Welcome Remarks

Assoc. Prof. Dr. Niane Sivongxay

It gives me great pleasure to extend to you all a very warm welcome on behalf of the Faculty of Natural Sciences and to say how grateful we are to Lao Ministry of Science and Technology (MOST) and the Japan science and technology (JST) who have accepted our invitation to convene this 5th Satellite of Core to Core Program (CCP), and the 2nd e-Asia Joint Research Program (JRP) Seminars, at Luang Prabang, the World's heritage City. In addition, on behalf of the organizing committees of these both seminars I would like to express my most sincere gratitude for all participants in this moment as the gateway to the initiation to our Scientific Schedule of the seminars.

Let me say what a great honor and satisfaction that National University of Laos, specifically Faculty of Natural Sciences to have been entrusted and honored to be the host of the 5th Satellite Core to Core Program A, Advanced Research Network on the Establishment of an International Research Core for New Bio-research Fields with microbes from tropical areas and the 2nd e-Asia Joint Research Program seminars on 23rd -24th October 2018. Many of the dignitaries who are also sharing their time with us in this event, have also changed previous commitments in their political and professional duties to find time to render stimulation and appreciation to this event represented here in Laos by scientists from 6 different countries.

It is gratifying to note that the agenda of the Seminar covers a wide range of very interesting items relating to the microbial Research and Biotechnology for biomass Utilization (in CCP). A glance through the list of presentations planned in these two days reveals the amazing diversity of these applications.

The Seminar is the result of a concerted effort and in this context I wish to record a word of thanks to all members of the CCP, e-Asia JRP, especially National Council Research of Thailand, Japan Science and Technology, Japanese and also Thai coordinators for their moral and financial support to these seminars and also to Lao researchers in conducting their experiments through the program.

In this occasion I also would like to give thanks to all scientists, researchers for kindly agreeing to be here in Laos to present your research progress and share your experiences. This will be invaluable benefit to related academic fields.

I personally believe we can achieve the objectives of cooperation by useful discussions to ensure a successful function.

Welcome all of you and please enjoy yourself.

Niane Sivongxay

Vice-Dean, Faculty of Natural Sciences

Message from Lao Coordinator

Assoc. Prof. Dr. Somchanh Bounphanmy

This is a great honor and privilege for me to extend my warm welcome all of distinguished guests and participants to **The 5th Satellite Seminar titled "International Symposium on Microbial Research and Biotechnology for Biomass Utilization" and The 2nd e-ASIA Joint Research Program Seminar,** hosted by the National University of Laos, during October 23-24, 2018 in Luang Prabang , the World Heritage City, Lao PDR.

The National University of Laos is very pleased to host the 5th or the last Satellite Seminar which is an important event of 5 years research cooperation of the Core to Core Program. This Seminar will certainly provide a great opportunity for all participants from the counterparts to meet and to share their research results.

The CCP Lao scientists are aware that we have been working in a difficult situation due to lacking of financial support from our side. Even though we have got financial assistance from the Japan Society for the Promotion of Science (JSPS) and the National Research Council of Thailand (NRCT) that have been provided a great chance for our some scientist representatives to conduct continuously their research both in Thailand and in Japan and have had quite a good result to share with you in this Seminar. On this occasion we would like to express our most heartfelt appreciation and most sincere acknowledgement for that kind and generosity of the assistance extended to Laos.

I would also like to express sincere thanks to the committees, in particularly Prof. Dr. Mamoru Yamada and Assoc. Prof. Dr. Gunjana Theeragool for kind cooperation and assistance to organize the Seminar. My thanks go to invited speakers and all oral presenters for their contributing research works. Thanks also to coordinators and all participants from Japan, Thailand, Vietnam, Indonesian, Germany, United Kingdom and Laos for kind cooperation in arranging the 5th Satellite Seminar and the 2nd e-ASIA Research Joint Program in Luang Prabang City.

Finally, let me wish you a fruitful seminar and have a nice time to stay in the memorable UNESCO Heritage Center, The Luang Prabang City.

Thank you.

Somchanh Bounphanmy

Lao Coordinator

Message from Japanese Coordinator

Prof. Dr. Mamoru Yamada

It is our great pleasure to hold the 5th Satellite Seminar in the Core-to-Core Program (Advanced Research Networks) entitled "Establishment of an international research core for new bio-research fields with microbes from tropical areas (World-class research hub of tropical microbial resources and their utilization)" joined with the 2nd e-ASIA JRP Seminar in Luang Prabang, Laos. I would like to take this opportunity to appreciate the enormous effort of the organizing committee of this seminar at the Laos side, especially Associate Professor Dr. Somchanh Bounphanmy and her colleagues, as well as the financial support of National University of Laos and the cooperation of Ministry of Science and Technology (MOST) in Laos.

The Core-to-Core Program (2014-2018) is a post-program of the JSPS-NRCT Core University Program (1998-2007) entitled "Development of thermotolerant microbial resources and their application" and the JSPS-NRCT Asian Core Program (2008-2012) entitled "Capacity building and development of microbial potential and fermentation technology towards new era", and is expected to create world-class research centers that partner over the long term with other core research institutions around the world in researches in leading-edge fields, on issues of high international priority. A large number of scientists are participating in this program, from seven countries of Thai, Vietnam, Laos, Germany, Indonesia, United Kingdom and Japan. Thus, we challenge the new bio-research fields with microbes from tropical areas in this program. There are five projects in this Core-to-Core Program as follows.

Project 1: Explorational Research of Useful Microbes
Project 2: Genome-based Research on Thermotolerant Microbes
Project 3: Research on Environmental Microbes Sustaining Tropical Ecosystem
Project 4: Research on Microbes Useful for Food, Packaging, Health, and Ecosystem
Project 5: Development of Next-generation Fermentation Technology for New Wave

I hope members at Lao side to work together with counterparts on original research topics related to these projects. During this seminar, you may obtain beneficial information or new ideas from presentation and discussion, which promote your further experiments. While, as we have the Young Scientist Seminar every year and some systems supporting student exchange, this program has contributed not only to microbial sciences but also to education to foster our successors.

Finally, I would like to thank all attendees and their contributions to this seminar, and the financial supports of National University of Laos, the National Research Council of Thailand (NRCT), Vietnam Ministry of Science & Technology (MOST), Beuth University of Applied Sciences, Ministry of Research, Technology and Higher Education (RISTEKDIKTI) and the Japan Society for the Promotion of Science (JSPS).

Mamoru Yamada
Japanese Coordinator
Professor, Yamaguchi University

Message from Thai Coordinator

Assoc. Prof. Dr. Gunjana Theeragool

I am delighted to welcome all of the distinguished guests and participants to the 5th Satellite Seminar of the Core to Core Program A. Advanced Research Networks on "Establishment of an International Research Core for Bio-research Fields with Microbes from Tropical Areas (World-class Research Hub of Tropical Microbial Resources and Their Utilization)".

Kasetsart University and Yamaguchi University established the Core University Program with financial support from the Japan Society for the Promotion of Science (JSPS). It took place over 10 years (1998-2007). The success of the 10 year core university program had the potential to be extended to the Asian Core Program. This program was created with financial support from JSPS and the National Research Council of Thailand (NRCT), ran for 5 years (2008-2012), and received collaboration from 4 active teams from Japan, Vietnam, Laos and Thailand, respectively. Following on this fruitful collaboration, we have established the Core to Core Program A. Advanced Research Networks. This 5 year (2014-2018) program receives financial support from JSPS, NRCT, the Vietnam Ministry of Science & Technology (MOST), the National University of Laos, The University of Brawijaya (Indonesia), Beuth University of Applied Sciences (Germany) and The University of Manchester (England).

This 5th Satellite Seminar is the second academic activity in 2018 arranged after the successful International Joint Seminar in Thailand Research EXPO (August 13, 2018). This seminar will provide a good opportunity for the participants to meet and discuss their future areas of collaboration in order to obtain the most fruitful results. In addition, I hope that the presentations and discussions which take place during this seminar will spur the participants towards the development of new research opportunities and productive collaboration.

On behalf of Thai Coordinator, I would like to express my sincere appreciation to National University of Laos especially Assoc. Prof. Dr. Somchanh Bounphanmy, Laotian Coordinator, for organizing the 5th Satellite Seminar. My thanks also go out to the invited speakers and all of the presenters for contributing their research work to this seminar. Thanks also to the Japanese, Vietnamese, Indonesian, German and English coordinators for their cooperation in arranging this 5th Satellite Seminar. Last, but not least, I would like to express my sincere gratitude to JSPS, NRCT, MOST in Vietnam, the National University of Laos, The University of Brawijaya, Beuth University of Applied Sciences and The University of Manchester for their continuing financial support.

Gunjana Theeragool
Thai coordinator

Message from Vietnamese Coordinator

Assoc. Prof. Dr. Ngo Thi Phuong Dung

It is a great honor and pleasure for me to preside over the message of the Vietnamese side on the occasion of the 5th Satellite Seminar of the Core to Core Program.

We have learned that this final Satellite Seminar of the Core to Core Program will be organized together with the 2nd e-ASIA Joint Research Program Seminar. The National University of Laos is as a host for such events that will be held on 23rd – 24th October 2018 in a meeting hall of Louanprabang's Assembly, Laos. During this event, all representative counterparts from Japan, Thailand, Laos, Germany, Indonesia, United Kingdom, and Vietnam will have a good opportunity to share the ongoings research and the finding results.

May I take this occasion to express a sincere thanks to the supporting institutions of all partner countries, and I would like to acknowledge the excellent effort of the organizing committee and team of The National University of Laos. We are also grateful to the invited oral speakers and the poster presenters as well as all participants who significantly contribute to the success of this seminar event.

We strongly believe that during this event all participants will learn the informative research findings and useful experiences, contributing toward the success of our Core to Core Program.

Ngo Thi Phuong Dung
Vietnamese Coordinator
Associate Professor
Biotechnology R & D Institute, Can Tho University

Message from German Coordinator

Prof. Dr.-Ing Peter Götz

Laos being the host of the 5th and final Satellite Seminar of the Asian Core-to-core Program on "Establishment of an international research core for new biological fields with microbes from tropical area" is a special occasion. Project partners will meet in the famous city Luang Prabang and we have the opportunity to intensify the contact to our research partners from Laos directly. For me personally, participation in the Asian Core-to-core Program was and is a great privilege which I enjoy very much. Therefore I want to thank all partners and colleagues and especially the organizing committee in Laos for making this possible.

Our network under the Core-to-Core Program is very well established for many years and although the distance between Asia and Germany is complicating the cooperation, we had a successful Satellite Seminar last year in Germany and we have increasing activities in student exchange. Worldwide political changes will very likely strengthen the ties between Europe and Asia, so exchanging and educating young researchers from different cultural backgrounds will be a future challenge. This challenge can be met by international networks, so it would be a mission for us to continue our network and intensify the people exchange.

I will happily follow invitations to join future network activities and proposals, for example for a continuation of the Core-to-Core initiative from the Japan Society for the Promotion of Science (JSPS). I am looking forward to our cooperation for the future and to the upcoming 5th Satellite Seminar in Laos. We will use this get-together as an opportunity to develop new ideas, new projects and new friendships.

Again I want to thank everybody in our Core-to-Core network for the great work and also the funding organizations, especially the JSPS, for the financial support as well as the National University of Laos for being our host.

Peter Götz

German Coordinator

Professor, Beuth University of Applied Sciences, Berlin

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Ms. Naoko Miyaji	Committee and Secretariat

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Scientific Program for CCP

The 5th Joint seminar of the new Core to Core Program (CCP), will be held in order to understand the progress of international collaboration on the followings 5 projects.

1. Explorational Research of Useful Microbes.
2. Genome-based Research on Thermotolerant Microbes
3. Research on Environment Microbes Sustaining Tropical Ecosystem.
4. Research on Microbes Useful for food, Food Preservation, Health, and Ecosystem Preservation.
5. Development of Next-generation Fermentation Technology for New Wave Industry.

Schedule and Program

Core-to-Core Program: 5th Satellite Seminar, 23rd – 24th October 2018
Venue: Meeting People' Council of Luang Phrabang Province, Laos

Time	Detail	Speaker
October 22, 2018		
Delegates from Japan (9), Thailand (6), Vietnam (1), Indonesia (1), Germany (1) arrive at Luang Prabang arrive airport***:*** Thai – 10:20 by TG2576, Japan – 19:50, Vietnam – 19:50, Germany – 10:20 (arrive on 19 October), Indonesia -		
Check in at Hotel (to be arranged)		
October 23, 2018 (October)		
08:30 – 09:00	Registration	
09:00 – 09:20	Opening Ceremony: - Welcome address - Opening remarks	 -President of NUOL - Dean FNS, NUOL
09:20 – 09:50	Current CCP to next CCP	Prof. Dr. Mamoru Yamada/ Assoc. Prof. Dr. Toshiharu Yakushi
09:50 – 10:00	**Group photo**	
10:00 – 10:20	***Coffee break***	
Session 1	Chairman – Prof. Dr. Poonsuk Prasertsan Co-Chairman - Assoc. Prof. Dr. Kumakiri	
10:20-10:40	***Invited speaker (Lao 1)***: Lactic acid bacteria utilization on ruminants feed in Lao PDR	Dr. Viengsakoun Napasirth
10:40-11:00	***Japan 1***: Conversion of biomass to ethanol by thermotolerant yeast	Prof. Mamoru Yamada
11:00-11:20	***Thai 1***: Ethanol production from sugarcane top by newly selected xylose fermenting yeast strains	Prof. Dr. Savitree Limtong
11:20-11:40	***Japan 2***: Cultutre-independent analysis of bacterial profiles in traditional fermented freshwater fish products in Laos.	Dr. Junichiro MARUI
11:40-12:00	***Laos 2:*** Analyses of quality-related factors in salt-fermented fish (pa-daek) and its fermentation process	Mr. Sayvisene (Ph.D. candidate)

12:00-13:30	**Lunch break**	
Session 2	Chairman: Assoc. Prof. Dr. Gunjana Theeragool Co-Chairman: Dr. Naoya Oku	
13:30-13:50	***Vietnam1***: Isolation and Selection of Indigenous Diesel Degrading Bacteria From Diesel Contaminated Soil in Vietnam..	Assoc. Prof. Dr. Nguyen Huu Hiep
13:50-14:10	***Thai 3:*** High purity of L-lactic acid production from food waste by thermotolerant *Bacillus* NF11	Assist. Prof. Dr. Vichai Leelavatcharamas
14:10-14:30	***Japan 3:*** Thermotolerance acquired through metabolic change induced by inactivation of a transporter in *Gluconobacter* sp. strain CHM43	Assoc. Prof. Dr. Toshiharu Yakushi
14:30-14:50	***Germany 1***: Process development for biobutanol production: From mathematical model to pilot plant	Prof. Goetz
14:50-15:10	***Thai 4:*** Co-digestion of palm oil mill effluent with oil palm wastes for production of hydrogen and methane	Prof. Dr. Poonsuk Prasertsan
15:10-15:30	**Coffee break**	
Session 3	Chairman - Prof. Goetz Co-Chairman - Assoc. Prof. Dr. Toshiharu Yakushi	
15:30-15:50	***Japan 3:*** A new antibacterial monoacylglycerol from an edible cyanobacterium *Nostochopsis lobatus*	Dr. Naoya Oku
15:50-16:10	**Thai 5:** Lactic acid bacteria from traditional fermented tea leaf and their biotechnological applications	Assist. Prof. Dr. Charchai Khanongnuch
16:10-16:20	**Closing Remarks and end of the seminar**	Assoc. Prof. Dr. Somchanh Bounphanmy
16:20-18:30	**Free (sightseeing)**	
18:30-21:00	**Reception Dinner**	Guest and all members

Lactic Acid Bacteria Utilization on Ruminants Animal in Lao PDR

Viengsakoun Napasirth[1,*], P. Napasirth[2], T. Soulinthone[1], C. Yimin[3] and S. Marui[3]

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The aim of present research were study the effects of Lactic acid bacteria (LAB, *Lactobacillus plantalum)* in ruminants feed as total mixed ration silage (TMRS) on chemical composition, voluntary feed intake and grow performance in Lao Native cattle (*Bos indicus*) and buffaloes (*Bubalus bubalis*). Experiment one three feed formulas were tested in twelve male of native cattle using completely randomized design (CRD). The results showed that non-significant difference in total feed intake in fresh and dry matter ($p>0.05$). However, the feed intake of cattle in % of body weight were found 3.42, 2.92 and 2.75 % of BW, in T3, T2 and T1, respectively ($p<0.05$). Nevertheless, There were non-significant difference among treatment in the growth rate ($p>0.05$), 0.72, 0.67 and 0.67 kg/head/day in T1, T2 and T3 respectively and Experiment two eight male buffaloes with 2-year old and 228kg average liveweight were randomly assigned to receive two dietary treatments (TMRS and TMRS+LAB) according to a completely randomized design. All animal were fed ad libitum and clean water drinking with 120 day fattening, animal were weight every 14day. The results of this experiment showed that chemical composition of treatments as dry matter (DM) = 22.99-23.26%, crude protein (CP) = 13.99-14.80%, nuetral detergen fiber (NDF) = 049.17-50.42%, pH value = 3.73-3.90. Volutary dry matter feed intake indicated that non-significant different ($p>0.05$) between TMRS and TMRS+LAB Lao27. However, LAB Lao27 in TMRS showed that higher %body weight intake, 7.47 %BWI and average dairy grain, 1.97 kg/h/d then TMRS, 5.45 %BWI and 0.98 kg/h/d, respectively ($p<0.05$). In conclusion a lactic acid bacterium (*Lactobacilus plantarum*) is potential to preserved feed well for ruminants feed in Laos.

Keywords: *Lactic acid bacteria, feed, cattle, buffalo, growth performance*

Conversion of biomass to ethanol by thermotolerant yeasts

Mamoru Yamada[1-3], Masayuki Murata[1], Tomoyuki Kosaka[1-3], Suprayogi[4], Sukanya Nitiyon[1], Chansom Keo-Oudone[5], Pornthap Thanonkeo[6], Nadchanok Rodrussamee[7], Noppon Lertwattanasakul[8] and Savitree Limtong[8]

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The worldwide demand of biofuels including bioethanol for transportation is predicted to be 10 folds from the current demand by 2050, and USA has initiated to utilize 2nd generation biomass for the further demand because of a lack of 1st generation biomass. Conversion from the former biomass to bioethanol is however very costly compared to that from the latter, and thus the development of efficiently convertible microbes in addition to establishment of energy-saving technologies for ethanol production is required. Here, we showed recently isolated yeasts and their characteristics. We targeted thermotolerant yeasts that can be applied for high-temperature fermentation (HTF) as an energy-saving technology. *Kluyveromyces marxianus* DMKU3-1042 that is one of most thermotolerant yeasts was mainly used for basic and applied researches as well as a control strain.

K. marxianus intrinsically possesses xylose metabolic pathway but the pathway is suffered from glucose repression. Thus, to avoid the glucose repression, DMKU3-1042 mutants that can utilize xylose and glucose at the same time were attempted to be isolated. A mutant causing amino acid substitution (G270S) in *RAG5* for hexokinase showed enhanced utilization ability of xylose in the presence of glucose [*Int Microbiol*, 18:235-244, 2015]. Further isolation of thermotolerant yeasts was carried out. Of these, *K. marxianus* BUNL-21 was found to have relatively high ethanol fermentation activity from xylose that is slightly lower and much higher than that of *Scheffersomyces stipitis* (*Pichia stipitis*) at 30 °C and at higher temperatures, respectively [*Springer Plus*, 5:185, 2016]. While, *K. marxianus* has intriguing activity of inulinase hydrolyzing inuline to fructose [*Antonie Van Leeuwenhoek*, 108:173-190, 2015]. DBKKU Y-102 that was isolated as inulin-utilizing yeast was examined with fresh Jerusalem artichoke tubers without inulin hydrolysis under consolidated bioprocessing (CBP). On the other hand, *Spathaspora passalidarum* can utilize xylose and glucose at the same time due to a weak glucose repression on xylose utilization. CMUWF1-2 isolated was shown to achieve efficient production of ethanol from various sugars and a high ethanol yield from xylose with little accumulation of xylitol [BMC Microbiology 18:73, 2018].

Keywords: bioethanol, thermotolerant yeast, xylose, inulin

Ethanol production from sugarcane top by newly selected xylose fermenting yeast strains

Savitree Limtong[1], Thitinun Sumyai[1], Noppon Lertwattanasakul[1] and Mamoru Yamada[2]
[1]Department of Microbiology, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand, [2]Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Ube, Yamaguchi 755-8505, Japan

Second generation (2G) ethanol, which produce from lignocellulosic biomass, offers an attractive means fuel ethanol production due to the fact that lignocellulosic biomass is a low-cost raw material. To use lignocellulosic biomass for ethanol production the lignin must be removed, and the cellulose and hemicellulose components have to convert to sugars, which will be fermented to ethanol. *Saccharomyces cerevisiae*, which is commonly used for industrial fuel ethanol production, ferment only glucose but not ferment xylose to ethanol. This will result in remaining of high xylose concentrate consequence to low ethanol fermentation efficiency. Therefore, to efficiently produce ethanol from lingo-cellulosic biomass yeast strain employ should ferment both glucose and xylose. Some yeast species show the ability to ferment xylose to ethanol in addition to glucose such as *Scheffersomyces stipitis* and *Sch. shehatae*, however, they cannot produce high ethanol concentration to meet the requirement for industrial scale. In Thailand, sugarcane tops, which consist of green leaves, bundle sheath and variable amounts of immature cane, are low-cost and abundance. Therefore, the objectives of this research were to screen for the effective xylose-fermenting yeast for better ethanol production from lignocellulosic hydrolysate and optimization of nutrients and fermentation conditions for ethanol production from hydrolysate of sugarcane top, a lignocellulosic raw material. One hundred yeast strains were investigated for their ethanol production from xylose by fermentation in yeast extract peptone xylose (YPX) broth containing 30 g/L xylose. The ability to ferment ethanol from xylose was found in 18 strains. Among these strains, two strains namely *Candida* sp. ST-958 and *Candida* sp. ST-2914 respectively produced 9.86 and 10.37 g/L ethanol from xylose which were higher than the other strains but slightly lower than that produced by the reference xylose fermenting strain, *Sch. stipitis* CBS 5773 (11.69 g/L). In yeast extract peptone glucose (YPG) broth containing 160 g/L glucose, *Candida* sp. ST-2914 produced 65.40 g/L ethanol that was higher than that produced by *Sch. stipitis* CBS 5773 (50.68 g/L), whereas, *Candida* sp. ST-958 produced nearly the same concentration (48.80 g/L). Optimization of nutrient compositions and cultivation conditions for ethanol production from sugarcane tops hydrolysate was performed by response surface method using Box-Behnken design. The optimum nutrient compositions, initial pH of the medium and cultivation temperature were 90 g/L total reducing sugar, 0.5 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4.7H_2O$ and 0.5 g/L KH_2PO_4, with pH 5, 100 rpm and 28°C. In these conditions, *Candida* sp. ST-958 and *Candida* sp. ST-2914 produced 37.79 and 37.70 g/L ethanol, respectively.

Keywords: ethanol, xylose fermenting yeast, lignocellulose, sugarcane top

References:
Sumyai, T. et al. 2017. Proceeding of The 29th Annual Meeting of the Thai Society for Biotechnology and International Conference. November 23-25, 2017. Bangkok, Thailand. pp. 133-144.

Cultutre-independent analysis of bacterial profiles in traditional fermented freshwater fish products in Laos

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Faculty of Agriculture, National University of Laos (FAG, NUoL) and Japan International Research Center for Agricultural Sciences (JIRCAS) have been engaged in collaborative research on microbiology of traditional fermented foods in Laos since 2012. The research project aims for the effective use and high value addition of the fermented foods for sustainable rural livelihood improvement. The research project has been focusing mainly on fermented freshwater fish products, such as sour fermented fish (*pa-som*) and salt-fermented fish paste (*pa-daek*) that represent Lao food culture utilizing various freshwater fish as an important food resource. We first conducted the culture-independent microbial identification, namely, PCR-DGGE to efficiently explore the microbial diversity in the products to elucidate the fermentation processes at the microbial level. Moreover, pH, lactic acid and salt concentrations were measured to see the correlation between bacterial profile and the taste components. In the *pa-som* production, lactic acid content and pH continued to increase and decrease, respectively, until 4 days of fermentation. Among the lactic acid bacteria detected, two *Lactococcus* and one *Weissella* species were detected in samples after 1–4 days of fermentation, suggesting these species play major roles in fermentation of *pa-som* tested in this study. In the analysis of salt-fermented fish paste, 13 lactic acid bacteria and four other indigenous bacteria species were detected in 10 *pla-ra*/*pa-daek* samples collected from Laos and Thailand. Salt-tolerant *Tetragenococcus* species were common in products with salt concentrations higher than 10% while *Lactobacillus* species were common in those with less than 10%. In short, salt concentration is the key factor that determines the representative lactic acid bacteria and lactic acid production in *pa-daek* fermentation. Simple measurement of salt concentration as well as pH therefore be useful in monitoring *pa-daek* production to ensure product quality and meet different consumer preferences in various regions.

Keywords: Fermented freshwater fish, Lactic acid bacteria, PCR-DGGE

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Analyses of quality-related facotors in salt-fermented fish (*pa-daek*) and its fermenation process

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Various traditional fermented foods made from local agricultural and fishery products have been part of Lao food culture, and are used to secure household livelihood in Laos. It is noteworthy that fish-fermentation techniques have been developed, which enabled perishable food resources particularly the seasonally available freshwater fish from ponds, paddy fields, rivers, and reservoirs to be furnished with value-added features such as long shelf life and palatability. The salt-fermented freshwater fish named "*pa-daek*" is the representative fermented freshwater fish product of Laos. A similar product called "*pla-ra*" is popular in Thailand. It is widely used as a shelf-stable, protein-rich cooking ingredient and almighty seasoning. In addition to in-house production for self-consumption, it is now commercially produced by small- and medium-sized local enterprises that are expected to contribute to the creation of regional food value chain for sustainable agricultural and rural development. In research collaboration between FAG, NUoL and JIRCAS, we have been conducting microbiological and biochemical analyses on *pa-daek* to elucidate the key factors of good fermentation practices for stable production as well as to develop a science-based fermentation control to obtain high-value added products. Variation in contents of lactic acid and glutamic acid that are related to storage property and flavor were observed between products. In our laboratory scale *pa-daek* fermentation, those compounds increased in a fermentation period dependent manner. The initial two months of the fermentation seemed to be critical to secure the growth of lactic acid bacteria and creation of acidic environment which is important for the storage stability of the products. Histamine, a possible cause of allergy-like discomfort was detected in *pa-daek* products, although the contents largely varied between products. Histamine producing bacteria were isolated from the products and their salt-tolerant properties were tested.

Keywords: Salt-fermented fish paste (*pa-daek*), Lactic acid bacteria, histamine

ISOLATION AND SELECTION OF INDIGENOUS DIESEL DEGRADING BACTERIA FROM DIESEL CONTAMINATED SOILS IN VIETNAM

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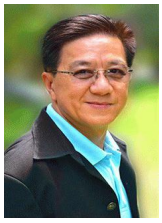
Diesel is one of important energy sources for our living. However, the environmental pollution of diesel is severe especially in developing countries. The bioremediation of diesel contamination is a major concern of scientists. The purpose of this study was to isolate and select diesel degrading bacteria from diesel contaminated soils in some provinces in Vietnam. The results showed that fifteen bacterial strains were isolated in minimal medium containing 1% of diesel as a sole carbon source. Bacterial colonies were circular, entire margin, convex elevation and white or yellowish color. Most of bacterial cells were rod shape, gram negative (13/15 strains). Strain D10, D13 and D15 could synthesized protease. Strains D10, D12, D3 and D15 could produce lipase. Strains D10L, D13 and D15 synthesized amylase. Strains D10, D12, D13 and D15 could produce cellulase. Three promising strains D10, D13 and D15 were chosen for 16S rDNA sequencing. Strains D10 and D13 were identified as *Bacillus licheniformis* and D15 was identified as *Achromobacter insolitus*. Interestingly, strain *A. insolitus* could grow in medium containing 1% of diesel and *B. licheniformis* could grow well in medium containing up to 1.5% of diesel. The result of this study contributed to the bioremediation of diesel contaminated soils.

Key words*: amylase, cellulose, diesel degradation, isolation, lipase, protease.*



High purity L-lactic acid production from food waste by thermotolerant *Bacillus* NF11

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Lactic acid was a monomer of polylactic acid (PLA) which was the raw material for biodegradable plastic production. Generally, mixture of D- and L-lactic acid was produced both in chemical and biological production processes and caused the problem of controlling the poly-lactide property. High purity of D- or L-lactic acid, thus, was a desire in PLA production. *Bacillus* NF 11, which could grow at high temperature, was screened from the thermotolerant bacillus stock culture and produced high purity of L-lactic acid. This strain, thus, could be used in low energy consumption fermentation process (without controlling the temperature). For industrial process, cost of lactic acid production depends on raw materials, fermentation and downstream process. In this study, food waste from Khon Kaen University canteen was used for decreasing raw material costs. Optimizations of food waste hydrolysis by enzyme glucoamylase and lactic acid production from food waste hydrolysate were studied. Glucoamylase 50 U g^{-1} total solid was found to be the optimum enzyme loading for food waste digestion, in which approximately 90 g l^{-1} of reducing sugars were obtained. Optimization of lactic acid production condition by *Bacillus* sp. NF11 was conducted by Central Composite Design (CCD) of Response surface methodology (RSM). The results exhibited that the optimum initial pH and yeast extract concentration for lactic acid production at 50 °C were 6.37 and 3.51%, respectively. Effect of aeration on lactic acid production was also examined. It was found that shaking at 150 rpm for 1 minute every 24 hours showed the best result. The final lactic acid concentration and % yield of 90.1 g l^{-1} and 98.4 % were obtained when these optimum conditions were used. The purity of the produced lactic acid was studied. The results showed that more than 95% of the produced acid was lactic acid and only L-lactic was found. These results suggested that the canteen food waste had a high potential for L-lactic acid production by thermotolerant *Bacillus* sp. NF11.

Keywords: L-lactic acid, food waste, thermotolerant *Bacillus*, *Bacillus* NF11

Thermotolerance acquired through metabolic change induced by inactivation of a transporter in *Gluconobacter* sp. strain CHM43

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Gluconobacter sp. is a group of acetic acid bacteria, which is industrially used for sorbose fermentation for vitamin C production. Thermotolerant microorganisms are beneficial to the fermentation industry because of a reduction in cooling efforts and other operational risks. Previously, we have obtained a thermally adapted *Gluconobacter* sp. strain CHM43AD by experimental evolution (1). In this study, we found only a single G insertion in the genome of the adapted strain, which causes a frameshift in a gene encoding a putative drug transporter SteP. A mutant derivative strain with the single G insertion in the *steP* gene (Wild-G) was constructed from the wild-type strain and showed increased thermotolerance. We found that the thermotolerant strains substantially accumulated intracellular trehalose and manifested a defect in sorbose assimilation, suggesting that the transporter is partly involved in the trehalose efflux and in sorbose uptake, and that the defect in the transporter can improve thermotolerance. Strain Δ*otsAB* constructed by elimination of the trehalose synthesis gene in the wild type showed no trehalose production but unexpectedly much better growth than the adapted strain at high temperatures. The Δ*otsAB* mutant produced more acetate as the final metabolite than the wild-type strain did. We hypothesized that trehalose does not contribute to the thermotolerance directly; rather a metabolic change including increased carbon flux to the pentose phosphate pathway may be the key factor. The NADPH/$NADP^+$ ratio was higher in strain Wild-G and much higher in strain Δ*otsAB* than in the wild-type strain. Reactive oxygen species levels in the thermotolerant strains decreased. We propose that the defect of the transporter causes metabolic change to generate more NADPH, which may enhance thermotolerance in *Gluconobacter* (2). This work was financially supported by the Advanced Low Carbon Technology Research and Development Program (ALCA) of the Japan Science and Technology Agency (JST).

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Process development for biobutanol production: From mathematical model to pilot plant

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Production of fuels from renewable resources is part of the European Strategic Energy Technology Plan (SET-Plan) to develop sustainable mobility and transportation. (SET-Plan Action 8, renewable fuels and bioenergy). Industrial production of biobutanol, being superior to other biofuels like bioethanol or biodiesel because of its physicochemical properties, is still hampered by low product concentrations, low productivities, complex biological product formation and challenging downstream processing. Combining a systems biology approach with process engineering principles, a systematic, model based development will allow the design of optimized production plants.

In order to overcome the abovementioned obstacles, research tools from biotechnology, ranging from molecular genetics to process engineering are applied in a joint effort within many research groups. In our group, we combine metabolic modeling and process design in a multi-scale approach to optimize the continuous acetone-butanol-ethanol (ABE) fermentation with *Clostridium acetobutylicum*. Starting from batch experimental data, mathematical models on various levels of complexity were developed. Aiming at harnessing the advantages of continuous processing, models were validated by comparison to experimental data from one stage continuous fermentations. Subsequently, a cascade of six continuous stirred tank reactors (CCSTR) was designed to allow steady state operation under physiologically defined conditions in reactor stages performing acidogenesis and solventogenesis.

We currently achieve a final butanol concentration of 8,2 g/L and a butanol productivity of 0,75 g/Lh in steady state of the CCSTR with *C. acetobutylicum* DSM 792. Modifications of the cascade setup have been implemented and evaluated: Recirculation loop within the cascade and an additional feeding point. Performance of the experimental modifications was in good agreement with the mathematical model prediction. Moreover, the operational stability of the CCSTR makes it a reliable tool for comparison and evaluation of solvent producing strains. Other wild types of solvent producing bacteria and a genetically modified strain are currently under investigation.

Utilizing the results and experience from lab scale, a scale-up of the continuous ABE fermentation to pilot plant scale is under way. The mobile pilot plant will process 1 m^3 whey per week and contains units for substrate preparation, fermentation, product recovery and waste water treatment. The fermentation unit combines one stirred tank bioreactor with four packed bed bioreactors into a cascade. Unit operations in downstream processing are solid/liquid separation and butanol recovery in adsorption columns. Main product is butanol, but added value will also be created from other product streams like whey proteins, gaseous products, biomass, acetone, ethanol, acetic acid and butyric acid. The pilot plant will allow to assess operating cost, product quality and attainable revenue from the products. An economic analysis of the multi-stage, continuous, whey-based ABE process will follow.

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Co-digestion of palm oil mill effluent with oil palm wastes for production of hydrogen and methane

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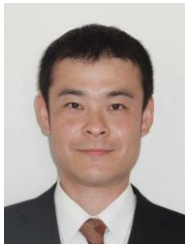
The solid waste residues from palm oil industry such as oil palm trunk, oil palm frond, empty fruit bunch and decanter cake were co-digested with palm oil mill effluent (POME) to enhance hydrogen and methane production via thermophilic two-stage reactors. In the first stage for biohydrogen production, co-digestion of POME with 10% oil palm frond gave higher hydrogen yield (54 mL-H_2/gVS) than with the other solid biomass as well as POME alone. In the second stage, the high content of volatile fatty acid in 20% decanter cake was a good substrate for methane production with a methane yield of 187 mL-CH_4/gVS. Co-digestion of POME with 10% oil palm trunk had potential to improve methane yield with about 1.8 fold increase of methane yield. During the two-stage anaerobic digestion, DGGE profile of bacterial community in the sludge revealed that *Clostridium* sp. was dominated in hydrogen production stage while the dominant strain in methane production stage was *Methanoshaera* sp.

Keywords: thermophilic fermentation, palm oil mill wastes, co-digestion, biogas, microbial community



A new antibacterial monoacylglycerol from an edible cyanobacterium *Nostochopsis lobatus*

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Cyanobacteria are oxygenic photosynthetic bacteria and known as prolific producers of bioactive secondary metabolites. Some cyanobacteria form macroscopic assemblages and are consumed as food, nutritional supplements, or folk medicines in many parts of the world.

Nostochopsis lobatus is a freshwater species which grows on the riverbed stones in shallow streams. This cyanobacterium is eaten as a dietary supplement in India and used for the treatment of fever and stomach ulcers in northern Thailand. Indeed, its medicinal validity against inflammation and ulcers has been confirmed by *in vivo* experiments using rodent models. Moreover, radical scavenging activity and inhibitory activity against hyaluronidase and tyrosinase were demonstrated by *in vitro* testings, suggesting its richness as a drug discovery resource. However, to date, only a single attempt was made to identify active ingredients in this alga, which prompted further chemical exploration.

A strain was established from a specimen collected in the Nan River, Thailand, and aqua-cultured. The collected algal mass (375 g) was extracted with EtOH, and a concentrate of the extract was partitioned into *n*-hexane-soluble, 90% MeOH-soluble, and *n*-BuOH-soluble layers, which were tested against a panel of microbes including fungi, yeasts, and bacteria. The second layer, inhibitory to the growth of two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* in the agar-diffusion assay, was further fractionated by a series of chromatographic separations, which yielded a new α-monoacylglycerol (0.7 mg).

The chirality of this metabolite was proven to be racemic as evidenced by a lack of Cotton effects in circular dichroism spectral analysis of its chromogen-labeled derivative and chiral resolution by recycle HPLC after derivatization with bis-(*R*)-methoxyphenylacetic acid. Its antibacterial activity was comparable to or slightly weaker than α-monolauroylglycerol, which is commercialized as a dietary supplement under a trade name of Lauricidin®.

Keywords: antibacterial, edible cyanobacterium, monoacylglycerol, *Nostochopsis lobatus*

Lactic acid bacteria from traditional fermented tea leaf and their biotechnological applications

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The microbiota of lactic acid bacteria (LAB) in thirty-five samples of Miang, a traditional fermented tea leaf product, collected from twenty-two different regions of eight provinces in upper northern Thailand was revealed through the culture-dependent technique. A total of 311 presumptive LAB strains were isolated and subjected to clustering analysis based on repetitive genomic element-PCR (rep-PCR) fingerprinting profiles. The majority of the strains belonged to the *Lactobacillus* genera with an overwhelming predominance of the *Lb. plantarum* group. Further studies of species-specific PCR showed that 201 of 252 isolates in the *Lb. plantarum* group were *Lb. plantarum* which were thus considered as the predominant LAB in Miang, while the other 51 isolates belonged to *Lb. pentosus*. The investigation via well-diffusion assay found that the culture broth of 106 isolates cultivated in MRS broth using glucose as a carbon source demonstrated the antimicrobial activity against the representatives of Gram-positive and Gram-negative bacteria. However, neutralized culture broth of only 5 isolates including *Lactobacillus plantarum* A9-2, *Lactobacillus pentosus* A14-6, *Lactobacillus pentosus* A26-8, *Lactobacillus pentosus* CMY46 and *Pediococcus pentosaceus* CMY9 and all are expected to produce the effective bacteriocins. All five strains also showed the tolerance with excellent survival rate of more than 90% after tested under stress of the simulated gastrointestinal conditions. The useful fermentative products including lactic acid, glycosidases their properties in novel oligosaccharides will be further investigated in the future.

Reference;

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