterium phlei* (*Mp*CAR), focusing on its structural and functional properties and its potential for industrial applications. CAR is known for its selective one-step reduction of carboxylic acids to aldehydes using ATP and NADPH. Despite being recognized as one of the most thermostable CARs characterized, the structure-function knowledge of *Mp*CAR remains limited, and its industrial relevance has yet to be explored through immobilization. Via computational and experimental approaches, this study aimed to gain insights into the *Mp*CAR enzyme. Molecular dynamics simulations revealed that *Mp*CAR exhibited structural stability at higher temperatures (30, 40, and 50 °C) compared to lower temperatures (10 and 20 °C), which was further confirmed through experimental validation. *Mp*CAR gene was co-expressed with a novel phosphopantetheinyl transferase from the thermophilic *Anoxybacillus geothermalis* D9 in *Escherichia coli* BL21(DE3). The enzyme exhibited optimal activity at 40 °C and pH 7 and was stable up to 50 °C and at pH 4-6. *Mp*CAR also displayed a high preference for polar solvents. Furthermore, the immobilization of *Mp*CAR onto polymeric Seplite LX120 using simple adsorption was successfully achieved. This work represents the first immobilization of CAR onto an ionic resin. The immobilized *Mp*CAR showed excellent immobilization yield (99%) and activity (184.4 U/g). The optimal activity of the immobilized *Mp*CAR was observed at 60 °C and pH 9, surpassing that of the free enzyme. The immobilized *Mp*CAR exhibited stability over a wide range of temperatures (10-100 °C) and pHs (4-11), as well as compatibility with various metal ions, surfactants, and polar solvents. The immobilized *Mp*CAR demonstrated long-term stability and good reusability, and was able to produce up to 2.6 mM of benzaldehyde at 60 °C. The successful immobilization of *Mp*CAR onto Seplite LX120 offers significant potential for its utilization in green industries.

Keywords: carboxylic acid reductase, structure, function, immobilization, application

ENGINEERED ACTIVE ZYMOGEN OF MICROBIAL TRANSGLUTAMINASE FOR ANTIBODY-DRUG CONJUGATION

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Abstract. Transglutaminase (TGase) is an enzyme that catalyzes crosslinking reaction between specific Gln and Lys residues of peptide/proteins. Microbial transglutaminase from *Streptomyces mobaraensis* (MTG) is more stable than mammalian TGase, does not require Ca^{2+} ion as a cofactor, and shows relatively broad substrate specificity especially for Lys residues located in conformationally flexible region of a target protein. It has been widely used from food to medical fields. In particular, it has recently attracted attention as a biocatalyst for the preparation of antibody-drug conjugate (ADC). In previous studies, efficient drug modification of antibodies was reported, however, it requires the exposure of reactive Gln residues ($Q298_H$) by enzymatic deglycosylation. Herein, we introduce a new chimeric protein composing of MTG zymogen and an antibody-binding domain, which enables the labeling of a specific Lys residues on IgG antibody (Trastuzumab) via the proximity effect. This novel chimeric protein will facilitate the design of new ADCs and will also enable the creation of new artificial proteins.

Keywords: antibody, bioconjugation, cancer, site-specific crosslinking, transglutaminase

EXPRESSION SYSTEMS FOR *BACILLUS SUBTILIS* AND THEIR POTENTIAL APPLICATIONS AS VACCINE DELIVERY VECTOR

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Abstract

Background: *Bacillus* species are essential hosts for the production of proteins at industrial levels; 60% of industrial enzymes are produced from these species. Regarding the host organisms to express heterologous genes, *Bacillus subtilis* has several advantageous features that can complement the most widely used host, *Escherichia coli*. This model Gram-positive bacterium is considered as GRAS, endotoxin-free, allowing high amounts of protein secretion. In terms of application, it can be used for waste treatment and as probiotics for animals. The recent development of molecular biology has potentially opened a new approach to exploiting this bacterium. In this study, we want to introduce the expression systems for these bacteria and the study to apply the vegetative cells as vaccine delivery vectors.

Methods: we used molecular biology methods, focusing on promoters to develop expression systems for *B. subtilis* to produce proteins in the cytoplasm and in the culture medium. We explore the sortase/sorting mechanism to anchor proteins on the surface of *B. subtilis* cells covalently. The authors generated *B. subtilis* strains expressing LTB in the cytoplasm, in the culture medium, and on the cell surface of the vegetative cells. The antigen-expressing *B. subtilis* strains were introduced orally into appropriate animals, and the immune responses and antibody levels were measured.

Results: We created expression vectors for the overexpression of recombinant proteins in the cytoplasm and the culture medium and on the cell surface of *B. subtilis* cells. The expression systems could be checked by using different reporters. Also, the target proteins could be expressed in inducible and inducer-free manners. The immune response study in appropriate animals showed that the antigen-expressing *B. subtilis* strains could be used to deliver the antigen and induce the immune response.

Conclusion: The results infer that the genetics tools would be potentially exploited to produce recombinant proteins and generate antigen-expressing *B. subtilis* strains for a potential multivalent vaccine.

Keywords: *Bacillus subtilis*, vaccine delivery vector, P*grac*, P*grac*01, P*grac*100, P*grac*212.

MACHINE-LEARNING-ASSISTED MOLECULAR EVOLUTION PLATFORM FOR ENZYME AND ANTIBODY ENGINEERING

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Abstract. Molecular evolution based on mutagenesis is widely used in protein engineering, where critical amino acid residues of a target protein are identified and mutated for alteration and maturation of function based on available structural information. Iterative saturation mutagenesis (ISM) is one of the principal molecular evolution methods. Beneficial mutations are accumulated in a step-wise manner. However, ISM does not always lead to the optimal sequence, because the effects of mutations on function are often synergistic or antagonistic. Here, we propose an integration of machine-learning prediction into molecular evolution. In this novel approach, we iterated mutagenesis, following machine-learning guidance: a Gaussian process to propose the next-round mutagenesis library. This enables to prepare a small library with high enrichment of functional proteins. First, a library of variants is generated to acquire the sequence-function relationship data. Second, the data are used for training a machine-learning model to create the second-round library. The library predicted by machine-learning is analyzed, and the data of positive variants are used for training a machine-learning model again. We show the potential of our approach as a powerful platform for accelerating the discovery of functional enzyme and antibody.

Keywords: antibody, enzyme, machine learning, molecular evolution

UNIQUE ENZYMES INVOLVED IN AMINO ACID METABOLISM IN HYPERTHERMOPHILIC ARCHAEA

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Abstract Archaea represent one of the three domains of life, and display features not found in eukaryotes and bacteria. *Thermococcus kodakarensis* KOD1 is a hyperthermophilic archaeon that displays an optimum growth temperature of 85˚C. The organism is an obligate anaerobe and heterotroph, and is able to utilize a wide range of carbon sources, including amino acids, peptides, pyruvate, and polysaccharides such as starch and maltooligosaccharides. *T. kodakarensis* utilizes elemental sulfur as a terminal electron acceptor when available, resulting in the generation of hydrogen sulfide. When elemental sulfur is absent, a fermentative mode of growth is observed in media containing polysaccharides or pyruvate, leading to the generation of molecular hydrogen.

Our group is interested in the metabolism of this organism and other archaea. In many cases, archaea utilize enzymes and pathways that are not found in eukaryotes and bacteria. We have previously reported novel pathways and enzymes related to chitin degradation, gluconeogenesis, pentose metabolism, coenzyme A biosynthesis, and lipoic acid biosynthesis.

Here we present results on enzymes related to amino acid metabolism in *T. kodakarensis*. We have previously characterized a number of enzymes related to the oxidative catabolism of amino acids, including amino acid aminotransferases, glutamate dehydrogenase, 2- oxoacid:ferredoxin oxidoreductases and NDP-forming acyl-CoA synthetases. We have also identified an ornithine w-aminotransferase that is necessary for the synthesis of proline in *T. kodakarensis*. Our recent results on studies related to the generation and conversion of ornithine will be presented.

Keywords: Archaea, amino acids, metabolism, enzyme

FUNCTION, STRUCTURE, AND ENGINEERING OF BIOSYNTHETIC ENZYMES

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Abstract.

Fe/2-oxoglutarate (2OG)-dependent oxygenases are involved in the diversification and complication of natural products by catalyzing a wide range of reactions, including desaturations, skeletal rearrange-ments, endoperoxide formation, and ring for- mations/expansions, in addition to the typical hydroxylation reaction. Due to their catalytic flexibility and high efficiency, Fe/2OG oxygenases have attracted keen attention for their application as biocatalysts. Here, we present our resent investigations on the structure- function analysis and engineering of Fe/2OG oxygenases in the biosynthesis of fungal meroterpenoids.

The non-heme iron and 2-oxoglutarate-dependent (Fe/2OG) oxygenase superfamily is one of the largest groups of oxidation enzymes. The enzymes in this superfamily are widely distributed in both primary and secondary metabolism. Here, we present the biochemical and structural characterizations and engineering of the unusually promiscuous and catalytically versatile Fe/2OG oxygenase SptF, involved in the biosynthesis of fungal meroterpenoid emervaridones. The in vitro analysis revealed that SptF catalyzes several continuous oxidation reactions, including hydroxylation, desaturation, epoxidation, and skeletal rearrangement. SptF exhibits extremely broad substrate specificity toward various meroterpenoids, and efficiently produced novel cyclopropane-ring-fused 5/3/5/5/6/6 and 5/3/6/6/6 scaffolds from other meroterpenoid compound. Moreover, SptF also hydroxylates steroids, including androsterone, testosterone, and progesterone, with different regiospecificities. Crystallographic and structure-based mutagenesis studies of SptF revealed the molecular basis of the enzyme reactions, and suggested that the malleability of the loop region contributes to the remarkable substrate promiscuity. Furthermore, we also demonstrate the structure-guided engineering of SptF to generate unnatural meroterpenoids. The site-specific saturated substitutions of the active site forming residues provided three new compounds, which are not produced by SptF wild type. SptF exhibits great potential as a promising biocatalyst for oxidation reactions.

Keywords: *Biosynthesis, enzyme engineering, natural products, meroterpenoids*

CONSTRUCTION OF ENZYME CASCADES WITH THERMOPHILIC ENZYMES

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Abstract. Metabolic engineering has matured into a practical technology to construct synthetic pathways within living (micro)organisms for the fermentative production of valuable chemicals. However, living cells equipped with such synthetic pathways often experience unexpected perturbations in the natural metabolic network, resulting in suboptimal growth and production of desired metabolites. To address this issue, our research group has developed an alternative approach involving the construction of enzyme cascades that mimic both natural and synthetic metabolic pathways outside of cells. Specifically, the use of thermophilic enzymes as building blocks facilitates easy purification through heat treatment of the cell lysate from recombinant mesophiles (e.g., *Escherichia coli*). This enables the rapid assembly of multi-step enzyme cascades. Through this approach, we have successfully created a variety of synthetic cascades and demonstrated the one-pot production of valuable chemicals. Additionally, we have developed supporting technologies to enhance the practicality of these enzyme cascades. In this presentation, the author will showcase selected studies from our project, with a particular emphasis on cofactor synthesis and regeneration.

Keywords: thermophiles, enzyme cascade, NAD^+, ATP

Biocatalysis: Transformative Solutions for Sustainability and Circular Economy Integration

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Abstract. Traditional chemical processes are characterized by environmental pollution, resource depletion, and high energy consumption. Biocatalysis has since emerged as a transformative technology, addressing these challenges by offering selective solutions for sustainable chemical transformations.

Biocatalysis explores enzymatic versatility through biomimicry, a promising avenue for sustainable chemical reactions. Modern tools such as directed evolution and machine learning further influence enzyme engineering, opening doors to novel chemical processes and applications.

In parallel, the global push for decarbonization and sustainability necessitates the utilization of waste products like lignocellulose, methane, and carbon dioxide within a circular economy framework. Recycling plastics at both the resin and monomer levels through biocatalytic events becomes essential in reducing reliance on fossil fuels and advancing sustainable pathways for a renewable economy.

Furthermore, addressing the climate crisis requires innovative carbon capture technologies capable of efficiently capturing CO2 from various sources through enzymatic action. Biocatalytic membranes show great potential for both capturing and utilizing CO2, providing a promising solution to this global challenge. Enzymes and microorganisms have also been engineered to convert biomass into biofuels such as bioethanol and biodiesel.

To bring out the full potential of biocatalysis, a synergistic partnership must be forged among nature, chemists, and protein engineers. This interdisciplinary collaboration capitalizes on the inherent enzymatic blueprints found in nature, empowering chemists to explore non-natural substances while allowing protein engineers to fine-tune and enhance enzymatic functionality.

Biocatalysis brings together integral components of a sustainable future (e.g., sustainable synthesis, circular economy principles, innovative technologies), offering solutions to pressing global challenges related to climate change, waste reduction, and resource conservation. As such, collaborative efforts across scientific disciplines and industries are essential to harness the full potential of these approaches.

CAN WE DESIGN A NEW FUNCTIONAL ANTIFREEZE PROTEIN FOR CRYOPRESERVATION?

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Abstract. Exotic properties of antifreeze proteins (AFP) and peptides have currently been widely used in cryopreservation due to their special functions; ice recrystallization inhibition (IRI) and thermal hysteresis (TH). Moreover, some antifreeze proteins have low function naturally (less activity) to be used in cryopreservation. An underexplored AFPIV from fish was found to show low AFP activity. Thus, this research aims to improve the activity of AFPIV for cryopreservation. In this *in-silico* study, the modified afp1m (first helix from Antarctic yeast) was fused to AFPIV using computational tools. Then, a new linker was designed to boost the ice interaction of AFPIV mutant (AFP1mb) and improve the activity. Next, bioinformatics tools such as ExPASy Prot-Param, Pep-Wheel, Swiss Model, Phyre2, ERRAT, PROCHECK and ProQ were used to validate and analyze the physicochemical, functional and structural properties. Furthermore, to evaluate the interaction between ice and the AFP1mb, the molecular dynamics (MD) simulation was executed for 100 ns at 260 K (0 ºC) using GROMACS software. The primary structure analysis showed that AFP1mb is hydrophobic in nature. The secondary structure analysis revealed that AFP1m2 is fully helix. The results of 3D modeling represented that this AFP with QMEAN of 0.48, confidence of 99.5% and coverage score of 22% had the best model. In addition, ProQ tool demonstrated the validity of the predicted model. The results of MD simulation illustrated that AFP1mb had more rigidity according to its proper helical structure, better ice interaction due to its stability of hydrogen bonds which mimic the freezing and thawing temperatures in cryopreservation condition. It also possessed high activity at the low rate of ice growth in different temperatures. In conclusion, the AFP1mb may shed light to improve its anti-freeze properties with better ice interaction to meet medical applications especially in cryopreservation of the cells and tissues.

Keywords: antifreeze protein, new multi-helices structure, computational tools Molecular dynamics simulation, Cryopreservation

YEAST CELL SURFACE ANCHORED RECOMBINANT PROTEIN: A POTENTIAL ENGINEERING FOR AQUACULTURAL APPLICATION

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Abstract. With the expanding of industrial biotechnology, number of apllied recombinant protein has increased rapidly. Besides therapeutic purpose, recombinant protein has been studied for other targets such as cosmeceutical, aquaculture, food and agriculture. The expression of a spectrum of recombinant proteins in different systems for a wide variety of purposes has been a major feature and challenge. Although many studies have provided comprehensive converage of various aspect of recombinant protein production, the challenge in application of recombinant protein for aquaculturing is still not fully addressed. The demands have driven the development of a variety of improvements in protein expression technology.

Yeast cell surface display system, in which a target protein is incorporated into the cell wall of the host cell, has become one of the most popular tools for protein engineering and is applied in various purposes, including oral vaccine. The yeast *S. cerevisiae* has been recognized as GRAS organism, which is authorized to use for food and pharmaceutical purposes. Furthermore, the yeast cell wall has a mass composition of 30 – 50% mannoproteins, 30 – 45% β-1,3 glucans, 5 – 10% β-1,6 glucans, and 1.5 – 6% chitin which may provide additional protection in the form of adjuvant activity. With the above potential, *S. cerevisiae becomes* to be a target host cell for anchoring heterologous protein. The yeast anchored recombinnant protein on its cell surface can stimulate immune system in the host when being applied as feed additivies for livestock.

APPLICATIONS OF MICROFLUIDICS IN NUCLEIC ACID EXTRACTION

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Abstract. Nucleic acid extraction is a critical step in molecular analysis. Traditionally, nucleic acid extraction methods require bulky equipment, complicated steps, a lot of reagents, and lengthy time. The process is expensive and usually takes place in highly equipped lab. With many benefits including fast, simple, and affordable operation, microfluidic bioseparation chip has offered an alternative tool to perform pretreatment processes of biological detection. Main focus on the design, material, and fabrication of the device is given. Their applications in bioseparation are also discussed. In addition, current advantages and remained limitations of the technology are also discussed to give a better understanding for future developments.

Keywords: Nucleic acid extraction, microfluidics, lab-on-a-chip, miniaturization.

ACETYLATION REACTION OF AMINES AND ALCOHOLS CATALYZED BY BACTERIAL ACETYLCHOLINESTERASE

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Abstract. Aminolysis and alcoholysis are reactions of an amino or hydroxyl group with a carboxylic acid derivative to form an amide or ester bond, respectively. The reactions are widely used for synthesizing compounds containing amide or ester bonds. Many serine hydrolases exhibit aminolysis of esters alongside their hydrolytic activity. The reaction is an example of the catalytic promiscuity of serine hydrolases that is distinctly different from their primary activity. Acetylcholinesterase (AChE) from *Pseudomonas aeruginosa* PAO1 has a catalytic Ser residue in its active site. In this study, we revealed that AChE from *P. aeruginosa* PAO1 exhibits aminolysis and alcoholysis as side reactions.

The recombinant AChE recognized ethyl acetate as a substrate. Therefore, we evaluated acetylation of the amine and hydroxyl group by AChE, using acetylcholine and ethyl acetate as the acetyl donor. AChE recognized diaminoalkanes with 4- to 12-carbon chains and aminoalcohols with 4- to 8-carbon chains as acetyl acceptors, resulting in their acetylated products. In the acetylation of 1,6-diaminohexane, AChE preferentially used ethyl acetate as the acetyl donor above pH 8.0 and the efficiency increased with increasing pH. It is suggested that the acetylation of the amine group by AChE was accompanied by an increase in the nucleophilicity of the amine group due to deprotonation at high pH. In contrast, the acetylation of 6-amino-1-hexanol was efficient with acetylcholine as the acetyl donor in the pH range of 4–10. In addition, acetylated 6-amino-1-hexanol was decomposed by AChE. Therefore, the acetylation of the hydroxyl group was presumably due to activation of the hydroxyl group by the His residue adjacent to the catalytic Ser. The kinetic study indicated that the acetyl donor and acceptor are competitively recognized by AChE as substrates. The function of AChE presented here is the first of its kind in these enzymes.

Keywords: Acetylcholinesterase, aminolysis, alcoholysis, acetylation, acetyl amino acids

ROLE OF CARBOHYDRATE BINDING MODULE FAMILY 5 IN GH FAMILY 19 CHITINASES FROM *CELLULOSIMICROBIUM* SP. NTK2

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Abstract. Biological degradation of chitin is one of the issues for utilizing chitin as a next-generation biomass resource. Recently, *Cellulosimicrobium* sp. NTK2 (NTK2 strain), an actinomycete that highly decomposes α-chitin, was isolated from crab shell compost, and its genome was sequenced. The NTK2 strain has eight chitinase genes, all of which are expressed and secreted into the medium to contribute to chitin degradation. Among them, three chitinase class I enzymes belong to Glycoside Hydrolase (GH) Family 19. By the comparison of these structures, we found that chitinase class I-2 (Chi class I-2) consists only of active domain and chitinase class I-3 (Chi class I-3) possesses carbohydrate binding module family 5 (CBM5) in addition of active domain. In this study, we constructed recombinant Chi class I-2, I-3, CBM5 deleted Chi class I-3 (Chi class I-3ΔCBM5), and Chi I-2 with CBM5 attached to the N-terminus (Chi class I-2+CBM5). By using them, we investigated the role of CBM5 in the function of GH family 19 chitinases.

All recombinant proteins were constructed as GST fusion proteins and purified from cell-free extracts using GST column. The activities of Chi class I-3 for α-chitin and chitosan decreased by the deletion of CBM5. In contrast, the activities of Chi class I-2 for α-chitin and chitosan increased by the addition of CBM5, indicating that the presence of CBM5 enhanced the activity of Chi class I enzymes for insoluble substrates. In the enzyme kinetics analysis using soluble ethylene glycol chitin (EGC), decrease in K_m value was observed by the addition of CBM5 to Chi class I-2, and increase in K_m value was observed by the deletion of CBM5 from Chi class I-3. The results indicate that the presence or absence of CBM5 may affect the affinity to substrate.

Keywords: chitinase, carbohydrate-binding module, actinomycete, recombinant protein

COMPREHENSIVE *IN SILICO* CHARACTERIZATIONS OF AGGLUTININ-LIKE SEQUENCES (ALS) FROM A LOCALLY ISOLATED YEAST AS POTENTIAL FUNGAL VIRULENCE FACTORS

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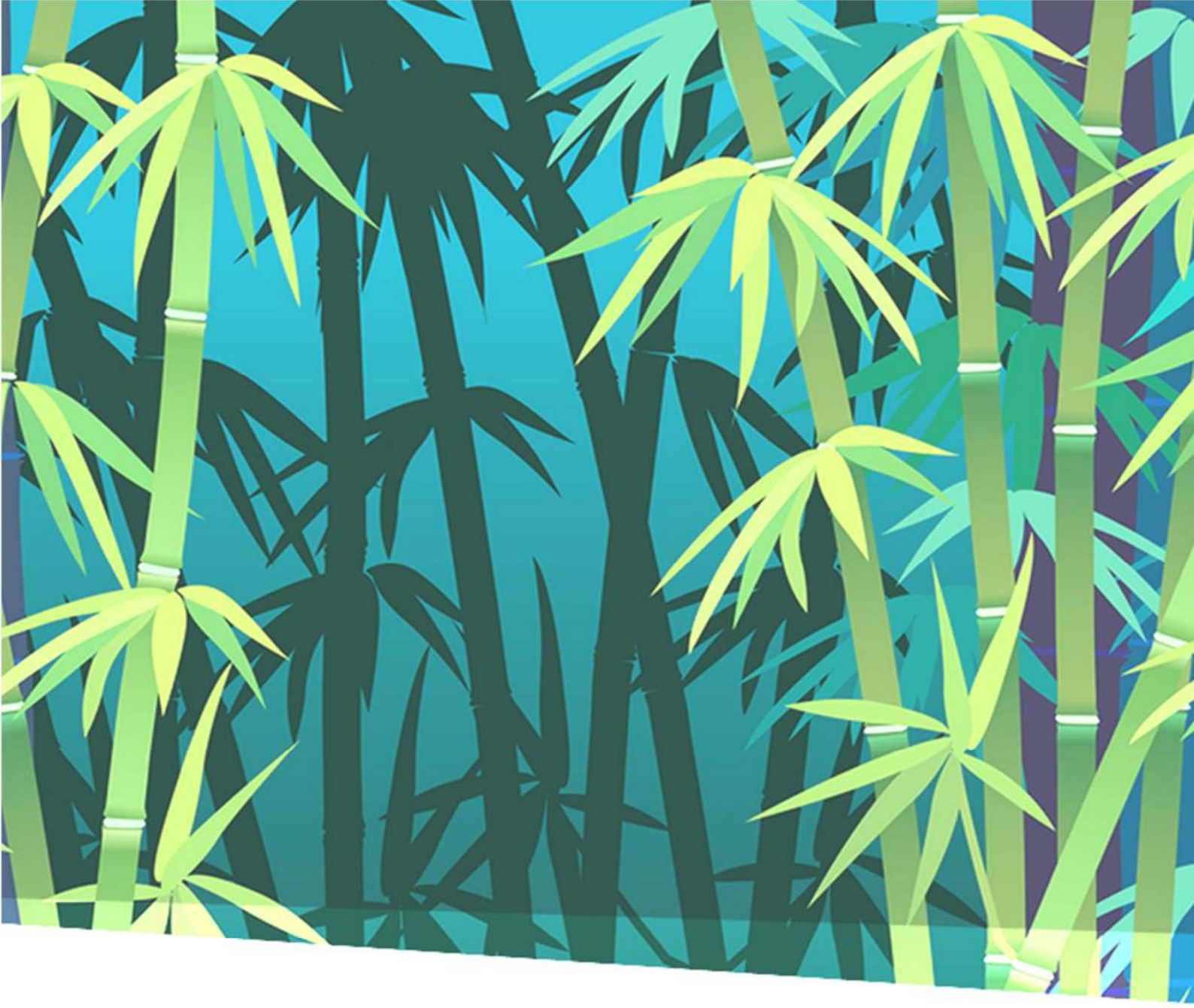
Abstract. Agglutinin-like sequence (Als) proteins from *Candida* spp. function as adhesins and invasins to elicit fungal pathogenesis towards infected hosts. The less pathogenic *C. guilliermondii* (teleomorph *Meyerozyma guilliermondii*) has been attended for its increasing antifungal resistance and isolation frequency. A local isolate of *M. guilliermondii* strain SO from spoiled orange was a cost-effective expression host for industrially important enzymes with whole genome sequenced recently. However, recent zebrafish embryo toxicity test has evident of its pathogenicity. Als, as membrane-bound adherence-modulating virulence factors (VFs), were least reported in *M. guilliermondii* especially their three-dimensional structures for virulence features. Thus, this study aimed to identify and characterize Als proteins in *M. guilliermondii* strain SO *via* comprehensive *in silico* approaches. Using profile Hidden-Markov model, 4 *Mg*Als with strong phylogeny and similar conserved domain with *C. albicans* Als3 (*Ca*Als3) were detected. *Mg*Als1056 was conserved towards *Ca*Als3 in its amyloid-forming region, tandem repeat domain, and protein binding cavity (PBC). The strain specific *Mg*Als sequential features were also highlighted. Molecular docking showed higher affinities of *Ca*Als3 mutant and *Mg*Als1058 towards hepta-threonine compared to the native *Ca*Als3, but the substituted critical K59M/Q residue only interacted hydrophobically with the ligand. Conversely, K59 in *Mg*Als1056 showed similar hydrogen bonding (< 3Å) with the crystallized structure of *Ca*Als3-hepta-threonine complex, with weaker interactions in *Ca*Als3, *Mg*Als1619 and *Mg*Als2471. *Mg*Als1056 was the most druggable among other members but at the pocket other than its PBC. The pathogenic roles of *Mg*Als1056 can further be corroborated experimentally through *MgALS* gene deletion *via* yeast genetic engineering and validation of virulence attributes through animal infection models. Herein, our comprehensive *in silico* characterizations of *Mg*Als provide conceptual advance on bioinformatics tools usage to identify and depict the pathogenic traits of VFs in rare *Candida* spp. pathogens besides contributing to the fields of medical biotechnology and applied microbiology.

Keywords: agglutinin-like sequence, Candida albicans, Meyerozyma guilliermondii, protein-binding cavity, amyloid-forming region, candidiasis



10. Nanobiotechnology, Biosensors, and Biochips

SENSING OXYGEN INSIDE PROTEIN NANOCAGES

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Abstract. Protein nanocages (PNCs) are well-organized structures formed through self-assembly widely found in the biosphere. They provide compartments for enzyme-catalyzed reactions, delivery, and storage and have also inspired the design of artificial PNCs. As one of the critical properties of some PNCs, oxygen-impermeability makes PNCs a promising platform for oxygen-sensitive or oxygen-responsive reactions and cargo delivery. However, few efforts have been devoted to oxygen sensing inside PNCs, an essential requirement in the characterization and development of PNC-based oxygen-impermeable compartments. In this work, we report a method for oxygen sensing inside PNCs by encapsulating an oxygen-sensitive quantum dot (QD). The PNC we used here is an artificial one assembled from the pentamer of the vertex protein of β-carboxysome, CcmL. The CcmL PNC encapsulated the QD sensor through self-assembly via protein-QD interfacial engineering. The high-resolution structure of the cage solved by integrating solid-state nuclear magnetic resonance (ssNMR) spectroscopy and cryo-electron microscopy (cryo-EM) shows an icosahedral assembly of the CcmL pentamers around the QD and highly conserved inter-pentamer interfaces. The structure also reveals two kinds of pores in the PNC shell, based on which we designed a molecular patch to the pores of the cage. When oxygen was detected inside the PNCs without or with the molecular patch by monitoring fluorescence quenching at different concentrations of oxygen, only the patched PNCs showed protection of QD fluorescence. The result indicates that the QD sensor can differentiate the oxygen-permeability properties of the CcmL PNCs under either status. Our oxygen-sensing method may be helpful in the experimental determination of the oxygen permeability of bacterial microcompartments (BMCs), BMC-derived shells, viral capsids, and other natural or artificial nanocontainers.

Keywords: protein nanocages, permeability, oxygen sensing, quantum dots, carboxysomes, self-assembly

MOLECULAR DESIGN FOR A HIGHLY SENSITIVE BIOMOLECULAR INTERACTION DETECTION SYSTEM BASED ON STRUCTURAL MODIFICATION OF CUTA1

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Abstract. Molecular recognition elements that bind specifically to target molecules are essential for reproducing, analyzing, and detecting biomolecular interactions *in vitro*. In this study, we aimed to establish a molecular platform for the development of multivalent molecular recognition elements that can improve interactions with target molecules. We designed molecules based on the trimeric basic structure of CutA1 from hyperthermophilic archaeon, *Pyrococcus furiosus*, which has an extremely stable structure, and verified the sensitivity of the interaction detection using these molecules. First, we designed a single-chained CutA1 (scCut) molecule consisting of three tandemly linked subunits, inserted StrepTagII (STII) as a ligand, and a conjugated an E-coil at the C-terminus (scCut_STII_E-coil). CutA1 was modified by inserting PS-tag and conjugating K-coil at the C-terminus (Cut_PS_K-coil). Each of these molecules was prepared using *E. coli* strain BL21(DE3) as a host. Since solubilization and thermostability were confirmed for both chimeric proteins, scCut_STII_PS and Cut_PS_K-coil were immobilized on the hydrophilic PS surface via PS-tag in an orientated manner with changing the mixing ratio of each protein. By the Enzyme-Linked Assay (ELA), the sensitivity of STII-ST interaction detection significantly improved with an increased ratio of Cut_PS_K-coil. Therefore, it was suggested that the protein complex designed with multivalent scCutA1 and a coiled-coil structure can alleviate steric hindrance in the interaction between the ligand (STII) and the analyte (ST) due to the rigidity of the coiled-coil structure and the flexibility of its linking groups, resulting in the improved sensitivity of the interaction detection. In conclusion, we discovered that the spatial arrangement of ligand molecules can enhance nano-biointerfaces, enabling more effective capture of target molecules.

Keywords: CutA1, molecular recognition element, immobilization, nano-biointerface, coiled-coil,

IMPROVEMENT OF OXIDATION STABILITIES OF VEGETABLE OIL AND BIODIESEL FUEL BY ADDING ULTRAFINE BUBBLES OF HYDROGEN

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Abstract. The consumption of carbon-neutral renewable fuels is recommended to reduce CO_2 in the atmosphere for global warming protection. However, those biomass fuels contain unsaturated alkyl groups and are very rapidly oxidized and change their fuel activities. In this study, we found that the ultrafine bubbles (UFB) of hydrogen can make extremely long-lasting oxidation stability of oil and biodiesel fuel (BDF) by dispersing UFB into them. UFB of H_2, CO_2 and Argon (Ar) to oil and BDF with a bubble diameter of about 100 nm were added to rapeseed oil and BDF using a UFB mixer and the number of UFBs in the liquid was measured using the light scattering UFB measurement device. Oxidative stabilities of oil and BDF were studied by Rancimat method. UFBs with a bubble diameter of about 100 nm in liquid is stable for more than 3 months. They have the surface charge and remain small without union of two. Because of their small diameter of approximately 100 nm, they exhibit BROWNIAN motion, and they remain in water for a long time without rising and escaping into the gas phase or atmosphere. Numbers of UFB dispersed in water was almost the same as that of the conventional ultrasonic UFB dispersion. On the other hand, when UFBs of hydrogen and oxygen were introduced into rapeseed oil and BDF, as much as 10^{13} –10^{14} UFBs/ml could be dispersed. Once the UFB is dispersed in the liquid, it is extremely difficult to remove them from liquid. THE OXIDATION STABILITY OF RAPESEED OIL AND BDF with dispersed hydrogen UFBs increased more than 5 times THAN THOSE WITH OTHER various UFBs. It is also strange that the oxidation stability does not change at all when Ar is dissolved in the UFB.

Keywords: Ultra Fine Bubbles (UFB), Oxidation stabilities of oil, Biodiesel fuel, Sustainable Aviation Fuel (SAF), Carbon Neutrality

NANOCELLULOSE, A PROTEIN-FRIENDLY DELIVERY SYSTEM FOR TOPICAL APPLICATIONS

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Abstract. Fibroblast growth factor 2 (FGF-2) is a multifunctional protein that plays an important role in wound healing. However, FGF-2 topical administration still has some limitations due to FGF-2 short half-life and instability. In this study, we prepared FGF-2-incorporated carboxymethyl cellulose nanoparticles (CMC:FGF-2 NPs) for FGF-2 stabilization and controlled release in burn treatment. Using ionic gelation method with Al^{3+} as cross-linking agent, the CMC NPs (at the optimum NaCl:CMC:$AlCl_3$ weight ratio = 66:10:1) were successfully prepared with spherical shape, non-clustered distribution, average size of 85.60 nm, and no cytotoxicity on NIH/3T3 cell line. In FGF-2 incorporation, the CMC:FGF-2 NPs exhibited an average size of 88.00 nm without aggregation, FGF-2 loading efficiency over 90 %, and FGF-2 release rate of approximately 30 %. Besides, the NPs showed an efficient preservation of FGF-2 biological activity and a remarkable FGF-2 protection against protease hydrolytic action. In the study on third-degree burned murine model, the CMC:FGF-2 NPs highly accelerated the wound closure, re-epithelialization, granulation tissue formation, and angiogenesis compared to naked FGF-2 and FGF-2-unincorporated CMC NPs. Generally, CMC:FGF-2 NPs could potentially become a novel strategy for clinical application in burn treatment.

Keywords: carboxymethyl cellulose, fibroblast growth factor 2, ionic gelation, nanoparticles, tissue repair and regeneration, wound healing

SOY LECITHIN BASED LIPOSOMES AS A POTENTIAL SYSTEM FOR DRUG DELIVERY

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Abstract. The utilization of liposomes as a versatile platform for efficient drug delivery has garnered substantial attention in pharmaceutical research. Among the various lipid-based formulations, soy lecithin-based liposomes have emerged as a promising system due to their biocompatibility, biodegradability, and low toxicity. Despite its numerous advantages, the drug carrier system encounters challenges related to its inherent stability, specificity for cancer cells, and controlled release of poorly water-soluble anticancer drugs (PWSADs). To enhance the efficacy of liposomes in delivering PWSADs and addressing these obstacles, a strategy was devised. This strategy aimed to passively target cancer cells and enhance drug availability by integrating polymer conjugates onto the surface of the liposomal system. Our research involved the creation of conjugates using biocompatible polymers and targeted ligands. These materials collectively formed a coating for drug-loaded liposomes through electrostatic interaction. The coated liposomes underwent comprehensive characterization, including assessments of size, morphology, zeta potential, drug loading efficiency, infrared structures, differential scanning calorimeter spectra, and drug release profiles. Further evaluations encompassed cytotoxicity experiments on cancer cells, along with an analysis of cellular uptake via confocal imaging. The findings revealed noteworthy size changes associated with increased coating materials. This alteration correlated with enhanced colloidal stability, elevated zeta potential, and improved toxicity toward cancer cells. Cellular uptake studies substantiated these results, showcasing the impact of the biopolymer coat on this phenomenon. Additionally, the data partly validated that targeted ligand could augment liposome uptake by cancer cells. Based on these outcomes, the liposomes coated with modified polymers represent a promising platform for delivering specific anti-cancer agents, particularly PWSADs.

Keywords: soy lecithin, liposome, drug delivery system, anticancer drug

NANO-IRON OXIDE REGULATES MICROBIOTA-GUT-INNER EAR AXIS FOR HEARING PROTECTION

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Abstract. Noise-induced hearing loss (NIHL) is a highly prevalent form of sensorineural hearing damage that has significant negative effects on individuals of all ages, and there are no effective drugs approved by the Food and Drug Administration for preventing or treating NIHL. In this study, we unveil the potential of superparamagnetic iron oxide nanoparticle assembly (SPIOCA) to reshape the dysbiosis of gut microbiota in NIHL by effectively suppressing the proliferation of harmful bacteria and the reduction of beneficial bacteria induced by noise. This modulation inhibits intestinal inflammation and oxidative stress responses, thereby protecting the integrity of the intestinal barrier. Consequently, it curtails the transportation of pathogens and inflammatory factors from the bloodstream to the cochlea, resulting in the attenuation of cochlear inflammation. On the other hand, gut microbiota modulated SPIOCA-induced metabolic reprogramming in the gut-inner ear axis mainly through the regulation of the sphingolipid metabolic pathway, which further contributes to the survival of cochlear hair cells and the restoration of hearing function. Our study confirms the role of the microbiota-gut-inner ear axis in NIHL and provides a novel alternative to protect hearing. The developed nano-iron oxide holds potential as a therapeutic nanomedicine for the treatment of NIHL and other microbiota dysbiosis-related diseases.

Keywords: Hearing loss; SPIOCA; Inflammation; Gut microbiota; Gut-inner ear axis; Sphingolipid metabolism.

SYNTHESIS OF GOLD NANOPARTICLES STABILIZED IN WATER-SOLUBLE *B*-GLUCAN BY GAMMA IRRADIATION WITH HIGH ANTIOXIDANT AND ANTICANCER ACTIVITIES

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Abstract. Colloidal solutions of gold nanoparticles (AuNPs) were successfully synthesized by γ-ray irradiation of 0.25, 0.5, 1.0 and 1.5 mM [Au^{3+}] solutions using water-soluble yeast *β*-glucan as a stabilizer. The optical characteristics of obtained AuNPs samples were confirmed by the appearance of a new peak with λ_{max} values at 521-523 nm in the UV-vis spectra. The determination from transmission electron microscope (TEM) images showed that the average particle sizes of AuNPs increased from 5.3 to 17.5 nm by the increase of [Au^{3+}] concentration from 0.25 to 1.5 mM, respectively. The crystalline structure of the AuNPs was also validated through X-ray diffraction (XRD) analysis. While the interaction of *β*-glucan with the AuNPs via steric (-Au…O-) coordination linkages was identified through Fourier transform infrared (FTIR) spectroscopy. The antioxidant activity of AuNPs investigated using free radical $ABTS^{\bullet}$ showed that this activity was strongly dependent on the concentration and particle size of AuNPs product. The results also revealed that the antioxidant activity of AuNPs was higher than that of ascorbic acid about 4.67 folds at the same tested concentration. In addition, the MTT (3[4,5 dimetylthiazol-2-yl]-2,5-diphenyltetrazol bromide) assay performed on a liver cancer cell line (HepG2) distinctly demonstrated that the treatment of 0.05 to 0.5 mM AuNPs strongly inhibited the growth of HepG2 cells and these cells could not survive at treatment concentration of 0.5 mM AuNPs. Meanwhile, the growth of the fibroblast cell line (L929) was almost unaffected by the same treated concentrations of AuNPs. Thus, due to the high purity, biocompatibility, high anticancer and antioxidant activity of AuNPs synthesized by the γ-rays irradiation method, this product may be potentially used as an antioxidant and anticancer substance.

Keywords: Anticancer, antioxidant, *β*-glucan, HepG2, gold nanoparticles, γ-ray irradiation

ORAL ADMINISTRATION OF NANO SILYMARIN, SILICA-INSTALLED REDOX NANOPARTICLES AS AN EFFECTIVE TREATMENT FOR INFLAMMATORY BOWEL DISEASE

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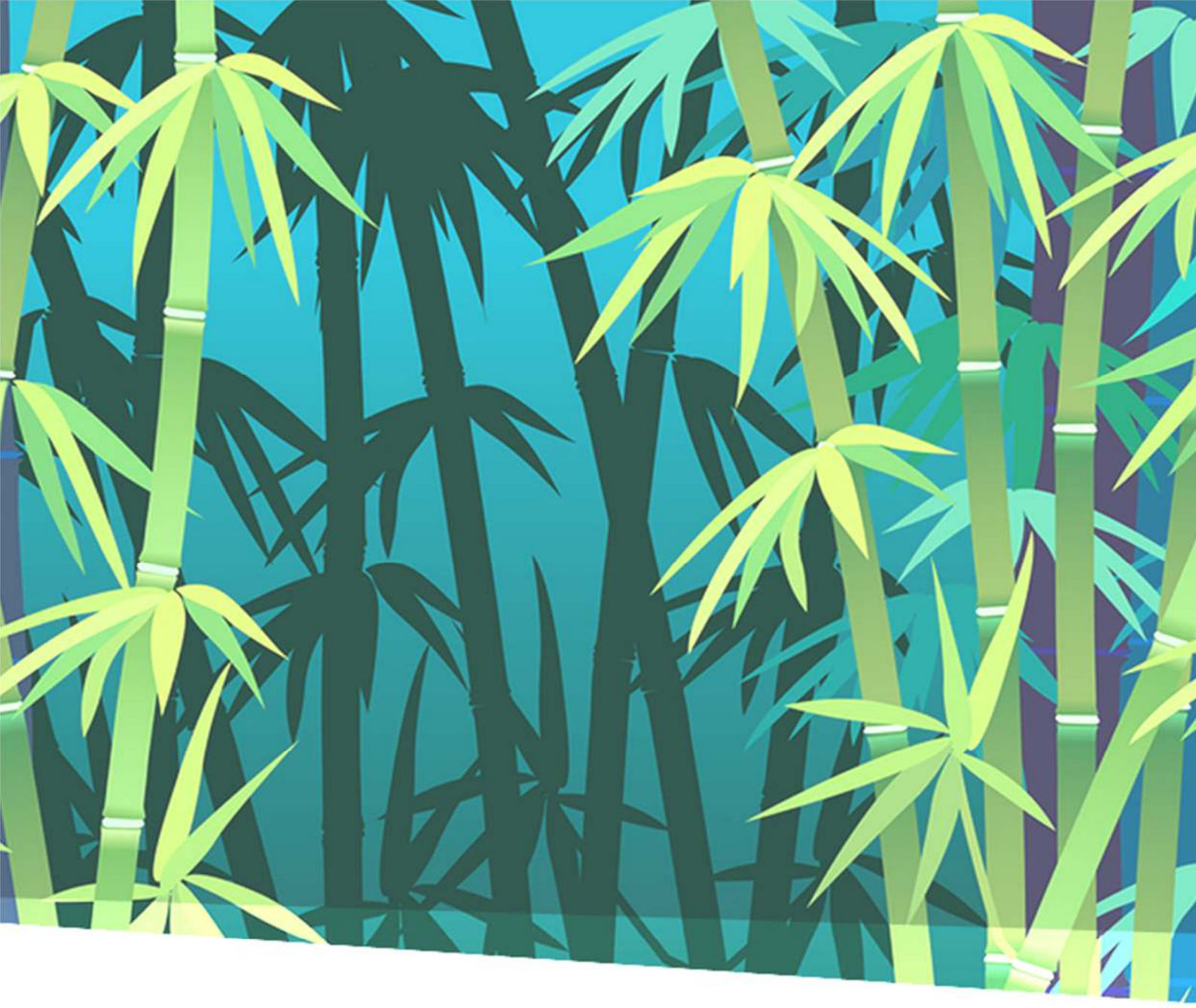
Abstract. It was reported that an estimated 10 million people worldwide were living with inflammatory bowel disease (IBD). While the exact causes of IBD remain unclear, the overproduction of reactive oxygen species contributes to oxidative damage in the intestines of affected individuals. Therefore, antioxidant therapy has emerged as a promising approach for IBD treatment. Silymarin (SM) is an essential active component of *Silybum marianum* (milk thistle). Nonetheless, SM faces challenges in bioavailability, metabolism and excretion, permeability, and water solubility. Thus, new approaches for the enhancement of bioavailability SM have been developing. One such approach involves the development of silica-containing redox nanoparticles (siRNP) measuring 50-60 nm in diameter, designed to improve the solubility and delivery of SM (Figure 1). SM-loaded siRNP (SM@siRNP) significantly increased antioxidant capacity in DPPH, ABTS, and FRAP assays compared to SM and siRNP-free. Besides, SM@siRNP also improved the anti-inflammatory efficacy *in vitro* by suppressing nitric oxide and inflammatory cytokines in the activated RAW 264.7 macrophages. *In vivo* pharmacokinetic studies involving oral administration of SM@siRNP demonstrated higher plasma SM concentration and prolonged retention in the colonic mucosa, surpassing the performance of free SM treatment in mice. Finally, the anti-inflammatory effect of SM@siRNP was investigated in dextran sodium sulfate-induced colitis mice. The results revealed that SM@siRNP significantly mitigated the severity of colitis, as evidenced by improvements in body weight loss, diarrhea, rectal bleeding, and colon length. This study indicated the potential of SM@siRNP as a promising therapy for IBD treatment.

Keywords: inflammation, chronic inflammatory disease, antioxidant, redox nanoparticles, silymarin.



11. Bio-Digital Convergence Technology

MULTIPLEXING CAPABILITY OF FIELD EFFECT TRANSISTOR FOR MICRORNA PROFILING DETECTION

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Abstract. A digital twin representing a human health status is not science fiction but a requisite medical approach for practicing precision medicine, provided that bioinformatic and translational medicine has fully developed.

To deal with such a complex and dynamic system of the human body and to provide linked information between bioinformatics and translational medicine, a next-generation biosensor with multiplexing capability, high sensitivity, and fast and digitizable is indispensable to target the system.

We have developed nanowire Field Effect Transistors (nwFET) for this purpose for years in our lab. This talk will demonstrate a new type of nucleic acid derivative (methylated phosphotriester oligo nucleotide) as a probe and surface modification chemistry for ultra-highly sensitive detection of biomarkers, such as miRNA 21, and most importantly, for multiplexing microRNA detection for prostate cancer diagnosis and treatment. The nwFET data were compared to the qPCR results of this research to validate that, without PCR amplification, the nwFET detection could substitute the qPCR panel approach in the future since the nwFET is much more cost-effective and time-saving to target a complex and dynamic system.

Keywords: Multiplexing; microRNA profiling; field effect transistor; prostate cancer.

BEYOND PERFORMANCE: CONSIDERATIONS FOR END-USER COMFORT IN THE DESIGN OF IN-SITU USER INTERFACES

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Abstract. Our reliance on computing technologies for making decisions or sense-making, has undergone a transformation from occurring in well defined settings (such as on traditional PCs) to taking place in-situ, for supporting everyday activities. In-situ user interfaces, have emerged largely from improved mobile and mixed-reality technologies and rely on mid-air input as the basis for interaction. Often, designers of such, on-the-go, user interfaces place an emphasis on enhancing end-user performance. However, we argue that supporting end-user comfort is as critical if in-situ interfaces are to become commonplace among the general population. In this talk, I will present some of our work on various aspects of end-user comfort for in-situ interactions. I will present models for estimating arm fatigue induced by mid-air input, and showcase interactive systems that have been specifically designed to circumvent such fatigue. I will also discuss elements of social comfort and present a framework for including such factors in the design process of end-user interfaces. I end my presentation with a discussion of some of the open problems in this space.

FUNCTIONAL ZWITTERIONIC SILANES FOR CONTROLLED SILANIZATION AND MEDICAL IMPLEMENTATIONS

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Abstract. Organosilanes are frequently applied for surface modification of oxide surfaces to afford finely control over the physical and chemical interfacial properties. With the development of nano-materials and advances in semiconductor technologies, the precise modulation of the functional coatings using self-assemblies become of importance. This presentation will introduce current works developed in my laboratory regarding to the functional organosilanes, particularly silatranes. Silatrane possesses a tricyclic caged structure and a transannular N → Si dative bond, making it chemically stable and controllable to avoid fast hydrolysis and aggregation in solution. Silatrane allows faster deposition and better formation of thin and homogeneous films than conventional organosilanes. We have demonstrated the excellent stability and antifouling properties of zwitterionic silatranes for silicon-based substrates. Moreover, the coating technology was extended to development of a new hemofiltration system for continuously capturing of circulating tumor cells (CTCs) from a large volume of whole blood. The system was equipped a column that was packed with antifouling zwitterionized silica microspheres (Figure 1). The silica microspheres were modified with sulfobetaine silane (SBSi) to inhibit fouling, resist clogging, and give a high surface wettability and prolonged operation time. Packed microspheres with different diameters formed size-controllable interstitial pores that effectively captured CTCs by ligand-free size selection. A large quantity of colorectal cancer cells was spiked into sheep blood, and the sample was circulated for 5 h with a total operational volume of 2 L followed by collection and culture in vitro. The results showed that the proposed hemofiltration device selectively removed abundant CTCs from in vitro circulatory blood. The viable cells were harvested for amplification and potential applications for precision medicine.

Keywords: Surface modification, zwitterionic materials, self-assembled monolayer, silane, silatrane

BIOMIMETIC APPROACHES TO ARTIFICIAL OLFACTION

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Abstract. Conventional approaches to machine olfaction are constrained due to the types of gas sensors currently commercially available. Most of these are hampered by their lack of selectivity, sensitivity to environmental disturbances, drift, and noise. A biomimetic approach to so called “electronic noses” has been adopted where increased understanding of biological chemical sensing has provided new insights into the design of artificial sensing systems. Olfactory receptor proteins are membrane bound proteins and Odorant-binding proteins (OBPs) are a family of soluble proteins found in chemosensory organs of vertebrates and insects and are associated with detection and release of chemical stimuli. These proteins can now be cloned, modified, and immobilized onto a variety of transducers. By expression and cloning OBPs from *Anopheles gambiae*, as well as major urinary proteins from the mouse that are closely related to vertebrate OBPs, we have constructed sensor arrays based on quartz crystal microbalance and surface acoustic wave transducers. These arrays function in a combinatorial way analogous to biological olfaction, and by suitable feature extraction combined with a neural network, these systems detect and discriminate many different chemicals. We have demonstrated outstanding stability of these devices over 2 years when applied to detection and discrimination of narcotics and explosives.

Keywords: Odorant binding proteins, sensor arrays, gas sensing, narcotic detection, explosive detection

STANDARDIZATION FOR BIOELECTRONIC NOSE BASED ON BIO-DIGITAL CONVERGENCE

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Abstract. Standards provide performance criteria that technical engineers can use to design products to optimize the reliability and safety of new product. Unlikely the electronic sensing devices for vision, audition, and touch, there are, however, no formal standards for electronic sensing device of smell that is designed to detect and evaluated odours. Recently, bioelectronic noses have been reported which use human olfactory receptors are primary recognition elements and nanomaterials as secondary transducers. The information obtained by bioelectronic nose should be eventually correlated with the human sensory evaluation data because the odour categories must be calibrated and defined by perceptual learning processes. It also needed to develop relevant standards for bioelectronic nose device, which has been studied by Bio-digital convergence group for standardization evaluation in IEC (International Electronical Commission). In this talk, we present the progress standardization for bioelectronic nose based on bio-digital convergence within IEC, discuss further actions to necessary standards that can provide such assurance must be created.

Keywords: Bio Digital Convergence, Bioelectronic Nose, Olfactory Receptors, Transducer, Signal Evaluation

INTELLIGENT INTEGRATION OF IMAGE PROCESSING, AI, AND LAB AUTOMATION TECHNOLOGIES FOR MORPHOLOGY-BASED CELL QUALITY ASSESSMENT

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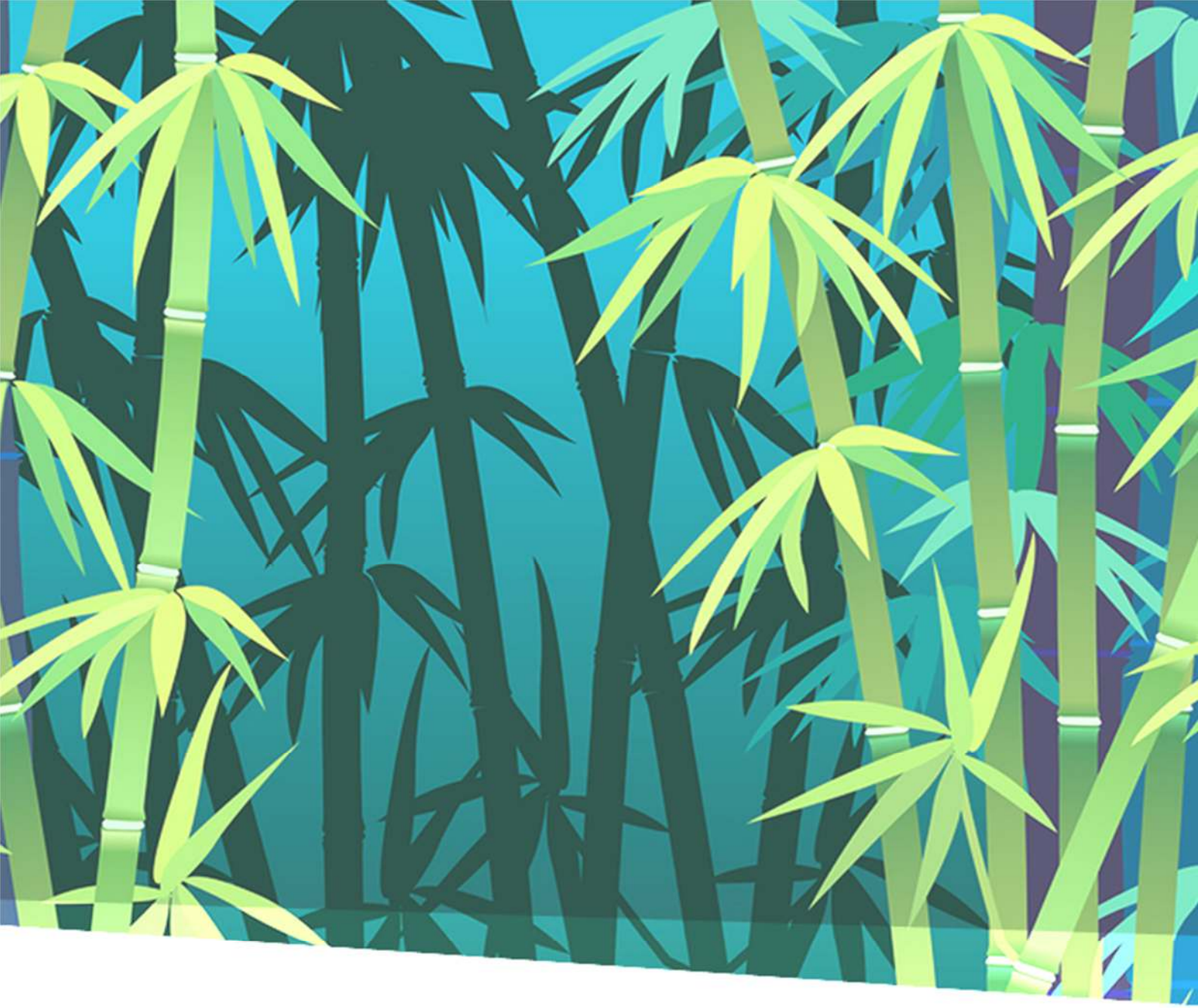
Abstract. Morphology of cells has been an important and trustworthy information to monitor cellular status non-invasively. Although such important information had long been evaluated subjectively with cell culture experts' eyes. By the technological advances in imaging and artificial intelligence (AI)-related technologies, such image-derived morphological information is now becoming to be expected as a new bio-marker to non-invasively evaluate cellular status. Our group has been reporting various applications of such AI-guided morphological cell evaluation methods as label-free real-time cell evaluation method for cellular quality evaluation and drug screening. By the technological combination with laboratory automation, simulation, image processing, and machine learning, we have been reporting "Super-early" cell quality prediction AI performance to support the reduction of unwanted cell culture cost and labor. Our AI applications indicate that adequately trained AI model can predict the cell quality within 1-2 days, which conventionally takes more than one moth to determine by classical experiments. Such real-time information of cellular status serves as in-process control data to clearly define the design space and enable data-driven critical parameter definition. To support the cell manufacturing industry, our method is accepted as powerful tool for cell culture process development. However, since such technology-based strategy is a novel challenge in cell manufacturing science which is still underdevelopment, it requires certain important points to be considered. In this presentation, we will present the technological advancements and challenges to build the combinational technology of "cell culture" and "AI technologies" for regenerative medicine industrialization.

Keywords: Regenerative cell therapy, quality control, image processing, deep learning, AI, cell manufacturing



12. Systems and Synthetic Biotechnology

HETEROLOGOUS EXPRESSION OF FUNGAL NATURAL PRODUCT GENES IN *ASPERGILLUS ORYZAE* AND *SACCHAROMYCES CEREVISIAE*

Waraporn Bunnak[1], Jaiyfungkhong Phakeovilay[2], Gunlatida Promsuk[3], Witcha Imaram[1], Supachai Vuttipongchaikij[4], Colin Lazarus[5], Passorn Wonnapinij[4], Pakorn Wattana-Amorn[1,2,3*]

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Abstract. Over the last decade, there has been a decrease in the rate of drug discovery, requiring new approaches to facilitate the process. Advances in omics technology have sped up the search for many silent natural product biosynthetic genes. In this work, heterologous expression of polyketide synthase and non-ribosomal peptide synthetase genes from *Menisporopsis theobromae* BCC 4162 and *Paecilomyces cinnamomeus* BCC 9616 was performed in *Aspergillus oryzae* and *Saccharomyces cerevisiae.* Several metabolites were produced including ascotrichalactone A, alternapyrone and an unexpected metabolite, anthranilic acid. Their biosyntheses were also investigated. Our results could provide a biological platform to produce fungal metabolites used in agricultural and pharmaceutical industries.

Keywords: Heterologous Expression, *Menisporopsis theobromae*, *Paecilomyces cinnamomeus, Aspergillus oryzae*, *Saccharomyces cerevisiae*

RECORDING BIOLOGICAL SURROUNDINGS FROM WITHIN

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Abstract. Complex and dynamic biological processes in microbial environments are often transient and difficult to access for direct and continuous measurement. Approaches that require destructive processing steps (e.g., RNA-seq) cannot provide temporal dynamics of the target information. Approaches that allow continuous monitoring (e.g., imaging) necessitate direct access to the target environment. On the other hand, DNA-based cellular recording offers the capacity to measure biologically relevant signals over time in places that are otherwise difficult to access, such as inside the body. Furthermore, the stored information in DNA can be coupled to gene regulation to directly report environmental states or control cellular logic operations. In this talk, I will describe a framework for storing temporal biological information directly in the genomes of a cell population. We developed a "biological tape recorder" in which biological signals trigger intracellular DNA production that is then recorded by the CRISPR-Cas adaptation system. This approach enables stable recording and maintenance of biological or digital data over multiple days and accurate reconstruction of temporal information by sequencing CRISPR arrays. Furthermore, I will describe my current efforts to improve temporal resolution and sensitivity of the system by directed evolution of CRISPR adaptation machineries. This emerging new modality of biological measurement will be key to gain novel insights in diverse biological processes and develop a variety of biotechnological applications.

Keywords: Molecular recording, Synthetic Biology, CRISPR

OPTOGENETIC SYNTHETIC BIOTECHNOLOGY USING LIGHT-DRIVEN MICROBIAL PROTON PUMP SYSTEMS

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Abstract. In microbial fermentative production, ATP regeneration, while crucial for cellular processes, conflicts with efficient target chemical production because ATP regeneration exhausts essential carbon sources also required for target chemical biosynthesis. To wrestle with this dilemma, we harnessed the power of microbial rhodopsins with light-driven proton pumping activity to supplement with ATP, thereby facilitating the bioproduction of various chemicals. We first demonstrated a photo-driven ATP supply and redistribution of metabolic carbon flows to target chemical synthesis by installing already-known delta rhodopsin (dR) in *Escherichia coli*. In addition, we identified novel rhodopsins with higher proton pumping activities than dR, and created an engineered cell for *in vivo* self-supply of the rhodopsin-activator, all-*trans*-retinal. Our concept exploiting the light-powering ATP supplier offers a potential increase in carbon use efficiency for microbial productions through metabolic reprogramming.

Recently, we also developed light-driven vacuole in yeast. In *Saccharomyces cerevisiae*, dR was specifically expressed on the vacuole membrane by conjugation with a vacuolar-localized protein Avt6. The localization of the Avt6-dR conjugating protein on the vacuole membrane was visualized by fluorescence microscope by fusion with GFP. The vacuoles harboring Avt6-dR were purified from *S. cerevisiae* and the light-driven proton pumping activity from outside the vacuole to inside the vacuole was evaluated from the rate of increase of pH outside the vacuole. Furthermore, light-induced increase in the intracellular ATP level was observed. The engineered *S. cerevisiae* with the light-driven vacuole has potential to be used as an energetic ATP supplying cell factory for various bioproduction applications.

Our concept exploiting the light-driven ATP supplier rhodopsin will become an essential common device for developing efficient cell factories to produce bulk and fine chemicals, and constructing a synthetic cell with optogenetic reprogrammed metabolisms. The results presented provide an opportunity for further improvement of carbon use efficiency in the key metabolite biosynthesis.

Keywords: rhodopsin, ATP, energy metabolism, metabolic engineering, systems and synthetic biotechnology, bioproduction.

PLANT-BASED PHARMACEUTICALS - FROM DISCOVERY TO FINAL PRODUCTS

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Abstract. Plants produce myriads of nitrogen-containing heterocyclic metabolites called alkaloids. These chemicals have served numerous eco-physiological functions in the plants as well as medicines for humans for thousands of years. For instance, semi-synthetic derivatives of camptothecin, an alkaloid from happy tree (Camptotheca acuminata), are potent anticancer agents such as topotecan (Hycamtin) and irinotecan (Camptosar). The presentation will focus on recent discoveries of new enzymes that are involved in biosynthesis of anticancer alkaloids, with a specific focus on camptothecin hydroxylases and the use of combinatorial chemoenzymatic C-H functionalizations to produce a suite of anticancer drugs, including topotecan (Hycamtin®) and irinotecan (Camptosar®). This work sheds new light on camptothecin metabolism and represents greener approaches for accessing clinically relevant camptothecin derivatives.

Synthetic Biological Circuits for Programming Cells to Compute, Sense and Manufacture

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Abstract. Cells live in an ever-changing environment and continuously sense, process and react to surrounding biochemical signals using their inherent sensors and gene regulatory networks. In this sense, cells can be viewed as replicating living computers with limitless potential. In this talk, I will introduce the design and engineering of new customized genetic circuits for the sensing and information processing of multiple cellular and environmental signals, with potentially disruptive applications in diverse areas including biosensing, biocomputin, biomanufacturin and biotherapies.

Keywords: synthetic biology, genetic circuits, logic gates, biocomputing, biosensing, biomanufacturing

TRANSCRIPTOME-BASED IDENTIFICATION OF POTENT PROMOTERS FOR BIOMANUFACTURING BY *ASPERGILLUS ORYZAE*

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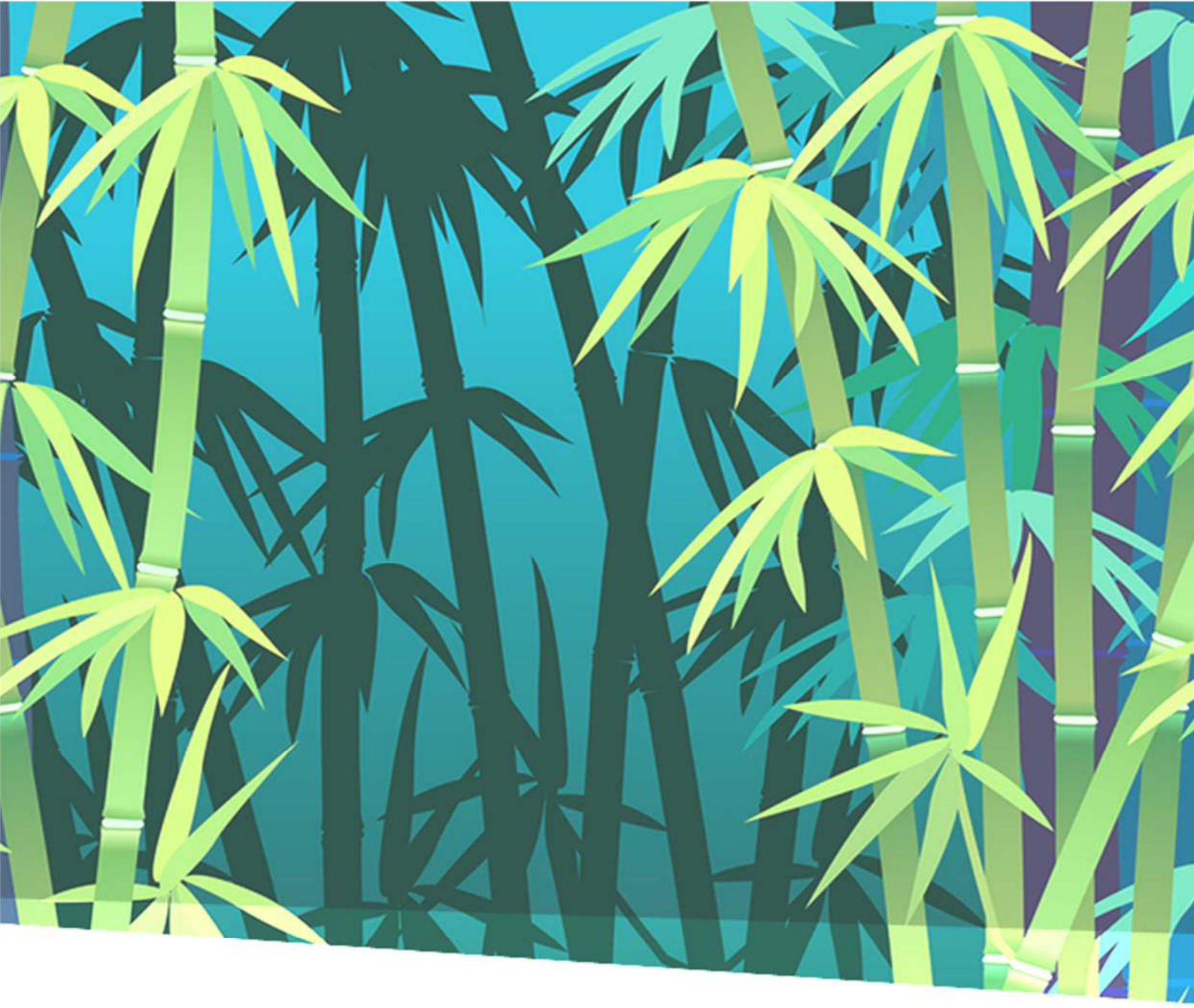
Abstract. Among the microbial cell factories, *Aspergillus oryzae* has been attracted as a potential cell platform to produce diverse primary and secondary metabolites with commercial interest. However, strain optimization remains a challenge for the production of economically feasible products. For rational optimization of this strain through synthetic biology, the potent regulatory promoters have gained attention. Using the transcriptome-based approach, *in silico* prediction, functional analysis, and deletion analysis of upstream promoter sequences, two potent constitutive promoters, glyceraldehyde triphosphate dehydrogenase promoter (*PgpdA1*) and ubiquitin promoter (*Pubi*), were identified for *A. oryzae*. The *PgpdA1* and *Pubi* displayed strong and moderate functions in driving the *uidA* (β-glucuronidase) as demonstrated by the GUS activity, respectively. Both promoters could function in the fungal cells grown on various carbon sources, in which glucose was the best carbon source for attaining the gene function. These regulatory promoters with different strengths and carbon source flexibility provide beneficial options for manipulating multiple pathways to allocate the metabolic capacity for producing desired bioproducts. This study has opened an opportunity for establishing the efficient strain improvement of Aspergilli via synthetic biology to accelerate progress in the production of functional ingredients and recombinant proteins by the fungal production system.

Keywords: Aspergillus oryzae, Synthetic biology, Constitutive promoter, Glyceraldehyde triphosphate dehydrogenase, Ubiquitin



13. Clinical and Public Health

TOTAL HOMOCYSTEINE IN BANGLADESHI PATIENTS WITH MYOCARDIAL INFRACTION STRONGLY CORRELATES WITH NEUTROPHIL-LYMPHOCYTE RATIO

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Abstract. Background: Elevated total serum homocysteine (tHcy) has been intensely associated with the risk of coronary heart disease (CHD). Interestingly, the level of tHcy is affected by genetic predisposition, such as, by methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism (rs1801133), as well as by the lifestyle. We, therefore, evaluated the association between tHcy, CHD, MTHFR 677C>T polymorphism and type of diet consumed (as a lifestyle marker) to identify their association with CHD. **Methods**: We enrolled patients with myocardial infarction (MI) who were confirmed by electrocardiogram, collected data by questionnaire and blood indexes. Correlation analysis of traditional cardiovascular risk factors and homocysteine was performed, and multivariate line regression analysis was conducted to explore the relevant factors for the myocardial infarction. A total of 133 patients and 48 healthy subjects were included in the final analyses according to the inclusion criteria. Different serum biochemical parameters were analyzed by chemiluminescence immunoassay techniques and genetic polymorphisms of the MTHFR gene were determined by polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) method. **Results**: There is no significant correlation found between MTHFR gene polymorphism and MI frequency in the Bangladeshi population. However, this population had an increased preference to intake rice compared to control. Therefore, an animal study was performed to study the impact of diet on the level of serum homocysteine. It was found that the level of homocysteine in control mice group, fed with normal chow based diet, was 1.1±0.09 µmol/L (n=4), which was significantly lower compared to the case group fed with rice-based diet (2.2±0.36 µmol/L, n=5, $P<0.05$). Additionally, correlation study showed a positive association between serum tHcy and Neutrophil/Lymphocyte Ratio (homocysteine vs NLR, $r = 0.413$, $R^2=0.17$, $P=0.03$). **Conclusion**: This study showed at least partially that dietary pattern, but not the MTHFR 677C>T gene polymorphism is associated with higher blood homocysteine levels in the Bangladeshi population, which can be easily diagnosed by neutrophil-lymphocyte ratio. Hence, neutrophil-lymphocyte ratio; which is an inexpensive, easily measurable, widely available biomarker; could provide a predictive index for diagnosing myocardial infarction in patients with the acute coronary syndrome.

Keywords: Myocardial infraction, Hyperhomocysteinemia, MTHFR gene, Neutrophil-lymphocyte ratio, PCR-RFLP.

INVESTIGATING CAPSULE TYPES, SEQUENCE TYPES, AND VIRULENCE DETERMINANTS FOR COMMUNITY-ONSET *ACINETOBACTER BAUMANNII* IN TAIWAN

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Abstract. *Acinetobacter baumannii* is a notorious global human pathogen. Moreover, *A. bauma*nnii strain is recognized as one of the most problematic pathogens outside the hospital setting and causes community-acquired *A. baumannii* (CAAB) infections. Unlike nosocomial infection, the molecular epidemiology of CAAB infections remains controversial and has rarely been reported. This study aims to investigate the epidemiology of capsular types (KL type), sequence types (ST type), and virulence determinants of CAAB strains in Taiwan. The KL types of 33 CAAB isolates were determined by *wzc/wzy* genotyping PCR, and we found that the KL types of the 33 strains were KL49 (n=6, 18%), KL14 (n=3, 9%), KL81 (n=3, 9%), KL9 (n=3, 9%), and others (n=18, 55%). Subsequently, the sequence types (STs) of all 33 CAAB strains were determined in the Oxford scheme. Notably, 21 STs were new and reported for the first time in this study; Specifically, 20 STs had a new allelic combination of previously known alleles, and one ST type had a new allele of the *gpi* gene, leading to new allelic profiles. We then selected six strains of KL49 and further identified ST type in the Pasteur scheme. Surprisingly, 5/6 strains of KL49 were ST10, which was predominant in America or Australia but has never been reported for CAAB in Taiwan. Interestingly, ST10 was reported as a hypervirulent ST type in a murine model. Additionally, in a serum resistance assay, we found that all KL49 (6/6), KL2 (1/2), KL9 (1/2), and other KL types (1/2) isolates could survive and re-grow up after serum treatment. The result indicated that KL49/ST10 strains against human serum could be an important determinant for the virulence of CAAB strains. Our study provided a newfangled foundation for understanding the molecular epidemiology of CAAB in Taiwan that would be critical for future virulence studies and disease control.

Keywords: Community-onset Acinetobacter baumannii, capsule typing, MLST, virulence traits

PATHOSENSE, A NEW WHOLE GENOME SEQUENCING DIAGNOSTIC PLATFORM FOR THE DIAGNOSIS OF VIRAL AND BACTERIAL DISEASES IN ALL ANIMALS - AN IDEAL TOOL FOR FAST IDENTIFICATION OF NEW EMERGING DISEASES

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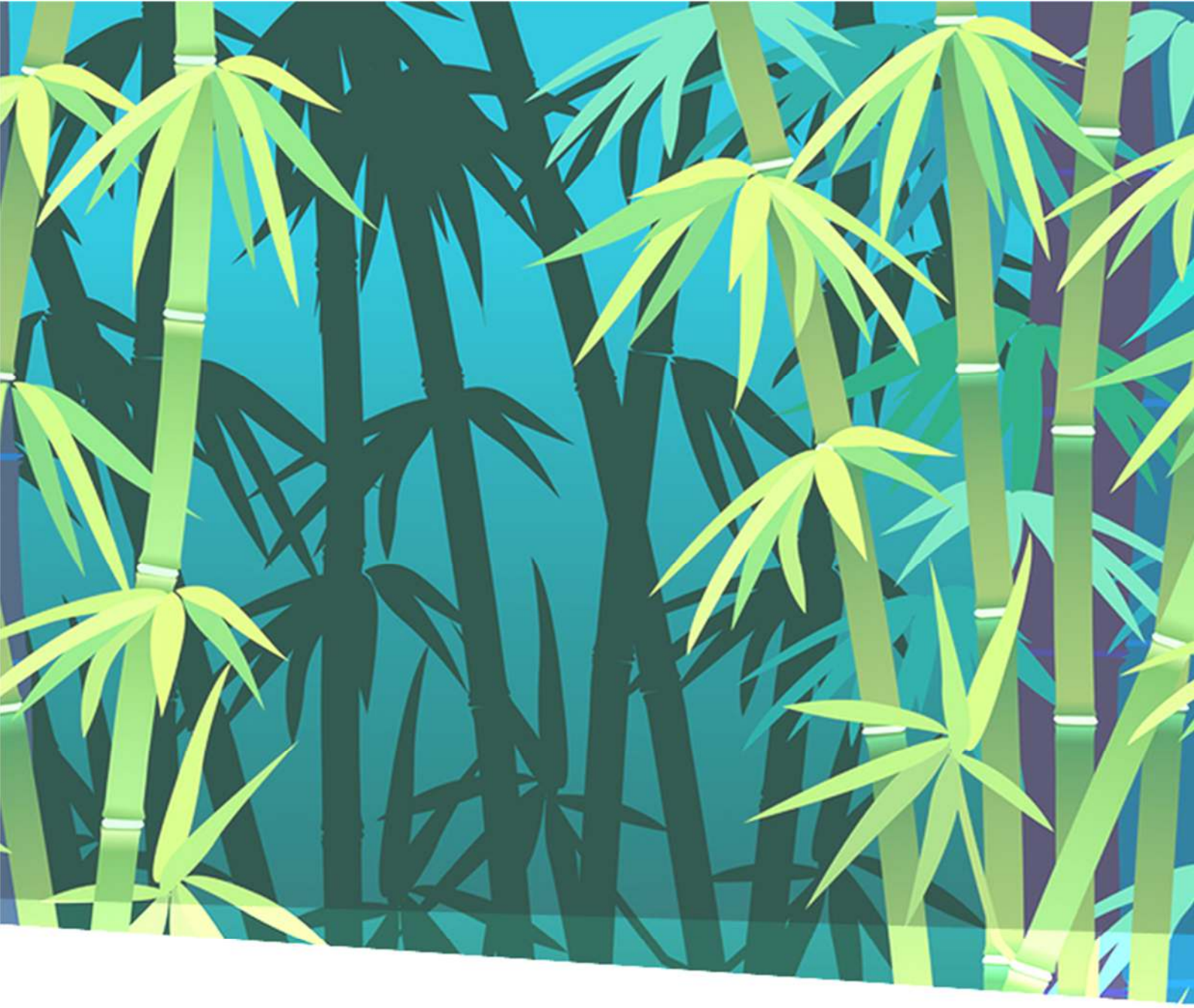
Abstract. Emerging infectious diseases caused by viruses and bacteria are regularly occurring. They can be devastating for animal production and if they jump to humans, they can be a real disaster for human health. Therefore, there is an urgent need for a fast identification of unknown pathogens. This was the basis for developing a new diagnostic platform that is not PCR based and does not require prior selection of the pathogens to test for. Samples are collected and purified with a patented swab as first step in pathogen genomic material enrichment before ad-random sequencing with nanopore sequencing (MinION or GridION; Oxford Nanopore Technologies). The reads are assembled and the obtained sequences are compared with known sequences from a database. This finally leads to the identification of all viruses and bacteria that are present in a sample. By enclosing an internal control we are able to semi-quantify the pathogens. An app on the smartphone allows the user to fill in the affiliation of the animal owner and the clinical signs and to register the sample by a QR code. After the analysis, the results are sent to the smartphone using the same app for informing the user. This new technology is now commercialized by a spinoff (PathoSense) and already on the market in Europe. For the future, we want to expand this technology in Asia and America. In this context we are looking for interested partners.



14. Medical Devices

TRENDS IN GLOBAL ORGANOID TECHNOLOGY AND INDUSTRY

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Abstract. “Trends in Global Organoid Technology and Industry" offers a deep dive into the captivating world of organoids—three-dimensional, miniaturized, and self-organized versions of organs produced in vitro. Originating from stem cells, these tiny organ models have become instrumental in bridging the gap between traditional 2D cell cultures and in vivo studies. Our exploration commences with a historical overview of organoid technology, mapping its emergence and the subsequent milestones that have propelled it to the forefront of biomedical research. As we transition into the modern era, we elucidate the prevailing global trends, underscoring how organoids are progressively shaping various sectors, from disease modeling to regenerative medicine. One of the cornerstone applications, organoid-based drug efficacy evaluations, is spotlighted, demonstrating how these mini-organs provide unparalleled insights into drug-tissue interactions, thereby paving the way for more targeted and effective therapeutic interventions. This comprehensive overview not only serves as an ode to the transformative potential of organoids but also paints a vivid picture of the current landscape and the infinite horizons yet to be explored in this dynamic industry. Join us for a holistic appreciation of organoids, where the past, present, and future seamlessly converge.

Keywords : Organoid, Trend, Drug screening , Application, ODISEI

MATURE INTESTINAL ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Abstract. The intestine is a complex organ with multiple histological and functional structures and uniquely adapted for performing specialized functions, including initial and selective barrier activity, nutrient absorption, host-microbiome interactions, and regulation of host defense and immune responses. A stepwise differentiation process from the groundbreaking publication by James M. Wells and colleagues in 2011 can efficiently produce three-dimensional (3D) human intestinal organoids (hIOs) derived from human pluripotent stem cells (hPSCs), including mainly human embryonic stem cells and induced pluripotent stem cells. This experimental model system is an important tool for studying the differentiation and maintenance of intestinal epithelial cells as well as pathophysiological processes underlying intestinal diseases. However, hPSC-derived 3D hIOs have several limitations, such as immature, fetal-like characteristics. Recently, we described an *in vitro* maturation methodology by co-culturing with immune cells or by exposing them to interleukin-2 for generating adult-like, mature 3D hIOs from hPSCs that closely resemble the *in vivo* tissue structure, cellular diversity, and functionalities. Here, we will discuss the impact of the maturation status of hIOs and present the potential usefulness of mature hIOs in various aspects of *in vitro* applications and their potential use *in vivo*.

Keywords: human intestinal organoid, in vitro maturation, human pluripotent stem cell, application

UNLEASHING PRECISION MEDICINE: ADVANCING SYSTEMS-BASED DRUG DISCOVERY AND BIOMARKER DEVELOPMENT FOR RARE CANCER

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Abstract. Remarkable progress has been made in biomedical research, leading to the development of more effective anti-cancer drugs. However, determining the optimal utilization of these drugs for specific patient subgroups and cancer types remains a challenge. Our laboratory has recently reported a systems-based drug discovery approach to identify repurposable therapeutic agents for Asian cholangiocarcinoma. A pan-omic database, coupled with comprehensive drug response profiles of 16 cholangiocarcinoma (CCA) cell lines from Thai and Japanese patients, has been made available to the scientific community. This systems-based approach is now being extended to investigate multiple myeloma and ovarian cancer. To enhance drug screening, we have adopted 3D culture techniques for both primary cells and cancer cell lines, creating a valuable biobank of patient-derived organoids. These organoids are proving to be invaluable tools in guiding clinicians toward suitable cancer drug choices. Additionally, I will present our latest findings on employing spatial biology technologies for biomarker discovery in rare cancer types such as cholangiocarcinoma, mycosis fungoides, and sarcoma. Our experiences demonstrate the potential of these cutting-edge techniques in uncovering valuable insights for precision medicine and targeted therapies. Overall, our research showcases the promising applications of systems-based drug discovery and advanced culture models, and highlights their potential in transforming cancer treatment strategies and improving patient outcomes.

Keywords : 3D culture techniques, cancer, biomarker, drug screening

TONSIL ORGANOIDS AS AN EX-VIVO DISEASE MODEL PLATFORM

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Abstract. Studies of human infectious diseases have been limited by the paucity of functional models that mimic normal human physiology and pathophysiology. Recent advances in the development of multicellular, physiologically active organotypic cultures produced from embryonic and pluripotentstem cells, as well as from stem cells isolated from biopsies and surgical specimens are allowing unprecedented new studies and discoveries about host–microbe interactions. The palatine tonsils (hereinafter referred to as “tonsils”) serve as a reservoir for viral infections and play roles in the immune system’s first line of defense. Recently, we achieved three-dimensional tonsil organoid culture from human tonsil tissues. The tonsil organoids successfully recapitulated the key characteristics of the tonsil epithelium, including cellular composition, histologic properties, and biomarker distribution. Notably, the outer layer cells of the organoids expressed receptor molecules essential for SARS-CoV-2 entry and were susceptible to the viral infection. Changes in the pattern of gene expression in tonsil organoids revealed that 1,098 genes associated with p38 MAPK, IL-6 and IL-17 signaling pathways were highly upregulated within 3 h after SARS-CoV-2 infection. Also, we investigated the morphological and genetic changes of tonsil organoids in response to HPV16 infection. For this aim, we generated human tonsil organoids stably expressing the HPV 16 whole genome via lentiviral transduction, as well as their HPV-negative counterparts with an empty vector, resulting that HPV whole genome transfection induced structural and morphological changes in the tonsil organoids. Next, single cell RNA sequence analysis was performed to explore changes in gene expression in the tonsil organoid after HPV whole genome transfection, resulting in numerous novel gene expression changes in tonsil organoid. Our study suggested that tonsil organoids could be available for investigation of infection-mediated pathology as an ex-vivo disease platform

Keywords : human tonsil organoid, infection, disease modeling,

RECENT ADVANCEMENTS IN ORGANOID-BASED REGENERATIVE MEDICINE

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Abstract. ORGANOISCIENCES stands at the forefront of biotech innovation, pioneering advanced organoid technology to address challenges associated with organ damage and shortages, thereby enhancing human health. Organoids are essentially three-dimensional, in-vitro constructs that mimic the micro-anatomy of actual organs, encompassing various organ-specific cell types that are spatially organized in a manner reminiscent of natural organs, allowing them to emulate certain organ functions. Such attributes render organoids as potent tools in regenerative medicine and transplantation due to their remarkable regenerative capacities. We take pride in unveiling our groundbreaking platform, "ATORM (Adult Tissue derived Organoid based Regenerative Medicine)," which is gearing up for its inaugural human clinical trials and is equipped with a GMP process tailored for organoid therapeutics. We're eager to share insights on the latest developments and industrial applications of organoids, alongside other cutting-edge organoid-based platforms.

Keywords : Organoid, Regenerative Medicine, Cell therapy , GMP, ATORM

A PRECLINICAL APPLICATION FOR PATIENT-DERIVED ORGANOIDS TO PREDICT CHEMOTHERAPY RESPONSE OF THE TARGETED FOLIC – GELATIN – PLURONIC P123 NANOGEL IN COLORECTAL CANCER.

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Abstract: There is the non-effective method to predict chemotherapy response and postoperative prognosis of colorectal cancer. Patient-derived organoid has become an important preclinical model. Previous study to show the targeted folic – gelatin – pluronic P123 nanogel can kill cancer cells (*in vitro*), and cells in the tumor (*in vivo*). This study aims to evaluate the effect of targeted folic – gelatin – pluronic P123 nanogel on patient-derived organoids in colorectal cancer. **Methods:** The patient-derived organoid is constructed from the tumor in colorectal cancer. The effects of dose-response of targeted folic – gelatin – pluronic P123 nanogel on patient-derived organoids are tested. **Results:** The patient-derived organoid is successfully constructed from the tumor in colorectal cancer. The targeted folic – gelatin – pluronic P123 nanogel reduces the size and number of patient-derived organoids in colorectal cancer. **Conclusions:** The patient-derived organoid can be an effective method to predict the chemotherapy response of the targeted folic – gelatin – pluronic P123 nanogel in colorectal cancer.

Keywords: preclinical, cancer, organoids, tumor

DEVELOPMENT OF POINT OF CARE TESTING DEVICES AT INTERNATIONAL UNIVERSITY – VIETNAM NATIONAL UNIVERSITY HO CHI MINH CITY

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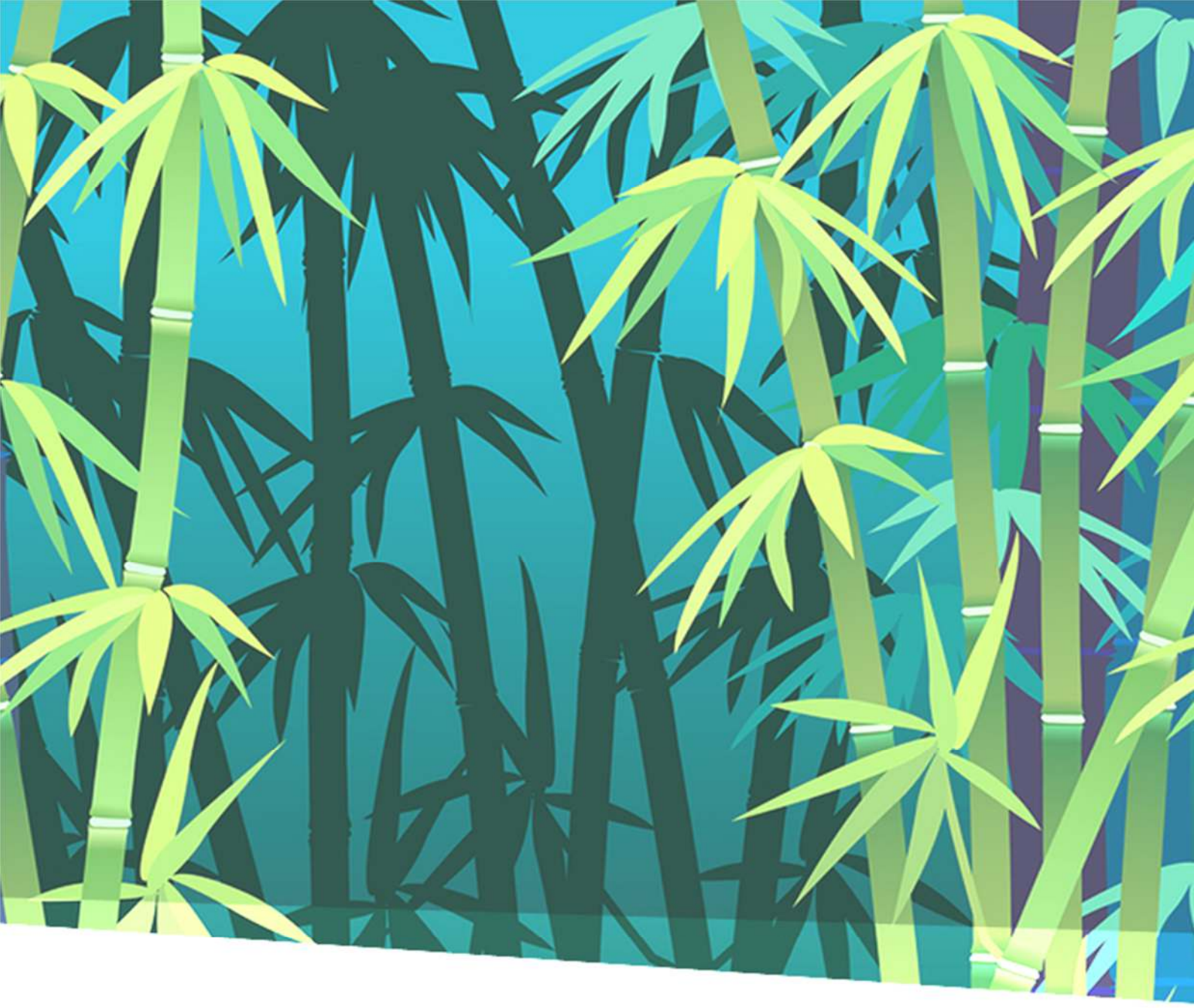
Abstract. Microfluidics are expected to revolutionize the healthcare industry especially in developing countries since it would bring portable, easy-to-use, self-contained point of care diagnostic devices to places with limited access to healthcare. To date, however, microfluidics has not yet been able to live up to these expectations. One non-negligible factor can be attributed to inaccessible prototyping methods for researchers in low-resource settings who are unable to afford expensive equipment and/or obtain critical reagents and, therefore, unable to engage and contribute to microfluidics research. Our research group has been developing low-cost techniques for microfluidic fabrication that require minimal capital, running costs, or minimal fabrication process. We then applied our techniques to design microfluidic chips for disease modelings and devices for point-of-care nucleic acid amplification testing applications. In this talk, we will present our experience, recent progress, and opportunities. In addition, we will present our recent research activities in developing lateral flow assays for diseases detection.

Keywords: Microfluidics, testing device, Viet Nam



15. Bioindustry Promotion and Bioeducation

BIOPROCESSING TECHNOLOGY TRAINING CENTER
(K-NIBRT PROGRAM)

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Abstract.

Demand for biopharmaceuticals has been increasing rapidly worldwide, and during the COVID-19 pandemic the biopharmaceutical industry became even more important. Consequently, as the biopharmaceutical market is growing significantly, corporate investment is expanding and demand for skilled professionals is increasing greatly.

Although companies are demanding more professionals who are fully equipped to work immediately in the field, there are not enough of them available. To solve this shortage, practical training needs to be carried out in GMP facilities, but due to contamination and security problems at facilities, there are demands for dedicated bioprocessing training facilities.

Accordingly, the government initiated a project to solve these problems and the 'Incheon City - Incheon Technopark - Yonsei University' consortium has been selected to construct a bioprocessing training center and set up a K-NIBRT(Korea-National Institute for Bioprocessing Research and Training). Incheon City, Incheon Technopark and Yonsei University are in charge of the center's construction, center management, and training operations, respectively.

The bioprocessing training center, which will be equipped with GMP-level facilities is being constructed in Songdo, Incheon. Construction was scheduled to commence in June 2023, and the center is expected to open in November 2024.

While the building is being constructed, the demo-training on production of antibodies and vaccines is being provided in practical training center of Yonsei University.

The curriculum will be provided by Ireland's NIBRT, an advanced institute for bioprocessing education and it will offer both degree and non-degree programs.

It is planned to offer training courses using digital transformation, and courses related to high-tech biopharmaceuticals including cell-therapy products and gene-therapy products. There are also plans to establish a separate corporate body to operate the center efficiently.

Keywords: bioprocessing, training, NIBRT, GMP

NOVEL NANOLIPOSOME ADJUVANT PLATFORM FOR INFECTIOUS DISEASES VACCINE DEVELOPMENT

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Abstract. Concerns about the emergence of new pandemics are growing as the incidence and cycle of new viral infectious diseases become shorter over the world. While dealing with new infectious diseases such as SARS, H1N1 flu, MERS, and most recently, COVID-19, it is critical to secure vaccine technologies and platforms that can quickly respond to new emerging infectious diseases. Eubiologics has been developing a novel nanoparticle adjuvant platform for use in the development of recombinant protein vaccines against various viral diseases. This liposome-based nanoparticle adjuvant consists of EcML (monophosphoryl lipid A (MPLA) derived from genetically engineered *E. coli*, TLR-4 agonist) and SNAP (Spontaneous Nanoliposome-Antigen Particleization) technology, which can display antigens on the liposome surface. EuCorVac-19, a COVID-19 vaccine developed by Eubiologics utilizing this novel nanoparticle adjuvant and SARS-CoV-2 receptor-binding domain (RBD) has shown remarkable efficacy and safety in phase 1/2 clinical trials. Furthermore, the interim results of phase 3 clinical trial demonstrate that the vaccine is superiority (immunogenicity) and non-inferiority (seroresponse rate) to the comparison vaccine. Moreover, by appropriately changing the primary vaccine antigens of different viruses, this adjuvant platform is advantageously adaptable to the generation of different viral vaccines. As a result, it is being used to develop other viral vaccines such as RSV, HZV as well as COVID-19 variant vaccines.

Keywords: Adjuvant, nanoliposome, vaccine, Covid-19, RSV, HZV.

16. Marine Biotechnology

THE DISCOVERY OF A NEW ORGAN IN SHRIMP THAT FORMS THE PORTAL OF ENTRY FOR PATHOGENS AND ALLOWS THE SHRIMP TO GROW, OPENS DOORS FOR A BETTER DISEASE CONTROL

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Abstract. Viruses, such as white spot syndrome virus, and bacteria, such as Vibrio species, wreak havoc in shrimp aquaculture. As the main portal of entry for various pathogens in shrimp remain unclear, infectious diseases are difficult to prevent and control. Because the cuticle is a strong pathogen barrier, regions that lack cuticular lining, such as the shrimp's excretory organ, "the antennal gland", are major candidate entry-portals. The antennal gland, up till now morphologically underexplored, was studied using several imaging techniques. Using histology based 3D-technology, we demonstrated that the antennal gland resembles a kidney, connected to a urinary bladder with a nephropore (exit opening) and a complex of diverticula, spread throughout the cephalothorax. Micro Magnetic Resonance Imaging of live shrimp not only confirmed the histology-based model, but also indicated that the filling of the diverticula is linked to the molting cycle and possibly involved therein. Based on the hemolymph filtration function and attached diverticle complex, we propose to rename the antennal gland as the "nephrocomplex". By an intrabladder inoculation, we showed high susceptibility of this nephrocomplex to both white spot syndrome virus and Vibrio infection compared to peroral inoculation. An induced drop in salinity allowed the virus to enter the nephrocomplex in a natural way and caused a general infection followed by death; fluorescent beads were used to demonstrate that particles may indeed enter through the nephropore. These findings pave the way for an oriented disease control in shrimp.

RECIRCULATING NUTRIENTS THROUGH BIOCONVERSION FOR IMPROVING PRODUCTION AND WITHOUT WASTE DISCHARGE IN SUPER-INTENSIVE SHRIMP CULTURE IN THE MEKONG DELTA, VIETNAM

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Abstract. This study aims to develop a shrimp culture technology without discharge and waste of shrimp production converts to valuable products. It used manipulating bacteria biomass, detritivores and seaweeds as bioconversion methods. The white shrimp farming was designed based on nutrients mass balance of input and output. Results showed that water quality in the shrimp pond was optimal for shrimp culture without discharge during the experiment. The shrimp production ranged 60 -70 tons/ha/crop. The shrimp culture indicated a high survival rate and improved quality shrimp production. Particularly, the maximal efficiency of feed utilization was recorded in the whole system. High valuable co-productions in shrimp farming increased income sources for culturists. Using bioconversion methods in white shrimp farming can be significantly improved sustainable indicators for shrimp culture such as an environmental issue, economic and shrimp production.

Keywords: white shrimp, waste, bioconversion, discharge, nutrients, recirculation.

ENZYMES FROM ACTINOBACTERIA OF SALINE HABITATS: CHARACTERISTICS, GENE EXPRESSION AND APPLICATIONS

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Abstract. We are investigating microorganisms of saline habitats of Coastal Gujarat for the last three decades. The diversity was studied based on cultural and morphological features, cell wall constituents, metabolic properties, extracellular enzymes, antibiotic resistance, 16S rDNA-based phylogeny, ARDRA, and DGGE. Proteases and amylases are prominent enzymes and in-depth investigations on proteases revealed unique features of salt-dependent temperature profiling, resistance against chemical denaturation, and function under multitudes of extremities. The genetic diversity of protease and amylase genes was probed by a range of designed primers. The diversity was reflected by the PCR conditions, length of the amplified products, and amino acid sequences of the enzymes. The effect of salt and induction on protease gene expression was studied. A protease gene revealed the highest homology with a *Bacillus megaterium* protease, corresponding to the peptidase domain of the S8 and S53 families. Further, a highly thermostable serine protease of *Nocardiopsis alba* Tata-5 was cloned, expressed in *E. coli*, and structurally elucidated by MALDI-TOF Mass Spectroscopy. The amylase genes of the haloalkaliphilic Bacteria and Actinomycetes from the saline desert and seawater were profiled by the PCR amplification using designed primers, revealing the diversity. Besides, metagenomically derived protease genes were also profiled, cloned, expressed, and characterized suggesting sequence and function-based diversity. The work was carried out by Jignasha Thumar, S.D. Gohel, M.K. Purohit, Amit Sharma, Foram Thakrar, D. S. Rathore, H.B. Bhat, M. Sheikh, Ankita Dobariya, Hasti Ramavat as part of their Ph.D. research.

Keywords: Saline habitats, Actinobacteria, Protease genes, Amylase genes, Gene cloning, Metagenomics

COMPARATIVE TRANSCRIPTOME ANALYSIS REVEALS SINGLE – NUCLEOTIDE POLYMORPHISM (SNP) AND DIFFERENTIALLY EXPRESSED GENES BETWEEN SURVIVED AND DEAD RED TILAPIA (OREOCHROMIS SPP.) POST THE CHALLENGE WITH TILAPIA LAKE VIRUS

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Abstract. Tilapia lake virus (TiLV) is reported as a threat to tilapia aquaculture in 16 countries from four continents with outbreaks causing up to 90% mortality. A TiLV isolate HB196-VN-2020 was propagated successfully from a diseased tilapia sample using an E-11 cell line and had 70% lethal dose of 9.1 x 10^4 $TCID_{50}$ $fish^{-1}$ in red tilapia. Liver, kidney, gut and spleen of three dead fish during the TiLV challenge and three survived ones at the end of the challenge experiment were collected for RNA extraction and sequencing. Total raw reads of each sample were over 20 million, Q30 was over 93% and alignment percent with the reference genome Orenil1.0 was more than 90%. In comparison with transcriptomic data of dead fish, those of live fish contained the number of unique single-nucleotide polymorphism (SNPs) in liver, kidney, spleen and gut at 1,460, 1,939, 6,217 and 896, respectively. By this study, we found 1019, 561, 127, 25 differentially expressed genes between survived and dead red tilapia post the TiLV challenge in liver, kidney, spleen and gut, respectively. This is the first study in Vietnam on the transcriptomic analysis of dead and survived red tilapia post the TiLV challenge. This study may help to discover novel prognostic markers for aquatic animal breeding and potential therapeutic targets against TiLV infection in tilapia.

Keywords: red tilapia, Tilapia Lake Virus, RNA-seq, single-nucleotide polymorphism (SNP), differentially expressed genes

Guided Bone Regeneration Using Phycocyanin-Loaded Micro/Nanofibrous Membranes with Hierarchical Structure

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Abstract. The natural regenerative capacity of bone tissue is impressive, but challenges arise when dealing with defects caused by osteoporosis, osteosarcoma, and certain congenital disorders. To address these challenges, research has explored a mix of synthetic and natural materials to produce scaffolds and hydrogels, aiming to expedite bone healing through the delivery of specific bioactive agents. In our research, we explored the potential of phycocyanin, a component extracted from Spirulina maxima, in promoting bone regeneration. Our evaluations included examining the effect of phycocyanin on MC3T3-E1 cells, focusing on cytotoxicity, the activity of alkaline phosphatase, and the capability for mineral deposition. We then developed a membrane combining poly lactic acid (PLA) and sodium alginate (SA), loaded with phycocyanin. This design was augmented by incorporating atelocollagen sourced from Paralichthys olivaceus. Various tests, including SEM, FTIR, XRD, and water contact angle measurements, were employed to scrutinize this new membrane's properties. Preliminary in vitro studies vouched for its biocompatibility and potential in mineral deposition. Furthermore, in vivo tests on bone defects underlined its potential in enhancing bone regeneration after 12 weeks of treatment. In essence, these phycocyanin-enriched membranes, with their intricate design, show significant potential for applications in bone tissue regeneration.

Keywords: Fibrous membrane, Phycocyanin, Atelocollagen, Bone regeneration

A DUAL-PHOTOCROSSLINKED HYDROGEL WITH GALLIC ACID-MODIFIED CSMA AND FGELMA FOR ENHANCED DIABETIC WOUND HEALING

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Abstract. Excessive inflammation and oxidative stress induced by trauma, burns, infections, and diseases are major contributors to chronic wounds. Thus, the development of materials capable of locally controlling the adverse effects due to excessive ROS production and persistent inflammatory responses is of paramount importance. This study presents a novel hydrogel with enhanced healing properties, designed from fish gelatin methacryloyl (FGelMA), which has recently emerged as a substitute for gelatin derived from land animals, and gallic acid-modified chitosan methacryloyl (GA-CSMA) polymers. The hydrogel was fabricated via a straightforward UV photocrosslinking method and its morphology, rheological properties, swelling behavior, and degradation potential were evaluated. The GM/G-CM hydrogels effectively scavenged the ABTS and DPPH radicals, and In vitro investigations demonstrated the cytocompatibility of hydrogels with human dermal fibroblasts (HDF) and RAW264.7 macrophages, substantially suppressed oxidative damage in ROS microenvironments. Furthermore, it inhibited the production of nitric oxide (NO), tumor necrosis factor-α (TNF-α), and Interleukin-1β (IL-1β) while enhancing the production of Interleukin-10 (IL-10) in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Finally, in a diabetic mouse model with chronic wound conditions, the hydrogel exhibited anti-inflammatory and angiogenic effects. These results suggest that the GM/G-CM hydrogel, with its enhanced anti-inflammatory and antioxidant properties, can potentially be utilized as a wound dressing to improve hard-to-heal chronic wounds.

Keywords: Fish gelatin, Chitosan, Gallic acid, Photocrosslinkable hydrogel, Diabetic wound healing

SUTAINABLE BIOMASS SOURCES FOR FUTURE WHITE BIOTECHNOLOGY

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Abstract.

Conventional biomass has a limited sustainability and could negatively affect the environment and food security. Replacing chemicals from fossil fuels requires a significant quantity of biomass and an enormous area due to the low photosynthetic efficiency. Furthermore, water scarcity and peak phosphorus are also of concerns for mass production of biomass feedstocks for white biotechnology. One of the viable options to resolve this situation is blue biomass or marine photosynthetic organisms culturing with seawater. Various types of algal culture systems have been developed to improve sustainability and economic feasibility of blue biomass production.

We have developed various floating microalgal culture systems using Selectively-Permeable Materials (SPMs) so that the microalgae can grow on dissolved eutrophic compounds in seawater. We've found that the ion permeability of SPMs is one of the key limiting factors and increasing ion permeability resulted in enhanced blue biomass productivity. In other words, our ocean culture systems are not light limited, yet, though we have achieved average biomass productivity of 20 g/m2/day using just seawater on our ocean test-beds. We will show recent results and SWOT analysis between conventional and blue biomasses. The future direction of blue biomass for sustainable feedstock for biorefinery will be discussed.

PATHOGENICITY CHARACTERIZATION OF NOCARDIOSIS IN ORANGE-SPOTTED GROUPER (*EPINEPHELUS COIOIDES*) VIA EXPOSURE TO NINE DISTINCT ISOLATES OF *NOCARDIA SERIOLAE* AND FOUR ADMINISTRATIVE ROUTES

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Abstract. *Nocardia seriolae* causes chronic nocardiosis in various marine and freshwater aquatic animals; however, grouper species have rarely been investigated. This study evaluated the pathogenicity of nocardiosis following *N. seriolae* infection in the orange-spotted grouper *Epinephelus coioides*. Nine identified genetic isolates of *N. seriolae* were tested *in vivo* using the intraperitoneal method and observed daily for 35 days. The most virulent isolate was then used to evaluate transmission through different routes (intraperitoneal IP, intramuscular IM, oral OR, and immersion IS) in the same fish model and was observed daily for 42 days. The results showed mild variation in virulence among *N. seriolae* isolates. AOD107132-2K and OT103003-N11 strains displayed the highest and lowest risk virulence, respectively, based on the accumulation and kinetics of mortality. IM and IP administrations showed an early phase response with early mortality by 5 dpc (30–100%), while slower kinetics of nocardiosis occurred in the OR and IS routes with slow mortality at 35 dpc (4–8%). Histopathology revealed typical granulomas, confirming the progression of nocardiosis in the diseased fish. These results provide the basis for further studies on the virulence profile of *N. seriolae* in Taiwan and a well-suited route of administration in orange-spotted groupers for further prevention development.

Keywords: nocardiosis, pathogenicity, chronic disease, orange-spotted grouper, administrative routes, granulomatous.

STATISTICAL OPTIMIZATION OF α-AMYLASE PRODUCTION ON RAW SUBSTRATES IN HALOALKALIPHILIC ACTINOBACTERIA, *NOCARDIOPSIS ALBA* KAM-13

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Abstract. This work relates to the amylase production in marine actinomycetes, *Nocardiopsis alba* Kam-13 using potato peel extract as a substrate for the growth[1&2]. The selected potential strain potentially utilized a raw starch substrate to grow and produce amylase at a significant level. Further, the amylase production was optimized using statistical methods. The amylase production was optimized using the 'one variable at a time (OVAT)' approach followed by the statistical methods to ascertain the interplay and combinational effect of significant parameters. The Placket Burman design followed by the next statistical approach, the Central Composite Design (CCD) was employed. Based on the Central Composite Design, the optimum combination of the significant factors was determined, leading to a three-fold enhancement in amylase production[3,4, 5 & 6]. The study, therefore, signifies the potential application of raw substrates without any other supplementation for the growth of extremophilic microorganisms to produce value-added metabolites and enzymes[2].

Keywords: Amylase, Response Surface Methodology (RSM), Raw substrate, Actinomycetes

Profiling of Amylase Genes from Haloalkaliphilic Bacteria and Actinomycetes of Saline Habitats

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Abstract. This study focuses on the gene profiling of amylases from haloalkaliphilic Bacteria and Actinomycetes from the saline habitats of Little Rann of Kutch and seawater of Alang and Dwarika Coast, Gujarat, India. The amylase-specific primers: BT-1, BP-2, BV-3, ND-4, ND-5, ND-6, NA-7 and OB-8 were designed using Primer3 software and then synthesized aiming at the amylases of bacteria and actinomycetes. While the primers BPL and BVL specific for bacteria were retrieved from the literature. Primers BT-1, BP-2, BV-3, OB-8, BPL and BVL were used for the amplification of bacterial amylase genes. While the primers ND-4, 5, 6 and NA-7 were used for the amplification of amylase genes of Actinomycetes. The PCR conditions with respect to annealing temperatures and other PCR steps were extensively investigated and determined. Gradient PCR and Touchdown PCR techniques were used to find out appropriate annealing temperatures for the amplification of the complete genes. The annealing temperatures were explored in the range of 47ºC to 65ºC. The amplified products generated in the range of 1500bp-1600bp were visualized on 1.2% Agarose gel and further analyzed quantitatively for purity, sequencing and cloning. Amplification profiling generated ideas about the effect of annealing temperatures, suggesting the diversity of amylase genes in haloalkaliphilic bacteria and actinomycetes of varied saline habitats.

Keywords: Haloalkaliphilic Bacteria and Actinomycetes, Amylase genes, Gradient PCR, Touchdown PCR, Gene Amplification Profiling

THE SUPPORT OF MARINE BIOTECHNOLOGY IN BOOST BLUE TRANSFORMATION

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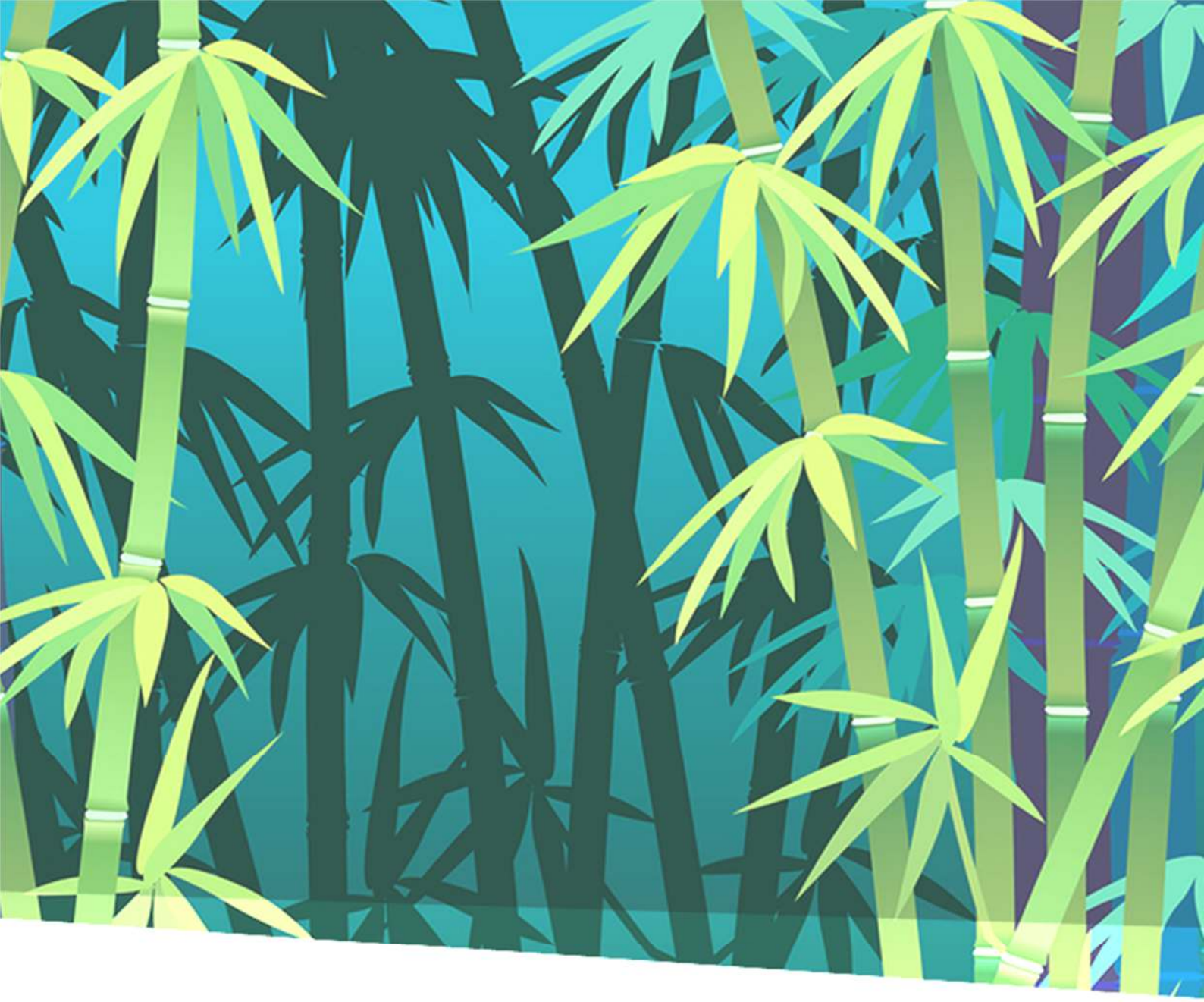
Abstract

The European Union had committed to meet the global sustainability agenda by 2030, putting sustainability at the core of its Blue Economy. Blue biotechnology produces nutraceuticals, cosmeceuticals, that promote human health and well-being; medicine also continues to benefit from the chemical diversity that is continuously being discovered, marine-inspired biomaterials and pharmaceutical drugs are already available on the market, marine algae and yeasts are second to none and several other industrial sectors have also been powered by blue biotechnology. However, the seafood industry has significantly boosted through blue biotechnology, being one of the areas where this technology has made the biggest progress towards the SDGs, especially to feed the population and meet the increasing fish demand.

This improvement is in progress thanks to the “blue transformation" which is a strategy for the transformation of aquatic food systems, making the system more sustainable and linked to the Farm to Fork strategy (F2F), which influences many aspects of the Blue Economy. The presentation will focus on the three mains objectives of the Blue Transformation and some specific related cases studies: sustainable aquaculture expansion and intensification; effective management of all fisheries; upgraded value chains. The blue transformation is the key to ensure the social, economic and environmental viability of aquatic food systems, and secure nutritional outcomes.

Keywords: marine biotechnology; blue transformation; fishery; aquaculture



Poster session

COMPARISON OF ITS, MATK AND RCBL DNA BARCODES FOR IDENTIFICATION OF ANOECTOCHILUS SPECIES IN NORTHERN VIETNAM

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Abstract. *Anoectochilus* are commonly known as jewel orchids that is a genus of about fifty species in the orchid family orchidaceae. Internal transcribed spacer (ITS), maturase (matK) and ribulose 1,5 biphosphate carboxylate (rcbL) regions sequence polymorphisms provide an efficient tool for discrimination of *Anoectochilus* species. The objectives of this study were to identify a molecular phylogenetic relationship of 32 jewel orchid accessions distributed in northern Vietnam using specific primers for ITS2, matK and rcbL regions. Results showed that the ITS2 region has higher potential identification than matK and rcbL markers. The findings of this study could provide a valuable information that is necessary for classification, identification and progation of medicinal valuable *Anoectochilus* species in Vietnam.

Keywords: Anoectochilus, DNA barcode, ITS, matK, rcbL.

IDENTIFICATION OF HERBAL MEDICAL PLANTS COLLECTED IN NORTHERN VIETNAM USING DNA BARCODE ITS2

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Abstract. Nowadays, the demand for using herb plants to treat and protect human health is increasing. Along with the growing herbal market, the adulteration of herbal medicinal materials is increasing in many countries, including Vietnam. It is difficult to determine the origin of herbal medicinal materials by morphological or chemical methods when they are substituted by herbs from closely related species or adulterated intentionally by materials from unrelated plants or processed materials. Therefore, it is necessary to use molecular methods based on DNA markers. ITS is a highly conservative nuclear **ribosomal** marker that has been widely used in the identification of plant species. In this study, the ITS2 marker was used to identify 8 herbal medicinal materials, including polyscias (DLTN, DLBG), morinda (BKTN, BKQN), black zingiber (GDTN, GDYB) and black curcuma (NDTN, NDYB). The results showed that the DLTN and DLBG were *Polyscias fruticosa*; BKTN and BKQN were different species, *Gynochthodes officinalis* (BKTN) and *Morinda officinalis* (BKQN); GDTN and GDYB were *Kaempferia parviflora*; NDTN and NDYB were *Curcuma caesia*.

Keywords: Curcuma, DNA barcode, ITS, morinda, polyscias, zingiber.

OPTIMIZATION OF CULTIVATION AND FORMULATION PROCESSING OF *SPHINGOBIUM* SP. FOR BIODEGRADATION OF ORGANOPHOSPHORUS INSECTICIDES

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Abstract. The global market for organophosphorus pesticides (OP) was estimated to be valued at $6.6 billion in 2022. It is projected that the market will reach $8.74 billion by 2028, with a compound annual growth rate (CAGR) of 4.8% over the forecast period. Because organophosphate insecticides are widely used, they can sometimes persist in crops. This is the reason why there are regulations on residual pesticides. *Sphingobium* sp. Cam5-1 exhibits activity in the biodegradation of OP insecticides. In this study, we established the mass culture conditions of Cam5-1, a strain of *Sphingobium* that degrades OP insecticides, in order to address the issue of residual pesticides. The culture temperature, carbon source, nitrogen source, and concentration were set according to the R2A medium, while trace elements were also included. The optimal medium was prepared with the following ingredients: 30g/L yeast extract, 20g/L glucose, 0.3g/L K2HPO4, 0.05g/L MgSO4, and 0.1g/L CaCl2. The optimal fermentation conditions were also developed as follows: 3L of the medium in a 5L JAR fermenter with 2% seed cultured medium (24h) under 150rpm, 0.5vvm for 32h at 30°C. The cell density reached 3.8 x 10^9 CFU/mL. Additionally, a formulation study was conducted to improve storage capacity. The liquid and powder formulations were developed and optimized for stability. Cryopreservation agents were compared and analyzed, and the protective agents that exhibited the highest level of cell stability were selected.

Keywords: *Sphingobium* sp., biodegradation, OP insecticides, microbial formulation

ESTABLISHMENT OF AN *IN VITRO* MICROPROPAGATION PROTOCOL OF PHILODENDRON 'PINK PRINCESS' THROUGH PROTOCORM-LIKE BODIES IN VIETNAM

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Abstract. Philodendron 'Pink Princess' known as a hybrid of *Philodendron erubescens* is one of the most popular houseplants containing dark green leaves with unique pink variegation. Due to its exceptional qualities listed above, Philodendron 'Pink Princess' has gained popularity in the ornamental plant market. In general, there is little research about this species' micropropagation protocols worldwide. Particularly in Vietnam, no studies about the micropropagation protocol of Philodendron 'Pink Princess' have been reported so far. The study aims to develop a regeneration-based micropropagation procedure from protocorm-like bodies (PLBs) viewed as an efficient approach for *in vitro* mass propagation. For the formation of PLBs, stem nodal segments were cultured on Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BA) alone or BA in combination with 1-naphthaleneacetic acid (NAA) at different concentrations. The development of PLBs-derived shoots was investigated in five various basal media like Murashige and Skoog (MS), Vacin and Went (VW), and Gamborg's B5. Roots were induced on Gamborg's B5 medium supplemented with different concentrations of Indole-3-butyric acid (IBA). The result showed that MS medium combined with 1,5 mg/L BA and 0,5 mg/L NAA optimized to create PLBs from stem node segments. Gamborg's B5 medium was appropriate for the development of the shoots derived from PLBs. In addition, the highest number of roots was recorded on Gamborg's B5 medium supplemented with 1 mg/L IBA. The established approach in this study significantly contributes to mass production as well as provides a fundamental protocol for variation breeding of the Philodendron 'Pink Princess'.

Keywords: basal media, Philodendron 'Pink Princess', protocorm-like bodies (PLBs), micropropagation

EFFECTS OF *ACTINOMUCOR ELEGANS* INCUBATION CONDITIONS ON ANTIOXIDANT ACTIVITIES OF TOFU ADDED WITH PURPLE SWEET POTATO

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Abstract. Fermented tofu (chao, sufu, furu,…) is a traditional product of Asian countries, which is produced by the process of tofu incubation with *Actinomucor elegans*. Besides of high nutrient value, fermented tofu is also a seasoning with special flavour. This study aims to evaluate the effects of *Actinomucor elegans* incubation conditions for tofu added with purple sweet potato (PSPTofu), including temperature (25, 30 and 35^{o}C), relative humidity (85, 90 and 95%), population of mold (10^4, 10^6 and 10^8 cfu/mL) and incubation time (18, 24, 30 and 36 hours) on the changes of total polyphenol content (TPC), total flavonoid content (TFC), anthocyanin, DPPH free radical scavenging capacity through inhibitory activity at IC_{50} of incubated tofu (pehtze). The results showed that to get the best quality of pehtze, PSPTofu should be incubated at 30^{o}C, with 95% relative humidity and the mold population of 10^6 cfu/mL during 30 hours. The pehtze represented the highest TPC (62.91 mg GAE/g db), TFC (17.32 mgQE/g db), anthocyanin content (308.88 µg/g db), DPPH free radical scavenging (85.52 µmol TE/g db) and IC_{50} value (6.61 mg/mL), respectively.

Keywords: Actinomucor elegans, antioxidant, purple sweet potato, tofu

IDENTIFICATION OF LACTIC ACID BACTERIA FROM TENDER JACKFRUIT (*ARTOCARPUS HEREOPHYLLUS* L.)

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Abstract. This study was carried out to isolate, select, and identify lactic acid bacteria (LAB) strains from tender jackfruit for application in the fermentation process. A total of 37 bacterial strains with physiological and biochemical traits resembling lactic acid bacteria were identified from two experimental samples of raw tender jackfruit (*Artocarpus hereophyllus* L.) and lactic acid fermented tender jackfruit. Three of those LAB strains—17R, 2F, and 10F—showed the highest levels of acidity and were selected for identification at the species level by the 16S rRNA technique. The findings of the identification revealed that the three lines 17R, 2F, and 10F exhibit more than 99% identity (with 100% coverage) with the gene sequences of *Weissella paramesenteroides*. Therefore, these lactic acid bacteria strains are recommended to be employed as starters in the pickling process of tender jackfruit in further studies.

Keywords: Fermentation, identification, lactic acid bacteria, tender jackfruit,

ECTOPIC EXPRESSION OF OLFACTORY RECEPTOR IN SKELETAL MUSCLE

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Abstract. Olfactory receptors (ORs) are the largest gene family in the mammal. ORs are mainly expressed in the olfactory sensory neurons; however, some ORs are ectopically expressed in extra-nasal tissues and play tissue-specific roles. We investigated the biological activity of OlfrA, one of the ORs, in the murine skeletal muscle. The OlfrA was significantly expressed in C2C12 myoblast and various types of skeletal muscle of C57BL/6N mice. OR signaling components, such as G protein subunit alpha L and adenylate cyclase 3 are also expressed in C2C12 myoblast. Next, we investigated the OlfrA activation by odorant R as a ligand of OlfrA. Induction of the second messengers by OlfrA activation was analyzed in transiently transfected Hana-3A cells with OlfrA and C2C12 myoblast, respectively. Activation of OlfrA increased intracellular cyclic adenosine monophosphate (cAMP), calcium, and inositol phosphate. The EC_{50} values of cAMP were 73.1 and 86.7 μM in Hana-3A cells and C2C12 myoblast, respectively. In the calcium assay, the EC_{50} values were 113.8 and 76.91 μM in Hana-3A cells and C2C12 myoblast, respectively. The EC_{50} values of inositol phosphate were 89.54 and 97.05 μM in Hana-3A cells and C2C12 myoblast, respectively. These findings demonstrated that OlfrA, an OR is a functional receptor in skeletal muscle and that the activation of OlfrA by odorant R transduces intracellular signals as a second messenger response in skeletal muscle.

Keywords: olfactory receptor, skeletal muscle, odorant, second messenger

BIODEGRADABLE POLYMER FILMS INCORPORATING FUNCTIONALIZED FEW-LAYER GRAPHENE

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Abstract. In this study, functionalized few-layer graphene (FFG) was produced using an environmentally friendly liquid exfoliation process employing coffee waste extract. The FFG was subsequently incorporated into four different polymer matrices: chitosan, polyvinyl alcohol (PVA), sodium alginate, and gelatin. Composite films were developed with varying concentrations of FFG ranging from 0 to 1 wt% of the respective polymers. The developed films were characterized for water content, swelling degree, thermal and mechanical properties, water vapor permeability, and indoor soil burial degradation. The tensile strength of the films made with pure chitosan, PVA, sodium alginate, and gelatine was greatly boosted by the addition of FFG, with improvements of 439%, 247%, 190%, and 195%, correspondingly. Furthermore, the Young's modulus of the composite films exhibited enhancements of 515%, 253%, 144%, and 341%, correspondingly. The elongation at break of chitosan and PVA films exhibited a decrease as the concentration of FFG increased. The sodium alginate/FFG and gelatin/FFG films demonstrated the highest elongation at break. The use of only 1% FFG resulted in a reduction of water vapor permeability in chitosan, PVA, sodium alginate, and gelatine composite films by 87%, 67%, 50%, and 55%, respectively. The inclusion of FFG resulted in a decrease in the water content and swelling degree of all composite films. Furthermore, the composite films exhibited comparable degradation following the introduction of FFG during the 60-day soil burial experiment.

Keywords: Functionalized few-layer graphene, biodegradable film, thermal and mechanical properties, indoor soil burial degradation.

PRODUCTION OF HUMAN ANTIMICROBIAL PEPTIDE LL-37 IN BARLEY

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Abstract: Cereal crops, such as maize, rice, and barley, represent one of the most often used platforms for the production of therapeutic biomolecules since their seeds offer an appropriate environment for a stable protein accumulation. Other advantages of cereal crops include relatively low secondary metabolite content and simple protein profile of grains, which facilitates downstream processing, and well-established infrastructures for large-scale grain production, harvest, transport, and post-harvest storage. Compared to microbial expression systems, plants offer several advantages, such as the absence of endotoxins or human pathogens, and lower cost of large-scale production.

Antimicrobial peptides are cationic amphipathic peptides containing less than 50 amino acid residues. They display broad-spectrum antimicrobial activity, which demonstrates their potential as a promising alternative to conventional antibiotics. Our group has recently established a feasible approach for producing recombinant human antimicrobial peptide cathelicidin (rhLL-37) in barley grains using endosperm-specific barley B1 hordein promoter, a protease cleavable maltose-binding protein fusion tag, and endoplasmic reticulum retention sequence (KDEL). Stable inheritance and expression of the rhLL-37 transgene were confirmed up to the T6 generation, which is essential for the future commercialization of the product. Nevertheless, further engineering aimed at cost-effective downstream processing avoiding the use of affinity chromatography purification and a protease-catalyzed tag cleavage is currently in progress.

Keywords: barley, grains, antimicrobial peptides, cathelicidin

EFFECTS OF SONICATION CONDITION AND RATE OF RED BEAN SPROUTS ADDITIONAL TO THE QUALITY OF MILK YOGURT RICH IN ANTIOXIDANTS AND FIBER

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Abstract. Milk yogurt is a type of coagulated milk product that is consumed worldwide. The goal of making a new milk yogurt product rich in antioxidants and fiber has been achieved at the laboratory scale. Survey parameters such as the ratio of milk: red bean sprouts (100 : 0, 90 : 10, 80 : 20, 70 : 30). Sonication time (3,5,7,9 minutes), sonication temperature (20, 30, 40, 50^{o}C) are the parameters used for survey in this paper. The results sensory evaluation found that a ratio of 90:10 is an appropriate mixing ratio for yogurt production. In addition, the addition of red bean sprouts increased the phenol and fiber content, while the protein content decreased slightly with the increase of red bean sprouts. When milk yogurt is sonicated at 40 °C for 8 minutes, it is the optimal temperature and time range. The results show that when gradually increasing the sonication temperature and sonication time, the viscosity of yogurt tends to increase from 516.67 to 673.33 dPa.s and the hardness also tends to increase from 19.33 to 24.90 g. At the same time, the SEM results also show that the fat particles of the sample using ultrasonic are smaller than that of the non-ultrasonic sample. Red bean sprout yogurt is rich in phenolic antioxidants and fiber suitable for making antioxidant-rich drinks.

Keywords: red bean sprouts, milk yogurt, sonication, SEM.

ECTOPIC ACTIVATION OF OLFRX THROUGH ODORANT N INCREASES THERMOGENESIS IN CULTURED ADIPOCYTES AND IN-VIVO.

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Abstract. Olfactory receptors are widely expressed in non-nasal tissues and the functionality of these ectopic olfactory receptors has been elucidated. Previously, expression of mouse olfactory receptor X (OlfrX) was demonstrated in brown adipocytes differentiated from C3H10T1/2 mesenchymal stem cells and stromal vascular fractions. This study investigated the function of OlfrX on brown adipocytes, which regulates energy metabolism, thermogenesis, and adiposity. Oral administration of odorant N for 10 weeks did not alter body weight and adiposity in mice. However, when mice were exposed to acute-cold exposure (4℃, 6 hrs) after oral administration of odorant N (10 mg/kg) for 2 weeks, the mice showed increased rectal and surface body temperature compared to those of control. These effects of odorant N were negated in OlfrX$^{-/-}$ mice. Activation of OlfrX by odorant N (2 μM) induced gene and protein expression of UCP1 and PGC1α in brown adipocytes both in-vitro and ex-vivo. These effects were abrogated by silencing OlfrX, demonstrating the effects of odorant N on UCP1 and PGC1α induction was OlfrX-dependent. Activation of OlfrX by odorant N did not affect second messenger productions including intracellular calcium, inositol phosphates, and cAMP; however, activated a signaling through ß-arrestin thus increased ERK1/2 phosphorylation. Inhibition of ß-arrestin by barbadin negated the effects, demonstrating that OlfrX stimulated ß-arrestin-ERK1/2 signaling axis. Ex-vivo experiments demonstrated increased UCP1 expression and phosphorylation of ERK1/2, CAMKII, and CREB by co-treatment of 0.1 μM norepinephrine and odorant N while OlfrX knockdown negated these effects. In addition, odorant N did not affect the expression of UCP1-independent futile cycles including SERCA2b-mediated calcium cycling, triglyceride lipid cycling, and creatine futile cycles. These results demonstrate that OlfrX increased thermogenesis of BAT by inducing UCP1 expression via activation of ß-arrestin-ERK1/2-CREB signaling axis. OlfrX may be a novel therapeutic target for obesity treatment and this possibility will be examined in human tissues.

Keywords: thermogenesis, ectopic olfactory receptor, adipocyte, brown adipocyte, *obesity*

EFFECTS OF NANO-ENZYME ON NPK FERTILIZERS ON GROWTH, YEILD AND ECONOMIC EFFICIENCES ON BIOMASS MAIZE IN THE SUMMER-AUTUMN CROP 2023 IN BINHDINH PROVINCE

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Abstract. The application of nano-enzyme technology in fertilizer production is a promising new direction to help improve economic efficiency in crop production in general and biomass maize production in particular. Large-scale trial with 3 treatments in Binhdinh province in the summer-autumn crop 2023: (i) T1 (Control): NPK16-16-8 fertilizer, apply once in the 5-leaf maize stage, the amount of application is 1,000 kg/ha; (ii) T2: NPK fertilizer containing nano-enzyme (NPK 18-14-6 + 6S), apply once at the stage of 5-leaf maize, the amount of application is 1,000 kg/ha; (iii) T3: NPK fertilizer containing nano-enzyme (NPK 18-14-6 + 6S and NPK 16-6-18 + 1Mg), apply 2 times, the first time apply to 5-leaf maize stage with the amount of 720 kg/ha NPK 18-14-6 + 6S, the second time fertilizes the stage of 8-10 leaves corn with the amount of 280 kg/ha NPK 16-6-18 + 1Mg. After 80 days of planting, harvest and determined that T2 had the highest biomass yield, reached 72.51 tons/ha and increased by 9.51 tons/ha, equivalent to an increase of 15.1% compared to the control, the profit of T2 was 40,031 million VND/ha, 10,311 million VND/ha higher than the control, equivalent to 34.7% increase. It is recommended to use NPK 18-14-6 + 6S nano-enzyme fertilizer once at the 5-leaf maize stage in biomass maize production instead of conventional NPK fertilizers.

Keywords: nano-enzyme, NPK fertilizer, 1 time fertilizing, biomass maize, summer-autumn crop, Binhdinh province

EFFECT OF AGRICULTURAL PRACTICES ON ENTOMOPHATHOGENIC FUNGI (*METARHIZIUM* SP. AND *BEAUVERIA* SP.) DENSITY IN THE RHIZOSPHERE SOIL OF BLACK PEPPER FIELD IN VIET NAM'S DAK LAK PROVINCE

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Abstract. Entomopathogenic fungi (EPF) play an important role in agricultural fields and help control insect pests. The high diversity of entomopathogenic fungi can manage insect pest populations and reduce their negative impact to below the economic threshold. However, these natural soil-born enemies may be adversely affected by agricultural practices including monoculture system, the use of chemical fertilizers and pesticides. Many different agricultural practices have been used on black pepper cultivation in Dak Lak province. The results show that black pepper monoculture system, nonliving supports have a negative impact on the diversity of *Metarhizium sp.* and *Beauveria sp.* Intercropping black pepper with coffee system, on the other hand, makes a significant contribution to increasing the diversity of *Metarhizium sp.* and *Beauveria sp.* The population of *Metarhizium sp.* and *Beauveria sp.* in organically fertilized black pepper fields was significantly higher than in chemically fertilized black pepper fields. Additionaly, fungicide use reduced the diversity of *Metarhizium sp.* and *Beauveria sp*. in black pepper field soil.

Keywords: Black pepper, *Metarhizium sp.*, *Beauveria sp.*,

CODING SEQUENCE ANALYSIS OF *EIF4-E* GENE IN *CUCUMIS SATIVUS* L. CULTIVAR 'NEP TA'

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Abstract. *eIF4-E* is a gene that plays an important role in infecting a crop of RNA viruses. Mutation of this gene prevents the virus from infecting the plant and therefore creates a virus resistant plant. In this work, the coding sequence of the *eIF4-E* gene in Nep ta cucumber (*Cucumis sativus* L cultivar 'Nep ta'). – a popular variety of cucumber in Vietnam, was isolated, sequenced and analyzed for structural characterization. The findings showed that the coding sequence of *eIF4-E* in Nep ta cucumber is homologous 99,44% to the published sequences of this gene. The coding sequence structure consists of five exons, with four nucleotides (at positions 430, 482, 530 and 603) changed compared to the original sequence (XM004147349.3), including one position in exon 2 and three positions in exon 3. This result is an important premise for creating virus-resistant varieties of cucumber in Vietnam.

Keywords: Coding sequence, cucumber, eIF4-E, gene isolating, sequencing

POLYPLOIDIZATION OF NGOC LINH GINSENG (*PANAX VIETNAMENSIS* HA *ET* GRUSHV.) BY COLCHICINE INDUCTION TO ACHIEVE MEDICINAL HERB DEVELOPMENT PROSPECTS

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Abstract. Polypoidization is one of the mainly desired pathways to gained potentially medicinal traits in several herb plants, especially with Ngoc Ling ginseng (*Panax vietnamensis* Ha *et* Grushv.). Chromosome doubling might lead to alter genetic materials coding or associating with the expected phenotype. Furthermore, Ngoc Linh ginseng is a rare and precious and an endemic species in Vietnam in terms of medicinal values. The aim of this study is to develop tetraploid Ngoc Linh ginseng from somatic embryos by colchicine treatment. Firstly, somatic embryos of Ngoc Ling ginseng were developed from petiole explants on ½ MS supplemented with different concetration of NAA and BA and then making tetraploid ginseng via cochicine-based induction from these globular embryos by immersed technique at various concentration and exposure time. Diploid and tetraploid ginsengs were accurately identified by flow cytometry and cytology methods. The results showed that the number of somatic embryos of Ngoc Ling ginseng gained from petiole explants on ½ MS supplemented with 0.2 mg/L NAA and 0.1 mg/ L BA were highest. These embryos could shoot-develop their best on MS medium supplemented with 0.75mg/L TDZ and root develop their best when subculturing onto the same medium added 1.5 mg/L NAA. The somatic embryos after cochicine treatment could increase secondary embryos. These secondary embryos gained in this study after colchicine treatment were identified tetraploid ginseng (2n = 4x = 48). Initially, tetraploid Ngoc Linh Ginseng is really succeeded when appearing morphological variation in comparison with diploid counterparts which promises potential values in introducing novel phenotypic ginseng for production and for further breeding purposes.

Key words: *in-vitro* propagation, Ngoc Linh ginseng, colchicine, polyploidization

THE ANTIBACTERIAL ACTIVITY OF THE COMPOUND IN THE ETHANOL EXTRACT OF *ADENOSMA BRACTEOSUM* BONATI.

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Abstract: *Adenosma bracteosum* Bonati. (*A. bracteosum*) is distributed mainly in Southeast Asia. In Vietnam, it is also found especially in Tay Ninh province and some provinces in the Mekong Delta. It is commonly used for medicinal purposes, such as treating colds, tumors, stomach disorders, digestive disorders, hepatitis, and skin problems. Some species are also used as insecticides and/or repellents against mosquitoes or fleas. Phenolic acids, flavonoids, and terpenoids are the main phytochemicals in this plants. Several bioassay-based assessments have demonstrated the biological activity of Adenosma in human health. In Vietnam, *A. bracteosum* has a long history of use in both conventional and modern treatments. It is used as a medicine to clear heat, lower blood pressure, and treat diseases related to liver function, because it contains a variety of compounds such as triterpenoids, flavonoids, thymol, linalool, and (E)-β-farnesene However, the antibacterial activity of the ethanol extract of *A. bracteosum* has not been thoroughly studied. In this study, the antibacterial activity of the ethanol extract of *A. bracteosum* was evaluated under in vitro conditions with six pathogenic bacteria strains: *Aeromonas hydrophila*, *Edwardsiella ictaluri*, *Erwinia aphidicola*, *Escherichia coli*, *Staphylococcus aureus*, and *Xanthomonas oryzae*. As a result, the ethanol extract of *A. bracteosum* was able to inhibit the growth of these six strains at a concentration of 0.9 g/mL. The diameter of the clear antibacterial circle (halo circle) was 11.5 mm (*Aeromonas hydrophila*), 10.5 mm (*Edwardsiella ictaluri*), 10.9 mm (*Erwinia aphidicola*), 14.25 mm (*Escherichia coli*), 11 mm (*Staphylococcus aureus*), and 12.25 mm (*Xanthomonas oryzae*) after cultivation for 24 hours. This conclusion may facilitate future studies on the direct inhibition of *A. bracteosum* extracts against these bacterial strains. At the same time, it also creates the potential to apply biotechnology in the interaction between compounds in plants and common pathogenic bacteria strains.

Keywords: *Adenosma bracteosum Bonati, antibacterial, bioactive, phytochemicals, extracts.*

SOMATIC EMBRYOGENESIS FROM STEM AND LEAF OF SANCHI GINSENG AND EVALUATION OF GENETIC STABILITY OF EMBRYO-DERIVED PLANTLETS BY SCOT MARKER

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Abstract. Sanchi is a highly valuable medicinal ginseng that is at risk of over-exploitation. This study aimed to authenticate, regenerate somatic embryos via stem- and leaf-derived callus, and to evaluate the genetic stability of plantlets. The initial sample was identified as *Panax notoginseng* based on the plant morphology and DNA barcodes. Callus was optimally induced from the stem and leaf explants on Murashige and Skoog (MS) medium supplemented 1 mgL-1 2,4 - Dichlorophenoxy acetic acid (2,4-D) and 0.4 mgL^{-1} thidiazuron (TDZ) or 0.2 mgL^{-1} kinetin, and well proliferated by supplementing 0.5 mgL^{-1} 2,4-D combined with 0.4 mgL^{-1} TDZ or 0.2 mgL^{-1} kinetin. The optimal somatic embryogenesis from stem- and leaf-derived callus was observed on Schenk and Hildebrandt (SH) medium containing 0.5 mgL^{-1} 2,4-D and 0.5 mgL^{-1} 1-naphthaleneacetic acid (NAA), 1.0 mgL^{-1} benzyl adenine (BA), and 30% coconut water (CW). The genetic fidelity of embryo-derived plantlets to the donor plant was proven through monomorphic banding of 10 Start Codon Targeted (SCoT) marker products. The genetically stable plantlets produced from stem- and leaf-derived embryos in the present study contributed to a significant improvement in propagation efficiency in this plant.

Keywords: Callus Induction, DNA Barcodes, Genetic Stability, *Panax notoginseng*, Somatic Embryogenesis

MICROPROPAGATION OF *ANUBIAS BARTERI* VAR. NANA PETITE, A VALUABLE ORNAMENTAL AQUATIC PLANT

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Abstract. The objective of this study was to establish an efficient protocol for the rapid micropropagation of *Anubias barteri* var. *nana* Petite, a valuable ornamental aquatic plant in the Araceae family. The basal bud explants were sterilized with a bleach solution at various ratios and durations. Next, the explants were placed on Murashige and Skoog (MS, 1962) medium supplemented with varying concentrations of BA to induce shoot formation and multiplication. The results showed that all explants (100%) survived when sterilized with a 40% bleach solution for 15 minutes. After six weeks of culture, in the presence of 3 mg/L BA, the maximum multiplication rate reached 5.33 shoots per explant, with an average of 4.92 leaves per shoot. To induce the formation of protocorm-like bodies (PLBs), shoot thin layer explants with a thickness of 0.2 cm were placed on MS medium supplemented with different combinations of BA and NAA. After four weeks of culture, the combination of 0.5 mg/L BA and 1.0 mg/L NAA yielded the highest number of PLBs per explant, with 6.57 PLBs observed. These primary PLBs were then transferred to MS medium containing 1.5 mg/L BA and 0.5 mg/L IAA, resulting in the multiplication of 2.5 secondary PLBs within two weeks. Subsequently, when the PLBs were transferred to an MS medium supplemented with 1.0 mg/L BA and IBA for the following two weeks, all PLBs produced shoots with an average height of 1.53 cm. Finally, the regenerated shoots were successfully rooted on an MS medium without plant growth regulators, leading to a 100% survival rate after four weeks when planted in the aquarium. Ongoing research is focused on further enhancing the multiplication of PLBs.

Keywords: *Anubias barteri* var. nana Petite, multiplication, protocorm-like-bodies (PLBs), shooting, thin layer.

SURVEY ON THE PRESENCE AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZA FUNGI IN THE ROOTS AND SUBSTRATE OF THREE ORCHID SPECIES *DENDROBIUM* SP., *CYMBIDIUM* SP. AND *PHALAENOPSIS* SP.

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Abstract: The symbiotic relationship between root fungi and plant roots plays an important role in the absorption of water and nutrients of plants. The study was conducted to survey the presence and distribution of Arbuscular Mycorrhiza fungi (AMF) in the roots and growing medium of three species of orchids *Dendrobium* sp., *Cymbidium* sp. and *Phalaenopsis* sp. grown on sphagnum moss, charcoal and pine bark in orchid gardens in Thu Duc City, Ho Chi Minh City. The AMF in the root samples were stained with trypan blue, the spores in the media samples were isolated by the wet sieving and decanting technique and stained with Melzer + PVLG to identify the spore morphology. The spores from orchid roots and growing media were observed under an optical microscope. The results indicated that in the roots and the media, there were presence of three fungal genera including Glomus, Acaulospora, Septoglomus. In addition, two spore types of Dr1, Dr2 in the roots and Ki4 spores in the medium were recorded. Glomus was the predominant genus in orchid roots. The number of AMF spores in the media fluctuated between 8 and 188 spores per gram of media. Meanwhile, the genus Acaulospora was the most commonly present genus in orchid media with the average total spore density of 188 spores per gram of media.

Keywords: Acaulospora, Arbuscular Mycorrhiza Fungi (AMF), Glomus, Orchid Mycorrhiza (OM), Septoglomus

EFFECT OF PLANTING DENSITY AND FERTILIZER LEVEL ON GROWTH AND YIELD OF KALI MUSLI *IN VITRO* CULTIVATED

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Abstract. Kali musli (*Curculigo orchioides* Gaertn.) is a precious medicinal plant of Asian countries with potential anticancer, antioxidant, aphrodisiac, immunostimulant, hepatoprotective, and antidiabetic activities. Various compounds, such as phenolics, glycosides, and saponins extracted from rhizomes have been reported. According to the Red Data Book of the IUCN, kali musli is a vulnerable plant because of its limited dispersal range of seeds and propagules. Planting density and fertilization are two crucial factors that affect crop growth and yield. Therefore, this study aimed to evaluate the growth and yield of *in vitro* kali musli plants at different densities and fertilizer levels. The field experiment was arranged in a split-plot design with three replications. The main factor was planting density at the three levels of 100, 50, and 33.3 plants m^{-2} with planting spaces 10 x 10, 20 x 10, and 30 x 10 cm, respectively. The subfactor consisted of three fertilizer levels: 15 tons ha^{-1} cow manure (control), 12 and 9 tons ha^{-1} cow manure combined with 25 kg N and 10 kg K_2O ha^{-1}. The results showed that the planting densities, fertilizer levels, and interaction between the two factors significantly affected the yield of kali musli. The highest rhizome productivity (29.05 tons ha^{-1}) was found when a planting density of 50 plants m^{-2} (20 x 10 cm) was combined with a fertilizer rate of 12 tons cow manure + 25 kg P_2O_5 + 10 kg K_2O ha^{-1}. In addition, the total flavonoid and curculigoside contents were also higher than those of the other combinations.

Keywords: *Curculigo orchioides*, planting density, fertilization, *in vitro* kali musli plants

QUANTIFICATION OF ALLANTOIN IN YAMS (*DIOSCOREA* SP.) USING A ^{1}H NMR SPECTROSCOPIC METHOD

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Abstract. Allantoin is an abundant component of yams and has been known as a skin protectant due to its pharmacological activities. In previous methods for allantoin determination via high- performance liquid chromatography (HPLC), the separation was unsatisfactory. We herein developed a ^{1}H quantitative nuclear magnetic resonance (qNMR) method for the quantification of allantoin in the flesh and peel of yams. The method was effectively performed through the relative ratio of signals integration of allantoin to a certain amount of the internal standard dimethyl sulfone (DMSO2) and validated regarding specificity, linearity (range 62.5–2000 µg/mL), sensitivity (limit of detection (LOD) and quantification (LOQ) 4.63 and 14.03 µg/mL, respectively), precision (RSD% 0.02–0.26), and recovery (86.35–92.11%). The method was then applied for the evaluation of allantoin in flesh and peel extracts of four different yams cultivated in Korea.

Keywords: Allantoin, yam, *Dioscorea*, NMR, qNMR, quantitation

EVALUATION OF GROWTH, YIELD OF RHIZOMES AND PHARMACEUTICAL QUALITY OF WILD TURMERIC (CURCUMA AROMATICA SALISB.) ACCESSIONS IN CAM PHA, QUANG NINH

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Abstract. *Curcuma aromatica* Salisb., a wild turmeric species of the genus Curcuma, is well-known for its antibacterial, antifungal and cosmetic properties. This study evaluated the growth, yield of rhizomes and pharmaceutical quality of six wild turmeric accessions, collected in six distribution including Yen Bai, Cao Bang, Quang Ninh, Quang Binh and Phu Yen, then planted in Quang Ninh. After arranging the experiment in a sufficiently randomized block (RCBD with 3) and harvesting the samples after 1 and 2 years planting for all accessions, the results show that highest individual plant yield of rhizomes and hectare yield of rhizomes per year were obtained from accession NT2 (collected in Trung Khanh district, Cao Bang province). First year, individual plant yield was 2.23kg/plant and hectare yield of rhizomes was 21.7 tons fresh /ha; the essential oil content and the starch content (dry weight basis) reached 0.62% and 44.3% respectively. Second year, individual plant yield was 3.12 kg/plant and hectare yield of rhizomes was 29.6 tons fresh /ha; the essential oil content and the starch content reached 0.69% and 45.4% respectively. Therefore, the NT2 accession is suitable for development as cosmetic and pharmaceutical raw materials.

Keywords: *Curcuma aromatica* Salisb., growth, pharmaceutical quality, yield of rhizomes

CHARACTERIZATION OF FOOD FUNCTIONALITIES OF A LAND CULTIVATED RED ALGA DULSE (*DEVALERAEA INKYULEEI*, FORMERLY *PALMARIA PALMATA* IN JAPAN)

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Abstract. Red alga dulse (*Devaleraea inkyuleei*, formerly *Palmaria palmata* in Japan) is a protein-rich eatable seaweed, with a protein content of approximately 40% of dry weight. The main proteins are phycobiliproteins with photosynthetic accessory pigments (chromophores). Dulse grows during the cold winter season of Hokkaido, which is located in the north of Japan, meaning that it cannot be harvested all year round. Therefore, a land cultivation system using cold deep-sea water was employed for year-round cultivation. The purpose of this study was to examine the functional ingredients (protein and carbohydrate) from dulse. The protein was extracted with water from dulse powder, consisting of mainly phycoerythrin (PE). The protein showed slight ACE inhibitory activity, whereas the inhibitory activity was extremely enhanced by thermolysin hydrolysis. Several ACE inhibitory peptides (VYRT, LDY, LRY, FEQDWAS) were isolated from the hydrolysates by reversed-phase HPLC. Among them, synthetic peptide LRY (IC50: 0.044 mol) showed a high ACE inhibitory activity. The PE has chromophores and showed strong radical scavenging activity. Recombinant PE β-subunit (rPEβ: apoprotein) and chromophores from PE were prepared to clarify the key constituent of antioxidant activity. The rPEβ showed lower radical scavenging activity than that of PE. The chromophores showed higher radical scavenging activity (90.4% ± 0.1%) than that of PE (17.9% ± 0.1%) on ABTS assay. The main component of cell wall of dulse is xylan. Xylooligosaccharide (XOS) was prepared by using commercial enzymes, showing that a unique trisaccharide (β-xylopyranosyl-(1→3)-β-(1→4)-xylobiose: DX3). The prebiotic effect of DX3 on enteric bacterium was evaluated. DX3 was utilized by *Bacteroides* sp. and *Bifidobacterium adolescentis*. *Bacteroides* sp. grew slowly as compared with β-(1→4)-xylotriose (X3), while *Bif. adolescentis* grew similar to X3. From these results, we showed that the main ingredients of dulse have health benefits.

Keywords: red alga, phycobiliproteins, land cultivation, xylan

STABILIZED CELLULASES – BIOCATALYSTS FOR CELLULOSE INDUSTRY

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Abstract. The cellulose industry, especially the bioethanol sector, needs stable and eco-friendly cellulases, with high enzymatic activity. Enzyme immobilization is an efficient method to enhance the stability and reusability of biocatalysts, obtaining versatile biosystems with improved operational parameters. The characteristics of the immobilized enzymes are settled by the porous structure and physico-chemical properties of the matrices. In our work 2 microbial *Trichoderma viride* and *Aspergillus niger* cellulases were used and different immobilization methods – physical adsorption on inorganic supports, entrapment in hybrid organic-inorganic matrices and mixed methods. The way in which the immobilization method and the type of support influence the catalytic efficiency were studied. The biocatalytic activity of the immobilized enzymes was modified very little by the supports network. The microbial cellulases showed an improved operational and in time stability for applications that require different reaction conditions. After one hour of keeping at 37ºC, pH 3.0, the relative activity of the immobilized CMCase was more than 98% of initial. The immobilized cellulases were reused successfully in 5 cycle of hydrolysis reaction. The cellulase entrapped in the organic-inorganic supports retains more than 13% of its activity after hydrolysis cycles of reuses.

Keywords: Cellulase, immobilization, organic-inorganic porous matrices, stable biocatalysts.

DEVELOPMENT OF A PROPAGATION PROTOCOL FOR EFFICIENT LARGE-SCALE PRODUCTION OF MD-2 PINEAPPLE (*ANANAS COMOSUS*)

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Abstract: Over the past three decades, the MD-2 pineapple cultivar has emerged as a principal cultivar in global pineapple production, owing to its exceptional resistance, nutritional richness, and tasty flavour. Recently, the demand for high-quality MD-2 plantlets has surged in Vietnam due to an increasing requirement for new plantations. However, conventional propagation methods for the MD-2 have been unable to satisfy the demand both quantitatively and qualitatively. To address this pressing need, this research was undertaken to establish an optimal protocol for the large-scale production of MD-2 pineapple plantlets. A series of sequential in-vitro and ex-vitro experiments were executed with the primary goal of refining culture medium compositions. The outcomes of this study unveiled that during the shoot multiplication phase, the employment of MS medium supplemented with 1 mg/l of Benzyladenine (BA) and 0.4 mg/l of Indole-3-butyric acid (IBA) resulted in the most prolific shoot formation (12.61 shoots per plantlet) and leaflet production (21.55 leaflets per plantlet). Furthermore, partly removal of apex meristems significantly enhanced shoot formation rates. In the subsequent root induction stage, half-strength MS medium supplemented with 1 mg/l of IBA exhibited superior efficacy in promoting root development, yielding an average of 10.60 roots per plantlet after four weeks of cultivation. During the acclimatization phase, optimal survival rates and growth of MD-2 pineapple plantlets were achieved through acclimation in a growth medium composed of 1/3 sand and 2/3 coconut coir, coupled with weekly application of a basic foliar nutrient composition. The entire protocol proved to be highly cost-effective and locally accessible, thus rendering it a feasible and pragmatic solution.

Keywords: Pineapple, micropropagation, MD-2 cultivar, In-vitro, shoot formation.

A STUDY ON THE MATERIALIZATION OF AGE-FRIENDLY HIGH VALUE-ADDED FOOD USING HEMP SEED OIL-PROCESSED BY-PRODUCTS

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Abstract. Hemp (*Cannabis sativa* L.) is a source of nutritious seeds that have been used as human food for thousands of years. The seeds contain nonmedicinal levels of the psychoactive compound called δ-9-tetrahydrocannabinol (THC) and, therefore, are different from medicinal marijuana. Currently, hemp seed is processed mainly by mechanical pressing to extract the valuable oil, while the residue is used to produce various protein-rich food products. Defatted hemp (*Cannabis sativa L.*) seed was extracted with 70% methanol solution. Then, fractionation was performed between hexane, ethyl acetate, butanol and water layers. As a result of comparing the antioxidant activity of the cannabis seed solvent fraction, it was confirmed that the antioxidant activity of the ethyl acetate fraction was the highest. Therefore, the ethyl acetate layer was subdivided into size exclusion chromatography and separated into semi-prep LC. After that, the polyphenol compound was separated using the semi-prep LC, and 1H-NMR was used to explain the structure of the components separated from the defatted hemp (*Cannabis sativa L.*) seed.

Keywords: Hemp seed, antioxidant activity, ethyl acetate layer, ^{1}H-NMR

DISCOVERING THE VALUE OF DOMESTIC SEAWEED RESOURCES AS FERMENTED FOOD RESOURCES AND VERIFYING THEIR POTENTIAL AS PREBIOTICS

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Abstract.

Marine species represent about one half of the global biodiversity, containing different and representative species. Among marine compounds, marine carbohydrates are considered important organic components of marine sediments. Carbohydrates extracted from marine algae, which have attracted the attention of several research groups because of their wide range of important biological activities with applications in the food, pharmaceutical and cosmetic industries.

Among them, *Codium fragile*(CF), which belongs to green algae, is found on the coasts of East Asia, Oceania, and Northern Europe, and in Korea, has been used in traditional medicine to treat enterobiasis, dropsy, and dysuria. Previous studies have shown that CF extracts have thrombolytic, anti-coagulant, anti-platelet, anti-inflammatory, and anti-cancer effects. Among them, anti-cancer showed activity in polysaccharide extracted from CF.

CF has also been added to kimchi in Korea or used as an ingredient in cold soup. It is known that the addition of CF improves the fishy smell of salted fish and slows down the kimchi's softening rate, thus preserving the fresh taste for a long time. However, no studies have been conducted on the effects of CF on kimchi.

In this study, polysaccharides and metabolites were extracted from CF to determine its potential as a substrate for the fermentation of lactobacillus, to confirm its inhibitory activity against harmful microorganisms, and to verify its potential as a prebiotic, and to produce high value-added products from CF.

For CF extraction, CF dried products were extracted with ethanol, and residues were extracted with distilled water. Distilled water extracts were deproteinized with 95% ethanol and sevag solvent, and deproteinized extracts were separated according to their molecular weight through dialysis.

Keywords: Codium fragile, prebiotics, polysaccharide, deproteinize

ANALYSIS AND CLONING OF THE LIGHT CHAIN FRAGMENT OF THE BOTULINUM NEUROTOXIN SEROTYPE E FROM A VIETNAMESE BOTULISM OUTBREAK

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Abstract: Botulinum neurotoxins (BoNTs) serotypes A, B, E, F cause the deadly botulism disease. Initial nucleic acid amplification test (NAAT) has pointed out the presence of BoNT/E in a recent outbreak in Vietnam in 2023 with a nearly 30% case fatality rate. However, the detailed sequence of the encoding gene has not yet been investigated. Thus, we conducted this study to amplify the entire light chain (LC) encoding region and clone the resulting fragment for further use as a positive control in diagnostic procedures. We first designed a pair of primers for BoNT/E-LC by aligning and comparing 20 published sequences. The primer pair optimized by Primer3Plus was checked for specificity, self-pairing ability, and cross-pairing using in silico PCR, OligoAnalyzer (IDT), Multiple Primer Analyzer (Thermo Scientific). The results showed that the target amplicon had a size of 1353 bp, including the ATG start codon required for future BoNT/E expression studies. The amplified fragment was subsequently cloned into the pLUG vector, sequenced, and compared with other strains using NCBI Blast.

Keywords: Clostridium botulinum, botulinum neurotoxin, cloning, sequence, serotype E.

OPTIMIZATION FOR SHOOT MULTIPLICATION AND ROOTING OF YELLOW APRICOT TREE (OCHNA INTEGERRIMA (LOUR.) MERR)

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Abstract. The Yellow Apricot tree (*Ochna integerrima* (Lour.) Merr.) is not only a popular ornamental plant but also possesses numerous valuable herbal properties. One of its prominent cultivars, the HD-01, stands out with its captivating characteristics. These include a high petal count exhibiting a vibrant yellow hue, big flower size (the diameter up to 4-6 cm), and an extended blooming period. This exceptional cultivar was selected by adept florists at the Binh Loi Apricot Village, an area situated in the suburban environs of Ho Chi Minh City. The primary objective of this research was to determine optimal phytohormone concentrations suitable for regenerating the HD-01 yellow apricot. The research findings indicated that the most favorable condition for regenerating shoots from yellow apricot callus consisting MS medium supplemented with 7 g/L agar and 30 g/L sugar, in combination with 1 mg BA/L and 0.25 mg NAA/L. Under these conditions, the height of shoot clusters reached 1.56 cm, with an average of 2.96 shoots per cluster and 3.17 leaves per shoot formed after 50-day of culture. In the subsequent phase, it was determined that a composition of a half-strength MS medium supplemented with 30 g/L sugar, 7 g/L agar, and 0.3 g/L activated carbon, along with the addition of 1 mg IBA/L, proved to be optimal for root induction. The highest number of roots achieved was 1.97 roots per plant. The plant height attained 1.82 cm, and an average of 4.0 leaves were observed per plantlet after 50 days of culture. Based on this determination, a premilitary protocol for HD-01 yellow apricot micropropagation was established.

Keywords: Ochna integerrima, Yellow apricot tree, micro-propagation, plant growth regulator, ornamental plants

ISOLATION AND STRUCTURAL ANALYSIS OF IMMUNOACTIVE POLYSACCHARIDES ISOLATED FROM *PROTAETIA BREVITARSIS SEULENSIS*

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Abstract. *Protaetia brevitarsis seulensis* is a larva of the chafer family and was approved as a food ingredient by the Ministry of Food and Drug Safety in 2014. Recently, interest and support for insect food are increasing, and various activation effects on *Protaetia brevitarsis seulensis* have been reported. It is a polysaccharide obtained from the hot water extract of *Protaetia brevitarsis seulensis* and has an immune-activating effect. However, the structure of the polysaccharide has not been analyzed. Therefore, in this study, in order to confirm that the polysaccharides of *Protaetia brevitarsis seulensis* have uniform information, a structural analysis was performed by selecting index sugars. Polysaccharides were obtained from hot-water extracts of *Protaetia brevitarsis seulensis* through deproteinization and dialysis. Afterwards, structural analysis was conducted through polysaccharide extraction yield experiments, sugar content analysis of polysaccharides, FT-IR, Monosaccharide analysis and methylation analysis. The main sugar of polysaccharides is glucose, and it includes various monosaccharides composed of rhamnose, xylose, mannose, and galactose. These results suggest that polysaccharides from *Protaetia brevitarsis seulensis* can be continuously obtained through selection of indicator sugars, and that polysaccharides from *Protaetia brevitarsis seulensis* can be usefully used as bioactive materials.

Keywords : Protaetia brevitarsis seulensis, Polysaccharide, Monosaccharide analysis, Methylation analysis

EFFECT OF LIGHTING CONDITIONS ON MORPHOLOGICAL DEVELOPMENT OF *IN VITRO* EMBRYO-DERIVED MERISTEM CULTURE OF ELITE XIEM XANH DWARF COCONUT (*COCOS NUCIFERA* L.) VARIETY

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Abstract. The current state of global coconut (*Cocos nucifera* L.) production is precarious and demands urgent concerted efforts to conserve the genetic materials of the crop. However, traditional coconut propagation methods are costly and time-consuming to save the ever-increasingly threatened coconut population worldwide. Alternative propagation methods through tissue culture are currently the frontier solution to solve these challenges. In this study, a novel protocol for direct meristem proliferation using Y3 medium supplemented with 1 µM TDZ was tested for longitudinally halved Xiem Xanh coconut apical meristem explants under two different lighting conditions i.e, 16 : 8 hr photoperiod light condition and completely dark condition. After 4 months of inoculation, four distinct morphology types were observed, which were classified into the following categories: cauliflower, regenerative, single-shoot and stunt morphology. Among which, the cauliflower morphology and regenerative morphology were observed to contain the bud precursors for developing into multiple shoots. Specifically, the cauliflower morphology was detected in 41.2 ± 15.2% of light-incubated cultures and in 26.1 ± 7.2% of dark-incubated cultures, while the regenerative morphology appeared only in light-incubated cultures (17.4 ± 7.3%). A withdrawal of TDZ was observed to result in multiple shoot regeneration 11 months post-inoculation. This study underscores the potentiality of this propagation method while calling for concerted efforts in further application onto more coconut varieties that require long-term conservation.

Keywords: coconut, conservation, shoot tip, apical meristem, Thidiazuron, proliferation

EFFECTS OF ORGANIC COMPOSITIONS ON THE FORMATION OF MICRO–RHIZOME NGOC LINH GINSENG (*PANAX VIETNAMENSIS* HA ET GRUSHV.) *IN VITRO*

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Abstract. Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.) belongs to the ginseng family (Araliaceae), is endemic to Vietnam known for 24 saponins dammarane with new structures not found in other ginseng species in the world. In this study, Ngoc Linh ginseng somatic embryos were cultured on SH mineral medium supplemented with 50 g/L sucrose, 8 g/L agar, 2 mg/L BA, 1 mg/L NAA, and individual organic compounds (casein hydrolysate, peptone, and yeast extract) with different concentrations (0; 0.5; 1.0; 1.5 and 2.0 g/L) to evaluate the effect of organic compounds source on the formation of micro-rhizome Ngoc Linh ginseng cultured in vitro. Research results after 8 weeks of culture showed that the highest number of micro-rhizome formed was 1.80 micro-rhizomes/sample in the culture medium supplemented with 50 g/L sucrose, 8 g/L agar, 2 mg/L BA, 1 mg/L NAA, and 1.0 g/L casein hydrolysate. This contributes to improving the efficiency of forming micro-rhizomes from embryos, an important stage to increase the rate of complete seedling formation per culture unit, and its applicability in ginseng biomass production under plant factory conditions.

Keywords: casein hydrolysate, micro-rhizomes, Ngoc Linh ginseng, peptone, yeast extract

OPTIMIZATION THE HYDROLYSIS PROCESS PARAMETERS WITH THE COMBINATION OF CELLULASE AND PECTINASE FOR INCREASING THE RECOVERY EFFICIENCY OF EXTRACT AND RETINOL ACTIVITY OF CAROTENOIDS FROM PUMPKIN FIBROUS STRANDS BY-PRODUCT (*CUCURBITA MOSCHATA*) BY RESPONSE SURFACE METHODOLOGY

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Abstract: Pumpkin (*Cucurbita moschata)* is a popular vegetable grown in many countries around the world, including Vietnam because of its easy-to-grow, high-yield characteristics, and contains many nutritional components. According to current processing methods, the fibrous strands from pumpkin fruits are almost unused. This study introduces a new approach to mining carotene pigments from plant tissues. It was shown that the assistance of combination of enzymes (pectinase and cellulase) could greatly enhance the recovery efficiency of the extract and carotenoids from the recovery process. This study focused on the optimization the enzymes hydrolysis by using the combination of cellulase and pectinase to improve the recovery efficiency of dry matter and bioactive compounds from the pumpkin fibrous strands. The cellulase/pectinase (C/P) volume ratios were investigated of 0/0, 0/1, 1/0, 1/1, 1/2, 2/1 at 0.1% concetration for the total amount of two the enzymes. Experimental design by response surface method (RSM) with central composite circumscribed (CCC) design was used to optimize the parameters of the hydrolysis process including: temperature (40-50 ^{o}C), enzymes concentration (0.1-0.2%) and time (60-120 minutes). Our finding showed that, with the C/P ratio of 1/2, there was a higher recovery efficiency in the extracts and carotenoids from the fibrous strands of pumpkin fruits compared with the other ratios and the control. The optimal hydrolysis parameters were found at the temperature of 44.65 ^{o}C, the enzyme concentration of total cellulase and pectinase of 0.14% and the time of 92.95 minutes. At this optimal condition, the filtration rate (66.83 ml/minute); the highest recovery efficiency of the extract (78.8%), lutein (54.92%), lycopen (58.57%); total retinol activity equipment (60.69 µg RAE/100g), these increased 25.82 ml/minute; 17.45%, 40.93%; 30.97%; 31.27 µg RAE/100g respectively compared with the control. This research result opens up the potential to effectively use of the pumpkin fruit by-product.

Keywords: pumpkin fibrous strands, hydrolysis, retinol activity, RSM.

EFFECT OF CLONED BOVINE EMBRYOS STORAGE METHOD DURING TRANSPORTATION ON THEIR PREIMPLANTATION DEVELOPMENT AND PREGNANCY RATE

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Abstract. Cloned bovine production has many practical applications, although the success rate remains low. Improving laboratory techniques have been investigated to overcome this problem. Embryo transportation has been considered that can affect embryo survival and quality. Although the storage materials, namely plastic straws and microtubes, were studied in many species, there are few reports in cloned bovine embryos, and no paper compares the efficiency of different transportation systems. Here we investigated the effects of transportation storage methods on preimplantation development and pregnancy rate of cloned bovine embryos. Briefly, each cloned bovine morula was stored in a plastic straw or a microtube without any hole or with two holes on its lid for two or four hours in the mini-incubator and then continued to culture in 5% CO_2 incubator until blastocyst formation. The results showed that cloned bovine embryos in microtubes with two holes maintained a higher rate of hatching blastocysts and average cell numbers after two- and four-hour incubation in the transportable mini-incubator. Furthermore, the pregnancy rate was higher for cloned bovine embryos stored in microtubes with two holes than the plastic straws' after embryo transfer. In conclusion, transporting cloned bovine embryos in microtubes with two holes could enhance the preimplantation development and pregnancy rate after embryo transfer.

Keywords: cloned bovine embryo, transportation, preimplantation development, pregnancy rate, plastic straw, microtube

YEAST METABOLIC ENGINEERING FOR CARBON DIOXIDE FIXATION

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Abstract. Industrial biotechnology based on yeast fermentation is a promising strategy to alleviate global warming and climate change. However, *Saccharomyces cerevisiae*, widely used in bioprocesses, emits a large amount of carbon dioxide (CO_2) during fermentation. In this study, we developed a mixotrophic CO_2-fixing *S. cerevisiae* to achieve carbon neutrality and sustainability in the bioprocess. By heterologous expression of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) and phosphoribulokinase (PRK), a CO_2-fixation pathway was constructed successfully in a xylose-utilizing *S. cerevisiae*. To improve the efficiency of CO_2-fixation, a delta-integration strategy was undertaken, and the RuBisCO gene copy number was increased to 10 copies. Additional Cas9-based genome editing was performed to overexpress other CO_2-fixation related genes. The resulting CO_2-fixing yeast, SJ03, displayed the highest RuBisCO activity. During anaerobic xylose fermentation, ethanol concentration was increased by 17% and ethanol yield was increased by 16% compared to control strain. In addition, CO_2 emission was decreased by 7%. These results suggest that overexpression of the CO_2-fixation pathway coupled with xylose utilization in *S. cerevisiae* might reduce CO_2 emission in bioprocesses.

Keywords: Xylose fermentation, bioethanol, Cas9, RuBisCO

IN VITRO PROPAGATION OF ROSE (*ROSA HYBRIDA*) CV. MON COUER

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Abstract. Rose is one of the most widely cultivated and commercial flowers in the world. In Vietnam, Rose '*Mon couer*' , with unique flower color and shape is one of the high value varieties preferred by the growers and markets. In conventional propagation, cutting and and grafting method have been rarely apply for the *Mon couer* cultivar due to their low budding and rootingability. In this study, to increase the multiplication rate of this cultivar, different environmental factors were investigated.. The results showed that treating surface nodes with Javen (50%, v/v)for 15 minutes got 93% survival and sterilized samples. Shooting medium for the auxillary buds was MS medium supplemented with 30 $g.L^{-1}$ sugar, 8 $g.L^{-1}$ agar, 3 $mg.L^{-1}$ BA, 0.2 $g.L^{-1}$ NAA. MS medium with the addition of 30 $g.L^{-1}$ glucose, 8 $g.L^{-1}$ agar, 1 $mg.L^{-1}$ BA, 0,1 $g.L^{-1}$ NAA was found that suitable for in vitro multiplicaiton, medium for the shoots was found on achieving an average of 10.51 shoots per sample at a height of 3.01 cm. The seperated shoots were suitaly rooted under MS medium supplemented with 1 $mg.L^{-1}$ NAA, resulting an average height of 4.71 cm and root length of 2.27 cm. Finally, the in vitro *Mon Coeur* planlets were transferred to ex vitro condition on 100% straw-decomposing substrate with 68.7% survial rate.

Keywords: *Mon Couer* rose, mediun, BA, NAA, *in vitro*, .

ANTIOXIDANT ACTIVITIES OF AMARANTH (*AMARANTHUS*) GRAIN FRACTIONS AND THE SEPARATION AND IDENTIFICATION OF ANTIOXIDANT COMPONENTS

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Abstract. Amaranth (*Amaranthus*) grain, widely cultivated and consumed in India, Nepal, and Africa, are one of the important similar grain because they have a lower carbohydrate content than other grain and are rich in proteins, especially essential amino acids. Amaranth (Amaranthus) grain contains phenolic acids such as vanillic acid, hydroxybenzoic acid, and gallic acid, and flavonoids such as rutin, which have antioxidant, anti-inflammatory, anti-cancer, gastro protective, and anti-diabetes. Amaranth (Amaranthus) grain was extracted with 100% methanol solution and winterization was performed to remove lipid components. Then, fractionation was performed between hexane, ethyl acetate, butanol and water layers. Among them, the ethyl acetate layer with the best antioxidant activity was subdivided into size exclusion chromatography. After that, the polyphenol compound was separated using the semi-prep LC, and ^{1}H-NMR was used to explain the structure of the components separated from the Amaranth grain.

Keywords: Amaranth, antioxidant activity, ethyl acetate layer, ^{1}H-NMR

BIOACTIVE COMPOUNDS ISOLATED FROM *CARTHAMUS TINCTORIUS*

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Abstract. *Carthamus tinctorius* (Safflower) is a dye plant belonging to Asteraceae, which is native in Egypt and widely distributed in China, India, and North America*. C. tinctorius* mainly is grown in warm environments and produces various bioactive secondary metabolites such as quinochalcones, polyphenols, alkaloids, and flavonoids. In addition, secondary metabolites from *C. tinctorius* are known to be useful for anti-obesitic, antidiabetic, antioxidant, anti-inflammatory treatment and neuroprotective. The purpose of this study was to isolate and analyze major bioactive compounds in *C. tinctorius. C. tinctorius* ethanol extract was fractionated with *n*-hexane, ethyl acetate, *n*-butanol, and water layer. The ethyl acetate and *n*-butanol extracts were investigated by prep-LC and analyzed by HPLC, ESI-MS, and ^{1}H NMR. The bioactivity effects of isolated compounds were studied. These results suggest that compounds from *C. tinctorius* can be usefully used as bioactive materials.

Keywords : Carthamus tinctorius, Safflower, Secondary metabolites, ^{1}H NMR, ESI-MS

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STUDY ON INSECT-BASED COMPOST AND ITS EFFECTS ON SOIL MESOFAUNA AND CROP PERFORMANCE

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Abstract. Bioconversion using insects recently attracts more and more attention and interest because of its great potential in fast treating agricultural wastes and producing more valuable materials. One of the cyclical applications of this process is to create environmentally friendly organic fertilizers for crops with black soldier fly larvae BSFL excrement and chitinous shell addition. BSFL fertilizer or zoocompost not only adds nutrients to plant growth but also contributes to the regulation of soil health through supporting a beneficial mesofauna system. This study aims at tailoring the impact of a BSFL compost and its applicable doses via mesofauna assessment in addition to discussing the zoocompost potential for sustainable agriculture in a pilot experiment on corn and spinach. The results showed that adding compost to the soil significantly changed the quantitative and qualitative composition of the nematode fauna at the optimal dose of 0.025 g/cm^2 without any phytotoxic impacts in another increased dose. For more details, plant parasites and plant disease associated nematodes were reduced by 45-50% while favoring beneficial species including bacteriophages, mycophages, and polyphages. Although within the short period trial scope, the BSFL compost treatment has shown its supportive influence on a photosynthetic chlorophyll b and overall plant growth, along with solving the problem of agricultural organic wastes in the long term. Hopefully, this study contributes to highlighting the importance of finding an effective and sustainable alternative towards agricultural waste management and circular economy where insect-based compost is very potential.

Keywords: Black soldier fly larvae, bioconversion, corn, spinach, nematode, organic fertilizer

RAPID *IN VITRO* MULTIPLICATION OF *POLYGONUM CUSPIDATUM* SIEB. *ET* ZUCC. BY USING TEMPORARY IMMERSION SYSTEM

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Abstract. *Polygonum cuspidatum* Sieb. *et* Zucc., belonging to the genus *Polygonum*, family Polygonaceae, is commonly distributed in mountainous regions and plains of China and Japan and is often cultivated for its medicinal roots. The roots of *P. cuspidatum* contain a significant amount of resveratrol, which has great potential in terms of antioxidant activity, cardiovascular protection, anti-inflammatory properties, anticancer effects, cholesterol reduction, and blood sugar regulation. During micropropagation on a commercial scale, temporary immersion systems facilitate partial automation and result in reduced production costs. The purpose of this study is to optimize the conditions for rapid *in vitro P. cuspidatum* on the TIS for high multiplication rate and good quality of plant. At 60 days of culture, the number of shoots per explant, shoot length and multiplication rate were evaluated. The resuls showed that the highest multiplication rate, number shoots per explant and shoot length were obtained in TIS with an immersion frequency of 15 minutes every 5 h and with a volume of culture medium of 300 mL per explants, which were 8.42 times, 47.60 shoots per explant and 6.65 cm, respectively. The bud's morphology is well-developed, strong, and the leaves show no signs of leaf twisting. The results of this study help to optimize the rapid *in vitro* proliferation process of *P. cuspidatum* and improve the quality control of this medicinal plant. In summary, the assessed immersion system offers a viable alternative for the commercial micropropagation of *P. cuspidatum.*

Keywords: Immersion frequency, *in vitro* multiplication, *Polygonum cuspidatum*, TIS (temporary immersion system), volume of culture.

THE INFLUENCES OF SUBSTRATE AND PLANTING METHODS ON THE DEVELOPMENT OF *AGARICUS SUBRUFESCENS*

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Abstract. *Agaricus subrufescens* is known as one of the most delicious, edible and highly medicinal mushroom species. It is effective in cancer prevention by up to 99% and has a treatment effectiveness of 90%. Despite its medicinal and economic value, the *Agaricus subrufescens* mushroom is not widely cultivated in our country. Research on the cultivation of *Agaricus subrufescens* focuses on investigating different sources of nutrients, types of soils, various substrate, and cultivation methods. The results show that the suitable nutrient medium for cultivating *Agaricus subrufescens* includes: 1% $CaSO_4$ + 1% $CaCO_3$ + 5‰ urea + 1% superphosphate + 1% lime, along with one of the following components: 10% rice bran, 15% cow dung, or 15% chicken manure. When combining the substrate of peat and one of the three nutrient formulas mentioned above, corresponding fructification time is: 23.2 days, 22.4 days, and 22.9 days respectively. Regarding the investigation of different substrate, six different mixing formulas were tested: 100% sawdust, 100% straw, 100% sugar bagasse, 50% sawdust + 50% straw, 50% sawdust + 50% sugar bagasse, and 50% straw + 50% sugar bagasse. The experimental results showed that the substrate consisting of 50% sugar bagasse + 50% straw, 100% sugar bagasse, and 100% straw had the highest yield. For the investigation of cultivation methods, the bag cultivation method and the removal of nylon bags and cultivation in trays were tested. The results showed that using the substrate of 50% straw + 50% sugar bagasse and the cultivation method of removing nylon bags and cultivating in trays with dimensions of 765 x 482 x 200 mm resulted in a yield of up to 38.62%, equivalent to 348.6g/6 spawn bags.

Keywords: Edible mushrooms, medicinal mushrooms, Agaricus subrufescens, cultivation substrate, planting methods.

FUNCTIONAL CHARACTERISTICS OF WILD-SIMULATED GINSENG LEAF PROCESSED BY SOLID-STATE FERMENTATION AND ENZYMATIC HYDROLYSIS

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Abstract. Wild-simulated ginseng (WSG), which belongs to *panax* species*,* is cultivated in the mountain without any artificial measures. This cultivation condition causes lower growth rate of WSG compared to normal ginseng, so the production and output of WSG is less than 10% of normal ginseng. To overcome incompetent production of WSG, using by-product of WSG including leaves can be desirable. WSG leaves involve diverse phytochemicals including ginsenosides which are helpful to suppress oxidative stress, inflammation, lipid accumulation, and senescence. In this study, WSG leaves were processed by solid-state fermentation by *R. oligosporus* and enzymatic biotransformation to enhance biological acitivities. Solid-state fermentation of WSG leaves newly produced L-carnitine which promotes lipid metabolism, and enhanced the amounts of rare ginsenosides in WSG leaves. Especially, rare ginsenoside compound K was newly produced and enhanced up to 32.3 ± 1.3 mg g^{-1} during this process. With developed biological properties, we could identify functional characteristics of WSG leaves on anti-inflammation in RAW264.7 cell, anti-lipid accumulation in 3T3-L1 cell, and anti-aging in HDF cells. These findings demonstrate that by-products of WSG processed by solid-state fermentation and enzymatic biotransformation could be utilized in nutraceutical and pharmaceutical fields as functional materials.

Keywords: Wild-simulated ginseng leaf, ginsenosides, L-carnitine, Senescence, Lipid accumulation, Inflammation

REPORT ON EMERGING FOLIAR ROT DISEASE ON GINSENG *PANAX VIETNAMENSIS* AND IDENTIFICATION OF THE CAUSAL FUNGAL PATHOGENS.

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Abstract. *Panax vietnamensis* is a valuable medicinal herbal resource in Vietnam. Having a long cultivation time, ginseng plants cultivated either in natural mountainous forests or under farm conditions are confronting infectious diseases that, in many cases, seriously threaten the harvest. In 2022, a disease outbreak in ginseng farms in the South-West mountain areas in Vietnam threatened more than 50,000 ginseng plants, leading to yield reduction and serious economic losses. In connection with this, a survey was performed in ginseng cultivating areas of two important indigenous ginseng varieties *P. vietnamensis* var. *vietnamensis* (Ngoc Linh ginseng) and *P. vietnamensis* var. *fuscidiscus* (Lai Chau ginseng). The two most significant diseases were noticed, the leaf spot disease (incidence of 2%-15%), and the foliar rot disease (incidence of 5%-18%). The foliar rot disease is reported here for the first time, having symptoms as brownish dry or water-soaked rot lesions on the leaves, more often at leaf tips, that can extend towards the leaf base. Two fungal strains isolated from the diseased leaves showed infection with symptoms similar to the original disease samples, and afterward, they could be recovered from the infections, thus confirming Koch's postulates. Taxonomic identification of the fungal isolates was achieved based on their morphological traits and comparative analyses of sequences of internal transcribed spacer (ITS) and elongation translation factor 1-alpha (TEF1-α). Two species *Fusarium boothii* and *Fusarium noneumartii* were pointed out as pathogens causing the foliar rot disease. The identification of the disease and its causal pathogens are of prime importance for the study of disease control, especially by antagonistic microorganisms.

Key Words: Foliar rot disease; *Fusarium boothii*, *Fusarium noneumartii*; Ginseng *Panax vietnamensis*.

ASSESSMENT OF GENETIC DIVERSITY IN VIETNAMESE *CYMBIDIUM* SPECIES BASED ON MORPHOLOGICAL AND MOLECULAR MARKERS

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Abstract. *Cymbidium* spp. are the most ornamental and cultivated orchids in Asia. In Vietnam, recently, due to the increasing demand of these orchids for horticulutre, many of these native plants have been overexploited with no concern on conservation or sustainable using. In this study, to clarify genetic diversity in Vietnamese *Cymbidium* species, 52 accessions were collected from different regions. Genetic relationships between and among these species were evaluated by using morphological and molecular markers. Based on 27 morphological characters, varieties in plant shape, leaf, flower size, flower color, etc. of the 37 of 52 orchids were recorded. For molecular marker, 11/32 species-specific SSR markers were seclected and tested, and a high level of genetic diversity with a polymorphic information content (PIC) value of 0.44 – 1.00, were discovered among these orchids. The DNA barcoding results for all assessions showed that 1 nulear region (ITS2) and 2 chloroplast regions (*rbc*L, and *mat*K) of were screened and sequenced. The DNA sequence and genetic distance analysis showed that low variety among species and clear differences between species were recorded in the Vietnamese *Cymbidium*. These results indicate that evaluation and conservation of Cymbidium genetic resources through morphological and molecular marker give valuable information for about utilization of the Cymbidium orchids, such as propagation and breeding.

Keywords: Cymbidium, DNA barcode, SSR, Morphological indicator.

IDENTIFICATION OF GENETIC ELEMENTS INVOLVED IN THE SYNTHESIS OF XOO-ANTAGONIZING COMPOUND PRODUCED BY THE RICE ENDOPHYTE *PANTOEA ANANATIS*

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Abstract. Leaf blight is one of the most destructive diseases of cultivated rice (*Oryza sativa* L.) caused by the bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae* (Xoo) leading to 30-50% yield loss. The endophytic bacterium *Pantoea ananatis* VY148 was isolated from the sterilized-surface stem of a healthy rice plant collected in Xoo heavily infected field in Vietnam. The strain showed a notable antagonistic ability against Xoo and thus presented a subject of interest for the study of compound biosynthesis. To study genes that participated in the Xoo-antagonistic compound biosynthesis, a library comprising 11.000 mutants of the VY148 strain was created by using transposon mutagenesis, and was used for screening the loss of their Xoo-antagonistic property. Out of the mutant library, a single clone without Xoo-antagonism was discovered, and its whole genome was sequenced and analyzed along with the genomic sequence of the wild-type strain to locate the transposon insertion site. It is revealed that the transposon disruption did occur within a cluster consisting of seven genes that very conserved among *Pantoea ananatis*. Further, the requirement of an *EanI/R* quorum sensing (QS) system for anti-Xoo compound formation in strain VY148 was also determined.

Keywords: rice, bacterial leaf blight, Xanthomonas oryzae pv. *oryzae, bacterial endophytes, Pantoea ananatis*

INVESTIGATE THE IMPACT OF β-CYCLODEXTRIN ON CHARACTERISTICS OF BITTER GOURD EXTRACT DURING THERMAL PROCESSING

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Abstract. Bitter gourd is cultivated as a vegetable crop in tropical and subtropical nations. The extract of this vegetable is high in antioxidants and anti-diabetic substances that support human health. However, the bitter taste and thermolability of bitter gourd make it difficult to use in food preparation. Although some studies showed that β-cyclodextrin (β-CD) may mask bitterness and preserve phytochemicals, it is still unclear how this agent will affect the properties of bitter gourd extract (BGE) during the thermal processing that most foods must go through before consumption in order to aid digestion and metabolism. The goal of this study is to evaluate the effects of β-CD on the properties of BGE under various heating conditions. The present study evaluated the bitterness, total saponin, polyphenol, antioxidant capacity, free amino acid, and browning intensity of BGE and BGE supplemented with β-CD (0.75%) after 20 minutes of heating at 60, 90, and 121 °C. It was discovered that β-CD reduced the impact of heat treatment on the BGE, specifically on saponins and color. Total polyphenol, amino acid, and saponin content all showed positive correlations according to principal component analysis, while these variables had negative correlations with browning degree. Additionally, it was shown that after the treatment period, the debittering ability of β-CD was still present. The molecular docking stimulation were also used to infer the bitter-masking mechanisms of β-CD. The results revealed that the thermal stability and sensory properties of BGE were enhanced by the addition of β-CD, providing the base for the use of BGE in heat-processed foods and beverages.

Keywords: Bitter gourd extract, β-cyclodextrin, Thermal processing, Saponin, Bitterness, Browning reaction

PLANT BIOMASS PRODUCTION RESEARCH AT THE UNIVERSITY OF SCIENCE (VNU-HCM): UPDATES AND LESSONS

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Abstract. Industrialization and population growth drive innovations in biomass production and plant engineering technologies. This data-driven statement is specifically difficult to execute under tropical climate and local technology challenges. We hereby illustrate our updates and practical lessons in plant biomass and bioengineering research at the University of Science (VNU-HCM). In an open cultivation system, we developed bioactivators that promote plant nutrient uptake, reduce chemical fertilizer, and improve yield without damaging soil health. Under a controlled environment, we developed a vertical biomass production system for recombinant protein production using lettuce (Lactuca sativa). The lettuces were transformed to express the SARS-CoV-2 receptor binding domain (RBD) protein. The vacuum pump transient transformation system has a capacity of 50 liters and can infiltrate 832 plants per hour. The vertical platform can produce at least 256 infiltrated lettuce plants per 1.25 m^2, more than four times higher than traditional farming. Both indoor and outdoor controlled environment systems for biomass production are the subject of study in our program. In vitro cell-line products such as *Nervilia plicata* biomass was successfully developed for commercial scale, yielding 10 kg/m^2 tubers within 3-4 months. Commercializing biomass-related research products is a crucial challenge requiring enormous investment and strategic planning.

Keywords: molecular farming, plant factory with artificial light (PFAL), sustainable agriculture, edible vaccine, renewable energy

SCREENING FOR 12.1KB DELETION WITHIN HMGA2 GENE SEQUENCE IN THE NETHERLAND DWARF RABBIT (*ORYCTOLAGUS CUNICULUS*)

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Abstract. In Vietnam, rabbit is traditionally bred for human consumption. However, it has been recently showed that several rabbit breeds are also kept as pets for human leisure. The Netherland dwarf rabbit is currently in an immense interest of many Vietnamese customers due to its personality and miniature stature. However, 12.1 kb deletion from position 44,709,089 bp to 44,721,236 bp in high mobility AT-hook 2 (HMGA2) gene on chromosome 4 was identified as the structural variant causing dwarfism and altered craniofacial development in this breed. It has been documented that HMGA2 plays an important role in regulating growth and individuals with genotype HMGA2 *del/del* are fatal several days after birth. Despite the economically high value of the Netherlands dwarf rabbit, there has been not any study on the genetic survey of lethal alleles in this breed in Vietnam. The aim of this study is to develop a fast and reliable method to screen the frequency of lethal allele of HMGA2 in the South of Vietnam. Rabbit saliva were collected, and DNA extraction were followed. Multiplex PCR with three primers were optimized and performed to detect the presence of 12.1 kb deletion within the HMGA2 sequence. Our data showed that the 12.1kb deletion in Netherlands dwarf rabbit population was detected by our optimized multiplex PCR. In 40 rabbit animals, the frequency of HMGA2 lethal allele carrier was 75% (30/40 individuals). Our results indicated that we successfully developed a fast, accurate multiplex PCR to detect carrier individuals. We recommend implementing our PCR procedure in genetic selection for carrier (*+/del*) and homozygous wildtype (+/+) animals in mating scheme to prevent the lethality of the rabbit offspring. Additionally, awareness should be raised to rabbit breeders to monitor the genetic makeup of the Netherland dwarf rabbit populations. It is recommended that more samples should be taken to obtain the genetic frequency of the HGMA2 lethal allele more accurately.

Keywords: Dwarfism, high mobility AT-hook 2, multiplex PCR, recessive lethal gene

USING CELLULOSE MEMBRANE LOADED WITH VIETNAMESE CORIANDER (*PERSICARIA ODORATA*) EXTRACT IN FOOD PRESERVATION

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Abstract. Vietnamese coriander (*Persicaria odorata*), an herb commonly used in Vietnamese cuisine, has been reported about antimicrobial capacity to foodborne pathogenic microorganisms. In food preservation, single-use plastics containing toxic substances have been polluting the environment and affecting living health. Microbial cellulose can be an alternative material in food packaging thanks to its biodegradability. This study aims to combine *P. odorata* extract and cellulose membranes from *Acetobacter xylinum* for food preservation and reducing single use plastic packages. We examined some natural products in *P. odorata* extract and their antibacterial capacity against 5 pathogenic microorganisms by agar diffusion assay. Phenolics, tannins, saponins, flavonoids are found in the fresh and dried *P. odorata* extracted by ethanol absolute and water. The dried *P. odorata* extract in ethanol absolute at the concentration of 500 mg/mL showed the highest antibacterial activity against *Escherichia coli* (with the diameter of inhibition halo of 7.47±0.12 mm), *Pseudomonas aeruginosa* (11.77±0.41 mm), *Klebsiella pneumoniae* (11.7±0.35 mm), *Salmonella typhimurium* (14.73±0.15 mm), and *Staphylococcus aureus* (16.5±0.26 mm). The minimum inhibitory concentrations (MIC) of *P. odorata* extracts against *E. coli*, *P. aeruginosa, K. pneumoniae*, *S. typhimurium*, *S. aureus* was at 50, 25, 25, 12.5, and 6.25 mg/mL, respectively. Bacterial cellulose membrane added the *P. odorata* extract could preserve beef up to 24 hours in organoleptic standards and 6 hours in aerobic microbial standard at room temperature according to Vietnam Technical Standards for fresh meat (TCVN 7046:2009).

Keywords: antimicrobial, bacterial cellulose, food preservation, herbal medicine, Vietnamese coriander *Persicaria odorata*, *Acetobacter xylinum*

OPTIMIZATION OF PROCESSING PARAMETERS FOR EXTRACTION OF TRITERPENOID SAPONIN FROM *CODONOPSIS JAVANICA* ROOTS USING ENZYMATIC EXTRACTION METHOD

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Abstract. Triterpenoid saponins from *Codonopsis javanica* (Blume) Hook f. & Thomson are an important ingredient in Vietnamese traditional medicine with many potent biological activities such as an anti-cancer, anti-inflammatory, anti-microbial, and anti-arthritic. The aim of this study focused on optimizing the process of total triterpenoid saponin extraction from *C. javanica* roots using the commercial enzyme extraction method and ethanol solvent. The experimental conditions were evaluated as the properties of the concentration of enzyme and incubation time, incubation temperature and pH, the root powder to solvent ratio, the extraction temperature and duration. The optimal conditions for Viscozyme L activity were at enzyme concentration (0.6%), temperature (50^{o}C), time (4 hr) and pH=5.0. The results showed that under the optimal conditions, including pretreatment of *C. javanica* root powder by Viscozyme L, root powder to 70% ethanol solvent ratio (1:7 w/v), extraction temperature of 70^{o}C for 2 hr, the maximum total triterpenoid saponin content was 1020.61 (mg/100g DW). The free radical scavenging activity of the triterpenoid saponin extracts from *C. javanica* roots were determined. The IC_{50} of triterpenoid saponin extracts without and with Viscozyme L treatment were 1066.69 and 1216.38 (µg/mL), respectively. Thus, a modified commercial enzyme process can enhance the extraction of triterpenoid saponin from *C. javanica* roots and reduce the time in the extraction process.

Keywords: *Codonopsis javanica*, extraction, enzyme, saponin, triterpenoid

EFFECT OF NATIVE *PENICILLIUM MENONORUM* STRAINS ISOLATED FROM RHIZOSPHERE SOIL IN VIET NAM ON THE GROWTH OF TOMATO PLANTS (*SOLANUM LYCOPERSICUM* L.)

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Abstract. As published by recent studies, some species of *Penicillium* promote plant growth by one of several different mechanisms, such as the production of PGPR phytohormones (gibberellins, auxins, cytokines, and siderophores), and mineral dissolution, substances and antagonize plant pathogens [1 - 3]. In this study, *Penicillium menonorum* LA02 - 2 was isolated from rhizosphere soil in Long An province and reported for the first time in Vietnam. The results of morphological characteristics and internal transcribed spacer gene sequence showed that the LA02 - 2 sample was *P. menonorum* with high similarity (99.96%) compared to the strain *P. menonorum* on Gen bank. Besides, the sample *P. menonorum* LA02 - 2 can secrete indole acetic acid (IAA) (13.7 mg/L), phosphate solubility reaches 421.9 mg of $Ca_3(PO4)_2$/L, 26.5 mg of $AlFO_4$/L, nitrogen fixation, siderophore production. Inoculating native *P. menonorum* LA02 - 2 strain into tomato plants in the greenhouse promotes plant growth in tomato plants such as leading to greater accumulation of biomass, increased height, and longer root length, with the isolated significantly ($p < 0.05$) increasing the dry biomass of tomato roots (26%) and shoots (35%). The results study showed the potential of *P. menonorum* to promote tomato plant growth and degrade insoluble phosphate.

Keywords: *PGPR (plant growth promoting rhizosphere), Penicillium menonorum, Tomato, Phosphate solubilization*

BLOOD BIOCHEMICAL PARAMETERS OF PHAN RANG SHEEP WHEN USING SILAGE FEEDS

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Abstract. The study aimed at evaluating the blood biochemical parameters of Phan Rang sheep when using some types of silage. The study was conducted on 24 sheep 6 months old, imported from Ninh Thuan province and raised at the Center for Livestock Biotechnology. The silage used includes corn stalks, wheat residue and fake cashews. Blood samples were taken by venipuncture from each animal at the same time before feeding in the morning on days 1, 45 and 90 of the experimental process. Blood biochemical indicators: Glucose, Total Protein, Albumin, Globulin, BUN, Creatinine, AST, ALT, ALP, electrolytes (Na, K, Ca, P) was measured with an Abaxis Vetscan 2 chemical analyzer. Biochemical parameters of three treatments and the control group on days 90 were respectively: Glucose (63.25 ± 11.32; 64.17 ± 10.25; 64.32 ± 10.51; 62.83 ± 9.82mmol/L), Protein (8.35 ± 1.33; 8.42 ± 1.21; 8.38 ± 1.27; 8.47 ± 1.18 g/L); Albumin (2.61 ± 0.72; 2.58 ± 0.53; 2.64 ± 0.66; 2.55 ± 0.47 gL) BUN (4.33 ± 1.18; 4.27 ± 0.88; 3.98 ± 1.23; 4.18 ± 0.72 mmol/L); Creatinine (1.44 ± 0.24; 1.47 ± 0.19; 1.51 ± 0.28; 1.48 ± 0.21 µmol/L); AST (133.72 ± 17.86; 127.52 ± 20.06; 137.28 ± 19.62; 135.25 ± 18.61 U/L); ALT (30.19 ± 4.35; 29.83 ± 4.8; 31.27 ± 5.14; 31.09 ± 5.03 U/L). The results evaluated that the blood biochemical parameters of sheep were normal when using silage diets. There was no statistically significant difference between the treatments and the control group. Our results validated that the use of additional silage in sheep production did not affect the blood biochemical indicators of Phan Rang sheep. These data could be useful for research, helpul to reduce livestock costs and limit environmental pollution from agricultural waste products.

Keywords: blood, biochemical parameters, Phan Rang sheep, silage.

ETHNOBOTANICAL AND CRITICAL APPRAISAL STUDIES IN *TITHONIA SPECIES*.

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Abstract. *Tithonia diversifolia* is a tropical woody herb or succulent shrub cultivated in many countries. It is an annual or perennial medicinal plant that has a subject of research due to its benefit in the treatment of different ailments. The plant material was extracted in different solvent like Ethanol, Methanol, Chloroform, Water and Diethyl ether to allow extraction of crude active compounds. This was used for Nutritional, Phytochemical, Antioxidant, Antibacterial, Antifungal, and Antidiabetic studies. Nutritional (Qualitative) analysis showed the presence of carbohydrate, Lipids, Proteins and Aminoacids, Methanol and Chloroform extract showed best activity. Phytochemicals such as Tannis, Flavonids, Saponins and Alkaloids are present. The phytochemical component for Tannin 85.91% , Saponins 42.85%, Phenols 39.86 % and Flavonoids 74.95%. Antibacterial activity was checked for Gram negative bacteria (*Escherichia coli*), Gram positive bacteria (*Staphylococcus aureus*) in *Escherichia coli* maximum zone of inhibition was observed at concentration 200µl and its percent inhibition was 1cm, in *Staphylococcus aureus* maximum zone inhibition was observed at concentration 80µl and its precent inhibition 0.8cm. Antifungal activity was checked for *Candida albicans* and *Aspergillus niger*. In *Candida albicans* maximum zone of inhibition was observed at concentration 160µl and its percent inhibition was 0.7cm. Rapid antioxidant screening was done by TLC and later confirmed by DPPH and FRAP assay. In DPPH assay IC_{50}Value is 66.80 and in FRAP assay IC_{50} value is 66.66. In vitro antidiabetic activity by α-Amylase inhibition assay and α-Glucosidase inhibition assay was performed at concentration 20µg/ml maximum α-Amylase inhibition was seen to be 85.71% and at concentration 40µg/ml maximum α-Glucosidase inhibition was seen to be 15.89%. Metformin is used as standard. All experiment was done in triplicate and its mean value is mention. *Tithonia diversifolia* can be used as an Antidiabetic and Antifungal source in pharma industry. There is much more scope for research in this medicinal plant material which in some part considered as alien invasive plant.

Keywords: Tithonia diversifolia, Antifungal, Antibacterial, Antioxidant, Antidiabetic.

INVESTIGATION OF ANTIBIOTIC-RESISTANT BACTERIA ISOLATED FROM WASTEWATER

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The pathogenic bacteria present in wastewater are the source of humans infection, and the aquatic environment is a favorable condition to help spread resistance genes in the microbial population. This research aimed to investigate the occurrence and distribution of antibiotic-resistant bacteria wastewater, identify and determine the antibiotic susceptibility of the isolates. The study was performed on wastewater samples in the South provinces of Vietnam. Isolates were obtained on selective media such as MacConkey agar, Cetrimide agar and Mannitol salt agar then evaluated antibiotic susceptibility by Kirby-Bauer disk diffusion test. The results indicated that microbiological contamination in wastewater was 33.3%, which the most predominant came from untreated sources (38.9%). The isolated bacteria was identified and distributed mainly in the genera: *Burkholderia* (23.5%), *Enterobacter* (21.6%) and *Pseudomonas* (27.5%). Others were *Staphylococci, Escherichia, Shigella* and *Vibrio*. Common pathogenic bacteria such as *E.coli, Shigella, Pseudomonas aeruginosa* were presented in industrial, pharmaceutical, food and municipal wastewater samples, mainly untreated samples. All tested antibiotics were resisted (resistance rates ranged from 10 to 67%): tetracycline had the highest rate (66.7%), followed by sulfamethoxazole/trimethoprim (33.3%) and ciprofloxacin (28.2%), the remaining antibiotics have resistance rates below 20%. Detected 13 isolates that are resistant to more than 1 antibiotic, accounting for 25.5%. Wastewater from hospitals, pharmaceutical and livestock were completely (100%) resistance to eight of the tested antibiotics was observed. Thus, the treatment of microbiological contamination in wastewater is an issue that needs to be taken care of and implemented to minimize the possibility of causing disease to humans and animals. Further studies are needed on the molecular biology of antibiotic resistance genes and how they are transmitted in the wastewater environment, especially from medical and livestock sources.

Keywords: *pathogenic bacteria, wastewater, antibiotic resistance*

EFFECT OF ASCORBIC ACID AND CULTURE TIMING ON IN VITRO GROWTH OF PORCINE OOCYTES DERIVED FROM PRE-ANTRAL FOLLICLES

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Abstract. The prevalent approach for fertility preservation in female cancer patients involves the biopsy of small ovarian cortexes containing pre-antral follicles for cryopreservation and then subject it to *in vitro* growth (IVG). However, the establishment of IVG process for oocytes derived from pre-antral follicles still poses numerous challenges. To address these problems, the aims of this study were to estimate the optimal timing of in vitro growth culture and evaluate the effects of ascorbic acid (AsA) supplementation in the IVG of porcine oocytes collected from pre-antral follicles (0.4–0.7 mm). The oocyte-granulosa cell complexes (OGCs) were cultured in IVG at three different time marks (7, 10, and 14 days) and at three different concentrations of AsA (0 µM, 50 µM, and 100 µM). In each treatment, morphology, survival rate of oocytes, diameter, antrum formation status, and chromatin configuration were examined. The results revealed that the oocytes significantly increased in diameter (approximately 105 µm) and developed chromatin configuration into germinal vesicle stage (as fully-grown oocyte) after 10 and 14 IVG culture days. However, the quality of oocytes slighly decreased on day 14. Furthermore, AsA at a concentration of 50 µM significantly increased the quality of oocytes with a higher survival rate (71.15%) and antral formation (32.18%) following the IVG process. On the other hand, AsA had no significant effect on oocyte size and chromatin configuration. In conclusion, in vitro growth with AsA at a concentration of 50 µM for 10 and 14 days were shown to be the optimal conditions for culturing the OGCs derived from pre-antral follicles.

Keywords: In vitro growth (IVG), porcine oocyte, pre-antral follicles, Ascorbic Acid, culture timing.

POTENTIAL OF GLUCURONIC ACID IN SUPPORTING THROMBOSIS DISSOLUTION BY NATTOKINASE ENZYME

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Abstract. Enhancing the efficacy of thrombus dissolution is a crucial concern in the management of thrombotic conditions. Natto, a fermented food that contains nattokinase enzyme, has been proven to be effective in dissolving thrombosis. During the fermentation process, *Bacillus subtilis* produces glucuronic acid, which contributes to the thrombus-dissolving capability of the nattokinase enzyme. In this study, the effect of glucuronic acid on the thrombus-dissolving ability of nattokinase enzyme was evaluated. To achieve this, 600 µg of glucuronic acid was introduced to 0.1 mL of extracellular nattokinase enzyme solution under physiologically relevant conditions, and the activity of artificial thrombin-fibrinogen dissolution was assessed. The highest activity recorded was 972.48 FU/mL, which indicates a 48.8% increase from the initial activity of 653.12 FU/mL. This finding highlights the potential of using glucuronic acid to enhance the activity of nattokinase enzyme and promote thrombus dissolution.

Keywords: Bacillus subtilis, glucuronic acid, thrombosis, thrombotic disorders, nattokinase enzyme.

ENGINEERING THE SANDWICH DOMAIN 1 OF A THERMOSTABLE PROTEASE FOR IMPROVED SOLUBILITY AND SOLUBLE EXPRESSION IN *E. COLI*

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Abstract. Affinity-based interactions is the most actively exploited field for recombinant protein products. The production of recombinant proteins in *E. coli* is greatly desired among the various hosts used because of its ease of handling, quick growth, inexpensive media, high target protein output, and ease of genetic manipulation. Furthermore, small scaffold proteins are easily expressed in *E. coli,* and these proteins are designed to supplement the limitations of antibodies, because of their greatly decreased size, higher mechanical and thermal stability, cell penetration potency, and long-term storage capabilities. The β-sandwich domain 1 (SD1) of a thermostable protease islandisin provides a suitable framework. It is a small protein (11.7 kDa) with a stable hydrophobic core composed of many hydrophobic residues. It is also structurally comparable to the variable domain of IgG, with surface loops that could be generated for specific target binding. However, given the rapid rate of SD1 synthesis and accumulation in *E. coli* cells, accumulation into inclusion bodies poses a challenge. We investigated how different modifications to SD1 amino acids affect its soluble expression, refolding yield, solubility, and stability. We used FoldX's ΔΔG calculation to identify mutational hotspots and Tango to identify aggregation-prone regions. We show that charged residues on the surface of SD1, specifically gate keeper residues (R, K, D, E, P) in aggregation-prone sites, enhanced protein solubility and reduced inclusion body accumulation in *E. coli*. The alterations had no influence on the protein's thermostability (Tm = 73). The heat denaturation in all mutants, including the wildtype, was irreversible. Our findings reveal that SD1 is stable and could be capable of binding to specific targets with minimal loss of thermal stability, making it useful in fields that require thermostable proteins.

Keywords: SD1, *E. coli*, soluble expression, solubility, stability.

DMSO-FREE, SERUM-FREE, CHEMICALLY DEFINED CRYOPRESERVATION MEDIA FOR MAMMALIAN CELLS

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Abstract. Many studies have shown that cells are likely to experience genetic or phenotypic variations over generations. And cryopreservation at -80 to -196℃ is believed to be the only way to keep cells unchanged in their properties. However, cryopreservation also can have a negative effect on cells since they are exposed to extreme environments (high osmotic pressure, physical and chemical stress, etc.). To minimize these negative effects and protect cells during cryopreservation process, CPAs (cryoprotective agents) are generally used in the cryopreservation media. Currently, DMSO (dimethyl sulfoxide) is the most commonly used cryoprotective agent. However, the use of DMSO is somewhat controversial as studies have shown that it affects cell viability and gene expression particularly in stem cells.

In this study, chemically-defined cryopreservation media for CHO cells and HEK293 cells, as model cell systems are developed without the use of DMSO and animal derived components such as serum. Cryoprotective candidates that can possibly prevent cell damage during cell freezing process are screened and evaluated systematically. And potential substances were classified into mainly effective cell-penetrating cryopreservatives, polymers, and antioxidants. Cell-penetrating agents and polymers can prevent intracellular or extracellular ice formation and minimize physical damage to cells by regulating osmotic pressure build-up across the cellular membrane. Recently, since it has been suggested that the chemical damage by ROS generation should be suppressed for effective cryopreservation, the additional supplementation of antioxidants in the freezing media has been also considered. We used Plackett-Burman design (PBD), and general full factorial design (GFFD) to develop and optimize serum-free, chemically defined formulations comparable to DMSO containing cryopreservation media. We have identified cell-penetrating substances that have the key roles to replace DMSO and other effective cryoprotective substances, and then optimized the formulation. When compared with commercially available DMSO-free cryopreservation media, the currently developed in-house DMSO-free cryopreservation media showed competitive performance in cryopreservation of CHO and HEK293 cells.

Keywords:: DMSO-free, serum-free, cryopreservation, mammalian cells

DEVELOPMENT OF A COVID-19 VACCINE CANDIDATE BASED ON THE SPYTAG/SPYCATCHER SYSTEM

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Abstract. Coronavirus disease 2019 (COVID-19) has become a worldwide public health emergency and effective and affordable vaccines are needed. Virus-like particle (VLP) vaccines are safe due to free viral genetic material, but still show effective immune responses. Recently, VLPs are used as novel vaccine platforms by presenting antigens on them[1)]. Especially, norovirus-like particles (noro-VLPs) show exceptional stability and have 3 loops that can be used for antigen presentation[2)]. However, there are several challenges for presenting antigen by genetic fusion, such as difficult VLP assembly or post-translational modification of the antigen. Therefore, producing the VLP and antigenic pathogen fragment separately and fusing by using modular system is attracting attention. SpyTag/SpyCatcher provides a covalent peptide-tagging system, which leads to simple modular decoration *in vitro*. The aim of this study is to develop a COVID-19 vaccine candidate by decorating noro-VLP with antigen of SARS-CoV-2, which are expressed in CHO cells, by using SpyTag/SpyCatcher system.

Keywords: virus-like particles (VLPs), norovirus-like particles, modular vaccine, CHO cells, SARS-CoV-2, SpyTag/SpyCatcher system

INVESTIGATION OF ALPHA-GLUCOSIDASE AND ALPHA-AMYLASE INHIBTION OF EXTRACTS ISOLATED FROM FLORA *HIBISCUS ROSA-SINENSIS* L.

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Abstract. *Hibiscus rosa-sinensis* L. is widely distributed in Vietnam. Flora *H.rosa-sinensis* is used to control blood sugar, treat dyslipidemia, treat inflammation of the lining of the stomach-intestines, bloody stools, dysentery, insomnia, pimple, itching, swelling. In addition, *H. rosa-sinensis* is also used to soothe dysmenorrhea, stimulate placental discharge after birth, treat cough, sore throat, treat gonorrhea in other countries. Glucosidase and amylase activity inhibition were investigated by method of Pistia et al. (2001) and Kim et al. (2020) on two extraction solvents, aqueous and 70% ethanol. The results of alpha - glucosidase inhibitory activity showed that IC_{50} of aqueous and ethanolic extract respectively were 1,4 ± 0,01 mg/ml and 0,93 ± 0,03 mg/ml compared with acarbose IC_{50} = 0,23 mg/ml. Aqueous and ethanolic extract exhibited inhibitory activity against alpha-amylase with IC_{50} value 1,40 ± 0,08 mg/ml and 0,95 ± 0,06 mg/ml was moderatly compared with acarbose 0,16 ± 0,02 mg/ml. These results revealed that extract was isolated with ethanol, the inhibitory activity of alpha-glucosidase and alpha-amylase enzymes was higher. Conclusion, flora *H. rosa-sinensis* is a potential medicinal source, which is significant in supporting blood sugar control. This study opens up a potential application of flora *H.rosa-sinensis* in the preparation of pharmaceuticals medicine to support diabetes treatment. The future prospects of developing antidiabetic drugs for preventing the hyperglycemia through using ethanolic extract from flora H.rosa-sinensis are demonstrated.

Keywords: Hibiscus rosa-sinensis, alpha – glucosidase, alpha – amylase, diabetes, flora

STUDY ON GRANULOCYTE COLONY-STIMULATING FACTOR RECEPTOR (G-CSFR) AFFECT HEPATOCYTES PROLIFERATIVE AND INFLAMMATORY SIGNALING EXPRESSION BY G-CSF TREATED BILE DUCT-LIGATED MICE.

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Abstract.

Objective: The study aimed to evaluate the effect of G-CSF on proliferative and inflammatory signaling, based on mRNA expression level of Interleukin-1β (IL-1β), Interleukin-33 (IL-33), G-CSF receptor (*csf3r*), Hepatocyte Nuclear Factor 4α (*hnf4α*) and Hepatocyte Nuclear Factor 6 (*onecut1*) on hepatocytes in bile duct ligation (BDL) mice.

Methods: BDL mouse model was induced in Swiss mice. On day 7 post-surgery, a 5-day G-CSF treatment course (61.5 µg/kg/day) was administrated, qRT-PCR method was performed to evaluate the gene expression level.

Results: Normal mice injected with G-CSF show a significant decrease in the mRNA expression level of IL-1β, IL-33, csf3r, and hnf4α genes compared with the control group at 2 days post-operation ($p<0.05$). Despite the downregulation of the onecut1 gene in the normal-treated model group, the difference was not significant ($p>0.05$).In the BDL model group, gene expression of IL-1β elevated in the BDL placebo group and significantly reduced in BDL + G-CSF ($p<0.05$), while there was no significant difference between the two groups for IL-33 and csf3r expression ($p>0.05$). In contrast, the hnf4α gene increased its expression in BDL treated model group, compared with the control group on the 26th day after surgery ($p<0.05$). We recorded the uptrend of onecut1 gene expression in both BDL groups compared with the control ($p>0.05$).

Conclusion: G-CSF reduces the mRNA expression of the Interleukin 1 subfamily, csf3r, hnf4α on normal-mice model hepatocytes. In the evaluation of BDL-treated G-CSF hepatocytes, the result shows that the mRNA expression of IL-1β was downregulated, while hnf4α rose, and csf3r remained consistent throughout the experiment.

Keywords: Cholestasis, Granulocyte Colony-Stimulating Factor, Hepatocyte, Hepatitis

OPTIMIZATION ASPERGILLUS NIGER SOLID-STATE FERMENTATION ON HYLOCEREUS UNDATUS PEEL TO ENHANCE BIOLOGICAL ACTIVITY

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Abstract. Solid-state fermentation (SSF) *by Aspergillus niger (A.niger*) was experimentally optimized on Hylocereus undatus Peel to reinforce the potential compounds as well as biological activity. It could be able to stimulate the expression of particular enzymes and metabolic pathways by studying media free of glucose. Additionally, the ideal duration time of 120 hours for the fermentation process was screened which had promoted released bioactive compounds. Material size, pH, temperature, and inoculum concentration were found to be possible options for optimization. Using Box-Behnken Design, the ideal condition was assessed and improved as follows: *A. niger* for CJ1 was incubated for 120 hours at 32 °C, 50% moisture content, 107.1 CFU/ml of inoculum concentration, pH of 3.1, and a material size of 3.3 cm. Total phenolic compounds (TPCs), Total Flavonoid compounds (TFCs), and antioxidant properties were dramatically elevated by 2.7, 4.52, and 8.45 times, respectively, under the optimal SSF conditions. The biochemical mechanism for TPCs enhancement via SSF was evaluated by using ferrous salt as inhibitor by evaluating TPCs correlated with enzyme released and the amount of biomass. The comparison of fermented and unfermented products using disk diffusion, spot assay, growth curve, minimum inhibitory concentration (MIC), and minimum bactericidal concentrations (MBC) revealed antibacterial characteristics on Escherichia coli and Staphylococcus aureus. The outcome demonstrated that the fermentation zone of the material was twice as large as the unfermented one. For fermented materials, the Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations (MBC) were 31.25 mg/mL and 46.87 mg/mL, respectively, while for unfermented materials, the values were 62.5 mg/mL and 93.75 mg/mL. Scanning electron microscope (SEM) identified the growth of *A.niger* on the rigid surface of Hylocereus undatus Peel with the presence of pectin, amylase and cellulase. LC-MS analysis illustrated the metabolism of *A.niger* to produce citric acid, oxalic acid, tannin, catechin, epicatechin, gallic acid, and gluconic acid. The potential in diabete treatment was initially justified by using Alpha-Glucosidase assay that the concentration of fermented substances was at 0.0488 µg/mL and the figure for unfermented one was at 1.5625 µg/mL.

Keywords: Optimization, Solid-state fermentation, Hylocereus undatus Peel, Aspergillus niger

CLEAVAGE-RESPONSIVE BIOFACTORY T CELLS SUPPRESS INFECTIOUS DISEASES-ASSOCIATED HYPERCYTOKINEMIA

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Abstract. Severe infectious diseases, such as coronavirus disease 2019 (COVID-19), can induce hypercytokinemia and multiple organ failure. In spite of the growing demand for peptide therapeutics against infectious diseases, current small molecule-based strategies still require frequent administration due to limited half-life and enzymatic digestion in blood. To overcome this challenge, a strategy to continuously express multi-level therapeutic peptide drugs on the surface of immune cells, is established. Here, chimeric T cells stably expressing therapeutic peptides are presented for treatment of severe infectious diseases. Using lentiviral system, T cells are engineered to express multi-level therapeutic peptides with matrix metallopeptidases- (MMP-) and tumor necrosis factor alpha converting enzyme- (TACE-) responsive cleavage sites on the surface. The enzymatic cleavage releases γ-carboxyglutamic acid of protein C (PC-Gla) domain and thrombin receptor agonist peptide (TRAP), which activate endothelial protein C receptor (EPCR) and protease-activated receptor-1 (PAR-1), respectively. These chimeric T cells prevent vascular damage in tissue-engineered blood vessel and suppress hypercytokinemia and lung tissue damages in vivo, demonstrating promise for use of engineered T cells against sepsis and other infectious-related diseases.

Keywords: COVID-19, engineered blood vessel, engineered T cell, hypercytokinemia, infectious disease

TREATING CISPLATIN-INDUCED ACUTE RENAL INJURY IN MICE WITH MESENCHYMAL STEM CELL-DERIVED EXOSOMES

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Abstract. Acute kidney injury (AKI) is a sudden loss of kidney function within hours or days. Recently, some regenerative therapies, including kidney transplantation, dialysis, and stem cell transplantation, have been used to treat AKI; however, these methods have disadvantages, such as high cost, rejection, tumorigenesis, and low survival rate. Therefore, this study aimed to evaluate the therapeutic effects of exosome infusion on AKI in mouse models. The AKI model was created by injecting cisplatin (10 mg/kg) into the mouse peritoneum. After four days, 10^8 exosomes from mesenchymal stem cells (MSCs) were injected into the tail vein. The efficiency of exosome therapy was evaluated through changes in blood and urine creatine and blood urea nitrogen (BUN) levels, tissue structure, and immunological changes. The sham group that received 0.9% sodium chloride and normal mice were used as controls. The results showed that cisplatin (10 mg/kg) successfully created an AKI mouse model. Exosome infusion is safe in mice at a dose of 10^8 exosomes per mouse. Exosome infusion can help recover creatinine and urea nitrogen levels and reduce the symptoms of AKI. Histological examination showed that exosome infusion significantly reduced tubular fibrosis and interstitial infiltration compared to those in sham mice. These findings showed that exosome infusion can be a promising therapy for AKI. However, future studies with more mice and long-term evaluations are required to obtain more information about the safety and efficacy of exosome therapy for AKI.

Keywords: Acute kidney injury (AKI), cisplatin, exosome, mesenchymal stem cells (MSCs)

METFORMIN REDUCES THE IMMUNE ESCAPE CAPACITY OF MOUSE BREAST CANCER CELLS IN HIGH GLUCOSE MEDIUM

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Abstract. Metformin has recently been identified as a type of medicine used to treat type 2 diabetes. Moreover, various proven mechanisms make it a potential drug candidate for cancer research. Numerous investigations have shown that high concentrations of metformin, particularly those of 5 mM or more, can have an anticancer effect via a variety of direct or indirect mechanisms. However, by using 2 mM metformin, mesenchymal stem cells, which are helpful for body regulation and regeneration, can undergo apoptosis. Interestingly, it has been demonstrated that mesenchymal stem cells are protected from metformin-induced apoptosis when used with high glucose levels of 4.5 g/L and 2 mM metformin. Hence, this study aimed to examine whether the combination of various metformin and glucose concentrations would affect the proliferation, migration, and gene expression of immune escape-related genes in 4T1 mouse breast cancer cells. The highlighted results revealed that 2 mM metformin with a high glucose concentration of 4.5 g/L inhibited the proliferation, migration, and gene expression of immune escape-related genes in 4T1 mouse breast cancer cells. Based on these findings, we suggest that optimizing glucose control is crucial for protecting favorable cell populations, such as mesenchymal stem cells, in cancer patients receiving metformin treatment.

Keywords: *immune escape, high glucose, metformin, breast cancer, metabolism*

INITIAL EVALUATION OF IMMUNE EVASION – RELATED GENE EXPRESSION OF 4T1 BREAST CANCER CELLS THAT ARE SELECTIVELY GRAFTED ON *BALB/C* MICE

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Abstract. Immune evasion is a strategy used by tumors to evade the host's immune response to adapt and continue growing in the body. We hypothesized that the cancer cells that display immune evasion are characterized by the upregulation of some particular genes related to immune evasion. Therefore, this study aimed to evaluate and compare immune evasion-related genes with and without an immune response. This study used the 4T1 breast cancer cell line. These cells were expanded *in vitro* to obtain enough cells for further experiments according to the manufacturer's instructions. The 4T1 cells were divided into 2 groups: without immune selection (*in vitro*) and with immune selection in which cells were injected into mice (*in vivo*). Under immune selection for 14 days, the live 4T1 cells grew and created tumors in mice. These tumors in mice were collected to evaluate the expression of the *ADAM17, CCL22, CD95L* and *RAET1* genes and compared to their expression *in vitro*. The results showed that the expression of *ADAM17, CCL22, CD95L* and *RAET1* was significantly higher in tumor-derived cells than in *in vitro* cells. These findings showed that the immune evasion of 4T1 cells was related to the upregulation of *ADAM17, CCL22, CD95L* and *RAET1*. Thus, *ADAM17, CCL22, CD95L* and *RAET1* play important roles in immune evasion. Therefore, they can be targets for advanced breast cancer therapy that inhibits immune evasion.

Keywords: Immune evasion, 4T1 cells, immune system, tumors, gene expression.

INDUCING CELLULAR SENESCENCE ON HUMAN FIBROBLASTS USING HYDROGEN PEROXIDE AS AN OXIDATIVE STRESS FACTOR

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Abstract. Various approaches have been used to study cellular senescence, such as exposure cells to ultraviolet-B light (UVB), Copper (II) sulfate ($CuSO_4$), *tert*-butyl hydroperoxide (t-BHP), and hydrogen peroxide (H_2O_2). Prolonged exposure to low doses of hydrogen peroxide (H_2O_2) causes DNA damage and decreases tissue repair capacity. H_2O_2 has been used to generate senescent models in human dermal fibroblasts (BJ, CRL-2522™) and human diploid fibroblasts (2BS), however, the effects of H_2O_2 on human fibroblasts (hFs) remain unclear. To understand the underlying mechanism of oxidative stress on human fibroblasts, we designed experiments to induce senescence by H_2O_2 treatment and observe involved biological pathways and discuss with published articles. The results showed that under H_2O_2 exposure condition of 400 µM with inoculate duration for 4 hours, human fibroblasts exhibit changes in morphology, slow down proliferate potency, increase level of the enzyme SA-β-Galactosidase (SA-β-Gal) and upregulate of cell-cycle related genes *p21, p53*. These results are similar to other publications that reviewed the hallmarks of cellular senescence. In conclusion, acute exposure to H_2O_2 at 400 µM within 4 hours can induce senescence in human fibroblast. Hence, this result could apply to induce cellular senescence in human fibroblast models for further experiments.

Keywords: *Cellular senescence, human fibroblasts, hydrogen peroxide, oxidative stress*.

DEVELOPMENTAL POTENCY OF SINGLE BLASTOMERE BIOPSIED FROM 8-CELL MOUSE EMBRYO IN DIFFERENT CULTURE MEDIA

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Abstract. In the present study, our main focus was evaluating the characteristics of preimplantation development of single blastomeres obtained from 8-cell fertilized mouse embryos and the formation of embryonic stem cells (ES cells) cluster. Specifically, two culture media, modified Chatot Ziomek Bavister (mCZB) and basic stem cell (SC) culture medium, were employed to optimize culture conditions to produce ES cell clusters from single blastomeres. Methodologically, a single blastomere was biopsied from an 8-cell embryo utilizing a micromanipulator system with the assistance of an XYClone laser to open a hole in the zona pellucida membrane. Single blastomere collected from 8-cell embryo was cultivated to the blastocyst stage in mCZB and SC medium (3 days after biopsy) then continued to culture in SC medium for production of ES cells cluster (6 days after blastocyst formation). The results indicated that mCZB medium provided more favorable conditions for the development of single blastomeres to blastocyst stage after 3 days compared with basic SC medium in the same interval. Furthermore, these blastomere-derived blastocysts shared similar morphological characteristics and the time of cell division from the morula stage to the blastocyst stage with the control group (fertilized 8-cell embryos). Besides, although the blastomere-derived blastocyst had the capacity to form ES cell clusters when subsequently cultured in a SC medium, single blastomeres continuously cultured in the basic SC medium witnessed a higher growth rate of ES cells cluster. The formation of these ES cells cluster can be envisaged as promising resources for producing ES cells lines for applications in regenerative medicine in the future. In conclusion, while the mCZB medium promoted the preimplantation development of single blastomeres until the blastocyst stage more effectively, the basic SC medium was superior to mCZB medium in terms of the establishment of ES cells cluster.

Keywords: basic stem cell medium, embryonic stem cells cluster, fertilized 8-cell mouse embryo, mCZB medium, single blastomere.

EFFECT OF SCRIPTAID ON PREIMPLANTATION DEVELOPMENT OF INTERSPECIES SOMATIC CELL NUCLEAR TRANSFER EMBRYO BETWEEN PORCINE RECIPIENT OOCYTE AND BOVINE DONOR CELL

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Abstract. Animal cloning by using somatic cell nuclear transfer (SCNT) technique has many applications. In preserving endangered animals, there are many species that no longer have any female individuals or oocyte source which makes it difficult to create offspring and build the population. Interspecies somatic cell nuclear transfer (iSCNT) is a technique that can help create embryo between 2 species by putting the nucleus of species A in the enucleated oocyte of species B. However, similar to studies about SCNT, iSCNT research also showed very low developmental rate of interspecies cloned embryos. In this study, we tried to improve the preimplantation development of the interspecies cloned embryo between pig oocyte and cow somatic cell by using a histone deacetylase inhibitor called Scriptaid (SCR). We carried out a one-step treatment with 250 nM SCR for the iSCNT embryos and yielded the following results. For the developmental rate, the one-step group reached blastocyst stage with a rate of 29.5% while the blastocyst rate of the control was 15.3%. To assess the blastocyst quality, we stained the blastocyst with DAPI and counted the number of cells per blastocyst. The number of cells per blastocyst of one-step group and control group being 50.3 ± 3.3 and 53.5 ± 11.5, respectively. These results suggested that with 250nM Scriptaid, the blastocyst rate of interspecies cloned embryo between porcine egg and bovine somatic cell was increased significantly, but the quality of blastocyst still not improved.

Keywords: interspecies somatic cell nuclear transfer (iSCNT), scriptaid (SCR), preimplantation development, interspecies cloned embryo

BONE MARROW MONONUCLEAR CELL TRANSPLANTATION CAN RECOVER SPINAL CORD INJURY IN MOUSE

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Abstract. Spinal cord injury (SCI) is a serious medical condition with complex pathological mechanisms that lead to sensory, motor, and autonomic dysfunction below the site of injury. Despite various medical developments, there are currently no effective regenerative treatments. Stem cell therapy in general and bone marrow-derived mononuclear cells (BM-MNCs) therapy in particular have been considered to be a promising treatment for SCI due to its multiple targets and reactivity benefits. The objective of this study is to investigate the safety and efficacy of BM-MNCs transplant in mouse model, in order to serve the trial programme of HCMC, Vietnam. The results showed that in both transection and compression mouse models (vertebrae D12-L2), there exists an improvement in locomotor test (Basso, Beattie, Bresnahan (BBB) locomotor rating scale), sensory test and sensory-motor test with dose 10^6 cells. The glial scar as well as myelin defragmentation were clearly reduced. The recovery effect of BM-MNCs is most evident within the first 7 days after injection, and the potential for motor recovery is more pronounced than the sensory. In conclusion, our results have initially recorded the recovery effects of BM-MNCs in 2 common SCI types in Vietnam.

Keywords: spinal cord injury, bone marrow mononuclear cells, SCI transection/compression mouse model

MULTIMODAL ANALYSIS OF METHYLATION AND FRAGMENTOMIC PROFILES IN PLASMA CELL FREE DNA FOR DIFFERENTIATION OF BENIGN AND MAGLINANT BREAST TUMORS

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Abstract. Breast cancer is the second leading cause of cancer deaths in women worldwide. Early detection of breast cancer has been demonstrated to improve patients' treatment outcomes and survival. Liquid biopsy based on detecting DNA shed by breast tumors into the circulation, known as circulating tumor DNA (ctDNA), has emerged as a promising non-invasive approach. However, differentiating benign breast lumps from malignant tumors remains a challenge in current clinical practice, and inaccurate detection may result in unnecessary invasive procedures. To address this challenge, we employed a multimodal analysis approach, namely SPOT-MAS (Screen for the Presence of Tumor by DNA Methylation and Size) to profile alterations in methylation and fragment length patterns of cell free DNA (cfDNA) from 133 breast cancer patients and 59 patients with benign breast lumps comprising cysts and fibroadenomas. We identified multiple distinct end motifs, differential methylation and fragment length patterns across 22 chromosomes, which were further exploited as input features to build machine learning models to discriminate early-stage breast cancer patients from patients with benign lesions. The models achieved an area under the curve of 0.87 (95% CI: 0.79 – 0.94) and a sensitivity of 64.1% at 90% specificity in detecting patients with malignant tumors. Therefore, our findings demonstrated that cancer-specific methylation and fragmentomic patterns in plasma cfDNA could serve as novel biomarkers for accurately differentiating malignant breast cancer patients from those with benign lesions.

Key words: Breast cancer, benign, liquid biopsy, machine learning, cysts and fibroadenomas

DISPARITIES IN IMMUNE EVASION MECHANISMS BETWEEN BREAST CANCER CELLS AND BREAST CANCER STEM CELLS

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Abstract. The concept of cancer stem cells (CSCs) gained prominence during the 1960s, denoting a subset of cells endowed with the capacity to engraft tumor masses, propagate under *in vitro* conditions, and exhibit resistance to conventional therapeutic modalities. The present investigation undertakes a comparative analysis of genetic expression patterns, focusing on a repertoire of genes implicated in escape mechanisms, differentiating the breast cancer stem cell subpopulation from its nonstem cell counterparts. This assessment is conducted under controlled *in vitro* cultivation conditions and subjected to the selective pressures exerted by the immune system within an experimental murine model. The findings unveil a substantial upregulation of a majority of scrutinized genes within the CSC population ($CD44^{+}CD24^{-}$) compared with the more differentiated cancer cells ($CD44^{-}CD24^{+/-}$). Notably, the genes CD95L, ADAM17, CXCL12, RAE1, TGFBR1, B7-H3, CCL22, and TAPBP exhibit heightened expression levels within CSCs, both in the controlled *in vitro* milieu and the dynamic *in vivo* context. Furthermore, in most instances, disparities in gene expression are more pronounced under *in vivo* selective pressures compared to the *in vitro* environment devoid of such selection. The outcomes of this investigation are poised to lay the groundwork for ensuing research endeavors aimed at intervening in the escape pathways inherent to BCSCs.

Key words: breast cancer, cancer stem cells, escape, immuno-surveillance

A COMPARISON OF IMIQUIMOD EXPOSURE TIME-DEPENDENT PSORIASIS MOUSE MODELS

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Abstract. Psoriasis is a common chronic and inflammatory skin disorder. It is characterized by a complex pathogenesis primarily involving the IL-17/IL-23 pathway. This study aims to compare the psoriasis characteristics of mouse models exposed to imiquimod (IMQ) in the long term and short term. In this study, *BALB/c* mice were divided into three groups (n = 5). Topical IMQ was applied on the shaved dorsal skin for 6 (Group 1) or 12 (Group 2) consecutive days to induce psoriasis lesions. Normal mice were used as controls (Group 3). Clinical symptoms (erythema, scaling, and thickness) were evaluated by measurement and observation. Histopathological features were assessed by H&E staining. These results were recorded on days 3, 6, 12, 18, and 24. The IL-17 and IL-23 expression levels were detected using quantitative RT–PCR and immunohistochemistry on days 3, 6, and 12. The results showed that mice exhibited distinct clinical and histopathological features of psoriasis-like lesions beginning from day 3 and showed typical severity on day 6 in both Groups 1 and 2. The expression levels of IL-17 and IL-23 in the skin lesion correlated with the clinical and histopathological alterations. These pathological features gradually diminished and were moderated to normal on day 24. However, the histological morphology and the expression of lesional cytokines, such as IL-17 and IL-23, still gradually decreased after day 6, even though IMQ was still applied up to day 12 in Group 2. Our study found that applying IMQ continuously for 6 days was the most effective method for establishing a mouse psoriasis model.

Keywords: *IMQ application, IL17/IL23 axis, psoriasis-like lesions, psoriasis mouse model*

ESTABLISHMENT OF AGED HUMAN DERMAL FIBROBLASTS BY ULTRAVIOLET IRRADIATION

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Abstract. Photoaging is a degenerative condition that leads to skin fragility, loss of function and cosmetic dissatisfaction. The senescence of human dermal fibroblasts (HDFs) plays vital roles in the pathogenesis of skin aging. Therefore, we aimed to develop an aged HDF model with an optimized acute UV irradiation protocol. HDFs isolated from abdominal skin were divided into groups UV1 and UV2. The irradiation dosages were UVB 780 mJ/cm^2+UVA 480 mJ/cm^2 for 10 mins in group UV1 and UVB 1170 mJ/cm^2+UVA 720 mJ/cm^2 for 15 mins in group UV2. The HDFs were then assessed with some markers for aging. The cell morphology and size were observed by microscopy. SA-β-galactosidase expression was measured using ImageJ software. The differentiation potential of HDFs into chondrocytes, osteocytes, and adipocytes was checked by inducible medium. The expression of some fibroblast -specific markers was evaluated by flow cytometry. Cell proliferation was measured by Alamar blue assay. Gene expression was quantified by real-time RT–PCR. The results showed that in the UV groups, the cell appearance became flattened and larger. Cell sizes were significantly increased in UV-irradiated HDFs ($p<0.001$). The integrated density of SA-β-gal signals was higher in the UV groups than in the normal group ($p<0.05$). The time for fibroblasts to differentiate into adipocytes and chondrocytes was longer in both UV groups compared to normal HDFs. The fibroblast markers CD 90 (94.64%), Vimentin (98.39%), and S100A4 (97.95%) were highly expressed. The division ability of cells significantly decreased in both UV groups at 72 hours and was maintained until 8 days ($p<0.001$), with a lower proliferation rate in the UV2 group. Moreover, UV light increased the mRNA expression of MMP-3, p16, p21, and p53 in the UV2 group ($p<0.05$). These results demonstrated that the UV2 dose is an appropriate protocol for establishing the HDF senescence model.

Keywords: human dermal fibroblast, ultraviolet, senescence

PRODUCTION OF TRANSGENIC CLONED BOVINE EMBRYOS EXPRESSING GREEN FLUORESCENT PROTEIN DERIVED FROM TRANSFECTED CELLS WITHOUT DRUGS SELECTION

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Abstract. Generating transgenic cloned bovine embryos that can express detectable markers such as green fluorescent protein (GFP) is considered one of the most important phases to make “pharming” animals for obtaining various recombinant human proteins through milk. In cattle, most of the current procedures are to establish the transgenic cell lines by drug selection and nuclear transfer of transgenic cells to produce transgenic cloned offspring. However, these selection methods showed limitations in toxicity and uncontrollable effects on the host genome. This study produced and evaluated transgenic cloned bovine embryos derived from GFP-transfected cells without using any drug selection. After 3 days from cell transfection, around 42% of culturing cells expressed the GFP signal under the UV light and this rate decreased gradually to 8.2% on day 10^{th}. Interestingly, the size of transfected fibroblasts was significantly bigger ($P<0.05$) than non-transfected fibroblasts (17.4 µm and 14.6 µm in diameter, respectively). After quick UV selection and nuclear transfer of non-transfected (control) and GFP-transfected cells to enucleated oocytes for cloned embryos production, there was no significant difference in development rates to the blastocyst stage between the control group and the GFP-transfected group but the quality of the blastocyst at day 7th of culture was significantly different in which the cell numbers of the control blastocysts were nearly two times higher ($P<0.05$) than the transgenic cloned blastocyst (100 cells and 54 cells, respectively). Remarkably, we observed the expression of GFP in the cloned blastocysts derived from GFP-transfected cells only. In conclusion, we succeeded in establishing the procedure by a combination of genome editing and animal cloning to produce transgenic cloned bovine blastocyst with stable expression of foreign GFP gene and free-drug selection requirement, which could be studied and applied for further production of genetically modified livestock with desirable traits.

Keywords: free-drug selection, GFP, pharming, transgenic cloned bovine embryos.

ANTICANCER ACTIVITY AGAINST *LIVER CANCER CELL* LINE HEPG2 AND IMMUNOSTIMULANT ACTIVITY IN CYTOXAN-INDUCED IMMUNOSUPPRESSION MICE OF RADIATION SYNTHESIZED SENPS/*β* -GLUCAN

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Abstract. Selenium nanoparticles (SeNPs) have attracted attention due to their high bioactivity and low toxicity compared to their inorganic and organic compounds. Selenium nanoparticle production (SeNPs) has been investigated in various fields, such as antioxidant, immunostimulant and anticancer activities. The objective of this study is to determine the anticancer and immunostimulant activity of radiation synthesized SeNPs/*β*-glucan. *In vitro,* results showed that SeNPs/*β*-glucan were able to inhibit the growth of HepG2 liver cancer cells, with an IC50 value of 6.511 ppm. Specifically at concentrations of 10 and 20 ppm, the survival rate of HepG2 cells was only 34% and 17 %, respectively. In addition, SeNPs/*β*-glucan SeNPs/β-glucan were not toxic to the L929 cell line, with a cell survival rate of nearly 70%. SeNPs/β-glucans are selective for normal and cancer cell lines, which is a suitable suggestion. The *in vivo* tests in cytoxan-induced immunosuppression mice revealed that the daily supplementation of SeNPs/β-glucan at concentrations of 2 – 6 mg per kg body weight of tested mice significantly stimulated the generation of cellular immune factors (white blood cells, neutrophil, lymphocyte, B cells, CD4+ cells, CD34+ cells and natural killer cell cells) and humoral immune indexes (IgM, IgG, TNF-α, IFN-γ and IL-2) in peripheral blood, bone marrow and spleen of cytoxan-induced immunosuppression mice. The suitable concentration to determine this product was about 6 mg SeNPs per kg body weight. The obtained results indicated that the radiation-synthesized SeNPs/β-glucan potentially applied as an agent for the prevention of immunosuppression and cancer in chemotherapy in the future.

Keywords: Anticancer, radiation, selenium nanoparticles, immunostimulant, immunosuppressed mice.

BIOCOMPATIBILITY AND BIOACTIVITY OF A PREMIXED TYPE BIOCERAMIC CEMENT FOR DENTAL USES

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Abstract. Direct pulp capping is a dental procedure for preserving vitality and function of the tooth pulp after pulp exposure. In this cases, a biomaterial directly covers the exposed pulp tissues and should induce differentiation of osteoblast-like cells to form a hard tissue barrier. Therefore, biocompatibility and bioactivity of the material is quite critical for direct pulp capping. The purpose of this study was to assess the biocompatibility and bioactivity of a premixed type bioceramic cement (Well Root PT). *In-vitro* samples of ProRoot MTA, Biodentine, and Well Root PT were prepared. To assess calcium weight volume of the samples, SEM/EDS mapping (n = 3 per group) were performed. ICE-OES analysis were proceeded to evaluate calcium ion release from the samples (n = 5 per group). For *in-vivo* analysis, a rat direct pulp capping model (n = 7 per group) was used. After the sacrifice, the samples were histologically evaluated. Calcium weight volume (%) was 42.83 ± 8.82 in ProRoot MTA, 47.05 ± 8.83 in Biodentine, 29.99 ± 4.94 in Well Root PT. Regarding calcium ion release at day 7, Biodentine (97.74 ± 16.85 ppm) released the largest amounts compared to ProRoot MTA (33.10 ± 11.25 ppm) and Well Root PT (20.87 ±8.98 ppm) with statistical significance (p < 0.017). At day 28, the amounts of calcium ion release were 271.91 ± 92.48 ppm in Biodentine, 124.16 ± 25.58 ppm in ProRoot MTA, and 72.37 ± 8.35 ppm in Well Root PT with statistical significance (p < 0.017). For in-vivo analysis, there were no significant differences in the inflammatory cell infiltration among the groups (p > 0.017). Within the limits of this study, Well Root PT is biocompatible and bioactive for direct pulp capping.

Keywords: bioactivity, biocompatibility, biomaterials, calcium silicate-based cements, vital pulp therapy

ISOLATION, CHARACTERIZATION, AND DIFFERENTIATION OF ONE-DAY-OLD PORCINE CARTILAGE STEM CELLS FOR TISSUE ENGINEERING

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Abstract: Extracellular matrix is produced by cells and has many applications in tissue engineering research. It is composed of various proteins and carbohydrates that form a complex network of fibers and molecules outside of cells. The extracellular matrix provides structural support to cells and tissues, as well as biochemical signals that regulate cell behavior and function. Engineered membrane from extracellular matrix has a good application in osteoarthritis disease. A new candidate stem cell source uses for the engineered membrane fabrication necessary. This study aims to investigate one-day-old porcine cartilage stem cells to build the engineered membrane for cartilage repair. **Methods:** Cells were isolated from one-day-old porcine cartilage tissue, cultured, and characterized their biological properties. Additionally, engineered membranes were fabricated based on cell sheet technology. Then, assessing this membrane for cellular toxicity and examining rabbit chondrocyte proliferation. **Results:** One-day-old porcine cartilage-derived cells expressed with stem cell markers. They had a population doubling time of approximately 1 to 2 days, maintained their polygonal morphology over 18 passages, and possess the potential for multilineage differentiation. The engineered membrane was not toxic to rabbit chondrocytes and the proliferation of rabbit chondrocytes in the engineered membrane extract medium increased. **Conclusions**: One-day-old porcine cartilage-derived cells have stem cell properties to some extent and offer potential as a cell source for extracellular matrix membrane fabrication.

Keywords: Extracellular matrix, engineered membrance, stem cells, tissue engineering

Enhanced Biocompatibility of 3D Scaffolds through Plasma-Assisted 3D Printing System

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Abstract. Fused deposition modeling (FDM) has established itself as a prominent three-dimensional (3D) printing technology in tissue engineering. However, the biocompatibility of 3D-printed scaffolds remains a challenge due to the inadequate cell affinity and biocompatible environment of the extruded materials. To address this issue, we propose a novel approach that leverages plasma treatment to enhance the biocompatibility of 3D-printed scaffolds. Our study introduces a plasma-based 3D printing system with dual heads, integrating a plasma device and a conventional FDM printer head to achieve layer-by-layer nitrogen plasma treatment. Through this innovative method, we systematically investigated the impact of plasma treatment duration on the wettability, roughness, and protein adsorption capability of 3D-printed scaffolds. Notably, extended plasma treatment times led to significant improvements in these parameters. Consequently, the layer-by-layer plasma-treated (LBLT) scaffold demonstrated remarkable enhancements in cell adhesion and proliferation, as evidenced by in vitro assays. Furthermore, our in vivo assays revealed that the LBLT scaffold exhibited superior tissue infiltration and reduced collagen encapsulation compared to non-plasma-treated scaffolds. This pioneering approach holds immense promise for diverse tissue-engineering applications, offering adaptability through the manipulation of gas or precursor levels. Crucially, the system's ability to impart biocompatibility across the entire 3D-printed structure sets it apart, overcoming the limitations of current biocompatible 3D scaffold engineering. In conclusion, this study introduces a comprehensive method for enhancing the biocompatibility of 3D scaffolds through plasma-based printing. By tackling the limitations of current techniques and offering a versatile platform for tissue engineering, our approach represents a significant step forward in advancing the field of 3D scaffold fabrication.

Keywords: 3D printing, Layer by layer deposition, Plasma treatment, Biocompatibility

ACELLULAR MATRIX FILM LOADED PHLOROTANNIN PREVENTS POST-IMPLANTATION INFLAMMATORY RESPONSE

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Abstract. Peritendinous adhesion mainly occurs between proliferating fibrous tissues and adjacent normal organs after surgery. Many physical barriers are applied to the implanted site to prevent peritendinous adhesion. However, these barriers often trigger inflammatory responses. Therefore, our study sought to develop phlorotannins-loaded cartilage acellular matrix (CAM) films as a physical barrier and investigate their inhibitory effect on inflammatory responses, which are associated with the induction of postoperative peritendinous adhesion (PAA). Our findings indicated that incorporating phlorotannin into the CAM film did not affect its unique characteristics including its thermal and spectroscopic properties. Moreover, the phlorotannins-loaded CAM films suppressed the expression of inflammatory mediators on RAW 264.7 macrophages stimulated using Escherichia coli lipopolysaccharides and exhibited an anti-inflammatory effect when implanted subcutaneously in rats. Therefore, our results highlight the potential of phlorotannins-loaded CAM films as a promising physical barrier to prevent PAA.

Keywords: anti-adhesion, phlorotannins, physical barrier

INVESTIGATION OF THE EFFECTS OF $CaCl_2$ AND HYALURONIC ACID CONCENTRATIONS ON FIBRIN GEL FORMATION FOR CELL CULTURE

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Abstract.

Objective: Fibrin gel has been demonstrated as an effective model for primary hepatic stellate cell (HSC) culture. However, fibrin gel exhibits some limitations, such as low transparency and poor stability. In this study, we aimed to address these drawbacks by investigating the influence of $CaCl_2$ and hyaluronic acid (HA) concentrations on fibrin gel transparency, stability, and suitability for HSC culturing.

Methods: Different concentrations of $CaCl_2$ (0.01, 0.025, 0.05, and 0.075 mM) and HA (0, 0.5, and 1 mg/mL) were employed to compare the properties of formed fibrin gels, including transparency, gel formation capability, stability, and stiffness. Additionally, HSC culturing experiments were performed on the formed gels.

Results: Fibrin gel supplemented with 0.025 mM $CaCl_2$ and 0.5 mg/mL HA exhibited the best transparency and stability after 7 days compared to other concentrations. Upon retrieval from fibrin gel, 3T3 cells display division rates comparable to those cultured on a plastic surface, accompanied by a high viability percentage, as evaluated through trypan blue staining. Moreover, HSC culturing on the formed gel revealed that HA promoted HSC activation by reducing lipid droplets and enhancing the expression of collagen markers.

Conclusion: The combination of 0.025 mM $CaCl_2$ and 0.5 mg/mL HA is suitable for generating fibrin gel for cell culturing purposes.

Keywords: fibrin gel, hyaluronic acid, gel transparency, gel stability, cell culture.

MICOPROPAGATION OF *DECASCHISTIA INTERMEDIA* CRAIB.

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Abstract. *Decaschistia intermedia* Craib, a member of the Malvaceae family, is a rare medicinal herb with significant applications in traditional medicine, owing to its diverse pharmaceutical properties such as promoting well-being, enhancing vitality, and addressing neurasthenia. This study was the very first attempt to establish a comprehensive micropropagation protocol for *Decaschistia intermedia* Craib. The findings from this research revealed that the optimal sterilization method obtained when treating the nodal segments with a 15% NaClO solution for 15 minutes. This procedure reached highest percentage uninfected and viable samples (82%). For the shoot multiplication phase, single nodal cuttings cultured on MS medium supplemented with 1.0 mg/l of Benzyladenine (BA) and 0.5 mg/l of Naphthaleneacetic Acid (NAA), led to remarkable outcomes. This hormonal combination demonstrated superior effects, yielded an average of 6.43 shoots per explant. Subsequently, the in-vitro shoots were transitioned to the rooting stage. The MS medium supplemented with 2.0 mg/l of NAA and 0.5 mg/L of Indole-3-Butyric Acid (IBA) resulted in the highest number of roots per plantlet (6.21 roots/plantlet), along with impressive root length (4.52 cm), tallest shoot height (6.39 cm), and the greatest leaf count (5.63 leaves/plantlet). Indeed, these findings significantly contribute to the successful establishment of a robust micropropagation protocol for *Decaschistia intermedia* Craib. This achievement facilitates large-scale production of the species and enhance efforts towards the conservation in its natural habitat.

Keywords: Decaschistia intermedia Craib; micropropagation; growth regulator, shoot and root induction

EVALUATION OF THE THERAPEUTIC EFFECTS OF MESENCHYMAL STEM CELL-DERIVED EXOSOMES IN STOMACH ULCER-INDUCED ANIMAL MODELS

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Abstract. One of the most common health conditions in the world is gastric ulcer. Different medications or drugs have already been used to treat gastric ulcers by primarily suppressing their symptoms; however, they also cause a variety of side effects. Hence, this study aimed to primarily evaluate the therapeutic potential effects of exosome-containing products (StomaHeal) in an acute gastric murine model. Mice were assigned into five groups: blank, nontreat, sham, positive, and exosome treatment groups. Gastric ulcers were induced by a single dose of 0.4 M HCl/60% ethanol. Blood was taken for total/differential leucocyte count. Stomach sections were obtained for macroscopic and histological evaluation. Mice were orally administered StomalHeal. The results showed that the mouse stomachs in the ulcerated control group displayed high macroscopic damage and remarkable gastric damage with high hemorrhagic and submucosal edema, disruption to the surface epithelium, inflammatory cell infiltration, and the appearance of granulate tissue at the ulcer base, forming the typical morphology of a gastric ulcer. The exosome treatment group showed obvious therapeutic evidence through the regeneration of damaged tissue and the acceleration of wound healing. This was not observed in the ulcerated control group. These results primarily showed that exosomes can be an alternative, noninvasive, and efficient therapeutic approach for treating gastric ulcers. However, more studies with larger sample sizes and long-term evaluations of safety and efficacy are needed to confirm the observations.

Keywords: Acute gastric ulcer, exosomes, histology, StomaHeal, wound healing.

EFFECT OF DIFFERENT CULTURE MEDIA ON CRISPR-ENGINEERED MOUSE EMBRYONIC STEM CELLS

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Abstract. Genetic engineering of stem cells is a novel field in regenerative medicine. With the advent of CRISPR-based system, stem cells are manipulated their genomes to serve for our purposes. However, one of the most challenging issues in stem cell culture is to maintain the pluripotency of these stem cells, especially during genome editing. Culturing stem cells under inappropriate conditions could lead to their differentiation and reduce reproducibility of experiments. The aim of this study was to check the effect of common stem cell culture media on genome editing efficiency together with pluripotency of stem cells. Using CRISPR/Cas9 system, two reporter genes were knocked-in into targeted alleles of mouse embryonic stem cells (mESCs). During the process of genetic engineering, different stem cell media were tested. mESCs responded differently to different media. In the most reliable condition for ESC culturing, 2i medium seemed to prevent random integration of reporter genens, which happens at high percentage using CRISPR system. However, this medium is too severe for the engineered cells. Most of the cells died. Interestingly, in the combination of 2i medium and common stem cell medium (DMEM+KSR+LIF), higher targeting efficiency was observed. This condition also supported the engineered cells in term of growing and maintenance of pluripotency.

Keywords: mESC, culture medium, pluripotency, CRISPR, cellular engineering, targeting efficiency

TOPICAL APPLICATION OF EXOSOMES FROM HYPOXIA-INDUCED ADIPOSE-DERIVED STEM CELLS AMELIORATES PHOTOAGING IN MICE

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Abstract. Exosomes are extracellular nanosized vesicles that can be utilized as cell-free therapeutics for many biological processes. In this study, we aimed to investigate the effects of exosomes from adipose-derived stem cells (ADSC-Exos) in ameliorating signs of photoaging in mice by topical application. Exosomes were extracted from hypoxia-induced ADSC culture medium by ultracentrifugation. Collected ADSC-Exos were identified by specific markers using flow cytometry. The vesicle morphology was determined by TEM. The photoaging mouse model was established according to a published protocol. The photoaged mice were divided into 2 groups with two exosome doses, 50 µg/mL (Exo1) and 100 µg/mL (Exo2). Exosomes (100 µL) were applied to aged skin 2 times/week for 6 weeks. The therapeutic efficacy of ADSC-Exos was assessed with some criteria. Skin wrinkles were evaluated based on the Bissette scale through microscopic photographs. Skinfold thickness was measured by a digital caliper. The elasticity of the skin was examined by pinch test. Skin hydration was quantified using a Corneometer® CM825 probe. Moreover, skin biopsies were performed to evaluate epidermal and dermal thickness by H&E staining. Collagen quantification by Masson's trichrome staining was analyzed by ImageJ software. In addition, the gene expression of MMP-1, MMP-2 and MMP-3 was detected using real-time RT–PCR. The results showed that after 6 weeks, skin wrinkles in the Exo2 group were markedly improved compared to those in the Exo1 and UV groups ($p < 0.0001$), nearly the score of the positive control. The skinfold thickness of mice in the Exo groups was decreased compared to that of mice in the UV group ($p < 0.05$). The snap back-time of the Exo groups was significantly lower than that of the UV group and similar to that of normal skin. ADSC-Exos led to an increase in the skin hydration of the treated group compared to that of the model group ($p < 0.05$). The epidermal thickness and dermal thickness in the Exo groups were observed to decrease significantly, and the intensity of collagen staining was elevated in the Exo2 group compared with the UV group. MMP-1, MMP-2 and MMP-3 mRNA expression levels were slightly downregulated in the Exo2 group compared with the UV and Exo1 groups. Our results demonstrated that topical application of hypoxia-induced ADSC-Exos could remarkably ameliorate skin solar aging.

Keywords: exosome, adipose-derived stem cell, photoaging, solar aging.

BROWN ADIPOCYTES DIFFERENTIATED FROM MESENCHYMAL STEM CELLS CAN CAUSE WEIGHT LOSS IN OVERWEIGHT MICE

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Abstract. Brown adipocytes contain a high number of mitochondria in the cytoplasm that can activate thermogenesis. Therefore, in the body, brown adipocytes can help regulate sugar and fat metabolism. This study aimed to establish the condition of differentiation of mesenchymal stem cells into brown adipocytes and try to treat overweight mice by brown adipocyte transplantation. Mesenchymal stem cells were isolated and expanded from human adipose tissues using a published protocol. Then, they were induced to differentiate into brown adipocytes under the effects of T3 and rosiglitazone. The differentiation efficacy was evaluated based on the expression of *UCP1, PPARg*, and *PGC1a*. The lipolysis assay was used to evaluate the thermogenesis potential of brown adipocytes. At the optimal condition of *in vitro* differentiation, the differentiated brown adipocytes were transplanted into overweight mice to check the weight loss efficacy. The results showed that the combination of T3 and rosiglitazone successfully induced the differentiation of mesenchymal stem cells into brown adipocytes *in vitro*. These brown adipocytes strongly expressed *UCP1, PPARg* and *PGC1a* and displayed lipolysis *in vitro*. Brown adipocyte transplantation causes weight loss in overweight mice compared to controls (PBS injection, white adipocyte transplantation). These findings showed that brown adipocyte transplantation is a promising therapy for weight loss.

Keywords: brown adipocytes, mesenchymal stem cells, weight loss, overweight

CELL ADHESIVENESS OF HYDROGEL SHELL COMPOSING CELL DOME: EFFECT ON THE CELLS CULTURED IN CELL DOME

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Abstract. Hydrogels are used for the fabrication of a variety of cell-laden structures. Since each hydrogel has unique characteristics derived from its components, it is important to select the appropriate polymer when preparing cell-laden structures. We recently reported on a cell culture and evaluation system called “Cell Dome”. Cell Dome is a hemispherical microdome with a diameter of 1 mm in which cells are enclosed in a cavity covered with a hemispherical hydrogel shell immobilized on a glass plate. To date, the effects of hydrogel shell materials on the cells enclosed in Cell Dome have not been studied. In this study, we reported the effects of cell adhesiveness of the hydrogel shell on the cells enclosed in Cell Dome. The hydrogel shells were prepared using alginate derivative hydrogel (AL-Cell Dome) or the mixed hydrogels of gelatin derivative and alginate derivative (GA-Cell Dome) via horseradish peroxidase (HRP)-mediated hydrogelation reaction. The effects of the hydrogel shells on enclosed cells were examined using human cervical cancer cell lines expressing fucci2 (Hela-fucci2). The results showed that Hela-fucci2 cells did not grow on alginate derivative hydrogel, but the cells were adherent, spread, and grow on the mixed hydrogels of gelatin derivative and alginate derivative. When the cells were cultured in GA-Cell Dome, the cells grew along the inner wall of the hydrogel shell. On the other hand, the enclosed cells in AL-Cell Dome did not adhere to the hydrogel shell and grew from the bottom. These results would be due to the cell adhesion ability of gelatin. These results indicate that the behavior of the cells cultured in Cell Dome changes depending on the material of the hydrogel shell.

Keywords: Cell Adhesiveness, Cell Dome, 3D culture, Gelatin, Hela-fucci2 cell, Tissue engineering

POLYHYDROXYBUTYRATE (PHB-CO-HHX) PRODUCTION BY ENGINEERED *RASLTONIA EUTROPHA* USING WASTE WOODY BIOMASS

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Abstract With the increasing global attention on climate change and carbon neutrality, the interest in the production and utilization of bioplastics, which can replace petroleum-based plastics and are biodegradable, has been growing. Among them, polyhydroxyalkanoate (PHA) is well-known as an environmentally friendly material that is 100% biodegradable in nature and can be synthesized by microorganisms, having diverse mechanical properties. In this study, it aimed to produce polyhydroxybutyrate-co-hydroxyhexanoate (PHB-co-HHx) using a engineered *R. eutropha*, utilizing waste woody biomass-derived hydrolysates. By employing a steam-explosion process, glucose and xylose-containing hydrolysates were obtained from waste woody biomass, with concentrations of 20 g/L and 2 g/L, respectively. When the engineered *R. eutropha* was cultured using these hydrolysates, the biomass concentration reached 18 g/L, PHA concentration reached 10 g/L, PHA content was 55%, and the HHx mol% was 10% within 72 hours. This study demonstrates the feasibility of producing PHB-co-HHx from waste woody biomass, and further research is expected to enhance the productivity of PHA through subsequent studies. This study was supported by R&D Program for Forest Science Technology [grant number: 2023473E10-2325-EE02] provided by Korea Forest Service.

Keywords: Waste woody biomass, Polyhydroxybutyrate(PHB), Biodegradable bioplastic, *Ralstonia eutropha*

SUSTAINABLE CULTIVATION OF MICROALGAE (*MICRACTINIUM* SP.) AND USE OF A DUAL-PURPOSE VANADIUM OXIDE NANOPARTICLE FOR BIOMASS HARVESTING AND BIODIESEL PRODUCTION

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Abstract. Microalgae are considered as a potential feedstock for the sustainable production of biofuel, several value-added compounds, and carbon-di-oxide sequestration. Improving algal biomass yield in a cost-effective way is the need of the hour. Various studies reported that microalgae require nutrients viz., nitrogen, carbon, phosphorous, potassium, and other trace elements for their growth. The formulation of a cheap and sustainable media composition for algae cultivation has gained much attention in recent times. Sustainable microalgae cultivation using readily available and affordable organic substances could address various environmental and resource challenges. The present study aimed to investigate the prospect of using Nano urea (NU), Groundnut De-oiled Cake (GDOC), and Organic Seaweed Extract (OSE) as media components for the cultivation of *Micractinium* sp. The investigation revealed that biomass yield was improved by 48% on using NU+GDOC+OSE medium compared to the control BBM medium. Moreover, with an optimized (NU+GDOC+OSE) medium, lipid content improved by 16%. A dual-purpose Vanadium oxide nanoparticle was developed to harvest algal biomass and catalyze Fatty Acid Methyl Ester (FAME) synthesis. The Vanadium oxide nanoparticle showed a flocculation efficiency of 98%. The Vanadium oxide nanoparticle also acted as a catalyst for the FAME synthesis by transesterification from the wet biomass. The FAME analysis showed the prevalence of palmitic (C16:0) and stearic (C18:0) acids. The study indicated that utilizing cheap nutrient sources could help in the sustainable cultivation of microalgae along with its harvesting and biodiesel production using a dual-purpose nanoparticle.

Keywords: Nano urea, Groundnut De-oiled cake, Organic seaweed extract, Vanadium oxide nanoparticle, flocculation, FAME

Algae Oil Extraction from Freshwater Microalgae *Chlorella* sp. by Soxhlet Extraction Method

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Abstract. Microalga has been regarded as a promising material source for biodiesel production due to a great amount of oil in their biomass which can replace petroleum diesel and are environmentally friendly. Microalgae *Chlorella* spp. and *Micractinium* spp. are available in diversified living environments and have a rapid growth ability. The biodiesel production process includes many different steps in which lipid extraction plays a very important role. The soxhlet extraction method was used to extract oil from microalgae strains with high dry-basis biomass and growth. Since then, the research investigated the optimal extraction conditions in terms of solvent usages, hexane/ethanol ratios (i.e. 1:1, 1.5:1, 2:1, 2.5:1, 3:1 v/v), and extraction time (i.e. a range of 1–5 hours) for retrieving the lipid-enriched oil. Results indicated that *Chlorella sorokiniana* CC02 had the dry biomass content (2.059 ± 0.088 $g.L^{-1}$) and the highest growth rate (μ = 0.537 ± 0.013 $days^{-1}$) under rearing conditions of 24/24 aeration at 0.5 $L.min^{-1}$, 28 °C, 16/8 light/dark regime. The optimal condition for their oil extraction was achieved with hexane/ethanol (1.5:1 v/v) at 4 hours, obtaining the highest extraction efficiency of 48.497 ± 0.605%.

Keywords: biodiesel, lipid, *Chlorella sorokiniana*, Soxhlet extraction method.

BIOHYDROGEN PRODUCTION VIA DARK FERMENTATION OF *CLOSTRIDIUM* SP. UTILIZING SPENT MUSHROOM SUBSTRATE AS FEEDSTOCK

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Abstract. With the global market growth of the edible mushroom industry, proper waste management must be carried out to mitigate potential environmental threats caused by spent mushroom substrate (SMS), as producing 1 kg of fresh mushrooms generates approximately 5 kg of wet by-products. Adopting the concept of "waste-to-fuel," SMS as a waste product of lignocellulose can be utilized as a low-cost and abundant feedstock for biologically producing hydrogen. In the pre-processing stage, to effectively decompose lignin, low-temperature alkaline treatment and high-temperature treatment were first applied to SMS of *Agaricus bisporus*. The effects of processing time, temperature, and solid-to-liquid ratio on reducing sugar yield were investigated using response surface methodology (RSM) to determine the optimized conditions. Then, a two-step sulfuric acid hydrolysis method was employed to convert hemicellulose and cellulose into reducing sugars (such as glucose, xylose, and arabinose). In the next stage, the feasibility of using SMS hydrolysate as a substrate for producing hydrogen via dark fermentation by different *Clostridium* strains was investigated, along with the utilization of activated carbon to reduce the inhibitory compounds produced by acid hydrolysis.

Keywords: Biohydrogen, *Clostridium* sp., Spent mushroom substrate, Circular economy

UTILIZING BICARBONATE-BASED INORGANIC CARBON SOURCE FOR THE GROWTH OF MICROALGA *CHLORELLA SOROKINIANA* SU-1 AS A PLATFORM OF CARBON CAPTURE AND UTILIZATION

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Abstract. As economic and industrial activities continue to grow, the severity of extreme weather events and climate change is increasing. Microalgae cultivation, a form of CCUS (Carbon Capture, Utilization, and Storage) technology, plays a crucial role in reducing net carbon emissions and promoting a circular economy. However, microalgae cultivation faces a technical challenge: a significant amount of CO_2 escapes into the gas-liquid culture system, which hinders efficient CO_2 capture and utilization. To address this issue, a novel method utilizing a bicarbonate-based culture medium has been proposed. This method involves absorbing CO_2 into an alkaline solution, facilitating its utilization by microalgae. During a 7-day culture period, *Chlorella sorokiniana* SU-1 effectively consumed almost all the bicarbonate, indicating the feasibility of cultivating microalgae in a bicarbonate-based medium if the alkalinity problem can be resolved. Therefore, the bicarbonate-based integrated carbon capture and microalgae production system represents a viable approach for enabling rapid CO_2 utilization by microalgae. This technique showcases the potential for future commercialization and offers a promising solution to the challenge of reducing CO_2 emissions through microalgae cultivation.

Keywords: microalgae, bicarbonate, carbon capture and utilization

CHARACTERIZATION OF BIOCHAR ACQUIRED FROM HYDROTHERMAL CARBONIZATION AND TORREFACTION OF ENERGY CROP, AGRICULTURAL RESIDUE, AND WOOD CHIP

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Abstract. Along with the increasing attentions on biochar, the confusion about the term 'biochar' is also growing. In general, biochar means solid phase product acquired from thermochemical conversion of a biomass. However its characteristics greatly differ by the process it went through. In this work, three types of biomass (energy crop, agricultural residue, and wood chip) were treated with two reactions (hydrothermal carbonization and torrefaction). The reactions were performed at same reaction temperature (220-300 °C) and time (30 min). The biochar acquired from hydrothermal carbonization was named hydrochar and that acquired from torrefaction was named torrefied char. With various analyses, the difference between hydrochar and torrefied char was investigated.

Keywords: Biochar, Torrefaction, Hydrothermal carbonization

GAMMA IRRADIATION-ASSISTED HOT WATER PRETREATMENT OF RICE STRAW FOR ENZYMATIC HYDROLYSIS

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Abstract. The conversion of lignocellulose to liquid fuels and chemicals via biochemical ways requires pretreatment to overcome its structural recalcitrance. Hot-compressed water, without adding any chemicals, holds promise as a way of pretreatment. Ionizing radiation such as γ irradiation can destroy molecular bonding in lignocellulose and loosen its structures. Radiation-assisted hydrothermal treatment may enhance the effect of biomass pretreatment, which has rarely been explored. In this work, γ irradiation followed by hot-compressed water treatment of rice straw was carried out. It was shown that radiation enhanced the hydrothermal solubilization of lignocellulose, and a higher radiation dose tended to induce greater solubilization. Radiation largely benefited the production of free sugars (glucose and xylose) in the liquid fraction, which achieved the highest concentrations at 180 °C. Radiation improved the production of total sugars (total glucose and total xylose) including monosaccharides and oligosaccharides in the liquid fraction. Total sugars achieved the highest amounts at 160 °C with total xylose dominating the sugar composition. The generation of xylose and xylo-oligosaccharides was more temperature-sensitive compared with that of glucose and glucosyl-oligosaccharides. Radiation enhanced enzymatic hydrolysis of rice straw pretreated by hot water from 140 to180 °C, and the highest sugar yields were obtained at 160 °C. The glucose yield by enzymatic hydrolysis was increased by 15-23% by radiation at 160 °C. 50 kGy of radiation plus hydrothermal treatment at 160 °C was able to achieve a sugar yield comparable to that of hydrothermal treatment at 200 °C. SEM morphology and FTIR spectroscopy of the pretreated biomass were examined for an insight of the radiation-assisted hot water pretreatment.

Keywords: lignocellulose, pretreatment, gamma irradiation, hot-compressed water

Non-strict Anaerobic Symbiotic TSH06 Fermentation to Produce Butanol Followed to Prepare Bio-jet Fuel

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Abstract. The consumption of jet fuel is increasing due to the development of aviation industry. Carbon dioxide produced by combustion of jet fuel in aircraft basic emitted in the atmosphere, which resulting in a strong greenhouse effect. Therefore, developing full-performance bio-jet fuel based on alternative feedstocks can effectively reduce the environmental impacts of aviation and ensure energy security in the industry. It has been reported that Alcohol to Jet was the more promising process for aviation bio-fuel production, so, bio-butanol become a prospective intermediate for bio-jet fuel production. Different from the traditional strict anaerobic fermentation of butanol. In our research, a new non-strict anaerobic symbiotic TSH06 was obtained, and a novel technology of butanol production was developed. The ratio of ABE and butanol concentration reached 1:8:1 and 19.2g/L, respectively in batch fermentation. Afterwards, bio-jet fuel was produced by series of chemical reactions with bio-butanol. The bromine index of product reached 153gBr/100g. At the same time, other performances of product such as water content, density and acid value, etc. were also analyzed, which satisfied the aviation fuel standards.

Keywords: Biobutanol; bio-jet fuel; fermentation; catalyst; production

DEVELOPMENT OF ADVANCED BIOHYDROGEN PRODUCTION USING FOOD WASTEWATER THROUGH INDUCED ENHANCEMENT OF ELECTRON TRANSFER

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Abstract. Biohydrogen production using organic waste is renewable energy, and waste treatment and energy production are possible at the same time. Food waste is one of the representative organic wastes and contains a large amount of carbohydrates, but hydrogen production can be inhibited by high concentrations of salt and volatile fatty acids (VFAs). In this study, the possibility of hydrogen production using food waste leachate (FWL) was investigated, and the effect of increasing productivity by supplementing a conductive material was confirmed. The total VFAs concentration of FWL used in this study was 40.2 g/L, which contained 27.8 g/L lactate, 5.9 g/L acetate, and 5.0 g/L butyrate. As a result of hydrogen production using 2-fold diluted FWL, hydrogen production, maximum hydrogen production rate, and lag time were 106.6 mL, 7.2 mL/h, and 8.1 h, respectively. In addition, when the magnetite (Fe_3O_4) was supplemented under the same conditions as the conductive material, it was 119.9 mL, 8.6 mL/h, and 6.3 h, respectively. From this, it means that FWL containing high concentrations of salts and VFAs can be used as a substrate for biohydrogen production, and the supplementation of conductive material can increase hydrogen productivity. This is expected to be applied as a technology for efficient energy production as well as reduction of organic waste.

Keywords: Biohydrogen, Conductive material, Food waste leachate, Magnetite, Organic waste

OPTIMIZING THE CULTURE CONDITIONS OF *RHODOPSEUDOMONAS PALUSTRIS* ISOLATED FROM THE PIGGERY WASTEWATER FOR TREATMENT EFFICIENCY IMPROVEMENT TOWARDS MALODOR-GENERATED HYDROGEN SULFIDE

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Abstract. Hydrogen sulfide (H_2S) is one of the most outstandingly malodorous and obnoxious odorants in breeding wastewater. Dealing with such malodors has been a major challenge for environmental technology. In this study, the native phototrophic purple bacteria from piggery wastewater were isolated and identified to be capable of removing H_2S (i.e. being a stinky and highly toxic agent), concurrently helping to treat the organic pollutants based on their autotrophic ability. Results indicated that *Rhodopseudomonas palustris* grew well on the SA media with 24/24-hour anaerobic conditions at 28–30^{0}C. Their biomass was reddish-purple, with a maximum absorption spectrum of 800nm as the specific characteristic of bacteriochlorophyll a. After 5-day cultivation, *R. palustris* reached the log phase at the rearing conditions of 2000-lux light intensity (24/24 hour), with the optimal pH of 7.0. They could grow well on the salinity thresholds of 1, 5, 10, 15, and 20‰. The appropriate sulfide concentrations for the best growth of such microorganism were 10−15mgS_2^-/L, achieving the H_2S removal efficiency of 99,9% after a 6-day culture. Conclusively**,** *R. palustris* was well adapted and proliferative towards the artificial culture medium. These results revealed a prominent utilization of such the native strain being isolated from pollutant wastewater for producing probiotics in order to treat the organic pollution and malodorous matters that impact human and animal health.

Keywords: phototrophic purple bacteria, *Rhodopseudomonas palustris*, breeding wastewater, organic pollution, malodor, odorants.

ANALYSIS OF ENVIRONMENTAL FACTORS AFFECTING AIRBORNE BACTERIA IN INDOOR AIR OF MULTI-USE FACILITIES IN KOREA

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Abstract: Since multi-use facilities are used by many people of various ages, it is important to strive to manage indoor air quality. There are numerous airborne bacteria in indoor air, some of which may be pathogenic and pose a threat to public health. In order to prevent this and effectively manage indoor air quality in multi-use facilities, it is important to investigate the interaction of microorganisms with the surrounding environment. Therefore, we analyzed bacteria by collecting airborne dust from the indoor air of multi-use facilities in cities in Korea. Sampling was conducted in summer and winter for a year, including kindergartens, libraries, stations, retails, subway compartment, train compartment. Bacterial DNA was extracted from the collected samples. Subsequently, the amount of bacteria was quantified using a quantitative polymerase chain reaction (qPCR), and the specific genera related to opportunistic infectious pathogens were qualitatively analyzed through next generation sequencing (NGS). At last, the correlation was analyzed by comparing it with surrounding environmental factors such as indoor temperature, outdoor temperature, relative humidity, user density, and land-use within a radius of 500m. As a result, it was confirmed that the total amount of bacteria was affected by the density of users of multi-use facilities ($p < 0.01$), and the total amount of bacteria differed depending on the facility group ($p < 0.01$). Subway compartments showed a significant difference from other facility groups particularly ($p < 0.05$). It was also confirmed that some of the genera of bacteria showed correlation with specific land use around them ($p < 0.05$).

Keywords: multi-use facility, indoor air quality, airborne bacteria, environmental factor, correlation

GENE EXPRESSION AND REDUCING TOXICITIES OF ZEBRAFISH EMBRYO EXPOSED TO NANO-TiO_2 AND NANO-POLYSTYRENE CAPTURED IN *AURELIA AURITA* EXTRACTS

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Abstract. Nanomaterials are produced intentionally or unintentionally during the life cycle from manufacturing to disposal, and they are known to be leaked and have potential risks to the environment and humans. In addition, an appropriate treatment and management method for unintentionally produced nanomaterials has not been established. Recently, interest in eco-friendly treatment of nanomaterials using biological materials has been increasing. In previous studies, we have confirmed that jellyfish (*Aurelia aurita*) extracts have the ability to capture and reduce nanomaterials in water. In this study, the risk before and after the capture of representative nanomaterials (Nano-TiO_2 and Nano-polystyrene) using jellyfish extracts was evaluated. The ability of jellyfish extracts to reduce morphological and genetic toxicity for both substances was analyzed through zebrafish embryogenesis and mRNA sequencing. Differentially Expressed Genes (DEGs) analysis was performed to select genes with significant expressed values (Fold change: ≥ 2 or ≤ 0.5). The major toxic pathways involved in DEGs were identified through GO enrichment analysis. As a result of the analysis, Nano-TiO_2 and Nano-polystyrene showed the most significant changes in gene expression in the metabolic process and developmental process, respectively. Jellyfish extracts have been found to reduce changes in gene expression in the toxic pathway, where the genetic change of each substance is the largest.

Keywords: jellyfish extracts, Nano-TiO_2, polystyrene nanoplastics, nano-toxicity, mRNA sequencing

INFLUENCE OF ENVIRONMENTAL FACTORS ON AIRBORNE MICROBIAL CONCENTRATIONS IN TRANSPORTATION FACILITIES

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Abstract. The concentrations of airborne microorganisms in indoor environments are generally significantly higher than in outdoor environments. Transportation facilities provide optimal conditions for microbial transmission due to their high population density and activity. Therefore, understanding airborne microbes in transportation facilities is crucial for promoting public health through the prevention of infectious disease transmission. We selected transportation facilities located in the Republic of Korea as sampling sites. Filtration methods were used for collecting airborne microbes. Amplicon sequencing was conducted to characterize the composition of the bacterial and fungal communities. In addition, we performed a quantitative polymerase chain reaction (qPCR) to quantify the concentration of fungi, bacteria, and five viruses associated with respiratory diseases. Using quantitative analysis data, regression equations were developed to analyze the relationship between airborne microbial concentrations and environmental factors. As a result of the multiple linear regression analysis, various factors, including population density, particulate matter, relative humidity, and temperature, showed a discernible influence on the concentrations of airborne microorganisms. The characteristics of the facility type had a more significant effect on airborne bacterial communities than seasonal factors, while seasonal factors had more significant effects on airborne fungal communities.

Keywords: Indoor air, Airborne microbes, Transportation facility, Microbial concentration

REGULATING CALCIUM LEVELS TO CONTROL THE PRODUCTION TIME AND MAINTENANCE OF BIOFILMS FORMED BY FILAMENTOUS CYANOBACTERIA.

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Abstract The biofilm system is a method of purifying water quality through the use of microalgae. It is gaining attention due to its ability to produce biomass while simultaneously purifying water. There are various types of microalgae that make up the biofilm. In many cases, filamentous cyanobacteria are initially attached to the material, followed by the attachment of other microalgae. Therefore, the attachment of cyanobacterial materials is the first step in constructing a biofilm and is crucial for its formation and maintenance. In this study, the correlation between calcium ions and biofilm formation time was investigated with the aim of enhancing productivity in filamentous cyanobacteria. Filamentous cyanobacteria *phormidium* sp., which were pure isolated cultures from nature, were used as the test spawn. The study confirmed that when calcium is adequately supplied, it grows extensively in a network structure. Conversely, when calcium is insufficient, it is characterized by self-aggregation. In particular, the mesh structure type exhibited approximately 47% higher biomass productivity and total nitrogen and total phosphorus removal efficiencies of 55% and 12%, respectively, showed higher water purification efficiencies within the same period. This study aims to propose an optimal culture medium that regulates the growth of filamentous cyanobacteria in a desired manner by controlling the calcium factor, leading to self-aggregation at a later stage. Through this, it is expected to increase biomass productivity while also improving the costly biofilm harvesting method.

Keywords: biofilm, filamentous cyanobacteria, calcium, biomass production, water treatment, microalgae.

CHITINOLYTIC PROTEINS SECRETED BY A MODERATELY THERMOPHILIC ACTINOMYCETES, *STREPTOMYCES THERMOLINEATUS* JCM6307

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Abstract. α-Chitin, the main component of crab shells, is a polysaccharide composed of N-acetyl glucosamine (GlcNAc). Because chitooligosaccharides have biological activity, chitin has attracted increasing attention as materials for biomass. The degradation of α-chitin currently relies on chemical hydrolysis that requires strong acid and high temperature. Therefore, the degradation process generates acidic waste and incurs high energy costs The issue can be addressed by replacing chemical hydrolysis with biological methods involving enzymatic chitin degradation. We have previously found that a moderately thermophilic actinomycete, *Streptomyces thermolineatus* JCM6307 (strain 6307), highly degrades α-chitin. In this study, we have identified the chitinolytic proteins secreted by strain 6307 by N-terminal amino acid sequence and genome analysis, and characterized them.

The growth of strain 6307 was remarkably improved when cultured in a chitin-supplemented medium, and efficient chitin degradation was observed. The reaction rate of reducing end releasing activity of culture supernatant for chitin powder was 0.04 μmol/h/mL, and high degradation activity for chitooligosaccharides was also confirmed. As a result of SDS-PAGE, the strain 6307 was found to newly secrete proteins of 45 kDa, 30 kDa and 20 kDa by culturing with the addition of chitin. N-terminal amino acid sequence revealed that the 45 kDa protein is a Glycoside Hydrolase (GH) family 18 chitinase, the 30 kDa protein is a GH family 19 chitinase, and the 20 kDa protein is a Lytic polysaccharide monooxygenase (LPMO). By genome analysis of the strain 6307, we clarified that this strain has 6 chitinase genes and 2 LPMO genes in the genome.

Keywords: *chitin, chitinase, Actinomycetes*

CONTROL OF PROTEIN SECRETION BY NUTRITIONAL CONDITIONS IN CULTURE OF CRAB SHELL DEGRADING BACTERIUM, *CELLULOSIMICROBIUM* SP. NTK2

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Abstract. *Cellulosimicrobium* sp. NTK2 (NTK2 strain) is a bacterium isolated as a crab shell- degrading bacterium from crab shell compost, and is expected to be used for the production of useful substances from unused chitin waste in the future. By the whole genome sequence analysis, NTK2 strain was found to have 8 chinases and 2 lytic polysaccharide monooxigenases in its genome. Having many chitinolytic protein genes enables efficient crab shell decomposition. However, the production of chitinolytic proteins and factors other than the presence of chitin has not been elucidated. To achieve chitinolysis and production of useful substances, more efficient production of chitinolytic proteins by the NTK2 strain is desired. In this study, we determined the nutritional conditions involved in chitin degradation in NTK2 strains and investigated the conditions for efficient chitin degradation.

As a result of investigating the effects of metals on the culture of the NTK2 strain, growth was remarkably improved by adding $MgSO_4$ to the medium at a final concentration of 1 mM. The growth of the NTK2 strain was enhanced by the addition of chitin, and the addition of 1 mM MgSO4 further enhanced the growth and dramatically increased the activity of the secreted chitinase. On the other hand, the addition of chitin increased the number of lysed cells due to the promotion of growth, resulting in significant release of DNA. By the addition of 1 mM MgSO4 to chitin-supplemented medium, NTK2 strain produced extracellular nuclease, and released DNA disappeared. These results suggest that degradation and recycling of released DNA induced by the addition of MgSO4 promoted further enhancement of proliferation of NTK2, leading to an increase in secreted proteins.

Keywords: Chitin, Chitinase, enzyme, magnesium

PRODUCTION OF POLYHYDROXYALKANOATES BY *SACCHAROPHAGUS DEGRADANS* FROM BROWN SEAWEED

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Abstract. The traditional method of producing polyhydroxyalkanoate (PHA) from waste biomass involves a pretreatment phase (using acid or alkali) to extract reducing sugars, followed by bacterial fermentation. This research aims to discover an environmentally friendly alternative for PHA production using brown seaweed. *Saccharophagus degradans* shows possibility as a bacterium capable of simultaneously producing reducing sugars and PHA, eliminating the need for pretreatment. By cultivating *S. degradans* in a membrane bioreactor to retain cells, PHA concentrations were approximately 4 times higher when compared to batch cultures that utilized glucose as a carbon source, and about 3 times higher when compared to cultures that used seaweed as a carbon source. X-ray diffraction, Fourier transform infrared spectroscopy, and nuclear magnetic resonance results revealed identical peaks for the resulting PHA and standard poly(3-hydroxybutyrate). This one-step process developed with cell retention culture of *S. degradans* has the potential to significantly enhance scalable and sustainable PHA production.

Keywords: polyhydroxyalkanoates, seaweed, cell retention culture, one-pot process

ISOLATION OF CELLULOSE-DEGRADING BACTERIA FROM SUGARCANE BAGASSE

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Abstract. The study isolated 8 strains of bacteria with potent cellulose degrading capability from bagasse samples collected from Bien Hoa Tri An Sugar Factory. The enzyme activity of the induced culture was observed on carboxymethyl cellulose (CMC) agar plates. Cellulase production was indicated by the appearance of a pale halo on agar medium containing CMC as substrate around the agar piece with colony on using lugol as an indicator. Cellulase activities of isolates were determined qualitatively by measuring the diameters of halo zones. The results showed that 3/8 bacterial strains had strong resolving power (halo zones ≥ 20 mm). The strain BH01 exhibited the maximum zone of clearance around the colony with diameter of 40mm was selected for species identification. The results showed that BH01 belongs to *Bacillus amyloliquefaciens* species with 100% similarity on NICB. The BH01 strain has the optimal growth conditions at pH 7 - pH 8, the temperature is from 35^0C – 40^0C.

Keywords: Bacillus amyloliquefaciens ,bagasse ,cellulose degrading, halo zones

STUDY ON CREATING PESTICIDE IMIDACLOPRID NANO ALGINATE AND SURVEY TO DECOMPOSITION TIME IN SOIL AND ON MUSTARD GREENS (*BRASSICA JUNCEA* L.)

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Abstract. Effective pest control and pesticide residues are two conflicting concerns in agricultural production and environmental protection. The rapid advancement of the nanotechnology industry has provided new perspectives for crop and environmental protection strategies. With the premise that it does not affect the insecticidal effect, it is crucial to facilitate the rapid decomposition of pesticide residues during the later stages. This study aim to deliver the insecticide imidacloprid in nanoformulation form to mustard greens (*Brassica juncea* L.). Composite nanoparticles, consisting of imidacloprid (IMI) in the outer layer and plant hormone 24-epibrassinolide (24-EBL) in the inner layer, were prepared by the W/O/W solvent evaporation method using a natural polymer as insecticide carrier. With this structure, the active element IMI is released very quickly under natural conditions. The pesticide nanoparticle alginate demonstrates relatively rapid decomposition time. On vegetables, IMI from alginate nanoparticles on vegetables decreased by 57% after 48 hours, whereas IMI from alginate nanoparticles combined with 24-EBL decreased by 72%; In soil, under natural conditions, IMI from alginate nanoparticles and IMI of alginate nanoparticles combined with 24-EBL after 48 hours reduced about 58%. Hence, the development of such pesticide nanoformulations will achieve sustained pesticide release, diminished the amount of residual pesticide levels in food, reduced the risk of environmental pollution, and enhanced safety for farmers.

Keywords: Nanoparticles, Imidacloprid, Environment, Decomposition time.

STUDY ON DIVERSITY AND ANTIFUNGAL ACTIVITY AGAINST PLANT PATHOGENS OF ACTINOMYCETES ISOLATED IN THAI NGUYEN, VIETNAM

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Abstract. The search for novel biological control strategies to inhibit the growth of plant pathogenic fungi has become widespread due to environmental concerns. Among them, actinomycetes have been studied extensively because they are recognized as an important source of antibiotic biosynthesis. In this study, actinomycetes strains were isolated from 40 soil samples in Thai Nguyen using selective media, after which they were evaluated for their antifungal activity against plant pathogens. The number of actinomycetes distributed in the soils ranged from 1,7 ± 0,67 × 106 to 12,07 ± 0,58 × 106 CFU/g soil. The proportion of strains in the gray group was 51,23%, the white group: 24,07%, the green group: 8,02%, the pink group: 5,56%, the purple group: 2,47%, and the yellow group: 1, 23%. Among the total actinomycetes isolates, the percentage of strains with antifungal activity was 70.73%. Two strains, TNA17.08 and TNA19.01, have strong antagonistic ability against all seven tested fungal strains. Strains TNA17.08 and TNA were studied for their morphological, physiological, and biochemical characteristics. Identification results based on 16S rDNA sequences showed that these two strains belong to *Streptomyces* and are scientifically named *Streptomyces* sp. TNA17.8 and *Streptomyces* sp. TNA19.01.

Keywords: antifungal activity, antibiotic biosynthesis, environmental concerns, plant pathogens, *Streptomyces*

ANALYSING MICROBIAL COMMUNITY DYNAMICS AND PHARMACEUTICALS DEGRADATION IN LAB-SCALE MBRS UNDER FLUCTUATING MICROPOLLUTANTS CONCENTRATION

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Abstract. Pharmaceutical are emerging contaminants (ECs), which are in some cases recalcitrant to biodegradation. The release of these biologically active compounds into the environment causes adverse effects on ecosystems and living organisms. Moreover, the pharmaceutical market is expected to grow, indicating increasing pollution scenarios. As bio-based solution, specific microbial communities have been identified as being able to degrade these persistent compounds and are representing a promising approach for a more sustainable removal of these compounds in wastewater. However, little is known regarding the adaptation of such microbial consortia for in situ implementation in wastewater treatment plants. One of the challenges in bioremediation is the fluctuation of wastewater composition and low concentration of pharmaceuticals in sewage inflow. Therefore, the goal of this study is to assess if a lab-adapted and efficient micropollutant- degrading microbial consortium is able to remove a combination of micro-pollutants under fluctuation stress which mimics the micropollutant concentration variability in a wastewater treatment plant. By using analytical methods combined with omics-technologies, we were able to demonstrate the degradation potential and microbial communities dynamics under fluctuating concentration of pharmaceuticals in wastewater. As matter of fact, microbial communities within membrane bioreactor increased the efficiency of pollutant removal of 100 mg/L pharmaceutical mixture over time and reached 100% after two months. Moreover, the adapted microbial communities were able to degrade 100% when the mixture of pharmaceuticals concentration was decreased to 1 mg/L and in five out of six MBRs when the concentration of pharmaceuticals was set again to 100 mg/L. Finally, the dynamic of the microbial communities boosted the pharmaceuticals mixture degradation over time. As the relative abundances of *Achromobacter* and *Burklholeida* decreases and of other genera increases, such as *Xanthobacter*, *Leucobacter*, and *Reynella*, the two more recalcitrant pollutants concentration decrease, showing a higher removal efficiency.

Keywords: pharmaceutical bioremediation, microbial communities, MBR, omics techniques.

16S-ONT-PROFILER, A COMPREHENSIVE PIPELINE FOR SPECIES- LEVEL MICROBIAL COMMUNITY PROFILING OF 16S RRNA NANOPORE SEQUENCING DATA

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Abstract. 16S metabarcoding, an approach allowing accurate and rapid identification of bacteria in a sample, is used widely in biotechnology, ecology, agriculture, environment conservation, and infectious disease diagnosis. Applying short-read sequencing, a current prevalent technology, on the 16S rRNA gene, only permits analysis of several sub-regions, which sometimes causes ambiguity in taxonomic classification. In a noteworthy stride forward, long-read sequencing with Nanopore technology has emerged as a promising solution, now we can yield reads covering the entire 16S rRNA gene. Nevertheless, comparatively high error rate of this technology seem to be a barrier. Here, we present 16S-ONT-Profiler, a comprehensive pipeline to produce precise taxonomic at species level from full-length 16S rRNA Nanopore reads. The pipeline consists of both alignment-based and alignment-free approaches with appropriate algorithms and a well-curated in-house database. Our performance evaluation on simulated datasets, mock community and environmental samples suggested an improved accuracy of our 16S-ONT-Profiler. The 16S-ONT-Profiler pipeline was written in Nextflow that can be deployed on any computing environment such as HPC, public clouds (AWS, Google Cloud, Azure), and even on a modest laptop..

Keywords: Metagenomics, 16S metabarcoding, ONT, Nextflow

BIOFILM INHIBITION EFFECT ON *PSEUDOMONAS AERUGINOSA* PA14 USING MUSHROOM EXTRACT

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Abstract. Aggregated bacteria grow on diverse environmental niches, industrial and clinical settings in the form of a three-dimensional biofilm structure. Biofilms are composed of a single or multiple species of bacteria embedded in extracellular polymeric substance (EPS), which affects increasing antibiotic resistance through restriction of the transport of antibiotics to the bacteria cells. An alternative approach to treatment with antimicrobial agents is using biofilm inhibitors that regulate biofilm development without inhibiting bacterial growth. In the present study, we found various mushroom extract's ability to inhibit *Pseudomonas aeruginosa* PA14 biofilm formation. The results showed that biofilm formation was reduced by mushroom extracts (*Lichtheimia hyalospora* and *Tyromyces kmetii*) in a concentration-dependent manner without affecting bacterial growth by static biofilm assay. Furthermore, mushroom extracts decreased production of extracellular polymeric substances, whereas increased bacterial swarming motility. Taken together, these finding suggest that mushroom extracts as a biofilm inhibitor have new potential for pharmacological and industrial applications.

Keywords: Mushroom extract, Biofilm Formation, Extracellular polymeric substances, Biofilm inhibitor

THE ROLE OF INTRACELLULAR METALS FOR RADIATION SENSITIVITY OF YEASTS RELATED TO ROS MANAGEMENT WITHIN THE CELLS

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Abstract. Manganese (Mn) plays an important role in activities of cells, including protects cells from oxidative damage. We isolated *Saccharomyces cerevisiae* IM3, which has four-to-five-fold Mn accumulation ability than the parental strain BY4741. IM3 showed significant manganese accumulation under the cultivation with manganese containing media and the less resistant to gamma-irradiation than BY4741. In the case of BY4741, Mn supplementation into nutrient medium showed significant resistant to ROS cause by some sources, such as gamma-irradiation, menadione, and hydrogen peroxide.

We also examined the gamma-ray sensitivity of other *Saccharomyces* strains, EC1118, AKU4102, IFO 2044 (Oriental yeast), NA87-11A, and DKD-5DH, in addition to Association yeast No. 7 from Brewing Society of Japan, in the presence of cadmium, and found that several strains showed increased radiation resistance against gamma rays. In this study, we will report ROS amount and antioxidation enzymes activities in relation to the gamma-ray sensitivity of these seven yeast strains cultured in the presence of copper or zinc. Copper accumulation within the AKU4102 strain increase the resistance to gamma rays while NA87-11A strain was more sensitive to gamma rays than the AKU4102 strain in the absence of copper. In the case of zinc, there was no change in resistance to gamma radiation compared to the no-additive medium. We are currently examining ROS ammout and activities of antioxidation enzymes in the yeast cells in relation with copper or zinc accumulation.

Keywords: Intracellular Manganese, Copper, Zinc, oxidative stress, *Saccharomyces cerevisiae*, Gamma irradiation, Reactive oxygen species (ROS)

THE ROLE OF ARYL HYDROCARBON RECEPTOR (AHR) IN THE MIGRATION OF GLIOBLASTOMA THROUGH AHR-IL24 AXIS

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Abstract. Cancer occurrence and development are closely related to the environment. Aryl hydrocarbon receptor (AhR) is an important receptor mediating the toxic effects of many environmental compounds and is also involved in regulating tumor cell migration. Glioblastoma is the most malignant glioma and exhibits high motility, but the effects of AhR on the migration of glioblastoma are still unclear. We aimed to understand the role of AhR in the migration of this type of tumor cell and to explore the underlying molecular mechanism. In cultured human neuroblastoma cells (U87), we found that AhR overexpression or knockdown increased or suppressed the migration ability of U87 cells, respectively. Furthermore, inhibition of basal activation of the AhR pathway suppressed migration ability, suggesting a positive correlation between endogenous activity of the AhR pathway and cell migration. When the AhR pathway was activated by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or 6-formyl [3,2-b] carbazole (FICZ), the migration of U87 cells was inhibited by inducing the expression of a tumor suppressor, IL24, which is a downstream responsive gene of AhR activation. Moreover, a similar AhR-IL24- dependent mechanism for migration inhibition of TCDD was documented in a breast cancer cell line and a lung cancer cell line. This study demonstrated that AhR plays important roles in regulating the migration of glioblastoma, and the induction of the AhR-IL24 axis mediates the inhibition of migration in response to TCDD or FICZ treatment.

Keywords: Aryl hydrocarbon receptor, Interleukin 24, Migration

DIOXIN INDUCED EXPRESSION AND ACCUMULATION OF NEUROFILAMENT LIGHT CHAIN IN NEURONAL CELLS

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Abstract. Dioxin and dioxin-like compounds form a group of persistent organic pollutants that can accumulate in humans and animals and persist for an extended period. Dioxin exposure is reported to affect nervous system development and increase the risk of neurodegenerative diseases. Generally, dioxin exerts its neurotoxicity via aryl hydrocarbon receptor (AhR). Neurofilament (NF) light (NFL) protein is a biomarker for both neuronal differentiation and neurodegeneration and its expression is controlled by the mitogen-activated protein kinase (MAPK) pathway. However, the effects of dioxin on NFL expression and involved mechanisms are incompletely understood. We aimed to investigate the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on NFL expression and elucidate the underlining signaling pathways and their potential crosstalk, specifically between MAPK and AhR pathway. We employed primary cultured rat cortical neurons to evaluate the effect of TCDD exposure on NFL expression. We also used nerve growth factor (NGF)-treated PC12 cells with specific inhibitors to investigate the involvement of and potential crosstalk between the MAPK pathway and the AhR pathway in mediating the effects of TCDD on NFL expression. After TCDD exposure, NFL mRNA and protein levels were upregulated in cultured neurons. NFL protein was preferentially found in the cell body compared with neurites of the cultured neurons. In PC12 cells, TCDD enhanced both NGF-induced NFL expression and phosphorylation of ERK1/2 and p38. The addition of MAPK-pathway inhibitors (PD98059 and SB230580) partially blocked the TCDD-induced NFL upregulation. CH223191, an AhR antagonist, reversed the upregulation of NFL and phosphorylation of ERK1/2 and p38 induced by TCDD. This study demonstrated TCDD-induced upregulation of NFL in cultured neurons, with protein retained in the cell body. TCDD action was dependent on activation of AhR and MAPK, while crosstalk was found between these two signaling pathways.

Keywords: Persistent Organic Pollutants; Dioxin; neurofilament; aryl hydrocarbon receptor (AhR); MAPK

CONTROL THE INFECTION OF *EDWARDSIELLA ICTALURI* USING *BACILLUS AMYLOLIQUEFACIENS* AGWT 13-031, AN EFFECTIVE SOLUTION AGAINST ENTERIC SEPTICEMIA IN TRA CATFISH (*PANGASIANODON HYPOPHTHALMUS*)

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Abstract. The aim of this study was to investigate the efficacy of *Bacillus amyloliquefaciens* AGWT 13-031 strain in inhibiting the growth of *Edwardsiella ictaluri* causing enteric septicemia (ESC) in catfish. *In vitro* results showed that *B. amyloliquefaciens* AGWT 13-031 effectively inhibited the growth of pathogens in agar medium with the inhibition zone diameter of 20 ± 1 mm. *E. ictaluri* was not detected after a 10-hour co-incubation in broth medium with *B. amyloliquefaciens* AGWT 13-031, both of which were added at the same concentrations. The pilot experiments showed that the addition of *B. amyloliquefaciens* AGWT 13-031 strain at a concentration of $1x10^5$ CFU mL^{-1} into two-month-old tra catfish rearing tanks for 72 h before the experimental challenge with *E. ictaluri* decreased fish mortality rates to 26.74%, compared to the control fish (86.07%), leading to a relative percent of survival (RPS) value of 68.93%. The use of *B. amyloliquefaciens* AGWT 13-031 in controlling *E. ictaluri* suspension for 48 h also significantly reduced catfish mortality rates in comparison with the control fish ($p<0.05$), proving RPS values of 88.52%. In the other hand, when applying *B. amyloliquefaciens* AGWT 13-031 in fry rearing tanks at day 5 and 10, this probiotic strain protected catfish against ESC after the experimental challenge with *E. ictaluri* at the RPS value of 54.14%. These findings suggested that *B. amyloliquefaciens* AGWT 13-031 could be used as a potential probiotic strain against ESC in catfish farms in Vietnam.

Key words: Bacillus amyloliquefaciens, enteric septicemia, probiotic in catfish

PARACETAMOL DEGRADATION IN DUAL-CHAMBER MICROBIAL FUEL CELL: EFFECT OF PARACETAMOL CONCENTRATION AND ANODE ENVIRONMENT

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Abstract. Paracetamol (PCT) is an analgesic drug that is currently used freely and widely by the public to reduce symptoms of pain and fever in the body. The COVID-19 pandemic has caused the use of PCT to continue to increase for healing. This increase in consumption led to the emergence of PCT compounds in the waters of Angke and Ancol of 0.42 µg/L and 0.61 µg/L. This study aims to study the potential for PCT degradation in the anode environment of the Microbial Fuel Cell (MFC) system using a consortium of the University of Indonesia Lake Mahoni mud bacteria. Experiments were carried out by varying the initial concentration of PCT and the pH of the anode environment. The results showed the PCT degradation in this system within 72 hours. PCT degradation of 13.47 ± 1.09%; 11.02±5.43%; and 28.54 ± 18.84% were obtained for variations in the initial concentration of 10.21; 20.24; and 31.45 mg/L, respectively. The trend of PCT degradation at higher concentrations was thought to be due to the different initial numbers of bacteria during the experiments. PCT degradation was also obtained at various pH of the anode environment 5.8; 7.0; and 8.2 of 31.31 ± 3.54%; 11.02±5.43%; and 48.69 ± 0.86%. Acidic pH conditions reduced the metabolic activity of the bacterial consortium in degrading PCT. A closed circuit system with a load resistance of 1000 ohms was used in this study to see the ability of the system to generate electric voltage. Microbial community analysis was also performed using the Next Generation Sequencing (NGS) 16s rRNA method. *Burkholderia* sp. is a bacterium that dominates the reactor containing PCT. These results can be used to develop technologies that are more efficient in removing PCT from pharmaceutical wastewater.

Keywords: COVID-19, dual chamber microbial fuel cell, microbial consortium, paracetamol, pollutant degradation

ENHANCED ANTIBACTERIAL ACTIVITIES OF TUNGSTEN OXIDE DECORATED WITH SILVER NANOPARTICLES

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Abstract. Nanoparticles have received significant attention in recent years for their potential to enhance the effectiveness of antimicrobial therapies. In this study, silver-decorated tungsten oxide (Ag@WO_3) nanoparticles were synthesized using a facile one-pot hydrothermal method, resulting in nanoflakes size of 70÷80 nm. These nanoparticles process a large surface area and exhibit higher visible-light harvesting ability, making them preferable for visible-light-driven, surface-driven, and antibacterial applications. The antibacterial ability of Ag@WO_3 nanoparticles was evaluated on the Gram-negative bacteria *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Antibacterial activity of WO_3 was enhanced by more than 3-times by decorating with 7% Ag. In addition, other factors such as concentration of nanomaterial and exposure time significantly affected antibacterial activity. Photocatalytic experiments revealed the light-absorbing properties of Ag@WO_3. Under visible light illumination, 100 ppm of Ag@WO_3 caused 100% cell dead for *E. coli* and 99.1% for *S. aureus*. Moreover, the evaluation of Ag@WO_3 nanoparticle's cytotoxicity on human hepatoma cell line (HepG2) showed that Ag@WO_3 was noncytotoxic to the cells. These results suggest promising potential of Ag@WO_3 nanoparticles for application in medicine and environmental aspects.

Keywords: Antibacterial activity, Ag@WO_3, Nanoparticles, cytotoxicity, hydrothermal, photocatalytic.

STUDY OF THE IMMOBILIZATION OF *TRICHODERMA VIRENS* ON BIOCHAR CARRIER DERIVED FROM RICE HUSK

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Abstract. Biochar possesses advantageous characteristics as a carrier for microorganisms, including a distinctive pore structure and large specific surface area. These properties enable it to provide protective shelter for microorganisms against detrimental environmental factors such as ultraviolet radiation and the competitive influences exerted by indigenous soil microflora. The aim of this study was to determine parameters for the immobilization of *Trichoderma virens* onto biochar carrier derived from rice husks, intended for the production of microbial products. All the experiments conducted within this study were carried out under controlled laboratory conditions. The study involved investigating the process of biochar pretreatment to create a microbial carrier for *Trichoderma virens*. In addition, the investigation of microbial immobilization parameters including: moisture content, bacterial density, incubation duration, and carrier composition. To immobilize *Trichoderma virens*, rice husk biochar treated with 0.07M HCl was used; the initial moisture content of the biochar was 50%; the initial bacterial density ranged from 10^6 to 10^7 CFU/mL; the incubation duration was 7 days; and the carrier composition comprised 50% (w) biochar and 50% (w) cornstarch powder. Results showed that after six months of storage, formulations of *Trichoderma virens* could remain the cell densities at over 10^6 CFU/g under room temperature conditions. These findings suggest that biochar can serve as a carrier to formulate and deliver microbial inoculants with potential for agricultural and environmental applications.

Keywords: Biochar, carrier, immobilization, rice husk, *Trichoderma virens*.

EVALUATION OF SPENT MUSHROOM SUBSTRATE AS REPLACEMENT OF PEAT IN PESTICIDE-DEGRADING BIOMIXTURE

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Abstract. Biobeds are bioprophylaxis systems to prevent pesticide point-source contamination. The original Swedish model comprises a clay layer at the bottom, a biomixture layer, and a grass layer on top. The composition of biomixture consisting of soil: wheat straw: peat (1:2:1 by volume) determines the efficiency of the biobed. Peat is a scarce material of high cost and may be subject to restrictions in the future, so a replacement material with similar properties must be found. This study evaluated the potential of replacing it with a locally available material, spent mushroom substrate (SMS). Three biomixtures containing different SMS (*Pleurotus eryngii*, *P. pulmonarius* and *Lentinus edodes*) were compared with a biomixture containing peat, as in the original Swedish design, on their microbial activity (measured as microbial population, respiration and ligninolytic activity) and degradation efficiency of pesticides (Chlorpyrifos, 2,4-D, Cartap and Cypermethrin). The microbial population was determined by the plate counting method, respiration was assessed by CO_2 evolution via the alkaline trap method and ligninolytic activity was determined using the MBTH/DMAB assay. The concentration of pesticides was identified by the QuEChERS method. The results showed that microbial activity and pesticide degradation efficiency were higher in the three biomixtures containing SMS than in the original type biomixture. The biomixture containing SMS from *P. pulmonarius* was the most biologically active. However, no significant difference in pesticide degradation efficiency was observed between SMS biomixtures. Based on the physicochemical characteristics, biological activity and preliminary results on pesticide degradation efficiency, SMS is suitable and can therefore be used as a replacement for peat.

Keywords: biobed, biomixture, peat, pesticide, spent mushroom substrate

EFFECTS OF DIFFERENT CELL TYPE CO-CULTURES ON THE PREIMPLANTATION DEVELOPMENT OF CLONED BOVINE EMBRYOS

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Abstract. Somatic cell nuclear transfer (SCNT) is a reproductive technique used to clone organisms by transferring the nucleus of a somatic cell into an enucleated oocyte. However, SCNT embryos have been found to have significantly lower developmental competence compared to *in vitro* fertilized embryos. To improve the development of cloned bovine embryos, researchers have explored the use of coculture techniques with different cell types. Among these techniques, coculture systems utilizing fibroblast cells have been found to enhance the development of SCNT embryos. Specifically, granulosa cells (GCs), cumulus cells (CCs), and mesenchymal stem cells (MSCs) cultured with synthetic oviduct fluid medium (mSOF) and fetal bovine serum (FBS) from day 3 to day 7 have been studied for their potential to promote embryo development. Studies have demonstrated that coculture with CCs can have a significant positive effect on the development of cloned bovine embryos. In one study, embryos cocultured with CCs from day 3 to day 7 displayed the highest blastocyst formation rates (45.83%) and the highest quality of embryos compared to those cocultured with GCs (41.45%), MSCs (42.00%), and free cells (21.00%). To analyze the differences between the means of the groups, a Tukey's test was employed for multiple pairwise comparisons in One-Way ANOVA. Overall, these findings suggest that coculture with CCs may be a promising strategy for improving the developmental potential of cloned bovine embryos. From there, it is not necessary to completely remove the surrounding cumulus cells of the matured oocyte, and retaining some cells is essential to create the culture conditions used to support the development of cloned embryos.

Keywords: Co-cultured, Cumulus cells, fibroblast, *in vitro* fertilized, mesenchymal stem cells, granulosa cells, Somatic cell nuclear transfer.

INVESTIGATION OF MULTIDRUG RESISTANT BACTERIA AT HOSPITAL WASTES AND AQUATIC HABITATS IN DHAKA, BANGLADESH

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Abstract. Multidrug resistance (MDR) has become a serious worldwide threat to public health as a significant number of antimicrobial agents are not working against disease causing bacterial pathogens which is alarming. Irrational use of antibiotics in hospitals provokes drug resistance leading to the dissemination of resistant gene in hospital wastewater (HWW). With increased worries about the threat to public health, the significance of the environment as a key reservoir for AMR transmission to both humans and animals is being acknowledged on a worldwide level. Unmetabolized antibiotics in low concentrations are released through urine and feces from treated patients and animals, of which a large proportion passes directly to the environment leading AMR in environmental microbiota. The present study was aimed to evaluate the frequency of multidrug resistant bacteria from HWW because of the possibility of antibiotics being released in wastewater indiscriminately and from freshwater habitats.

Wastewater and sediment samples were collected during August to December 2022 from six government and five private hospitals and from 11 sampling sites including 7 ponds, 3 lakes and 1 river in Dhaka city. A total of 22 samples were processed with Peptone Yeast Glucose (PYG), Eosine Methylene Blue (EMB), Xylose Lysine Deoxycholate (XLD) and cetrimide agar media for eneumeration and isolation of bacteria. Bacterial load of wastewater samples on PYG, EMB, XLD and cetrimide agar media for both types of hospitals ranged 7.5 × 10 - 1.8 × 10^5, 9.2 × 10^2 - 1.1 × 10^5, 4.2 × 10^3 - 8.1 × 10^4 and 1.6 × 10 - 6.1 × 10^3 cfu/ml, respectively. In the case of sediment samples, the range of bacterial load was 4.9 × 10^5 - 2.09 × 10^7, 8.2 × 10^4 - 7.4 × 10^6, 4.1 × 10^4 - 5 × 10^6 and 1.45 × 10^3 - 2.1 × 10^5 cfu/g, respectively. During this study, a total of 397 bacterial colonies were isolated of which 51 isolates were screened for further research. All of the isolates were gram negative, aerobic, facultative anaerobic, non-spore former. The most frequently identified bacteria based on their biochemical test were *Enterobacter* spp. (18), *Pseudomonas aeruginosa* (7), *Salmonella* spp. (6), *Citrobacter freundii* (5), *Klebsiella pneumoniae* (3), *Salmonella typhi* (3), *Proteus vulgaris* (3), *Enterobacter cloacae* (1), *Escherichia coli* (1), *Citrobacter* spp. (1), *Serratia marcescens* (1), *Proteus mirabilis* (1) and *Providencia alcalifaciens* (1). Molecular identification of 16S rRNA gene was also carried out to 4 of the isolates.

Fifty isolates were assessed against 15 antibiotics of different disk concentrations for antibiotic susceptibility test following Kirby-Bauer disk diffusion method. All the isolates except 6 showed multidrug resistance (88%). The prevalence of MDR in government hospitals was found to be 23/25 (92%) while in private hospitals was found to be 22/25 (88%). The isolates (96%) showed highest resistance to flucloxacillin, followed by vancomycin (90%), penicillin (90%), tobramycin (90%), clindamycin (88%), kanamycin (88%), gentamycin (86%), cephalexin (84%), cefuroxime (82%), moxifloxacin (80%), azithromycin (80%), ciprofloxacin (76%) and erythromycin (74%). Tetracycline (62%) and chloramphenicol (54%) were found to be the most susceptible antibiotics compared to other antibiotics. Among the isolates, one *Escherichia coli*, one *Salmonella* sp. and one *Citrobacter freundii* showed 100% resistance to all the used antibiotics highlighting the indiscriminate use of antimicrobials. The results indicated that maximum identified bacteria developed resistance to multiple classes of antibiotics. To avoid dissemination of multidrug resistant bacteria, hospital wastewater should be treated properly before released into the environment as well as implementation of antibiotic stewardship programs is necessary to ensure appropriate use of antibiotics.

Key words: Bacteria, hospitals, aquatic habitats, MDR

INHIBITION OF *MICROCOCCUS LUTEUS* BIOFILM FORMATION BY BACTERIOPHAGE ISOLATION FROM AQUACULTURE PONDS

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Abstract. The bottom of aquaculture ponds, there are many different species of bacteria, besides pathogenic bacteria and biofilm-forming bacteria. Bacteria living inside biofilms are resistant to antibiotics at concentrations thousands of times higher than free-living bacteria, and this is also a hiding place for many pathogens. The study isolated bacteria STM1 from the bottom slime of aquaculture ponds intercropping with shrimp and saltwater fish in My Xuyen district, Soc Trang province, Vietnam. Bacteria STM1 are tetrad cocci, Gram-positive, non-motile, colonies are yellow on TSA medium, positive for catalase, oxidase, do not ferment glucose and mannitol. Through morphological observations and biochemical reactions, STM1 bacteria have been identified with the species *Micrococcus luteus*. The study also isolated 4 bacteriophage strains capable of infecting *Micrococcus luteus* STM1. All were able to inhibit biofilm formation at salinities between 5 and 20 ppt. In addition, when observed under the microscope bacteriophages pTV2, pTV5, pST9Δ1 and pST9Δ2 both reduced the number and size of microcolony of STM1 bacteria compared with the control treatment. The phage pST9Δ2 strongly inhibited the biofilm of *Micrococcus luteus* STM1 and reached 82% at 15 ppt salinity. Observation of phage morphology under electron microscope (SEM) has similar morphology with short-tailed phage of family Podoviridae, with average measured size ranging from 55 to 60 nm.

Keywords: bacteriophage, *biofilm, Micrococcus luteus, Podovirida*

SYNTHESIS OF IR780-CHLORAMBUCIL AS A PHOTOSENSITISER FOR TREATING MCF-7 AND HEPG2 CANCER CELLS

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Abstract. Heptamethine Cyanine dye have been used for detection and real-time monitoring of cancer. IR-780 iodide has been used as an ideal platform to construct theranostic agents for cancer imaging and therapy. Chlorambucil (CHL) is an effective chemodrug for cancer therapy which have been ultilized for conjugation to synthesis new drug. Thus, in this study we report the new synthesis drug IR780-CHL for phototherapy purpose. The new synthesis IR780-CHL has been confirm the structure by liquid chromatography – mass spectrum (LC-MS) and 1H nuclear magnetic resonance (1H NMR). The LC-MS findings indicated a mass of 616.35 MW, which corresponded to our predicted fraction. While the ^{1}H NMR demonstrated the presence of resonant peaks in IR780-CHL that are exclusive to CHL and IR780. UV-Vis measurements have revealed that the absorbance of IR780-CHL has moved to 756 nm instead of 780 nm. Before and after laser activation, the toxicity of IR780-CHL was comparable to that of CHL, indicating that the synthesis did not influence the in vitro effectiveness of the drug treatment. The development of IR780-CHL has considerable potential for cancer detection and treatment.

Keywords Phototherapy, IR780, Chlorambucil, Cancer, Drug release

DEVELOPMENT OF ORALLY pH-SENSITIVE REDOX NANOTHERAPEUTICS FOR TREATMENT OF GASTRIC ULCER

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Abstract. Gastric ulcer, a gastrointestinal disorder leading to both morbidity and mortality, has widely spread and impacted millions of lives around the world. Although the pathophysiological mechanism is not fully understood, the oxidative imbalance caused by the excessive production of reactive oxygen species (ROS) has been considered a central driving factor. In this study, two novel redox nanoparticles, including pH-sensitive RNP^N, and pH-insensitive RNP^O with several tens of nanometers in size, have been designed as oral nanotherapeutics for gastric ulcer through scavenging activities of ROS. Both RNPs exhibited different free radicals scavenging capacities *in vitro*. In addition, the acidic pH resulted in the protonation of amino linkages in the hydrophobic core and the disintegration of RNP^N structure. Consequently, the enhancing exposure of nitroxyl radical groups (ROS scavengers) led to the stronger antioxidant capacity of RNP^N under gastric pH. In contrast, RNP^O containing ether linkages stabilized under different pH environments. Moreover, orally administered RNP^N *in vivo* showed greater accumulation and retention compared to that of RNP^O owing to its pH-responsive properties. Hence, RNP^N indicated more effective protections against gastric inflammation and lipid peroxidation in aspirin-induced gastric ulcer in mice. Furthermore, toxicity and morphology changes were not observed in zebrafish embryos during 120 h of exposure to both RNPs. Therefore, RNP^N with more effective *in vitro* and *in vivo* antioxidant activities is a promising oral nanotherapeutics for gastric ulcer therapy.

Keywords: ROS, pH-sensitive, redox nanoparticles, gastric distribution, gastric ulcer, zebrafish toxicity.

CREATION OF A NON-INVASIVE MALARIA VACCINE AND ELUCIDATION OF THE IMMUNE MECHANISM

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Abstract

Introduction. Malaria is an infectious disease caused by the *Plasmodium* species, which invade the human body during blood sucking of mosquitoes. Vaccination of malaria can decrease the risk of malaria, however, the areas threatened by malaria are mainly in developing countries. In such countries, the medical system is often inadequate, and there are problems such as a lack of medical personnel to administer the injections.

In this research, we aimed to develop a non-invasive transdermal malaria vaccine. We used solid-in-oil (S/O) technology to deliver antigens into the body through the skin and attempted to develop a transdermal formulation of malaria vaccine. In this presentation, we report on the antibody production capability of the S/O formulation encapsulating Pvs25, a malaria antigen.

Experiments. Pvs25 solution in water was added to surfactant/cyclohexane solution and homogenized to form emulsion, which was then lyophilized. The lyophilized Pvs25/L-195 complex was dispersed in isopropyl myristate to prepare the S/O formulation encapsulating Pvs25. To evaluate the vaccine efficacy of S/O formulation of Pvs25, the backs of BALB/c mice were dehaired and patches containing the prepared S/O formulation were applied for 24h for transdermal administration. For comparing with injection, Pvs25 solution including Alum, immunostimulant was injected to back of mice by subcutaneous injection. Blood samples were collected over time from the last administration, and IgG against Pvs25 in serum was quantified by enzyme linked immunosorbent assay.

Result & Discussion. The results of antibody titer measurement, injection group showed high antibody titer from the early stage of blood collection. On the other hand, S/O patch group showed significant increase in antibody titer from the day4 after immunization, and the serum titer on the day46 was comparable to that of injection group. These results suggested that the S/O formulation of Pvs25 could be used as a transdermal malaria vaccine.

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Keywords: Transdermal Drug Delivery System, Vaccine

ENHANCED OVEREXPRESSION OF SECRETED ENZYMES BY DISCRETE REPEAT PROMOTERS IN *STREPTOMYCES LIVIDANS*

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Abstract. *Streptomyces lividans* is an efficient host for extracellular overproduction of recombinant proteins. To enhance the overexpression strength of *S. lividans*, we designed several kinds of expression plasmid with different positioning of repeat promoters. The effect of repeat promoters was evaluated by measuring the accumulated amounts of a stable transglutaminase or an unstable carboxypeptidase that was secreted into the medium. In this study, we used our strong promoter of *scmp*Pc, which was from *S. cinnamoneus* TH-2 (M. Uraji et al., *Biosci. Biotech. Biochem.* 86(8) 1122-1127(2022)), and another strong promoter of *kasO**P. Successive tandem position of repeat promoter upstream the normal promoter did not enhance the expression of transglutaminase. Discrete position of repeat promoters both upstream and downstream the normal promoter enhanced the expression of transglutaminase to two-fold; and the downstream ones also enhanced the expression of carboxypeptidase to 1.7-fold. On the hand, there were still some constructs of plasmids with tandem and discrete promoters that did not promote the expression of the target enzymes, indicating the complexity of the mechanisms of repeat promoters working on gene expression. To further improve the usability and productivity of *S. lividans*, we constructed a dual gene expression vector of “pTSKr duet” containing the two strong constitutive promoters. The “pTSKr duet” vector can realize the co-expression of two genes simultaneously and independently (L. Yang and T. Hatanaka, *Biosci. Biotech. Biochem*. 87(3) 349-357(2023)).

Keywords: *Streptomyces*, recombinant protein, extracellular overproduction, gene expression, repeat promoters

ENZYME PRODUCTION FOR LEATHER PROCESSING

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Abstract. Proteases are one of the most important enzymes in industrial application; they hydrolyze peptide bonds between two amino acids of a polypeptide chain. Because leather manufacturing is one of the most polluting industries, enzymes are studied and applied in the industry as a greener alternative to the conventional process. This study aims to use protease obtained from recombinant *E. coli* BL21(DE3)-pET32a-asp and *B. halodurans* BCRC 910501 for leather processing. After bacterial enzyme cultivation, protein concentration, activity and molecular mass were evaluated. Crude enzymes were used for leather soaking and dehairing to evaluate the possibility of enzyme use in leather processing. Results showed different protein concentrations in the supernatant (extracellular protein) depending on bacteria. Enzyme activity assay indicated lower B. *halodurans* specific caseinolytic and keratinolytic activity compared to *E. coli*. SDS PAGE showed protein molecular masses. Also, after induction it was observed that protein with 48 kDa molecular mass were still in the E. *coli* cells membrane. According to the soaking findings, protease from *B. halodurans* might be suitable for leather processing, however, extracellular protease from recombinant E. coli did not show any effect in dehairing.

Keywords: enzymes, leather, B. *halodurans*, E. *coli*.

THE EFFECT OF *ORTHOSIPHON ARISTATUS* EXTRACT AND *PERILLA FRUTESCENS* EXTRACT ON GUT MICROBIAL COMMUNITIES BY FLOW CYTOMETRIC FINGERPRINTING

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Abstract. Traditional medicine develops significantly nowadays in Vietnam, some remedies support digestion and increase health. *Orthosiphon aristatus* extract and *Perilla frutescens* extract are used widely, and there is a lot of research on plant extract's pharmacological effect. However, the insight into the *Orthosiphon aristatus* and *Perilla frutescens* extract impact on gut microbiome composition is limited. Conventional methods such as heterotrophic plate count, selective plating, and molecular techniques could be difficult to distinguish the fecal samples of mice using plant extracts from mice using saline, and those methods are time-consuming and labor intensive. In this study, a flow cytometry (FCM) based approach was used for its fast and objective comparison of microbial communities. The method consists of two steps, firstly mouse *Swiss albino* used plant extracts for a period of time, and its fecal samples were collected each day. Secondly, the analysis of the community of gut microbiota by flow cytometry. The data indicate differences in alpha or beta diversity of *Swiss albino*'s fecal samples that used plant extracts compared to mice that did not use plant extracts. Our results show the potential of the flow cytometric fingerprinting method in understanding the impact of plant extracts on the composition of microbial communities.

Keywords: flow cytometry, gut microbiota

A STUDY ON ANTI-INFLAMMATORY EFFECTS OF *PSORALEA CORYLIFOLIA* STIMULATED BY *ENTEROBACTER AEROGENES* DERIVED FROM HUMAN SKIN FLORA

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Abstract. The *Psoralea corylifolia* L. is an important medicinal plant with thousands of years of clinical application. It has been widely used in many traditional Chinese medicine formulas for the treatment of various diseases. So we present work was methylene chloride fraction from *P. corylifolia* (MCF-P) and quantified this indicator bakuchiol. As a result of HPLC analysis, it was confirmed that the content of bakuchiol was 27.6%. Remarkably, we studied the anti-inflammatory effects of MCF-P and Bakuchiol after treating ferment of *Enterobacter aerogenes* J2K-739 strain, derived from skin flora, to induce inflammatory factors. In order to verify the inflammatory relief effect of MCF-P and bakuchiol, that measurements of nitric oxide (NO) by using Griess reaction assay. The mRNA expression level of the inflammatory factors was confirmed through real-time PCR analysis. And mRNA expression level of iNOS, IL-6, IL-1β, COX-2, and TNF-α were significantly decreased in the concentration - dependent manner during MCF-P treatment. Through these results, it is believed that the *P. corylifolia* can be used as a natural cosmetic ingredient that has the effect of inhibiting inflammation.

Keywords: Psoralea corylifolia, bakuchiol, *Enterobacter aerogenes*, skin flora, anti-inflammation

GENOME SEQUENCING AND RNA-SEQ ANALYSES OF NITRILE RUBBER-DEGRADING ACTINOMYCETE, *GORDONIA* SP. J1A

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Abstract. Nitrile rubber (NBR) is a widely used petroleum-derived material in various applications such as globe and seal. Since most NBR waste has been incinerated, the development of a recycling technology was desired. In this study, we focused on the development of a bio-recycling method of NBR waste. An NBR-degrading actinomycete, *Gordonia* sp. strain J1A, was isolated by a screening from activated sludge of wastewater treatment of an NBR manufacturing plant. To elucidate NBR degradation mechanism, we predicted NBR-degrading enzyme gene by genome and RNA-seq analysis. Three candidates, DUF, CYP, and linc, were selected. DUF was expressed by *E. coli* and the lysate showed a little NBR-degrading activity. In this presentation, we report on gene clusters associated with NBR degradation, their functions, and enzymatic NBR degradation process.

Keywords: Nitrile rubber (NBR), NBR degradation enzyme, gene expression, degradation mechanism, bio recycling

IDENTIFICATION OF DOMINANT MICROORGANISM DURING FERMENTATION OF CACAO GROWN IN TIEN GIANG

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Abstract. Tien Giang is one of provinces having the largest cacao yield in Viet Nam. Cacao is usually fermented at farms or cooperatives before selling to the commodity market. Microbial population has a vital role during fermentation and effect to quality of products processed from fermented cacao beans such as chocolate or cacoa. Microorganism in 3 fermented cacao samples (1 from farmer, 2 samples fermented in lab with and without banana leaf) was isolated in YPD, MRS, YGPD media every day during 7 days of fermentation. There were 39 yeast strains, 40 acid acetic bacteria strains and 43 lactic acid bacteria strains isolated. The yeast dominated from 48h to 72h of fermentation (from 5,49 to 7,86 log CFU/g), acid acetic bacteria dominated from 72h to 96h and reduced gradually while lactic acid bacteria retained high density at 144 h of fermentation. The results of fermenting ability and viability of the dominant strains showed that 2 yeasts, 1 acid acetic baterium and 1 lactic acid bacterium exhibited high performances. Molecular identification results revealed they were *Kluyveromyces marxianus, Pichia kudriavzevii, Acetobacter pasteurianus* and *Lactobacillus plantarum.* Trial fermentation with those strains (2 mixtures) were conducted and the fermented cacao showed better sensory features than the cacao without microorganism application.

Keywords: cacao, dominant microorganism, fermentation, Kluyveromyces marxianus,.

CORRELATION OF RHIZOSPHERIC AND ENDOPHYTIC BACTERIA IN THE ROOTS OF *ADENOSMA BRACTEOSUM*

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Abstract: Endophytic bacteria are known as stimulant factors for plant growth. Their role is to improved plant resilience to numerous diseases and environmental stressors. *Adenosma bracteosum* has been shown to have antibacterial properties and inhibit to develop the cancer cells in human. But where do the endophytic bacteria come from? The soil, contact with plant roots, the leaves, the flowers and the seeds of plant, may be original of endophytic bacteria. This study investigated the relationships between the endophytic and rhizospheric bacteria of *Adenosma bracteosum* collected at Lo Go Xa Mat National Park in Tay Ninh province by 16S rDNA gene-based metagenomic analysis. Illumina MiSeq sequencing revealed rhizospheric and endophytic bacteria had approximately equal amounts of the *Chloroflexi* (20% of population), while the *Proteobacteria* were more abundant in the endosphere (33% of population) than rhizosphere (17% of population). In addition, *Acidobacteria* (21% of population) detected in the rhizosphere as well as *Actinobateriota* (13% of population) in the endosphere showed the diversity and correlation between them in the *Adenosma bracteosum* root zone. Mostly of endophytes come from rhizopheric soil of plants because they presented both of roots and rhizosphere. In contrast, certain endophytic genus or species are not found in soil, while some rhizopheric genus or species are not found in plant roots. Parallely, by using the PICRUSt2 tool, the endophytes from *Adenosma bracteosum* have been predicted metagenomes which were involved in biosynthesis, degradation/utilization/assimilation, generation of precursor metabolite and energy, glycan pathways, macromolecule modification, and super pathways in the host plant. It is clear that these processes are closely related and have important functions in the growth and development of the host plant. This conclusion could facilitate the future research of the ecological functions as well as biotechnological potential in agriculture.

Keywords: *Adenosma bracteosum, endophytic bacteria, metagenomics, rhizosphere bacteria.*

ISOLATION AND SCREENING OF *STREPTOMYCES* SP. TRAINS FROM NEW SOURCES IN VIET NAM FOR MICROBIAL TRANSGLUTAMINASE PRODUCTION

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Abstract. The protein glutamine γ-glutamyl transferase, known as microbial transglutaminase (MTGase, EC 2.3.2.13), catalyzes the acyl transfer reaction between the γ-carboxamide group of the glutamine residue moiety bound to the primary ε-amine of different compounds, thereby forming isopeptide bonds. In addition to the application of restructured proteins to increase the brittleness of food products, MTGase is also used as a protein–protein, protein-peptide, or peptide-peptide binding catalyst in the field of research on biomedical and pharmaceutical products, such as antibody–drug conjugates. This study aimed to isolate and screen *Streptomyces* sp. capable of producing MTGase in Binh Duong and Dong Nai. Thirty-eight isolated actinomycetes were screened for the presence of transglutaminase. The results show that the study successfully screened actinomycetes CT1 for the ability to biosynthesize MTGase by hydroxamate assay, with an activity of 0.1378 U/mL. Through molecular biology identification methods and morphological comparison, actinomycete CT1 has been proven to belong to the genus *Streptomyces* and has the closest match with the strains of *Streptomyces angustmyceticus*. The crude broth contained MTGase with an estimated molecular weight of 38 kDa, the same as the standard by SDS–PAGE.

Keywords: Actinomycetes, hydroxamate assay, microbial transglutaminase, SDS–PAGE, *Streptomyces angustmyceticus.*

ISOLATION AND SCREENING FOR PLANT GROWTH-PROMOTING RHIZOBACTERIA FROM WILD LEGUME *ARACHIS PINTOI*

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Abstract. Plant growth-promoting rhizobacteria (PGPR) plays an important role in sustainable agriculture in terms of biofertilization, biocontrol, and bioremediation. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticide use is a big challenge nowadays. The use of PGPR has been reported to be an environmentally sound way of increasing crop productivity by facilitating plant growth through either a direct or indirect mechanism. Wild legume, Pinto peanut (*Arachis pinto*) also known by its scientific name *Arachis pintoi*, is a perennial tropical legume with high botanical resistance. So, pinto peanut (*Arachis pintoi*) without the influences of the cultivation system is considered as a potential source of beneficial microbes.

This present study reports on the isolation and screening for plant growth-promoting rhizobacteria from the wild legume *Arachis pintoi*. Soil, root, and root nodule samples from *Arachis pintoi* were collected, and a total of 32 bacterial isolates were obtained. Thirty-two isolates from *Arachis pintoi* were studied for their plant growth-promoting characteristics. Among isolated bacteria, 28 (87.5%) strains show the ability to fix nitrogen, and 3 (9.375%) isolates show phosphate solubilization ability with the highest SE% at 122.9% for NS8. In addition, all isolated strains are able to produce Indole-3-acetic acid production, and extracellular polymeric substances (EPSs) with the highest quantity at 5.53 g/l for R3 and 6.07 g/l for R13.

As a result, the bacteria researched in this work might provide a significant, ecologically friendly replacement to chemical fertilizers to improve plant growth, which would support the development of sustainable agriculture.

Keywords: Arachis pintoi, PGPR, indole-3-acetic acid production, phosphate solubilization, and EPS.

ANTIOXIDANT ACTIVITY OF ENDOPHYTIC BACTERIA IN *EUPHORBIA HIRTA* L.

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Abstract. Research on extracts endophytic bacteria of *Euphorbia hirta* L. which have the antioxidant activity was conducted. Samples of *E. hirta* L. were collected from Ninh Kieu District, Can Tho City. In this study, the antioxidant activity of extracts endophytic bacteria of *E. hirta* L. had been determined by the antioxidant abilities against 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), Fe^{2+}-2,4,6-tri(2-pyridyl)-1,3,5-triazine (FRAP) free radicals. The result showed that 115 endophytic bacteria strains that are found in all parts of *E. hirta* L. such as leaves, stems and roots. Among 115 strains the study revealed 27 endophytic bacteria ones in *E. hirta* L. with antioxidant activity. The results showed that the ethyl acetate extract from selected endophytic bacterial fluid culture (CS-RE9) had higher antioxidant activity than Trolox on DPPH ($EC50_{CS\text{-}RE9}$ = 110.36 µg/mL), NO ($EC50_{CS\text{-}RE9}$ = 112.5 µg/mL) and FRAP ($EC50_{CS\text{-}RE9}$ = 67.8 µg/mL). CS-RE9 was identified as *Bacillus* sp. CS-RE9 based on their 16S-rRNA gene sequences.

Keywords: antioxidant, *Bacillus* sp., endophytic bacteria, *Euphorbia hirta* L.

METABOLIC ENGINEERING OF *ESCHERICHIA COLI* FOR THE PRODUCTION AND DEGRADATION OF MICROBIAL-POLYMER

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Abstract. Microbial metabolic pathway engineering is a potent strategy used worldwide to produce various value-added chemicals. We drastically rewired the primary metabolic pathway of *Escherichia coli* to produce maleic acid, which is one of the most important dicarboxylic acids and is used to produce various polymer compounds and pharmaceuticals. The production, yield against the theoretical yield, and production rate reached 12.0 g/L, 67% (mol/mol), and up to 4-fold compared to that in previous reports, respectively. These results are the highest values of maleate production in microbes to our knowledge. The results reveal that our strategy strongly promotes the production of valuable chemicals. In addition, we tried to perform the production of microbial polymer involving aromatic residues and the creation of the polymer-degrading enzyme anchored *E. coli*. 4-Hydroxymethylbenzoic acid, 2,5-pyridinedicarboxylic acid, and 2,5-furandicarboxylic acid were adopted as the candidate aromatic chemicals. Several compounds including aromatic residue inhibited the polymerizing reaction of some polyhydroxybutyrate synthase (PhaC). This result implied the interaction between that PhaC and the aromatic chemicals and this enzyme get a prototype one to polymerize aromatic chemicals.

Keywords: *Escherichia coli*, maleic acid, aromatic polymer, enzyme

KT-WGS-FUNGI: A PIPELINE FOR FUNGAL GENOME ASSEMBLY AND ANNOTATION FROM SHORT-READ SEQUENCING DATA

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Abstract. Fungi as eukaryotes are most abundant on the earth with high diversity in morphology, phylogeny metabolism and ecology. They are well known for a variety of bioactive molecules of agricultural, industrial and pharmaceutical significance. The reconstruction of fungal genomes from next generation sequencing (NGS) data by bioinformatics pipelines and the consequent functional annotation of their genes' repertoire are fundamental steps in studying their biological mechanisms, such as metabolism, virulence factors, and drug resistances. We have developed a bioinformatics pipeline**, kt-wgs-fungi,** that is compatible with short-read sequenced data. The pipeline starts with a quality control step to remove low-quality and contaminated reads. Then, the genomes are assembled using SPAdes and MaSuRCA. The output assemblies are filtered for contigs of fungal origin using BLAST, and the best assembly is chosen based on contiguity and completeness using BUSCO and Quast. The genome is then annotated automatically using Funannotate, and the CDS are further assigned using eggNOG-mapper and fungiSMASH. The results are then visualized and using an in-house KTest script. The **kt-wgs-fungi** pipeline was able to assemble and functionally annotate the genomes of *F.oxysporum , G.lingzhi*, and *G.lucidum* with good quality. The results has enabled the identification of candidate genes or gene clusters for their virulence or bioactive compound production. The pipeline is scripted in Nextflow and Bash language, making it scalable, high-throughput, and capable of deploying on multiple platforms such as HPC or public clouds. In conclusion, the **kt-wgs-fungi** pipeline provides a good solution for scientists who wish to gain a better understanding of the genetics and function of interest fungi through NGS approaches.

Keywords: Fungi, genome assembly, functional analysis, NGS

EVALUATION OF BETA-GLUCOSIDASE ACTIVITY FROM SOME FUNGAL STRAINS AND THEIR APPLICATION IN CONVERTING RUTIN FROM *SOPHORA JAPONICA* FLOWERS TO QUERCETIN

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Abstract. Beta-glucosidase is a hydrolytic enzyme reported to degrade flavonoid glycosides into aglycone. In this study, *Rhizopus arrhizus* isolated from rice wine starter and *Trichoderma reesei* were cultured separately or in a mixed culture to evaluate β-glucosidase activity, as well as the ability to degrade rutin from *Sophora japonica* flowers into quercetin, a flavonoid with strong antioxidant activity and widely used in pharmaceuticals and cosmetics. The results showed that the β-glucosidase activity of *R. arrhizus* was the highest, reaching 3333.3 U/mg, followed by *T. reesei* at 769.2 U/mg and the lowest was 666.7 U/mg in mixed culture. Beta-glucosidae production measured in these samples respectively was 177.5; 128.5 and 43.2 U/ml. Under solid fermentation conditions, flower powder were mixed with spore solution at the ratio of $1.3x10^6$ spores/g. Thin layer chromatography (TLC) results clearly showed the appearance of quercetin spots with increasing density during eight days of fermentation, especially in the *R. arrhizus* sample. This result shows the potential of the *R. arrhizus* strain in converting rutin to quercetin, increasing the value of medicinal materials.

Keywords: beta-glucosidase, quercetin, rutin.

ENHANCEMENT OF FIBRINOLYTIC ACTIVITY IN NEW NATTO DERIVED FROM BLACK TURTLE BEANS (*PHASEOLUS VULGARIS*)

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Abstract. Natto, a soybean-derived fermented product renowned for its robust fibrinolytic properties, has gained widespread popularity in recent years. However, soybeans have been globally acknowledged as a significant source of allergens. Consequently, there is a growing emphasis on replacing the fermentation substrate of high-fibrinolytic activity natto with an alternative medium, while simultaneously optimizing its nutritional composition, to cater to the increasing demand from individuals with soy allergies. This study introduces a novel approach by utilizing black turtle beans as a new fermentation substrate for natto production. By employing a combination of one-factor-at-a-time experiments, Plackett-Burman design, and central composite design, the optimal fermentation conditions for *Bacillus subtilis* MS5, a strain known for its production of the nattokinase enzyme, were determined. The highest enzyme activity observed in this study was 418.32 FU/mL, which is equivalent to the nattokinase enzyme activity found in 5g of natto. This level of activity is sufficient to meet the daily requirements of an adult consuming 25g of natto. The key factors identified to significantly impact the fermentation process included a peptone concentration of 2.8%, a bacterial density of 10^4 CFU/100g of *Bacillus subtilis*, an initial pH of 5.5, and a fermentation duration of 33 hours. Furthermore, the black turtle bean-based natto demonstrated remarkable free radical scavenging ability, as evidenced by a DPPH activity of 81.21 µg/mL. These findings underscore the considerable potential of black turtle bean-based natto in enhancing fibrinolytic activity and effectively scavenging free radicals. Such attributes not only contribute to the treatment of atherosclerotic blood clots but also hold promise for improving overall human health through tailored nutritional interventions. By providing an alternative to soybean-derived natto, this research opens up new avenues for individuals with soy allergies to benefit from this highly regarded fermented food product.

Keywords: Bacillus subtilis, black turtle beans, fibrinolytic activity, nattokinase enzyme, soy allergies.

IN VITRO PROBIOTIC POTENTIAL OF LACTIC ACID BACTERIA (LAB) ISOLATED FROM VIETNAMESE PICKLE WITH ANTIBACTERIAL ACTIVITY AGAINST *HELICOBACTER PYLORI*

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Abstract. Vietnam among the top three highest rate of *Helicobacter pylori* (*H. pylori*) infection in Southeast Asia. There are few studies that investigated the use of probiotics as non-chemical compounds which are safer than some drugs for alternative therapy. The purpose of this study is to evaluate the effectiveness of Lactic Acid Bacteria (LAB) isolated from Vietnamese pickle and their antibacterial activity against *H. pylori* ATCC 43504 *in vitro*. A total of sixteen (16) LAB was isolated from Vietnamese pickle based on their morphological characteristics, physiological, biochemical properties. Twenty LAB isolated strains were confirmed as probiotics by using in vitro methods, including tolerance to acid, bile salts and safety experiments. Antimicrobial activity of LAB strains was screened using agar-well diffusion method and the anti-urease activity effect of LAB was determined by the phenol red method. The results show that 8 strains have a high probiotic potential. These selected strains were chosen for further investigation of resistance to *H. pylori* and ability to reduce urease activity. No.16 and No. 8 strains had the largest zones of inhibition and significantly reduced *H. pylori* urease activity. This study had shown that 2 strains (No.16 and No.18) had strong probiotic potential and can effectively inhibit the growth of *H. pylori* with reducing urease activity. It expected to be used as an adjuvant therapy for *H. pylori* eradication and to enhance the probiotic value of Vietnamese fermented products.

Keywords: Probiotics, antimicrobial activity, Lactic Acid Bacteria, *Helicobacter pylori*

EFFICACY EVALUATION OF *BACILLUS* SP. RP16 AGAINST *FLAVOBACTERIUM COLUMNARE* CAUSING TAIL ROTTEN DISEASE IN TRA CATFISH (*PANGASIANODON HYPOPHTHALMUS*)

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Abstract. *Flavobacterium columnare* is the causative agent of white patch disease with a high catfish loss rate, similar to that of enteric septicemia and Motile Aeromonas septicemia caused by *Edwardsiella ictaluri* and *Aeromonas hydrophila*, respectively, in pangasius. These diseases frequently occur and cause lots of damage during pangasius farming in the Mekong Delta, Vietnam. Recently, the use of antagonistic bacteria against pathogens has gained increasing interest in order to reduce the antibiotic use in fish farm for disease prevention and treatment. Therefore, this study screened *Bacillus* spp. strains expressing the resistance ability against *F. columnare* by the agar well diffusion test and the protective effect of these *Bacillus* spp. strains were approved in catfish model. There were eight *F. columnare* strains collected from diseased catfish cultured in An Giang and Long An provinces. The species identification was confirmed using PCR and sequencing. In the other hand, from the collection of 12 *Bacillus* spp. strains preserved at Biotechnology Center of Ho Chi Minh City, which were concurrently resistant to *E. ictaluri*, *A. hydrophila*, and *S. agalactiae*, three *Bacillus* spp. strains strongly antigonistic to *F. columnare* were selected with inhibition zone diameters more than 25 mm. The lethal dose LD_{50} of *F. columnare* LA4 strain in Tra catfish (at the mean weight of 1.2 – 1.5 g) was determined at 2.8×10^4 CFU mL^{-1}. The results of evaluating the protective effects (RPS) of the *Bacillus* sp. RP16 strain at the concentration of 10^5 CFU mL^{-1} using immersion route in pangasius reached the highest value of 53.1%.

Keywords: *Bacillus*, catfish, antagonize, *Flavobacterium columnare*, tail rot disease

MUTATION IN THE S-RIBOSYLHOMOCYSTEINASE (*LUXS*) GENE INVOLVED IN QUORUM SENSING AFFECTS MOTILITY, PROTEASES, N-ACYL HOMOSERINE LACTONE, BIOFILM FORMATION AND VIRULENCE IN HYPERVIRULENT *AEROMONAS HYDROPHILA*

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Abstract. Hypervirulent *Aeromonas hydrophila* strain (vAh) has been identified as a primary pathogen associated with high mortality in Tra catfish (*Pangasianodon hypophthalmus*) and was firstly identified as a fish pathogen in Vietnam in 2022. Quorum sensing is a form of "bacterial communication" that regulates gene expression in response to an increase in bacterial population through signal carriers. In this study, the role of the S-ribosylhomocysteinase (*luxS*) gene-based autoinducer (AI-2) system of the hypervirulent *A. hydrophila* strain was described via the mobility, biofilm-forming ability, capacities of producing exoprotease and N-Acyl homoserine lactone (C4-HSL, C6-HSL), and virulence on experimental Tra catfish. Compared with the vAh wild type, *luxS* knockout mutant of the vAh strain (mLuxS) obtained by homologous recombination exhibited a decreased mobility ($p<0.05$), enhanced ability to produce exoproteases and N-Acyl homoserine lactone (C4-HSL, C6-HSL) ($p<0.05$). The experimental immersion challenge showed that mLuxS strain caused no fish mortality at the concentration of 1.9×10^7 CFU/mL, while the wild-type vAh led to fish mortality of 55% at the bacterial concentration of 1.12×10^7 CFU/mL ($p<0.05$).

Keywords: Hypervirulent *Aeromonas hydrophila* strain, Quorum sensing, *luxS*,

DECIPHERING THE INTERACTIONS OF CHEMICALLY DISTINCT AMPICILLIN AND S-D-LACTOYLGLUTATHIONE SUBSTRATES IN A PROMISCUOUS BLEG-1 B3 METALLO-β-LACTAMASE

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Abstract. Metallo-β-lactamases (MBLs) are class B β-lactamases that inactivate a broad- spectrum, clinically prevalent β-lactam antibiotics. In our recent study, a novel, evolutionary divergent MBL of B3 subclass from *Bacillus lehensis* G1 termed BLEG-1 was found to exhibit both β-lactamase and glyoxalase II (GLXII) activities, which involves hydrolysis of β-lactams and S-D-lactoylglutathione (SLG) respectively. We hypothesized that the catalytic promiscuity of BLEG-1 could be contributed by its structural resemblance to B3 MBL and GLXII, as well as the adoption of active site architecture which allows the recruitment of both ampicillin and SLG. However, as this postulation was based on the static crystal structure of BLEG-1, its bifunctional properties under a dynamic environment is still vital to be investigated to identify the key structural and chemical features that govern dual substrates binding and catalysis. For this purpose, molecular dynamics (MD) simulations of BLEG-1 and its substrate complexes were carried out using YASARA software, whereby their topological changes and substrates interactions throughout the MD trajectories were analyzed. Five key residues which could be crucial for binding, orientation, and stabilization of substrates were selected for alanine scanning. MD data showed that the active site loops of BLEG-1 are flexible, thereby forming a plastic pocket that could mould around the structurally distinct substrates and bring them near the catalytic group. The hydrophobic vicinity in the N-terminal domain and the distributions of polar residues over the active site are specifically critical for the interactions of ampicillin and SLG respectively. This was further evidenced through the decrease of MBL and GLXII activities of BLEG-1 upon alanine substitutions of the five key residues which consist of isoleucine, phenylalanine, leucine and arginines. This study has provided molecular insights into the dual functionality of BLEG-1, laying valuable foundation for the evolutionary studies and design of inhibitors against BLEG-1 and its homologs.

Keywords: antibiotic resistance, metallo-β-lactamases, enzyme promiscuity, substrate interactions, molecular dynamics, mutagenesis

EFFECTS OF HEAT TREATMENT ON THE PSYCHROPHILE-BASED SIMPLE BIOCATALYST

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Abstract. We constructed the Psychrophile-based Simple bioCatalyst (PSCat) for efficient bioconversion using by the psychrophilic bacterium *Shewanella livingstonensis* as the host microorganism. The synthetic pathway of the target compound by mesophilic enzymes expressed in psychrophilic bacteria is dominated by moderate temperature heat treatment because the heat treatment can inactivate almost all psychrophilic metabolic enzymes that consume substrates and energy materials for by-product formation. Heat treatment also increases the membrane permeability of the substrate by partially disrupting the cell membrane. In our previous study, we had constructed an efficient bioconversion process to produce polymeric materials with PSCat. It had achieved high-yield production of polymeric materials such as 3-hydroxypropionaldehyde, 1,3-propanediol, 3-hydroxypropionic acid, aspartic acid, and itaconic acid. In addition, PSCat has also been used for sustained reaction by immobilization with alginate. Recently we have focused on the production of aromatic compounds, valuable chemicals for engineering polymer materials with PSCat. To evaluate the influence of heat treatment at moderate temperature on the cell structure of psychrophilic bacteria (*S. livingstonensis* and *Shewanella frigidimarina*), we observed the cells after heat treatment by electron microscopy. The cells after heat treatment had protrusions in the cell surface, it showed partial disruption of the cell structure to increase the membrane permeability. We also studied the leakage of the proteins from PSCat by the bacterial strains expressing green fluorescent protein (GFP) fused enzymes. The leakage decreased depending on the molecular size of the proteins. Psychrophilic cells could be used as biocatalyst for the production by optimizing the heat treatment condition and the size of the expressing enzymes.

Keywords: psychrophilic bacteria, *Shewanella livingstonensis*, heat treatment, green fluorescent protein

ENHANCED FOLDING OF PET DEPOLYMERASE ALLEVIATES ENZYME CONCENTRATION-DEPENDENT INHIBITION FOR EFFICIENT PET DEGRADATION

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Abstract. The accumulation of polyethylene terephthalate (PET) waste is a serious environmental problem. The PET-degrading enzyme from *Ideonella sakaiensis* (*Is*PETase) is a promising enzyme for biodegradation of PET owing to its exceptional activity and specificity toward PET. However, *Is*PETase, as also other PET-degrading enzymes do, suffers from enzyme concentration-dependent inhibition (ECDI), preventing the use of a high concentration of enzyme for faster PET degradation. Herein, we show that improved folding of *Is*PETase can alleviate ECDI via increased substrate binding affinity. Although *Is*PETase has two native disulfide bonds, most studies have been conducted using a recombinant enzyme (*Is*PETase^{BL}) produced from *Escherichia coli* BL21(DE3). We produced and characterized the enzyme (*Is*PETase^{SH}) from *E. coli* SHuffle T7 Express, a strain suitable for the expression of proteins with disulfide bonds. Due to the improved folding, the production yield, solubility, activity and stability of *Is*PETase^{SH} were all significantly improved compared to those of *Is*PETase^{BL}. Notably, *Is*PETase^{SH} showed an alleviated (but not completely resolved) ECDI with a 4-fold increase in the optimal enzyme concentration. The alleviation of ECDI was strongly correlated with increased substrate binding affinity of *Is*PETase^{SH}. A similar result was obtained when an engineered *Is*PETase variant (FAST-PETase) was produced in *E. coli* SHuffle T7 Express. This study may provide a clue to explain why ECDI occurs and a guide how to alleviate ECDI for a more efficient PET biodegradation.

Keywords: polyethylene terephthalate, IsPETase, disulfide bond, concentration dependent inhibition, Escherichia coli

SALT-ASSISTED STABILIZATION OF A CO_2-CAPTURING ENZYME VIA ONE-STEP *IN-SITU* IMMOBILIZATION IN DIATOM-INSPIRED SILICA NANOSPHERES

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Abstract. Carbonic anhydrase (CA), an enzyme that catalyzes reversible CO_2 hydration, has been considered a powerful and green catalyst for CO_2 reduction owing to its ultrafast kinetics and biobased nature. For industrial utilization, immobilization of CA is needed to increase thermal stability, lifespan, and enzyme recovery. Diatom-inspired silica provides a green platform for the efficient immobilization and stabilization of enzymes in a fast and facile manner. Herein, the effect of salt supplementation on the thermal stability of bovine CA (bCA) loaded in bioinspired silica was investigated. Silica synthesis was facilitated by the silica-forming R5 peptide fused to the bCA; bCA-R5 was immobilized *in situ* in the synthesized silica nanoparticles with an excellent immobilization yield. The thermal stability of bCA-R5 was improved via salt supplementation, which was controlled by the cation-assisted increase in silica synthesis with a high packing density. The salt effect on enzyme stabilization was dependent on the pH and the enzyme's electrostatic nature. The immobilized bCA-R5 was 12,700-fold and 11-fold stabilized via the salt-assisted method over the free enzyme and the immobilized enzyme without salt supplementation, respectively, representing the highest thermal stability (a half-life of 577.6 h at 60 °C) among CAs immobilized in biobased silica. The green catalyst efficiently synthesized and reinforced via the simple and robust route can be successfully used as a powerful agent for CO_2 capture under high-temperature conditions. In addition, this simple, green, and robust strategy can be widely applicable to immobilizing and stabilizing various proteins, maximally exploiting the potential of diatom-inspired silicification.

Keywords: Enzyme immobilization, bioinspired silica, salt, carbonic anhydrase, stability, carbon dioxide capture

ARTIFICIAL PROTEIN LIPIDATION AND ITS DYNAMICS ON LIPID MEMBRANES

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Abstract. Protein lipidation contributes to increasing their affinity for the plasma membrane. Palmitoylated proteins localize to phase-separated domains (rafts) and play important roles in signal transduction through both two-dimensional dynamics (raft localization) and three-dimensional dynamics (vesicular transport). In this study, we constructed the artificial protein lipidation system and investigated the effect of the alkyl chain length of lipidated proteins on their dynamics on lipid membranes. Specifically, we evaluated their localization behavior on cell-sized liposomes (Giant unilamellar vesicles, GUVs), their diffusion behavior on supported lipid bilayer (SLB), and their intracellular trafficking into living cells (Jurkat cells). Utilizing a cross-linking reaction catalyzed by microbial transglutaminase, EGFP-LQ, which is LQ-tag fused to the *C*-terminus of green fluorescent protein (EGFP), was cross-linked to lipid-G_3SRHK for preparing EGFP-lipids (EGFP-C16, EGFP-C18, EGFP-C20, EGFP-C22). Firstly EGFP-lipids were mixed with phase-separated GUVs ([DOPC]:[DPPC]:[Cholesterol] = 4:4:2). We observed the localization of EGFP-lipids to liquid ordered phase, which is a raft model phase on GUV enriched with DPPC and cholesterol. Secondly, EGFP-lipids were anchored to SLB ([DPPC]:[Cholesterol] = 6:4) followed by fluorescence recovery after photobleaching (FRAP). We observed the decreasing of diffusion rate with elongation of the alkyl chain length of the EGFP-lipid in FRAP experiment. This could be explained by increasing intermolecular interactions in lipid membranes with longer alkyl chain. Finally, intracellular trafficking of EGFP-lipids was observed using activation of Jurkat cells by Concanavalin A. We found the internalization of EGFP-lipids into the cells and in particular EGFP-C16 was most efficiently internalized. The internalization pathway could be cholesterol dependent endocytosis. These results also indicate that changing of lipid moieties can significantly affect the dynamics of lipidated proteins on lipid bilayers.

Keywords: Protein lipidation, Palmitoylation, Raft, Vesicular transport, Enzyme

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STUDYING THE EFFECT OF MUTATIONS ON THE STRUCTURE AND FUNCTION OF *TAQ* DNA POLYMERASE

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Abstract: *Taq* polymerase is a heat-resistant enzyme isolated from the bacterium *Thermus aquaticus*, which thrives in hot springs with temperatures ranging from 70-75°C. However, it still retains significant activity at temperatures of 20-37°C, which is a limitation for certain PCR reactions as it can lead to non-specific primer binding. In this study, the structures of five reported hot-start *Taq* DNA polymerases, having no activity at room temperature, were predicted using homology modeling and Artificial Intelligence technology. This structural ensemble was compared with the natural structure of *Klentaq* in order to analyze the impact of the mutations on the structures. Apart from the mutant amino acids, an additional nine amino acids (in the mutant structures) were found to alter the side chain dihedral angles compared to the natural structure. The changes in these residues expanded the conformational spaces of the structures in which the DNA template could be jammed in the active conformational space of the structure, thereby obstructed the conformational transition between closed and open states at room temperature. Moreover, this study also analyzed the directionality of the reported mutations. The findings of this study significantly contribute to a deeper understanding of the mechanism and functional features of the mutant *Klentaq* structures and provide important insights for developing an enhanced enzyme with superior features to serve the scientific community.

Keywords*: Taq* DNA polymerase, *Klentaq*, directionality of mutations, hot-start*, mutant structure*

ANTIOXIDANT AND ANTIFUNGAL ACTIVITIES OF PROTEINS EXTRACTS FROM THE EARTHWORM (*PERIONYX EXCAVATUS*) IN HO CHI MINH CITY, VIETNAM

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Abstract. *Perionyx excavatus* is dominated species of earthworm for farming in Ho Chi Minh City, especially are very suitable for earthworms grown, in temperature and humidity conditions. Recently earthworm protein extracts are rich natural sources of antioxidant activities. In this study, the extract obtained earthworm proteins by ethanol and water solvents. Most crude extracts were purified using hexane solvents and filtration through a 0.22 µL-sized membrane. The active extracts were characterized by TLC, SDS-PAGE and FTIR. The antifungal activity was tested by the agar plate diffusion technique and through the MIC value. The antioxidant activities had determined by radical scavenging activity with DPPH and cation ABTS* reagents. The results showed that TLC and FTIR analysis of active extracts showed the presence of amino acids and peptides. SDS-PAGE of those extracts also revealed proteins with a molecular weight of less than 15 kDa (ethanol 80 % extract) and more than 50 kDa (ethanol 50 % and water extracts). In the extracts, crude proteins from ethanol 80% extract showed the highest activity with a MIC value of 3 mg mL^{-1} (*Candida albicans* strain) and IC_{50} value of 0.278 mg mL^{-1} (DPPH assay) and 0.201 mg mL^{-1} (ABTS assay). This study suggested a new way of utilizing *Perionyx excavatus* as an antifungal and antioxidant applied to the cosmetic industries.

Keywords: earthworm, antifungal, proteins, antioxidant, ethanol 80%, SDS-PAGE

LOW PRESSURE DEAD-END NANOFILTRATION FOR SODIUM GLUCONATE RECOVERY: COMPARATIVE STUDY ON FERMENTATION BROTH AND SYNTHETIC SODIUM GLUCONATE SOLUTION

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Abstract. Sodium gluconate (SG) is a commonly used organic salt in the construction industry, primarily exploited as a cement additive and metal chelating agent. Synthesis of SG through fermentation is widely recognized as a more cost-effective and environmentally friendly approach compared to other approaches. The current study was conducted as an introductory on the recovery of SG from fermentation broth using low-pressure dead-end nanofiltration with a pressure range of 3–9 bar. The effect of pressure and membrane type was evaluated on synthetic medium fermented broth (SMFB) and pure SG solution (PSGS). The findings of the research indicate that the NF245 membrane exhibited superior rejection and recovery capabilities, but the NF270 membrane had a larger permeation flux. Increases in pressure resulted in an increase in flux, concentration, and rejection, while simultaneously decreasing the recovery. A notable disparity in results was seen as a consequence of distinct retention mechanisms arising from the low pH of the fermented broth. Consequently, a just weak contact between SG molecules and the membrane ensued. The results of the experiment indicate that the NF245 membrane, when subjected to a 30-minute nanofiltration process at a pressure of 9 bar, achieved a recovery rate of SG molecules of 96.63%. In comparison, the NF270 membrane only achieved a recovery rate of 92.52%.

Keywords: Fermentation broth, low-pressure nanofiltration, recovery, separation, sodium gluconate.

DECOLORIZATION OF TEXTILE DYE BY SPORE SURFACE DISPLAYED SMALL LACCASE FOR THE ENHANCED THERMAL STABILITY AND ROBUST REPEATED REACTION

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Abstract. In this study, we tried to decolorize synthetic dyes using small laccase (SLAC) from *Streptomyces coelicolor*, which is resistant to pH, temperature change, and traditional inhibitors for the actual industrial applications using spore surface display system. We inserted SLAC-His6 tag at the C-terminal of CotE anchoring motif. The proper surface expression of CotE-SLAC fusion protein on the surface of Bacillus subtilis spore was verified with flow cytometry using FITC labeled anti-His6 tag antibody. After 6 h of reaction, more than 90% of Indigo carmine was decomposed using recombinant SLAC displaying Bacillus spore, whereas less than 10% of Indigo carmine was decomposed with wild type spore. Over 70% of laccase activity was retained with recombinant SLAC displaying spore, which was heat- treated for 3 h at 90oC. For eight rounds of repeated decomposition of Indigo carmine, no significant decrease of enzymatic activity was observed. This showed the robust characteristics of spore display format for repeated and harsh condition reactions.

Keywords: Bacillus subtilis, spore display, small laccase, decolorization

EXPRESSION, PURIFICATION, AND CATALYTIC ACTIVITY AT LOW TEMPERATURE OF A RECOMBINANT TEV PROTEASE

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Abstract. Tobacco Etch Virus (TEV) protease is a highly site-specific cysteine protease that recognizes the amino acid sequence ENLYFQG/S and cleaves the peptide bond between Q and G/S. TEV protease is commonly used to remove tags or an undesired fusion partner from recombinant fusion proteins. Particularly, for target proteins that are enzymatic in nature, the process of tag removal needs to be carried out at low temperatures (4 °C) to preserve the enzyme's activity for subsequent research steps. However, studies have so far not focused on assessing the activity of TEV protease under this condition. In this study, we harvested TEV protease as an enzyme and the fusion protein harboring the TEV site as an enzymatic substrate by expressing *sfGFP-TEV protease-(His)$_6$* in *E. coli* BL21(DE3) cells and *MBP-Cas9-eGFP-(His)$_6$* in *E. coli* C41(DE3) cells, respectively. Both Histidine-tagged proteins were successfully purified by Ni^{2+} affinity chromatography and used for setting up cleavage reactions. The results showed that TEV protease could efficiently cleave the fusion protein MBP-Cas9-eGFP at the specific recognition site (ENLYFQG/S) at 4°C under tested reaction conditions. These results could serve as reference data for establishing an efficient cleavage of other fusion proteins containing a TEV protease recognition site under low temperature conditions.

Keywords: Cas9, fusion protein, low temperature, tags, TEV protease

IMMOBILIZATION OF CUTINASE ON MAGNETIC NANOPARTICLES AND ITS APPLICATION IN COTTON BIOSCOURING

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Abstract. The hydrophobic nature of greige cotton fabric induced by its surface lipid barrier hinders its wettability during processing. Cotton scouring is carried out to remove the wax and lipid barrier and facilitate any wet processes. It is carried out by treatment of greige fabric with sodium hydroxide. The present study is aimed at providing eco friendly approach of cotton bioscouring as compared to the traditional toxic effluent producing chemical methods for removal of wax barrier without damaging the fibre. Magnetic nanoparticles were synthesized and cutinase was immobilized onto them to make it more stable, reusable and easier product recovery. Further, conditions were optimized to maximize immobilization %. Cutinase immobilized nanoparticles were characterized in terms of size, crystallinity, magnetic behavior, chemical composition and thermal stability. The effect of immobilized cutinase and its reusability was observed on cotton bioscouring.

Keywords: Immobilized enzyme; Reusability; Greige cotton fabric; SEM; FTIR

BIO-NANOVESICLES FOR TUMOR DIAGNOSIS AND TREATMENT

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Abstract. Nano-sized extracellular vesicles (EVs), such as exosomes, offer tremendous potential in cancer diagnosis as important biomarkers and serve as a novel and promising drug delivery platform due to their endogenous origin, stability, biocompatibility, and other unique attributes.

We have made significant advancements in this field. Firstly, we developed a highly sensitive fluorescent biosensor that utilizes dual signal amplification for the detection of leukemia cell-derived exosomes [1]. Additionally, we successfully demonstrated a multiplex immuno-PCR (mI-PCR) assay, which enables precise molecular typing of acute myeloid leukemia (AML) by simultaneously detecting multiple surface CDs on AML exosomes [2].

Addressing the challenge of efficiently loading and delivering long RNA to target tumor cells for therapeutic purposes, we put forth a groundbreaking exosome-based RNA delivery system. This system employs a light-inducible RNA enrichment and releasing protocol by reshaping exosome producer cells [3]. Furthermore, we engineered a novel cancer cell membrane-derived nanocarrier (mCas9-sGNRs) capable of efficiently and simultaneously delivering various therapeutic agents (CRISPR/Cas9 and gold nanorods). This innovative approach enables synergistic photothermal/gene therapy for cancer treatment [4].

Our research not only facilitates the sensitive and specific detection of tumor-derived EVs, contributing to precise cancer diagnosis, but also presents versatile and safe strategies for constructing bio-nanovesicles-based vehicles. These vehicles efficiently load and deliver therapeutic cargos to specific targets, thus paving the way for more advanced and personalized cancer medicine.

Keywords: cancer cell membrane, exosomes leukemia, Nanovesicles

ENHANCED STORAGE STABILITY AND EFFICACY OF INHIBITORY PEPTIDES VIA CONJUGATION WITH ZEOLITIC IMIDAZOLATE FRAMEWORK-8 (ZIF-8)

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Abstract. Previously, two specially designed inhibitory peptides were found to be effective in inhibiting B3 subclass metallo-β-lactamase (MBL) termed Bleg1_2478 (currently named as BLEG-1) from *Bacillus lehensis* G1 which has a broad activity spectrum against β-lactam antibiotics. However, the efficacies of the inhibitory peptides when exposed to various conditions, even at physiological ones, decreased over time. Therefore, this study aims to maintain or prolong the efficacy of the inhibitory peptides through their conjugation with zeolitic-imidazolate framework-8 (ZIF-8), a porous and thermostable nanomaterial. Successful conjugation of the peptides with ZIF-8 was confirmed through X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Brunauer-Emmet-Teller (BET), thermogravimetric analysis (TGA) and transmission electron microscopy (TEM) analyses. Inhibitory assay of ZIF-8@PEPTIDE conjugates on purified BLEG-1 revealed their ability in inhibiting the enzyme. Storage stability assays of the ZIF-8@PEPTIDE nanoconjugates showed stable inhibitory activities on BLEG-1 for up to two weeks under the tested temperature range of 4-37°C when compared to their non-encapsulated peptide counterparts. In conclusion, conjugation of the potent inhibitory peptides into ZIF-8 potentially improved their efficacy and stability under prolonged storage. This serves as an effective system to maintain the efficacy of therapeutic molecules that otherwise are unstable when subjected to various conditions.

Keywords: ZIF-8@PEPTIDE, BLEG-1, efficacy, stable, storage

STUDY ON BIOCOMPATIBLE NANO-SCALED METAL ELECTRODES FOR BIOMETRIC SIGNAL MEASUREMENT

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Abstract. The study focuses on the development of biocompatible metal electrodes for biometric signal measurement, particularly in the EEG (Electroencephalography) signals. The goal is to create the metal electrodes that can be safely and comfortably placed on the user's prefrontal cortex to measure brainwave signals without causing any adverse reactions or allergies. One of the major concerns with the conventional metal electrode structure, which includes Noble Metal/Ni/Cu circuits, has been known as the risk of nickel (Ni) leakage. When these electrodes come into contact with the user's skin, the released nickel can trigger allergic reactions in sensitive individuals. In this research, a novel electrode structure technology was proposed to overcome the issue of nickel presence between the Noble metal (which comes into contact with the human body) and the electrical signal connection circuit, Cu. The main objective was to develop a biocompatible metal electrode structure that eliminates or substitutes nickel, ensuring it poses no harm to the human body. To achieve this, the researchers utilized semiconductor processing techniques, enabling them to create metal electrodes with superior characteristics compared to conventional designs. The resulting electrodes exhibited excellent biocompatibility, making them a safe and reliable choice for biometric signal measurement when in contact with the human body.

Keywords: Biocompatible, Nano-Scaled, Metal Electrodes, Biometric Signal, Electroencephalography,

AN INTRANASAL MULTIVALENT EPITOPE-BASED NANOPARTICLE VACCINE CONFERS BROAD PROTECTION AGAINST DIVERGENT INFLUENZA VIRUSES

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Abstract. The development of a universal influenza vaccine to control public health threats from circulating and emerging influenza viruses is highly desirable. Here we reported an intranasal multivalent epitope-based nanoparticle vaccine with broad protection against divergent influenza A and B viruses. Three highly conserved epitopes consisting of the A α-helix of hemagglutinin (H), the ectodomain of matrix protein 2 (M) and the HCA-2 of neuraminidase (N) are presented on a self-assembling recombinant human heavy chain ferritin cage (F) to generate the HMNF nanoparticle. Intranasal immunization of mice with HMNF has mobilized potent immune responses, including high levels of antigen-specific antibodies and T cell-mediated responses, which exhibited cross-reactivity to various antigen mutations. Vaccination with HMNF conferred full protection against lethal challenge with divergent influenza A and B viruses. The broad protection of HMNF nanoparticles could be attributed to the synergetic function of antibodies and T cells. Moreover, the induced immune responses are long-lasting, and protection is maintained six months after vaccination. Our constructed HMNF nanoparticle can serve as a promising candidate for a universal influenza vaccine.

Keywords: nanoparticle vaccine, influenza viruses, broad protection, multivalent epitopes

GENETICALLY ENCODED FLUORESCENT BIOSENSORS

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Abstract. Genetically encoded fluorescent biosensors can directly image various molecular events in living cells and have become powerful tools in studies of cell biology. This presentation introduces their categories, designs and applications. Based on our investigation in study on redox biology using a number of genetically encoded fluorescent biosensors, we also systematically discuss their advantages, existing problems and solutions, and highlight the future development.

Keywords: genetically encoded fluorescent sensing probes, fluorescence resonance energy transfer (FRET), circularly permuted fluorescent protein (cpFP)

BIOELECTRONIC TONGUE FOR IDENTIFYING AND MASKING BITTERNESS BASED ON BITTER TASTE RECEPTOR AGONISM AND ANTAGONISM

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Abstract. Among the basic tastes of humans, bitterness is assumed to be the sense of avoidance of toxic substances and food spoilage. In the field of medicine, bitterness disturbs medication adherence. Therefore, identifying and masking bitter taste are important to develop palatable foods and promote medication compliance in the food and pharmaceutical industries. Agonism and antagonism of bitter taste receptors can serve as strategies for identifying and masking bitter taste at the molecular level. In the present study, we developed a bioelectronic tongue to characterize the agonism and antagonism of bitter taste receptors. Human bitter taste receptors, namely hTAS2R16 and hTAS2R31, were produced using an *Escherichia coli* expression system and reconstituted into nanodiscs (NDs). Subsequently, hTAS2R16-NDs and hTAS2R31-NDs were immobilized on the surface of graphene field-effect transistors to construct bioelectronic tongues. The developed system sensitively detected their agonists, salicin and saccharin, up to approximately 100 fM with high selectivity in real time. Antagonists of hTAS2R16 and hTAS2R31 decreased the value of equilibrium constant (K) and resulted in a shift in the dose-dependent curve, indicating antagonism-based masking of bitter taste. Therefore, the developed bioelectronic tongues can be used to identify and mask bitter tastes based on the agonism and antagonism of hTAS2Rs.

Keywords: bitter taste receptor, nanodiscs, field-effect transistor, graphene, bitter tastes masking

LUMINESCENCE QUENCHING OF CARBON DOTS DERIVED FROM WASTED PAPER CUP FOR DETECTION OF HEMOGLOBIN

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Abstract. Hemoglobin is a tetrameric metalloprotein consisting a protein part (globin) and four iron-containg parts (heme), which exists in red blood cells. The hemoglobins carry oxygen and carbon dioxide between the lungs to various part of body through blood. The concentration of hemoglobin is highly related with clinical diseases, such as anemia, heart disease, leukemia, etc. To estimate the hemoglobin concentration, cyanmethemoglobin method is the most accurate and widely utilized method of hemoglobin detection. However, this method is not suitable for low-concentration measurement of hemoglobin. In order to solve these problems, fluorimetric measurement with carbon dots can be solution. Fluorescent carbon dots (CDs) have been widely studied owing to their water-soluble properties, bio-compatibility, high quantum yield, and strong chemical and optical stability. Also, as the dominant composition of carbon dots was based on carbon atoms, various organic composition can be utilized as a precursor of the CDs. Among organic components, the wasted paper cup is favorable candidates as the precursor of CDs, due to their annual disposal amount and easy separation.

In this study, CDs were synthesized by using hydrothermal method. The structural, morphological, luminescent properties and surface chemistry of the CDs were investigated. Also, the detection of hemoglobin was performed by analyzing the luminescence quenching of CDs for different hemoglobin concentration.

Keywords: carbon dot, luminescence, wasted paper cup, hemoglobin

APPLICATION OF A READY-TO-USE CELL SENSOR FOR DIOXINS AND DIOXIN-LIKE COMPOUNDS SCREENING IN MEAT SAMPLES

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Abstract. Dioxins and dioxin-like compounds (DLCs) in foodstuffs are closely related to human health. As China is the largest food-consuming country, there is a potentially large demand for screening bioassays that are rapid, cost-effective and capable of determining dioxins and DLCs in foodstuffs. CBG2.8D is a reporter gene-based recombinant cell sensor that was recently developed for determining dioxin and DLCs in ambient and seafood samples. In this study, we established a bioanalytical method with this ready-to-use cell sensor for the bioanalysis of dioxins and DLCs in different types of meat samples. Twenty-nine samples from three typical types of meat (beef, pork and fish) were collected and subjected to both instrumental analysis and a CBG2.8Dbioassay. The intra- and inter-lab reproducibility of the bioassay was investigated and the coefficients of variation (CVs) were lower than 25%, suggesting that the cell sensor had a good reproducibility for the meat samples. Based on the correlation equation and coefficient obtained by comparing the data from the instrumental analysis and CBG2.8D bioassay, we found that this method had better performance with pork and fish than with beef. The compliance rate was also determined by comparing the results from the instrumental analysis and there were no false results for the pork and fish samples. Lastly, a complete operation procedure was summarized as a guideline for practical application. In conclusion, the CBG2.8D cell sensor exhibits excellent stability and is capable of screening dioxins and DLCs in meat samples.

Keywords: AhR, Cell sensor, Dioxin, Food, Bioassay screening

EFFICIENT PRODUCTION OF HUMAN OLFACTORY RECEPTORS FOR PATTERN-BASED ODOR ANALYSIS USING CELL-FREE SYSTEM

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Abstract. Human odor perception relies on the intricate human olfactory receptor (hOR) response patterns that encode the identity of odors. Analyzing these patterns, resulting from the binding of odorants to hORs, is crucial for understanding how humans perceive odors. However, the complexity of approximately 400 hORs in humans presents significant challenges in deciphering the binding information. In this study, we present a method for efficiently producing hORs using a cell-free protein synthesis (CFPS) system. The CFPS system not only enables rapid production of multiple hORs but also facilitates simultaneous translation and solubilization of hORs due to its open nature. To enhance hOR expression, we utilized AT10 tag, previously reported by our group, and optimized the CFPS conditions to achieve the maximum hOR production yield. Additionally, we employed magnetic beads and separation racks for high-throughput purification. The proposed approach facilitates odor analysis based on response patterns by measuring hOR activation in response to various odors using a non-cell-based assay. This system represents a significant breakthrough in the study of human olfaction and, notably in standardizing odors for future research and applications.

Keywords: Human olfactory receptor, Cell-free protein synthesis, Pattern-based odor analysis, High-throughput

EXTRACTION OF DIFFERENT PROTEIN FEATURES AMONG MULTIPLE DEEP LEARNING MODELS FOR PROTEIN ANNOTATIONS

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Abstract. Protein sequence information is registered in biological databases, and then a large number of protein sequences are being sequenced by next generation sequencing technology. Accordingly, the number of unannotated protein sequences is more explosively increasing against annotated sequences. To efficiently determine the annotations, the extractions of new protein features which are different from existing knowledge are required. Deep learning can extract important protein features based on training data and then predict protein functions. Many studies have reported deep learning models with high accuracy for predicting protein annotations. However, in the reports, which amino acid sites in protein are important for the prediction of the annotations have not been discussed among multiple deep learning models. Here, 3 deep learning models for the prediction of the proteins included in a protein family were analyzed using an explainable artificial intelligence method to explore important protein features. As a result, the models regarded different sites as important for each model and for each sequence, and all models also recognize different amino acids from the secondary structure, conserved regions and active sites as important features. These results suggest that the models can interpret protein sequences through different perspectives from existing knowledge. Overall, building and evaluating models using multiple deep learning methods are more important to extract various protein features.

Keywords: deep learning, protein sequence, feature extraction, explainable artificial intelligence

IDENTIFYING IMPORTANT FLUXES BY FLUX VARIATION GENERATION

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Abstract. In addition to the conventional cultural data, omics data have been necessary and acquired for the research and development of metabolic analysis and simulation. However, it has been still a laborious task to (re-)acquire such experimental data each time the conditions such as host organism, strain, cell, medium composition, and/or culture conditions/or target organism or strain change. If the important variables to be measured are known in advance and the number of variables is small, the experimental burden can be expected to be reduces. Therefore, in this study, we aimed to extract important variables and necessary numbers to predict metabolic flux distribution for reducing the experimental burden. For this purpose, generation of flux variations by flux sampling with genome-scale metabolic model (GSM) and its analysis were performed. As a case study, acetate production from glucose in *Escherichia coli* with GSM iJO1366 was used. Flux sampling by OptGP using additional 1000 pattern constraints on substrate, product, and growth fluxes produced more variety samples than the default case. The analysis of the result suggested that iron ions, O_2, CO_2, and NH_4^+ fluxes were important for predicting the metabolic flux distribution. Additionally, metabolic flux distributions extracted from flux sampling results using a literature value of CO_2 flux as a query were compared with the literature result of ^{13}C metabolic flux analysis. As a result, it was suggested that the important flux obtained by this method was valid for the prediction of flux distribution. Therefore, the proposed method of this research was useful for extracting important variables for predicting metabolic flux distribution and suggested the possibility of contributing to reducing measurement variables in experiments.

Keywords: genome-scale metabolic model, flux sampling, flux distribution prediction, important flux extraction, reduction of the experimental burden

DIFFERENTIAL EXPRESSION ANALYSIS REVEALS KEY GENES ASSOCIATED WITH RESPONSE MECHANISMS AGAINST STRAINS OF *XANTHOMONAS ORYZAE* INFECTION IN RICE *(ORYZA SATIVA* L.*)*

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Abstract. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)*, and bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola (Xoc)*, are severe diseases devastating tremendously on rice crops globally. In this study, we conducted a comprehensive bioinformatics analysis to identify key genes involved in response mechanisms against *X.oryzae* infection through available transcriptomic data. Specifically, we analyzed GSE36272, a microarray expression dataset, from GEO-NCBI database, including 65 samples of two susceptible rice cultivars *(Nipponbare, IR24)* inoculated with 7 strains *of Xoo* (3 pathogenic wild-type, 1 non-virulent, 1 pathogenicity-reduced and 2 mutant strains) and 1 strain of *Xoc*, together with controls: untreated condition and reduced water-added condition. All samples have been subjected to in-depth analysis, including *quantile normalization*, *principle component analysis*, *differential expression analysis* implemented in RStudio combined with GEO2R tool to find a common set of differentially expressed genes (DEGs) for 2 tested cultivars. As a result, significant DEGs (p-value ≤ 0.05) compared to the controls were found in all bacterial-inoculated samples. Separate analysis of samples inoculated with pathogenic wild-type *Xoo* strains *(PXO99A, T7174, PXO86)* identified 3500 downregulated genes (logFC ≤ -1) and 2497 upregulated genes (logFC ≥ 1), 2595 downregulated genes and 2032 upregulated genes that are common responses of both cultivars to *Xoc (BLS303)* infection. KEGG Pathway analysis classified DEGs into 13 different pathways, with *phenylpropanoid biosynthesis* pathway being the highest proportion in *Xoo* infection (4.1%). Furthermore, we constructed protein-protein interaction (PPI) network using STRING database and MCODE algorithm in Cytoscape was applied to predict hub genes and modules. Gene encoding *ribose-5-phosphate isomerase 3* and *adenylate kinase 5* were found as key genes in response pathways against *Xoo*, while *chloroplastic photosystem I reaction center subunit XI* was shown as hub gene in response pathways against *Xoc*. The identified genes may have a vital role in elucidating molecular mechanisms against *X.oryzae* infection in rice.

Keywords: X.oryzae, bioinformatics, microarray expression, R, DEGs, KEGG, PPI

SYNTHETIC SIGNAL PEPTIDE LIBRARY FOR THE ENHANCED PRODUCTION OF RECOMBINANT PROTEINS IN *CORYNEBACTERIUM GLUTAMICUM*

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Abstract. *Corynebacterium glutamicum* stands out as an appealing candidate for biotechnological applications in recombinant protein production. Its non-sporulating, non-pathogenic, and Generally Recognized as Safe (GRAS) characteristics make it a favorable choice. Being a gram-positive bacterium with a single cell membrane and minimal endogenous secretory proteins, it enables the efficient and pure overproduction of target proteins through secretory expression. Despite these advantages, challenges persist in its effective utilization. One key aspect is the need for an appropriate signal peptide to successfully secrete specific proteins. To address this, we introduce an innovative approach to enhance the extracellular production of recombinant proteins in *C. glutamicum*. We initiated this strategy by constructing a library of synthetic signal peptides (SPs), facilitating sec-dependent secretory production within *C. glutamicum*. Through screening this SP library, we identified the optimal signal peptide for achieving efficient secretory production of target proteins. Leveraging this optimized signal peptide, we achieved significantly effective secretory production of the desired protein for secretory production in *C. glutamicum*.

Keywords: *Corynebacterium glutamicum*, Secretory production, Synthetic peptide library, Recombinant protein

ENGINEERING OF *ESCHERICHIA COLI* TO INCREASE *DE NOVO* PRODUCTION OF *P*-COUMARIC ACID

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Abstract. *p*-coumaric acid, a phenolic acid of significant commercial value, finds a range of practical applications in the fields of nutraceuticals and pharmaceuticals due to its notable antioxidant, antibacterial, and anticancer properties. Furthermore, *p*-coumaric acid is gaining attention for its role as a central intermediate in the phenylpropanoid biosynthesis pathway. For the production of *p*-coumaric acid in *Escherichia coli*, we first developed *E. coli* able to overproduce L-Phenylalanine and *trans*-cinnamic acid, both primary precursors of *p*-coumaric acid, through intensive modifications to metabolic pathways. Subsequently, a biosynthesis route for *p*-coumaric acid was established from *trans*-cinnamic acid by concurrently expressing cinnamate-4-hydroxylase and its reductase sourced from *Arabidopsis thaliana*. Additional enhancements were made by eliminating acetate production pathways and improving the NADPH pool to amplify production yields. With the engineered strain, we engaged in fed-batch cultivation using a 5-L scale bioreactor. By optimizing the bioprocess, including the induction timing, we successfully achieved the production of gram-scale quantities of *p*-coumaric acid.

Keywords: Escherichia coli, *p*-coumaric acid, *trans*-cinnamic acid, metabolic engineering

BIOLOGICAL EFFECTS OF Γ-IRRADIATION CAUSING LOW-DOSE HYPER-RADIOSENSITIVITY IN HUMAN FIBROBLAST CELLS - RELATION WITH NRF2 GENE EXPRESSION

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Abstract. Effects of low-dose radiation (LDR) on living organisms including human beings are still being debated, despite of LDR being ubiquitous in our environment. To elucidate the molecular mechanism of hyper-radiosensitivity (HRS), which is one of prominent phenomenon of LDR, the precise dose eliciting HRS was determined using human normal fibroblast cells. The cells were treated with γ-rays irradiated from a cobalt-60 source by 20-3,000 mGy and after two days, the dose of HRS was determined as 60 mGy by the assay of living cell number. Because gene expression of NF-E2 (Nuclear Factor, Erythroid 2)-related factor2 (Nrf2) in various biological systems is known to increase against many stresses, such as exposure to various toxins or irradiations, the real-time RT-PCR (reverse transcription polymerase chain reaction) targeted on the Nrf2 gene was performed. The levels of Nrf2 mRNA at 40 and 60 mGy were less than those of 20 and 80 mGy, which corresponded to the radiation doses for HRS. These results suggest that the HRS phenomenon in human fibroblast cells is strongly related to the reduced response of the Nrf2 gene expression.

Keywords: low-dose radiation, hyper-radiosensitivity, γ-rays, human fibroblast cells, Nrf2

IN SILICO ASSESSMENT FOR HIGH-RISK CARDIOVASCULAR VARIANTS IN THE VIETNAMESE POPULATION FROM THE PUBLIC GENOME DATABASE

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Abstract. Cardiovascular diseases (CVDs) are multifactorial diseases with multiple genetic factors, which increase the risk of cardiovascular diseases. This study aims to identify high-risk cardiovascular variants in the Vietnamese population. Using an in-house bioinformatic pipeline, we attempt to identify the prevalence of potential genetic variants associated with cardiovascular diseases and possible novel cardiovascular variants in 122 Kinh people datasets from the 1000 Genomes Project. This result shows cardiovascular mutations can be identified in Vietnamese datasets with 238 genes, with two high risk variants. In the Vietnamese population, the distribution of cardiovascular mutations is expressed by *FTO*, *ABCC8*, and *PPARG*. These genes are the most potentially mutagenic genes. Conversely, *NPPA*, *LPA*, and *CNNM2* are the genes with the lowest mutagenicity. In addition, the prevalence of genetic variants is classified into four variant consequences in coding regions, such as missense variant (73%), stop-loss variant, and in-frame deletion variant (1%). Furthermore, cardiovascular mutations identified 25 possible novel genes associated with cardiovascular diseases, with two potentially novel high-risk variants at the *NPPA* gene in the 122 KHV datasets. Two mutations of *NPPA* are stop-lost with coding sequence (CDS) positions such as NPPA:c.304T>C and NPPA:c.464T>C, which have not been provided evidence in OMIM databases or Vietnam studies. These mutations are predicted for clinical significance with benign clinical impact through Clinvar and the Variant Effect Predictor (VEP), which affect cardiovascular diseases (atrial fibrillation familial type 6). These results present the genetic diversity related to cardiovascular diseases in healthy people in the Vietnamese population and provide the prevalence of possible genetic variants associated with cardiovascular diseases. In addition, these findings assess the clinical significance of mutations in the *NPPA* gene with two high-risk variants. These findings of the research project can contribute to the cardiovascular disease screening program by enhancing the understanding of cardiovascular variants and operating better in the experimental laboratory for future research.

Keywords: *high-risk cardiovascular, population genetic, Genome Database, in silico sreening*

THE PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTIONS AND RELATED GENES AMONG STUDENTS IN HO CHI MINH CITY

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Abstract. Vietnam has seen the emergence of antibiotic-resistant bacterial strains such as *Staphylococcus aureus* as a result of the irresponsible and undirected use of antibiotics. This pathogen can cause more severe opportunistic infections, including bloodstream infections and even life-threatening conditions like endocarditis. Numerous studies have demonstrated that *S. aureus* can develop mechanisms of antibiotic resistance or evolve into highly resistant strains, particularly Methicillin-resistant *S. aureus* (MRSA), posing treatment difficulties. In this study, the prevalence of multidrug-resistant MRSA strains and the Panton-Valentine leucocidin (PVL) virulence genes of *S. aureus* isolated from 350 nasal swabs was determined and updated. The results of the analysis revealed that 63 (54.7%) of 115 *S. aureus* strains contained the methicillin resistance gene, MecA. Moreover, the PVL virulence gene was found in 25 strains (7.1%). The prevalence is consistent with previous research on the prevalence of MRSA in the community, which indicates that there was no change after the implementation of Covid-19.

Keywords: Multi-resistant, MecA, MRSA, Panton Valentine leucocidin, *Staphylococcus aureus*.

EVALUATION OF THE EFFECTIVENESS IN CYTOLOGICAL DIAGNOSIS AND USABILITY FOR DNA EXTRACTION OF TOPPURE ® HPV PAP SMEAR COLLECTION KIT

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Abstract: Currently, Liquid-Based Pap Test (LBPT) is a common method in the early screening and detection of cervical cancer in Viet Nam. TopPURE ® HPV Pap Smear Collection Kit is a gynecological cell collection kit made in Viet Nam, optimized for LBPT. The purpose of this study is to evaluate the effectiveness in cytological diagnosis and the usability for DNA extraction of TopPURE ® HPV Pap Smear Collection Kit. The evaluation result of 30 random swab samples collected from clinics shows that the kit effectively removes mucus and blood in the sample and the treated cells are still well stained with PAP stain. Cells are stored for a month that keeps their shade and the nucleus is clearly visible when stained with PAP stain. The high diagnostic of the kit is shown by the Kappa value = 1, which is completely similar to the diagnostic results compared to the control kit. The real-time PCR test results show that the number of samples that are positive for HPV account for 76.7% and positive for the internal control (hRP gene) account for 100% of the total samples, showing that the samples treated with the kit can be used for DNA extraction.

Keywords: Cervical cancer, HPV, LBPT, TopPURE ® HPV Pap Smear Collection Kit

PRE-CLINICAL RESEARCH OF DENTAL MEDICAL DEVICES; DENTAL IMPLANTS, BONE GRAFTING MATERIALS, AND MEMBRANES

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Abstract. K-MEDI hub (Daegu-Gyeongbuk Medical Innovation Foundation) is a public institution established to support the improvement and diffusion of medical industry research achievements. Through this connection, we are supporting the development of new medical technologies and various medical companies. The medical device support team of K-MEDI hub pre-clinical research center (PRC) uses animal models to perform basic research related to various medical devices such as dental medical devices, vascular connection medical devices, and therapeutic auxiliary devices, as well as performance evaluation necessary for product licensing and commercialization. We are constantly researching to present reliable results, and in this presentation, we would like to introduce representative effectiveness evaluations conducted for the approval of dental medical devices.

Keywords: dental medical devices, pre-clinical research, experimental animals, dental materials

INVESTIGATING ON GENETIC DIVERSITY OF *NOCARDIA SERIOLAE* CAUSES CHRONIC DISEASE IN FINFISH IN TAIWAN

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Abstract. Chronic disease following *Nocardia seriolae* infection in a wide range of aquatic animals has been reported in many Asian countries and recently in America and Mexico. This study aimed to investigate genes and gene variants associated with the disease. A total of 66 strains isolated from 14 known and four unknown host fish from five sites (in Taiwan) were characterized using two combined methods – pulsed-field gel electrophoresis PFGE and repetitive extra-genic palindromic amplification rep – PCR. Macrorestriction analysis using PFGE depends on the number and distribution of restriction sites throughout DNA, while rep-PCR uses outward-facing primers (BOXA1R) to amplify multiple segments of DNA located between conserved repeated sequences**.** High genotypic diversity was recognised among the isolates with 10 pulsotypes being identified from the PFGE method and 21 reptypes from the rep-PCR method. Pulsotypes A8 and RI analysed by PFGE and repPCR, respectively, were found to be predominant within five sites in Taiwan over 17 years of isolation. This study provides potential epidemiological data, which will aid the fish farming activities and prevention method development.

Keywords: Nocardia seriolae, aquatic animals, genotype, Pulse-field Gel Electrophoresis, Repetitive extragenic Panlindrome PCR

PATHOGENICITY OF NOCARDIOSIS IN MODEL ORANGE-SPOTTED GROUPER, *EPINEPHELUS COIOIDES,* VIA EXPOSURE TO NINE DISTINCT ISOLATES OF *NOCARDIA SERIOLAE*

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Abstract. This study investigates on pathogenicity of nocardiosis following *Nocardia seriolae* infection with different pulsotypes in model orange-spotted grouper *Epinephelus coioides*. Grouper is a highly evaluated produce fish species in Taiwan aquaculture and has been recorded that facing the risk of devastation by *N. seriolae* infection. Therefore, it is important to understand the pathogenic nocardiosis in grouper to establish well-suited prevention and practice strategies in fish farming. Nine pulsotypes of *N. seriolae*, which were characterized in our previous study, were tested in vivo by intraperitoneal method and daily observed for 35 days. The examination was performed on clinical signs, cumulative mortality, gross test, and histopathology. We found a mild variation in virulent manifestation among pulsotypes of *N. seriolae* via *in vivo* tests. Isolates AOD107132-2K (A3) and OT103003-N11(A2) displayed the highest and lowest risk-virulent based on their results on percentages and kinetics of mortality within 10 days. Histopathology confirmed that nocardiosis progressed in disease fish with typical early or/and newly granulomas appearing all over inner organs. This investigation brings aid to further studies in the pathogenicity of nocardiosis and virulence profile of *N. seriolae* in Taiwan.

Keywords: nocardiosis, *Nocardia seriolae*, pathogenicity, orange-spotted grouper, pulsotypes, granulomatous.

A NOVEL COMPLEX NANOCAPSULE PREPARED BY IONIC GELATION OF CHONDROITIN SULFATE AND CHITOSAN USED FOR ENCAPSULATION OF FISH OIL

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Abstract. In the current study, a biocompatible nano-carrying platform using chitosan (ChI) and chondroitin sulfate (ChS) was developed for the encapsulation of cobia liver oil (CBLO) in order to prevent oxidation and improve its absorption. An ionic gelation method was applied to encapsulate CBLO with different weight ratios (from 1.0 to 1.5) to obtain the ChS-ChI nano-capsules (ChS-ChI@CBLO NCs). SEM and TEM characterization of the nano-capsules displayed a spherical shape and diameter around 267-381 nm. The loading capacity (LC) and encapsulation efficiency (EE) for ChS-ChI@CBLO NCs estimated by TGA (Thermal Gravimetric Analysis) and DTG (Derivative Thermal Gravimetric) analysis were 16.5-25.7 % and 56.2-33.4 %, respectively. The structure stability of ChS-ChI@CBLO NCs was confirmed through DSC and XRD analysis, moreover DSC also further confirmed the anti-oxidation stability of ChS-ChI@CBLO NCs. FTIR spectra confirmed excellent stability of ChS-ChI@CBLO NCs against high temperature and sunlight exposure. Nano-degree of ChS-ChI@CBLO NCs has great loading capacity and encapsulation efficiency for encapsulation of CBLO. In addition, characterized results also can indicate the ChS-ChI@CBLO NCs have high anti-oxidation ability to against long-term, hyperthermia, and ultraviolet exposure. Therefore, this study demonstrates that nano-capsules are beneficial to preserve sensitive compounds to prevent metamorphosis, and non-toxicity in safety. These materials are suitable to expand their applicability for human health in the food and pharmaceutical industries.

Keywords Anti-oxidation; complex-nutrition; fish oil; nano-capsule; chondroitin sulfate; chitosan

RADIATION DEGRADATION OF YEAST β-GLUCAN WITH NOVEL POTENTIAL APPLICATION AS A NATURAL IMMUNOSTIMULANT ADDITIVE FOR SHRIMPS

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Abstract. β-glucan has been well known as an immunostimulant and growth-promotion additive in animal husbandry as well as in aquaculture. However, the main disadvantages of this natural polymer such as the high molecular weight (Mw) and water-insoluble properties lead to a low permeability into cells and low bioactivity. In this study, the low Mw and water-soluble *β*-glucan products were successfully prepared by γ-ray and electron beam (EB) irradiations in combination with 1% hydrogen peroxide. Both γ-ray and EB irradiation are useful methods for the degradation of *β*-glucan . However, the γ-absorbed dose for the degradation of this natural polymer was lower than by EB to obtain the same degradation level. Particularly, for inducing water-soluble *β*-glucan with Mw~15 kDa from the native product with Mw~64 kDa, the required dose for degradation by γ-irradiation was found about 50 kGy, while EB irradiation required approximately 70 kGy. The structural characterization results of water-soluble *β*-glucans analyzed by Fourier transform infrared spectra (FTIR) and X-ray diffraction (XRD) showed that the irradiation did not cause any change in basic structure of *β*-glucans, except for the reduction in degree of polymerization. In addition, the immunostimulant effect of the obtained water-soluble *β*-glucans was tested on the Pacific white shrimp (*Litopenaeus vannamei*) and Tiger shrimp (*Penaeus monodon*) challenged with *Vibrio parahaemolyticus*. The results showed that the cumulative mortality of tested shrimps in the diets supplemented with water-soluble *β*-glucan significantly decreased compared to that of the control ($p<0.05$). The water-soluble *β*-glucan with Mw of 15 kDa was found to be the most effective, which displayed protective effects on tested Pacific white shrimps and Tiger shrimps about 56.5 and 60%, respectively. These results revealed that the radiation degraded *β*-glucan with Mw~15 kDa prepared by γ-ray or EB is potentially promising to be used as a natural immunostimulant additive for shrimp culture.

Keywords: γ-irradiation, electron beam, low molecular weight, shrimp, *Vibrio parahaemolyticus*, water-soluble *β*-glucan

DUAL CROSS-LINKED PVA/METHACRYLATE HA/COS-SINAPIC ACID HYDROGEL FOR WOUND DRESSING

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Abstract. The pursuit of an optimal wound dressing hydrogel, balancing antioxidant, antimicrobial, mechanical, and biological attributes, remains a formidable challenge in the field. Our current investigation involved synthesizing chitooligosaccharides (COS) conjugated with sinapic acid (SA) through an H2O2-triggered grafting polymerization. Concurrently, we enhanced hyaluronic acid using methacrylation, yielding a compound termed "HAMA". A series of analytical methods, including Fourier-transform infrared spectroscopy and proton nuclear magnetic resonance spectroscopy, validated the synthesis of COS-SA and HAMA. Merging these components with polyvinyl alcohol (PVA) led to the development of a dual cross-linked composite hydrogel, purposed for full-thickness wound treatment. Our evaluations spanned from its physical characteristics to its in vitro biocompatibility. Preliminary findings indicated that the hydrogel exhibited consistent porosity, high absorbance, and mechanical resilience. Intriguingly, integrating the COSs-SA conjugate notably amplified the hydrogel's biocompatibility, antioxidant, and antimicrobial traits. In vivo trials further illustrated its efficacy in promoting wound closure, tissue regeneration, and collagen synthesis. Conclusively, our work suggests that the COSs-SA augmented PVA/HAMA hydrogel holds considerable promise for advanced wound care applications.

Keywords: Fibrous membrane, Phycocyanin, Atelocollagen, Bone regeneration

FABRICATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY HYDROGEL BASED ON FISH GELATIN/OXIDIZED HYALURONATE FOR DIABETIC WOUND TREATMENT

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Abstract. Persistent inflammation is a significant challenge in the chronic wound healing such as diabetic wounds or open trauma infections. In this study, we report a hydrogel that promoted wound healing under chronic inflammation conditions. The hydrogels were fabricated fish gelatin (FG), which has recently emerged as a substitute for gelatin derived from land animals, cross-linked with oxidized hyaluronate (OHy) as a natural macromolecular crosslinker. FG was extracted with hot water from *Paralichthys olivaceus* skin and identificated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), hydroxyproline content, fourier transform infrared (FTIR) spectroscopy. In addition, fabricated hydrogels of different ratio were assessed for their swellin, gel fraction, morphological and rheological properties. In an *in vitro* studies, hydrogels were non-cytotoxicity on human dermal fibroblasts (HDF) and RAW 264.7 macrophages. The hydrogels also exhibited antioxidant and anti-inflammatory effects by inhibiting the reactive oxygen species (ROS) and nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and prostaglandin E_2 (PGE_2) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Furthermore, the *in vivo* diabetic wound model showed that the hydrogel can reduce inflammatory responses and promote re-epithelialization, angiogenesis and collagen deposition. These results demonstrate that hydrogels can be successfully used for chronic wound healing applications.

Keywords: Fish gelatin, Oxidized hyaluronate, Diabetic wound healing

THE COMPLETE MITOCHONDRIAL GENOME OF SIGANUS VIRGATUS VALENCIENNES (SIGANIDAE: ACANTHURIFORMES)

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Abstract. *Siganus virgatus* Valenciennes 1835 is an essential species for examining reef ecosystems; however, its mitochondrial genome has not been studied. In this research, the mitogenome of *S. virgatus* was sequenced and characterized. The results revealed a circular genome of 16,505 bp that was composed of A (28.1%), C (31.3%), G (14%), and T nucleotides (26.6%). The genome contained 13 protein-coding genes, 22 transfer RNA genes, and two ribosomal RNA genes. Most genes of the mitogenome were transcribed on the heavy strand (H-strand), whereas *ND6* and eight tRNA genes (including *tRNA-Ala, -Asn, -Cys, -Gln, -Glu, -Ser* (1)*, -Pro,* and *-Tyr*) were transcribed in the light strand (L-strand). Comparative analysis revealed high a degree of conservation of gene content and order among the Siganus mitogenomes. Phylogenetic analysis inferred from whole mitogenomes exhibited a close relationship between *S. virgatus* and *S. guttatus*. The newly completed mitogenome of *S. virgatus* provides genomic data for further studies on population genetics and the evolution of the *Siganus* genus and the Siganidae family.

Keywords: mitogenome, phylogenetic relationship, Siganudae, rabbitfishes.

PROTECTIVE EFFICACY TESTING OF HERBAL PRODUCT AGAINST EARLY MORTALITY SYNDROME – EMS AND WHITE SPOT SYNDROME -WSSV ON WHITELEG SHRIMP (*LITOPENAEUS VANNAMEI*)

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Abstract. Early mortality syndrome (EMS) caused by *Vibrio parahaemolyticus* pVPA3-1 and white spot syndrome caused by White Spot Syndrome Virus (WSSV) cost the global shrimp production industry billions of dollars annually. In current shrimp farms, the use of chemicals and antibiotics are preferred for treating and controlling many diseases, including white spot disease, thus resulting in many obstacles for shrimp export and consumption. Using herbs in aquaculture has been considered as potential alternatives to antibiotics. Extracts of 30 indigenous herbs were evaluated for their antibacterial and antiviral activities. The results showed that there were two herbal extracts at a feeding dose of 3.000 mg kg^{-1} providing a protective effect of 52% against *V. parahaemoliticus* pVPA3-1 and at a feeding dose of 100 mg kg^{-1} with the protection of 40-50% against WSSV. These extracts were mixed to develop a safe herbal product for shrimp and protected up to 40-50% shrimp from EMS and WSSV when feeding continuously with the feed mixed with the herbal product within 21 days at the laboratory scale. The pond trial at Duyen Hai Fishery and Service Cooperative in Can Gio District showed that shrimps raised in ponds and fed with feed mixed with our herbal product showed bright and immediate intestinal tracts than the control shrimp. At week 4, shrimp in the control pond were infected with white spot syndrome and died completely, while the shrimp in the experimental pond still grew normally and reached the survival rate of 75% after 90 days of culture. These results demonstrated that the herbal procduct developed by our team had an ability to prevent EMS and WSSV infection in shrimp ponds and also increased shrimp digestibility.

Keywords: White Spot Syndrome Virus, White Spot Syndrome-WSSV, Early mortality syndrome (EMS), *Vibrio parahaemoliticus*, Herbal extracts, white leg shrimp (*Litopenaeus vannamei*)

FABRICATION OF BILAYER NANOFIBER COMPOSITE FISH COLLAGEN/PCL/CHITOOLIGOSACCHARIDES SCAFFOLDS FOR FULL-THICKNESS WOUND-HEALING

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Abstract. The development of tissue-engineered biodegradable artificial tissue substitutes with extracellular matrix-mimicking properties that govern the interaction between the material and biological environment is of great interest in wound-healing applications. In the present study, novel bilayer nanofibrous scaffolds composed of fish collagen (FC) and poly(ε-caprolactone) (PCL) were fabricated using electrospinning, with the covalent attachment of chitooligosaccharides (COS) via carbodiimide chemistry. The architecture and fiber diameter of the non-cross-linked nanofibrous scaffolds remained consistent irrespective of the polymer ratio under different electrospinning conditions, but the fiber diameter changed after cross-linking in association with the FC content. Fourier-transform infrared spectroscopy analysis indicated that the blend of biomaterials was homogenous, with an increase in COS levels with increasing FC content in the nanofibrous scaffolds. Based on cytocompatibility analysis (i.e., the cellular response to the nanofibrous scaffolds and their interaction), the nanofibrous scaffolds with high FC content were functionally active in response to normal human dermal fibroblast-neonatal (NHDF-neo) and HaCaT keratinocyte cells, leading to the generation of a very effective tissue-engineered implant for full-thickness wound-healing applications. In addition to these empirical results, an assessment of the hydrophilicity, swelling, and mechanical integrity of the proposed COS-containing FC-rich FC/PCL (FCP) nanofibrous scaffolds confirmed that they have significant potential for use as tissue-engineered skin implants for rapid skin regeneration.

Keywords: Fish collagen, PCL, Chitooligosaccharide, Electrospune nanofiber, Full thickness wound healing

FABRICATION OF TRIPLE CROSS-LINKED METHACRYLATE KAPPA-CARRAGEENAN/POLY(VINYL ALCOHOL)/CHITOOLIGOSACCHARIDE WOUND DRESSING FOR WOUND HEALING

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Abstract. To develop an effective and mechanically robust wound dressing, a poly (vinyl alcohol) (PVA)/methacrylate kappa-carrageenan (κ-CaMA) composite hydrogel encapsulated with a chitooligosaccharide (COS) was prepared in a cassette via repeated freeze/thaw cycles, photo-crosslinking, and chemical cross-linking. The chemical, physical, mechanical, in vitro biocompatibility, in vivo wound-healing properties, and antibacterial activity of triple-crosslinked hydrogel were subsequently characterized. The results showed that the PVA/κ-CaMA/COS (Pκ-CaC) hydrogel had a uniformly thick, highly porous three-dimensional architecture with uniformly distributed pores, a high fluid absorption, and retention capacity without disturbing its mechanical stability, and good in vitro biocompatibility. Macroscopic images from the full-thickness skin wound model revealed that the wounds dressed with the proposed Pκ-CaC hydrogel were completely healed by day 14, while the histomorphological results confirmed full re-epithelization and rapid skin-tissue remodeling. This study thus indicates that the composite Pκ-CaC hydrogel has significant potential for use as a wound dressing.

Keywords: PVA, Kappa-carrageenan, Chitooligosaccharide, Hydrogel

DEVELOPMENT OF PHLOROTANNIN-COATED POLY (ε-CAPROLACTONE) FILMS WITH ANTI-INFLAMMATORY PROPERTIES FOR UTILIZATION AS A POSTOPERATIVE ANTI-ADHESION AGENT

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Abstract. Phlorotannins are known to possess several therapeutic properties such as anti-inflammatory and anti-oxidant effects, both of which are related to prevent tissue adhesion. Therefore, this study sought to fabricate phlorotannin-coated PCL films for the prevention of post-surgical adhesion. Phlorotannin-coated PCL films were fabricated via the solvent casting and coating methods. After which the fabricated film was characterized using water contact angle analysis, Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and drug release ability analysis. Furthermore, the anti-inflammatory and cell proliferation-inhibiting properties of the fabricated film were assessed in LPS-stimulated RAW264.7 macrophage cells and NHDF-neo cells using the nitric oxide production assay, the WST-1 assay kit, and Hoechst 33342 dye. The results indicated that the phlorotannin-coated PCL film significantly inhibited nitric oxide production and reduced NHDF-neo cell proliferation. These results suggest that phlorotannin-coated PCL films constitute a promising anti-adhesive bioactive compound with applicability in the field of DDSs.

Keywords: Anti-adhesion, Anti-inflammation, Coating, Film, Phlorotannins, Poly (ε-caprolactone)

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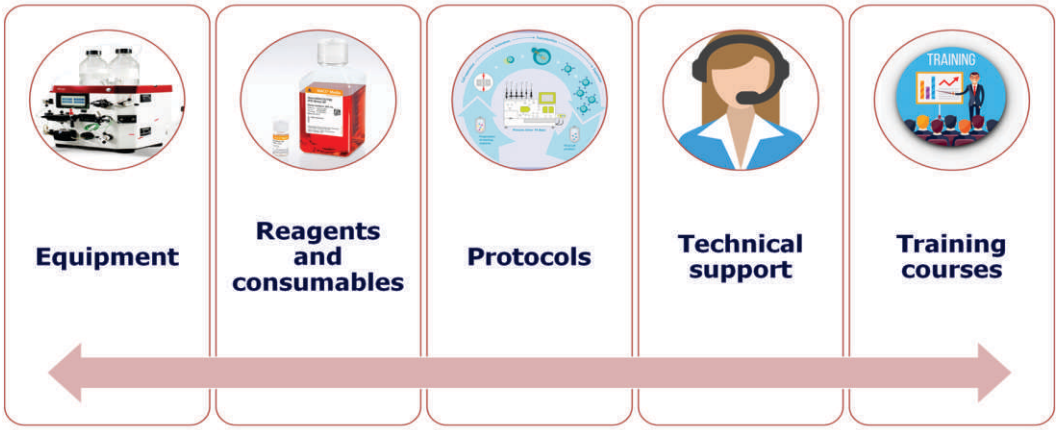

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- ✓ Nucleic Acid extraction
- ✓ PCR/ Realtime PCR
- ✓ Hybridization
- ✓ NGS library preparation
- ✓ MLPA
- ✓ ...

Cell & Gene Research and Therapy

- ✓ Cell biology research
- ✓ Drug testing/ QC
- ✓ Stem Cell therapy
- ✓ Immune Cell therapy
- ✓ ...

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Ultra II DNA PCR-free input amounts: 250 ng – 1,000 ng sheared DNA

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- Total time:* ~2:30 – 3:00 hrs

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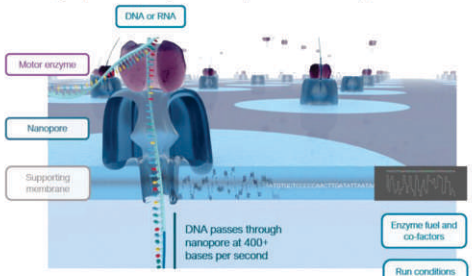

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VEMEDIM IS A PIONEERING ORGANIZATION IN VIETNAM THAT HAS INVESTED IN CONSTRUCTING A BIOTECHNOLOGICAL FACILITY SPECIALIZING IN INTERFERON AND PROBIOTICS PRODUCTION

To proactively engage in research and development of biotechnological products, Vemedim has invested in specialized facilities for probiotic and interferon products equipped with state-of-the-art fermentation processes. Furthermore, we conduct selective research on microbial strains with the most promising probiotic characteristics to promote sustainable livestock farming practices.

Our products, such as Bactozyme, Lactozyme, LacProbi, Probiotic, Probi P, and Vime-Subtyl, have consistently exceeded expectations through clinical trials and practical applications in various farming regions and facilities. They promote robust animal health, rapid weight gain, reduced food consumption, and decreased foul odors in livestock environments. Additionally, these formulations effectively inhibit and eliminate intestinal pathogenic bacteria like *E. coli, Salmonella, Klebsiella, Vibrio,* and *Edwarsiella,* reducing reliance on antibiotics and addressing the growing antibiotic resistance issue.

With the trend toward bio-safe livestock farming, Vemedim plans to continue its research and invest in cutting-edge equipment. Our goal is to introduce the best biotechnological products to the market, capable of completely replacing antibiotics and contributing to the supply of clean food for consumers in the future.

tabc

Vietnam Technical And Applied Biology Joint Stock Company

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Email: Sales@tabc.com.vn; Phone: 024 628 44446
HCM Office: 3/35, Binh Gia Street, 13 Ward, Tan Binh District, HCM
Email: Thermobio@gmail.com; Phone: 028 6682 1019

* Nucleic acid isolation kits
*Reverse Transcriptase Enzymes and cDNA kits
*Enzymes and Master mix for PCR; RT-qPCR
*Modified Enzymes and Electrophoresis reagents
*Chemicals used in ELISA, WESTERN Blot...
*Synthesis: oligonucleotide, probe, plasmid, gene...

*Microbial culture medium
*Mediums and Chemicals for cell culture
*Antibody for research
*Basic chemicals of high purity
* Reference standards used in HPLC, GC, LC analysis...

* Providing high-quality plastic ware for Life Science Laboratory Consumables
* Choosing our plastic consumables for everyday research as well as ground breaking discoveries.

ThermoFisher SCIENTIFIC

Thermo Scientific™ molecular biology workflow solutions:
* High-quality essentials for everyday applications
* Supporting great science through innovation in molecular biology
*Molecular biology portfolio has represented leading technology, enabling reliable performance for every step of the traditional molecular biology workflow.

Scepter™ 3.0 Handheld Automated Cell Counter
The handheld device allows for quick, accurate and easy cell count measurements

Millicell® DCI Digital Cell Imager
Rapid and objective assessment of confluency, morphology and growth status in cell culture

thermo scientific
Authorized Distributor

Merck KGaA,

SYNBIO TECHNOLOGIES

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

BIO BASIC INC.

SIGMA-ALDRICH

SPL
SPL LIFE SCIENCES

WATSON BIO LAB

SSIbio

SARTORIUS

ABT BIOLOGICAL SOLUTIONS COMPANY LIMITED

ABT is one of the leading biotechnology companies, specializing in manufacturing and providing products, services and total solutions in the field of Molecular Biology Diagnostics.

ABT always focuses on product research and development activities, applying scientific and technical achievements to create the best products, services and best quality solutions, contributing to affirming the Vietnam brand in the international market.

In addition, ABT's human resources are elite and experienced, operating with the motto "Dedication - Responsibility - Creativity" combined with modern production processes that meet international standards (ISO 9001: 2015, ISO 13485:2016) will bring the best products, services and solutions to customers.

Customers coming to ABT will receive free technical training and consulting support, products with clear warranty policy, stock in hand and fast delivery.

NUCLEIC ACID EXTRACTION KITS

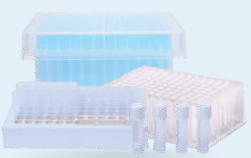
REAL-TIME PCR KITS

EQUIPMENTS

ABT
The Next Generation of Biotech Solution

REAGENTS

MOLECULAR LABORATORY SET-UP SERVICE & OLIGO SYNTHESIS SERVICE



CONSUMABLES

http://www.abtvn.com
sales@abtvn.com
0903 307 258

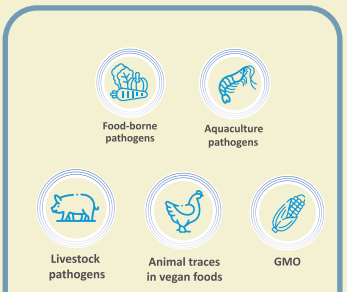
KHOA THUONG BIOTECHNOLOGY COMPANY LIMITED

KT BIOTECH

Since being established in 2004, the origin from The University of Science, KT biotech has become the leading company in Vietnam in developing and commercializing real-time PCR kits. We offer an extensive range of Diagnostic reagents includ - ing real-time PCR kits and ELISA kits. Our extensive product portfolio is tailored to assist you to detect pathogenic factors in aquaculture, human health, livestock, GMO and bacteria in food. On the other hand, scientific services such as research and development, research cooperation is also one of our important considerations.

As the combination of Science and Commercial, we have an experienced R&D team, a commitment sales force, and a quick response customers supporting team. Through out of nearly 20 years of development, we have built a complete network with distribu- tion partners across South East Asia and end-users from various organizations all over Vietnam.

We provide total solutions with high quality and reliability to our customers, and prompt responses to your urgent need to deal with new challenges.



Real-time PCR Kits

DNA/RNA Extraction Kits

Scientific services

ELISA Kits

CÔNG TY TNHH CÔNG NGHỆ SINH HỌC KHOA THƯƠNG

https://kt-biotech.com/ sales@kt-biotech.com (+84) 028.3761.2606

MEDIWORLD LIMTITED COMPANY

Mediworld BIOTECHNOLOGY

Mediworld Co., Ltd. has always been the pioneer in the field of applying Biotechnology in **Dermatology and Aesthetic Skincare.** Up to now, Mediworld has always tried to cooperate with Vietnamese scientists especializing in Biotechnology, which aims at creating and developing aesthetic skincare products and solutions that are scientifically suitable for Vietnamese skincare industry.

Biotechnology Skincare Solutions aim at supporting the prevention and slowing down of the skin aging process, supporting the recovery of damaged skins which are caused by the chemicals overuse in the skin care process, which is proved to be the company strengths and makes a difference in today's cosmetics market.

For example: The solutions of skin nourishing, restoring and regenerating.
STEM CELL EXTRACT Serum is one of a generation of Serums which is improved with the use of deer antler stem cell extract cultured in an environment that does not contain FBS (Fetal Bovine Serum), helping to eliminate IgG factors (Fetal Bovine Serum) that are considered a cause of immune irritation) and prevent infectious factors that may come from animal sources.

Solutions of health improving and skin nourishing:
Mediworld Company applies Biotechnology to develop a lot of products such as the **MEDINANO** products applying Nano Silver technology to protect health and to get optimal skincare, for instance **MEDINANO SHOWER GEL, MEDINANO FEMININE WASH**,...

In addition to the application of Biotechnology - plant extract technology, the company also launches the product of Herbal Shampoo to which can nourish and stimulate hair growth by using natural and safe methods.

* Internal documents

Topical application to improve skin healing process and avoid scarring.

TBR TECHNOLOGY CORPORATION

TBR
Top Biotechnology Research

ABOUT TBR

TBR Technology Corporation stands as one of the leading business in Vietnam, with a strong emphasis on the provision of comprehensive solutions for the field of biology, particularly in the area of Molecular Biology.
These solutions embrace a diverse range of products, focus on providing equipment, instruments, consumables, chemicals, laboratory setup service, oligonucleotide synthesis,...Notably, TBR remains at the vanguard of progress by consistently updating its repertoire with cutting-edge and widely adaptable testing methodologies.
We specialize in providing biotechnology products and solutions, especially molecular biotechnology solutions in three main areas: Research, Analysis - Testing, and Testing
We position our brand bases on 2 main criteria: Product quality and customer service. Along with our core value is “Creativity and Integrity”, TBR company has earned the trust and loyalty of over a thousand esteemed customers as their preferred partner, because of our quality product commitment, competitive prices, quick delivery and enthusiatic technical support.
Moreover, we proudly become the exclusive distribution partner for esteemed brands: Bioer, Gunster, Bioline by Meridian Life Science and Biowe

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BIOER · BIOWET · meridian BIOSCIENCE · GUNSTER

FUJIWA VIETNAM COMPANY LIMITED

FUJIWA VIETNAM COMPANY LIMITED was established in May 2018 with a total initial investment of 300 billion VND on an area of more than 40,000m2. The factory is equipped with modern Japanese automatic production lines to produce FUJIWA VIETNAM high-quality alkaline ionized water, high-quality Hydrogen-rich drinking water, FUJIFUL hand sanitizer spray, multi-purpose cleaning solution bottles. FUJION, SALT ION mouthwash.

- **Company name:** FUJIWA VIETNAM CO., LTD
- **Office:** 57A Trung My Tay 13, Trung My Tay Ward, District 12, City. Ho Chi Minh
- **Headquarters + Factory:** 286 Ho Van Tang, Tan Phu Trung Commune, Cu Chi
- **Hotline:** 19006759 - 0901 661 789
- **Email:** info@ionfujiwa.vn

Fujiwa®
Tinh hoa từ Nhật Bản

6 CORE VALUES

Integrity
Integrity in every action, respect for every transaction and verifiable

Follow
Committed to complying with laws, codes of conduct and state regulations.

Respect
Always respect colleagues, partners, customers and the company's core values

Fair
Treat employees, customers and partners fairly

Morality
Always uphold the true value of your products and act ethically.

Dream
I succeed, you succeed, we succeed together.

1 BIOGROUP VIETNAM'S CAPACITIES:

- Consulting and providing the total technology/equipment solutions at a reasonable cost.
- Performing equipment technical services such as: Design Qualification (DQ), Installation Qualification (IQ), Operation Qualification (OQ), Performance Qualification (PQ), Preventive Maintenance (PM), etc.
- Technology transfer.

2 WE PROVIDE BIO-SOLUTIONS:

01 Biological Sample Processing & Cryopreservation

02 Cell Culture

03 Bioassays

04 Biological Products Purification

05 High Security Sperm & Embryo Cryopreservation

06 Biological Samples Cryoshippers & Cell Thawing

BIOGROUP®
Passion for Serving Bio-solutions

BIOGROUP VIETNAM CO.,LTD

Floor 14, HM Town Building, 412 Nguyen Thi Minh Khai, Ward 5, Dist 3, HCMC, Vietnam

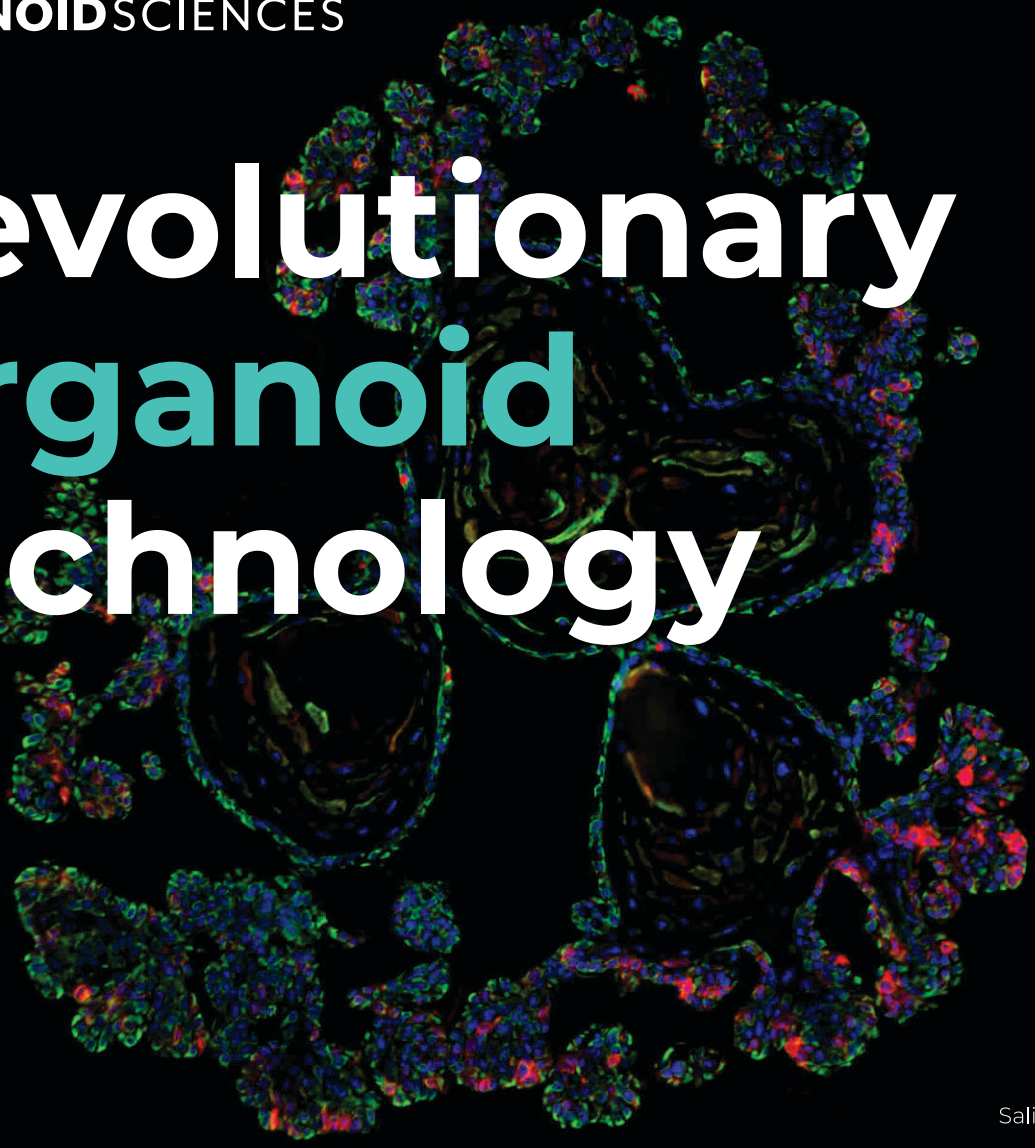
84 28 22 534856

info@biogroupvietnam.com

https://biogroupvietnam.com/en/

ORGANOIDSCIENCES

Revolutionary Organoid Technology



Salivary Organoid

ORGANOIDSCIENCES, founded in 2018, is a leading biotech company that develops innovative organoid technology to address organ damage and shortage. Organoids are miniaturized versions of organs produced in vitro, showing realistic micro-anatomy, and are promising in the fields of regenerative medicine and transplantation.
The recent shift by the U.S FDA away from animal testing requirements has made organoids an ideal in vitro tool for drug discovery with patient heterogeneity.
Patient-derived organoids have great potential for drug development and personalized medicine, with a specialized focus on evaluating patient-specific immune checkpoint inhibitors using the company's ODISEI platform.
Organoids generated from adult tissue-specific stem cells have the ability to directly regenerate damaged lesions, providing a fundamental treatment solution for unmet medical needs in an aging society, in contrast to the limited efficacy of current commercialized cell therapies due to paracrine effect.

VOS DISCOVERY was established to globally expand organoid technology, building upon the expertise of ORGANOIDSCIENCES. With a focus on innovative technology within Vietnam, VOS DISCOVERY aims to lead the biotech industry and drive advancements in the field of bioscience.

Established by

ORGANOIDSCIENCES

VOSDISCOVERY

vosdiscovery.vn vosdiscovery@organoidrx.com

2F, Incubation Center B Lot D.01 Tan Thuan St. Tan Thuan EPZ, Tân Thuận, Dong W, Quận 7, Thành phố HCMC 756200