JSPS-NRCT Vientiane Symposium on Bioproduction Platform

13 January 2014,
Settha Palace Hotel in Laos

JSPS-NRCT
Vientiane Symposium
on
Next-Generation
Bioproduction Platform Leveraging
Subtropical Microbial Bioresources

13 January, 2014

At Settha Palace Hotel, Vientiane

JSPS-NRCT Vientiane Symposium on Next-Generation Bioproduction Platform Leveraging Subtropical Microbial Bioresources

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Organized by Biotechnology and Ecology Institute (BEI) and ICBiotech (OU)

Opening remark	10:00-10:05	Director, ICBiotech	**Takuya Nihira**
Address	10:05-10:15	Director, Biotechnology and Ecology Institute	**Sourioudong Sundara**
1	10:15-10:45	Isolation and characterization of freshwater microbial opsins from Tonle Sap Lake by PR recombinant method	**Choun Kimleng**, Kwang-Hwan Jung
2	10:45-11:15	Exploiting natural bioactive compounds produced by actinomycetes	**Shigeru Kitani**, Yasuhiro Igarashi, Watanalai Panbangred, Takuya Nihira
3	11:15-11:45	Derivative-xylooligosaccharide production from extracted xylan by direct grown culture, *Bacillus clausii* SPP20	Ketsara Phoemsuk, Patthra Pason, Rattiya Waeonukul, Akihiko Kosugi, Khanok Ratanakhanokchai, **Chakrit Tachaapaikoon**
	11:45-13:30	Lunch at Settha Palace	
4	13:30-14:00	Characterization of thermotolerant yeast isolated in Thailand and improvement towards cost-effective bioethanol production	**Yu Sasano**, Thipa Asvarak, Choowong Auesukaree, Kun Wang, Minetaka Sugiyama, Yoshinobu Kaneko, Chuenchit Boonchird, Satoshi Harashima
5	14:00-14:30	Characterization of oleaginous yeasts and lipid production from crude glycerol by the selected strain, *Rhodosporidium fluviale* DMKU-RK253	**Savitree Limtong**, Pirapan Poiburee, Wichien Yongmanitchai, Takao Ohashi, Kazuhito Fujiyama
6	14:30-15:00	*In vitro* metabolic engineering for on-demand bioproduction	**Kohsuke Honda**
	15:00-15:30	Coffee break	
7	15:30-16:00	Development of integrated hydrolysis process for sugar platform biorefinery on conversion of lignocellulosic biomass to biofuels and chemicals	Surisa Suwannarangsee, Jantima Arntong, Aphisit Poonsrisawat, Navadol Laosiripojana, Lily Eurwilaichitr, Yasuo Igarashi, **Verawat Champreda**
8	16:00-16:30	Update on current status of Vietnam microbial resources at Vietnam Type Culture Collection	**Thao Kim Nu Nguyen,** Trinh Thanh Trung, Le Thi Hoang Yen, Dao Thi Luong, Duong Van Hop
9	16:30-16:40	Diversity of Thermotolerant Acetic Acid Bacteria in Lao PDR	**Chanhom Loinheuang,** Gunjana Theeragool, Kazunobu Matsushita, Toshiharu Yakushi
10	16:40-16:50	Isolation and Characterization of Xylose Utilizing Yeast for Ethanol Fermentation	**Chansom Keo-Oudone,** Kongsamai Sayavongsa, Soulitar Sonethongkham, Nataly Chandala, Noppon Lertwattanasakul, Napatchanok Yuangsaard, Savitree Limtong, Mamoru Yamada
11	16:50-17:00	Surveying the Invertebrate Pathogenic Fungi Diversity in The Huay Yang Forest Reserve, Vientiane Capital	**Toulaphone Keokene,** Chanhom Loinheuang, Chansom Keo-Oudone, Manichanh Sayavong
Closing remark	17:00-17:05	Professor, Mahidol U	**Watanalai Panbangred**

	Poster presentation	Multi-cell wall degrading enzyme from *Aspergillus aculeatus* for viscosity reduction in very high gravity cassava fermentation	**Aphisit Poonsrisawat**, Sittichoke Wanlapatit, Atchara Paemanee, Lily Eurwilaichtr, Kuakoon Piyachomkwan, Verawat Champreda

Major Members Involved in the Asian CORE (2009-2013) Project

Team/Unit	Member in Japan	Member in Thailand	Member in Other Countries
Coordinator	Prof. Takuya Nihira (Osaka U)	Prof. Watanalai Panbangred (Mahidol U)	Dr. Duong Van Hop (Vietnam National U Hanoi, Vietnam) Mr. Hang Chan Thon Mr. Sokunthia Thao (Royal U Phnom Penh, Cambodia) Dr. Sourioudong Sundara (BEI, Laos)
Research Area	**Member in Japan**	**Member in Thailand**	**Member in Other Countries**
Low Mr bioactive compounds	Prof. Takuya Nihira (Osaka U) Prof. Yasuhiro Igarashi (Toyama Pref U) Prof. Kenji Sonomoto (Kyushu U) Prof. Kazumasa Hirata (Osaka U)	Prof. Watanalai Panbangred (Mahidol U) Dr. Wonnop Visessanguan (BIOTEC)	Dr. Duong Van Hop (Vietnam National U Hanoi, Vietnam) Mr. Sokunthia Thao (Royal U Phnom Penh, Cambodia) Dr. Sourioudong Sundara (BEI, Laos)
Industrial enzymes	Prof. Kazuo Sakka (Mie U) Prof. Kozo Asano (Hokkaido U)	Dr. Khanok Ratanakhanokchai (KMUTT) Prof. Vithaya Meevootisom (Mahidol U)	
Yeast strain improvement	Prof. Satoshi Harashima (Osaka U) Prof. Kazuhito Fujiyama (Osaka U) Prof. Eiichiro Fukusaki (Osaka U)	Dr. Chuenchit Boonchird (Mahidol U) Prof. Savitree Limtong (Kasetsart U)	
Whole cell catalyst	Prof. Hisao Ohtake (Osaka U) Prof. Yasuo Igarashi (2009-2012, U Tokyo) Prof. Masaharu Ishii (2013, U Tokyo)	Dr. Thunyarat Pongtharangkul (Mahidol U) Dr. Alisa Vangnai (Chulalongkorn U) Dr. Verawat Champreda (BIOTEC)	

(Presentation 1)

Isolation and characterization of freshwater microbial opsins from Tonle Sap Lake by PR recombinant method

Choun Kimleng, Kwang-Hwan Jung

Department of Life Science and Institute of Biological Interfaces, Sogang University, Seoul, 121-742, Korea

Microbial rhodopsin is a seven-transmembrane helix protein that has a retinal as chromophore. Rhodopsins have function as light-driven ion transport, photosensing activities (type I rhodopsin) and function as visibilities in animal eyes (type II rhodopsin) (Atamna-Ismaeel et al, 2008). Proteorhodopsin (PR) is an abundant microbial rhodopsin, which has a function as light-driven proton pumps and was found in many different marine environments (Koh, Atamna-Ismaeel et al.). Recently, it has been reported for existence of opsins in non-marine environment with homologous to PR. This study was carried out for further understanding of those partial existing non-marine origin of opsins containing the conserved region sequence of microbial rhodopsin (Choi et al). Ten distinguished partial sequences of opsins homologues from *Tonle Sap Great Lake* in Cambodia were isolated and constructed chimerical proteins by insertion of those partial sequences to green light absorption proteorhodopsin (GPR) containing N-terminal and C-terminal regions. The partial sequences are containing conserved region between helix C and helix F. The cassette insertion as previous report has called "CFR, Chimeric Freshwater Rhodopsin". To characterize these chimera freshwater rhodopsin from Tonle Sap (CFR-TS), were overexpressed in E. coli and purified for observing absorption spectra, different spectra, pK_a value of proton acceptor, proton pumping activities and photocycle properties. In this study, five partial sequences were characterized and named as CFR-TS1, CFR-TS2, CFR-TS3, CFR-TS4 and CFR-TS5. Those chimeric proteins were studied about their absorbance spectra showing different absorbance spectra (λ_{max}) at 518 nm, 527.5 nm, 525.5 nm, 544.5 nm, and 548.5 nm, respectively. The other characteristics of those chimeras Rhododopsin were also revealed.

References:

1. Atamna-Ismaeel, N. et al.: *ISME J.*, **2**, 656-662 (2008).
2. Choi, A. R. et al.: *Appl. Microbiol. Biotechnol.*, **97**, 819-828 (2013).

(Presentation 2)

Exploiting natural bioactive compounds produced by actinomycetes

Shigeru Kitani[1], Yasuhiro Igarashi[2], Watanalai Panbangred[3], Takuya Nihira[1]

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Natural products have a major commercial impact in the fields of medicine and agriculture, where they continually give rise to novel applications and new modes of action and targets. To this end, actinomycetes, especially those belonging to the genus *Streptomyces*, have often been exploited because of their ability to biosynthesize an impressive array of novel metabolites. As the search for producers of novel compounds continues, it becomes apparent that many terrestrial actinomycetes including *Streptomyces* species isolated from different area produce the same compounds, and the ratio of finding genuinely new biologically active molecules is greatly declined. Thus, to overcome these problems, new concepts for exploiting novel compounds are necessary to be established.

In the presentation, we will show the potential ability of Thai actinomycetes to produce novel compounds, which has been analyzed based on the physical characters reflecting the chemical structures of the compounds. Furthermore, another strategy to discover novel compounds, namely, activation of cryptic metabolites with modification of *Streptomyces*-hormone pathway, will be demonstrated.

The production of *Streptomyces* secondary metabolites is often regulated by low-molecular-weight bacterial hormones called autoregulators. Although 60% of *Streptomyces* strains may use γ-butyrolactone-type molecules as autoregulators and some use furan-type molecules, little was known about the signaling molecules used to regulate antibiotic production in many other members of this genus. Recently, the laboratory of Prof. Nihira has successfully purified a signaling molecule (avenolide) from *Streptomyces avermitilis*-the producer of the important anthelmintic agent avermectin with annual world sales of $850 million-and determined its structure, including stereochemistry, by spectroscopic analysis and chemical synthesis as a class of *Streptomyces* autoregulator. Exogenous addition of *Streptomyces* autoregulators chemically synthesized has unleashed the production of cryptic metabolites in our preliminary experiment, and thus this finding could lead to the discovery of novel natural products including clinically useful bioactive compounds.

Reference:

1. Kitani, S. et al.: *Proc. Natl. Acad. Sci. USA*, **108**, 16410-16415 (2011).

(Presentation 3)

Derivative-xylooligosaccharide production from extracted xylan by direct grown culture, *Bacillus clausii* SPP20

Ketsara Phoemsuk[1], Patthra Pason[2], Rattiya Waeonukul[2], Akihiko Kosugi[3], Khanok Ratanakhanokchai[1], Chakrit Tachaapaikoon[2]

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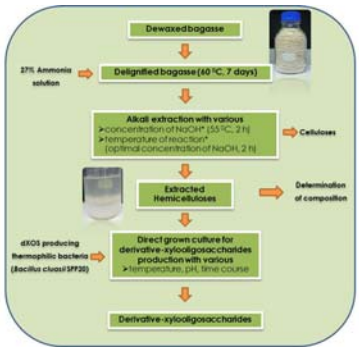
[3]*Japan International Research Center for Agricultural Sciences, Tsukuba 303-8686 Japan*

Xylan is a major component of hemicellulose in lignocellulosic biomass. It can be used as the raw material for produce valuable bio-product *e.g.* xylose, xylitol, and xylooligosaccharides (including derivative-xylooligosaccharides, dXOS). Recently, dXOS are applied to functional foods such as dairy, tea, energy drinks and raw material of pharmacy and cosmetic industries (Carvalho, et. al. 2013). Generally, xylan comprise approximately 23.0-44.5% in lignocellulose materials, it depend on type of the plants. Therefore, it interest to use xylan as the renewable source for dXOS production. In this study, dXOS production from a cheap raw material bagasse via unique characteristic microorganism was reported. Xylan extraction procedure was shown in Fig. 1. Xylan was extracted from bagasse at various concentrations of sodium hydroxide and temperatures. Optimal condition for xylan extraction was 15% sodium hydroxide at 55°C, which 0.25 g of xylan/g bagasse was extracted (80% recovery). Thereafter, extracted xylan was used as the sole carbon source for cultivation. Over 100 bacteria strains were isolated from soils on mineral salt medium with 0.5% (w/v) extracted xylan from bagasse as sold carbon source and incubated at 55°C at 200 rpm for 48 hours. The result revealed that only one strain (given namely, SPP20) was produced and accumulated major product of dXOS (molecular size nearly xylopentaose) (Fig. 2). The 16S rRNA gene sequence analysis revealed that the strain SPP20 similar with *Bacillus clausii*. This research not only success to extract xylan which high recovery yield, but also can be isolate a unique characteristic thermophilic bacterium for dXOS production.



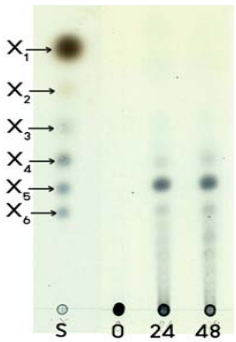


Fig. 2. TLC of dXOS in culture supernatant of *B. clausii* strain SPP20 when cultured at time interval on extracted xylan at pH 7.0 and 55°C. (X_1-X_6 = standard xylose and xylooligosaccharides).

Fig. 1. Scheme of xylan extraction from bagasse.

Reference: 1. Carvalho, A. F. A. et al.: *Food Res. Int.*, **51**, 75-85 (2013).

(Presentation 4)

Characterization of thermotolerant yeast isolated in Thailand and improvement towards cost-effective bioethanol production

Yu Sasano[1], Thipa Asvarak[2], Choowong Auesukaree[3], Kun Wang[1], Minetaka Sugiyama[1], Yoshinobu Kaneko[1], Chuenchit Boonchird[2], Satoshi Harashima[1]

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For achievement of sustainable society, bioethanol is now regarded as a promising fuel. Because bioethanol fermentation is very stressful condition for yeast, a lot of properties are required in yeasts for efficient bioethanol production such as thermotolerance, ethanol tolerance, acid tolerance, fermentation inhibitor tolerance, and xylose fermentation ability. Especially, the most important property is thermotolerance, because thermotolerant yeast enables simultaneous saccharification and fermentation (SSF) and reduction of cooling cost. We isolated from Thailand novel *Saccharomyces cerevisiae* strains that can ferment at 41°C (Htg^+ phenotype). One of these strains, named C3723 was used as a starting strain for further improvement. C3723 was crossed with TISTR5606 strain, a high ethanol producer, to construct TJ14 strain. TJ14 strain produced 45 g/l ethanol from 100 g (w/v)/l cellulose at 39°C. Genetic analysis revealed that six *HTG* genes (*HTG1-HTG6*) are responsible for Htg^+ phenotype. We have cloned *HTG2* and *HTG6* genes as *CDC19* encoding pyruvate kinase in glycolytic pathway (1) and *RSP5* encoding E3 ubiquitin ligase (2), respectively. We found that C3723 strain has higher pyruvate kinase activity than laboratory strain at high temperature and transcription level of the *RSP5* gene in C3723 strain was increased, suggesting that global alteration of cell physiology through ATP levels and protein degradation is associated with the Htg^+ phenotype. We have also tried to improve TJ14 more by UV irradiation, generating TJ14-U54. TJ14-U54 strain exhibited confluent growth at 42°C and displayed an ethanol productivity more than 10% higher (65 g/l) than that of TJ14. Now, we are attempting to clone genes that are responsible for thermotolerance at 42°C. We are also trying to identify genes conferring acid tolerance using *S. cerevisiae* multicopy genomic tiling library. We clarified that overexpression of *HAA1* encoding a transcription factor conferred lactic acid tolerance, and *SNA4* encoding an unknown protein or *GAS1* encoding β-1,3-glucanosyltransferase conferred sulfuric acid tolerance. We also created a strain showing significant improvement of ethanol tolerance by applying a genome reorganization technology that we have recently developed. Deletion of the 25 kb left terminal region of chromosome VIII and 35 kb right region of chromosome XIV resulted in significant improvement of ethanol tolerance. When these finding are combined, it would lead to breeding of multiple stress tolerant yeast strain useful for cost-effective bioethanol production.

References:

1. Bejaphokee, S. et al.: *N. Biotechnol.*, **29**, 166-176 (2012).
2. Shahsavarani, H. et al.: *Biotechnol. Adv.*, **30**, 1289-1300 (2011).

(Presentation 5)

Characterization of oleaginous yeasts and lipid production from crude glycerol by the selected strain, *Rhodosporidium fluviale* DMKU-RK253

Savitree Limtong[1], Pirapan Polburee[1], Wichien Yongmanitchai[1], Takao Ohashi[2], Kazuhito Fujiyama[2]

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This study aims to use oleaginous yeast and crude glycerol from biodiesel production for the production of microbial lipid to be used as an alternative lipid source in biodiesel production. A total of yeast 323 strains were isolated by an enrichment technique in medium containing pure glycerol as a sole carbon source. These newly isolated strains and the other 99 red yeast strains in private culture collection were screened for the strain accumulated high lipid cultivated in crude glycerol. Strain DMKU-RK253 accumulated the highest lipid, 65% of biomass, was selected for optimization. The experimental design based on statistic methods was used. Before using Plackett-Berman design to screen important condition with respect to their effects on the lipid accumulation, optimization of nitrogen source was separately carried out. Both inorganic and organic compounds were used for optimization and ammonium sulfate with monosodium glutamate were selected to be used as the combined nitrogen source for statistical screening. The Plackett-Berman design was used to screen important conditions with respect to their affect on the lipid accumulation. A total of nine variables *viz.* temperature, pH, shaking speed, and concentration of curd glycerol, monosodium glutamate, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$ and KH_2PO_4 were determined. Three factors *viz*. temperature, crude glycerol concentration and $(NH_4)_2SO_4$ concentration were found to be the factors significantly influence accumulation at the confidence level of 95%. While the factors which significantly effects on lipid production were the same except temperature was replaced by shaking speed. Therefore, four factors *viz.* temperature, shaking speed, crude glycerol concentration and $(NH_4)_2SO_4$ concentration will be subjected to identify the optimal conditions for lipid accumulation from crude glycerol using a Box–Behnken factorial design.

Identification of the 34 oleaginous strains accumulation lipid higher than 20% of biomass based on the analysis of the sequence of the D1/D2 domain of the large subunit rRNA gene revealed that the 13 strains were identified to be six basidiomycete yeast species i.e. *Rhodotorula mucilaginosa*, *Rhodotorula taiwanensis*, *Rhodosporidium fluviale*, *Sporidiobolus ruineniae*, *Cryptococcus* cf. *podzolicus* and *Cryptococcus laurentii*. Eight strains were belonged to six ascomycete species i.e. *Kodamaea ohmeri*, *Pichia guilliermondii*, *Pichia galeiformis*, *Lindera subsufficiens*, *Lindera mrakii* and *Tetrapisispora namnaoensis*. Ten strains were similar to *Rhodotorula* sp. CBS 8885, whereas the other three strains could be identified as two novel ascomycete yeast species. The result implied that oleaginous yeasts were distributed in both phylum Ascomycota and phylum Basidiomycota. The strain DMKU-RK253 was identified as *Rhodosporidium fluviale*.

(Presentation 6)

In vitro metabolic engineering for on-demand bioproduction

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The integration of biotechnology into chemical manufacturing has been recognized as a key technology to build a sustainable society. Considerable research effort has been exerted to improve the economy of current microbial-fermentation-based production processes for biofuels, bioplastics, and other industrially important chemical compounds. The optimization of the metabolic flux of microbial cells by enhancing the expression levels of desired genes and/or by depleting those of undesired ones has emerged as a powerful strategy to improve microbial cells. However, these approaches, the so called “metabolic engineering”, often suffer from flux imbalances as artificially engineered cells typically lack the regulatory mechanisms characteristic of natural metabolism. One of the possible strategies to overcome this limitation is to avoid the use of living microorganisms and to use only enzymes involved in the metabolic pathway. The heat treatment of recombinant mesophiles (*e.g., Escherichia coli*) having heterologous thermotolerant enzymes results in the one-step preparation of highly selective biocatalytic modules. The assembly of these modules enables us to readily construct an *in vitro* artificial metabolic pathway. This approach is, in principle, applicable to all thermophilic enzymes as long as they can be functionally expressed in the host, and thus would be potentially applicable to the biocatalytic manufacture of any chemicals or materials on demand. In this lecture, our latest achievements on *in vitro* metabolic engineering for the production of various compounds, including lactate, malate, and *n*-butanol, will be presented.

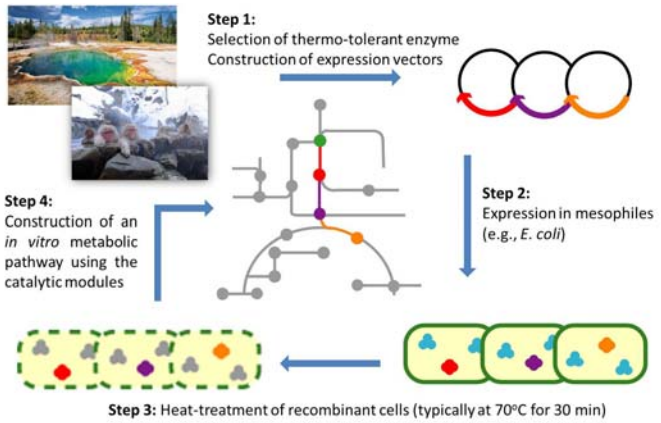

FIG. Schematic illustration of the basic procedure for *in vitro* metabolic engineering with thermo-tolerant biocatalytic modules.

References:
1. Ye, X. et al.: *Microbial Cell Fact.*, **11**, 120 (2013).
2. Krutsakorn, B. et al.: *Metab. Eng.*, **20**, 84-91 (2013).

(Presentation 7)

Development of integrated hydrolysis process for sugar platform biorefinery on conversion of lignocellulosic biomass to biofuels and chemicals

Surisa Suwannarangsee[1], Jantima Arntong[1], Aphisit Poonsrisawat[1], Navadol Laosiripojana[2], Lily Eurwilaichitr[1], Yasuo Igarashi[3], Verawat Champreda[1]

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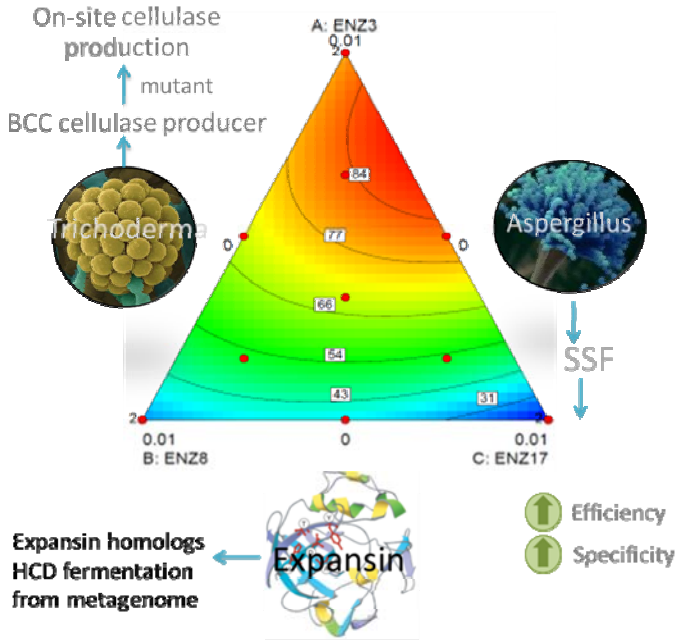
Bioconversion of lignocellulosic plant biomass to biofuels, commodity chemicals, and biomaterials as an alternative to conventional production from non-renewable petroleum resource is the basis of the sustainable biorefinery industry. Establishment of an economically viable and technologically practical hydrolysis process for efficient saccharification of biomass is thus considered as a platform for applicability of bioconversion processes in industry. Currently, R&D at the NSTDA-JGSEE Integrative Biorefinery Laboratory is focusing on development of integrated sugar platform biorefinery process for conversion of various local lignocellulosic plant biomass to valorised chemicals using multi-disciplinary catalytic and bio-processes. The concept for low-temperature and green pretreatment based on chemical and high-pressure liquid hot water has been applied for increasing biomass digestibility for subsequent enzymatic hydrolysis. Fractionation of lignocellulose components was also explored using one-step aqueous-organosolv process for value maximization of biomass components. The efficient cellulolytic enzyme system for saccharification of the lignocellulosic substrates has also been studied based on the synergistic action of catalytic and non-catalytic auxiliary components obtained from local microbial strains and environmental metagenomes. The ternary enzyme formulation using a core cellulase, accessory enzyme and bacterial expansin has been developed, aiming to increase efficiency and specificity of the enzyme systems for production of composite biomass sugars which are the key starting point for further conversion to biofuels and value-added chemicals. Consolidated bioprocessing using lignocellulolytic microbial consortia has also been explored for direct conversion of biomass to valorised products. The work will lead to the establishment of an efficient biomass hydrolysis technology for potential local lignocellulosic substrates which is an important platform for further study on biomass conversion to target products of industrial interest.

References:

1. Klamrassamee, T. et al.: *Bioresour. Technol.*, **147**, 246-284 (2013).
2. Suwannarangsee, S. et al.: *Bioresour. Technol.*, **119**, 252-261 (2012).
3. Wongwilaiwalin, S. et al.: *Appl. Microbiol. Biotechnol.*, **97**, 8941-8954 (2013).



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(Presentation 8)

Update on current status of Vietnam microbial resources at Vietnam Type Culture Collection

Thao Kim Nu Nguyen, Trinh Thanh Trung, Le Thi Hoang Yen, Dao Thi Luong, Duong Van Hop

Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, 144 Xuan Thuy, Hanoi, Vietnam

Vietnam Type Culture Collection (VTCC) is the biggest culture collection in Vietnam. Our mission is to preserve and manage the microbial genetic resources for the sustainable use of valuable microorganisms. VTCC is currently holding 9670 strains including 1873 bacteria, 3247 actinomycetes, 1292 yeasts, 2750 fungi among others. Beside the regular work in isolation and identification of microorganisms, in 2013, VTCC has carried out the following main projects:

- Investigating the biodiversity of aerobic endospore-forming bacteria isolates from Con Dao National park.

- Investigating the diversity of antibiotic-producing aerobic bacteria isolated from Hoang Lien National park and surrounding agricultural lands.

- Publishing a novel hyphomycete species, *Condylospora vietnamensis*, isolated from fallen leaves in Bach Ma National park.

- Biologically characterization of mangrove microalgae isolated from Xuan Thuy National park.

- Evaluating the secondary metabolites produced by 50 actinomycete strains using HPLC for the discovery of novel compounds.

- Characterization of anti-*Xanthomonas oryzae* pv. *oryzae* compounds produced by Vietnamese actinomycetes for the biological control of bacterial blight disease in rice.

(Presentation 9)

Diversity of Thermotolerant Acetic Acid Bacteria in Lao PDR

Chanhom Loinheuang[1], Gunjana Theeragool[2], Kazunobu Matsushita[3], Toshiharu Yakushi[3]

[1]Faculty of Science, National University of Laos; [2]Faculty of Science, Kasetsart University, Bangkok, Thailand; [3]Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan.

Thermotolerant microorganisms including acetic acid bacteria (AAB) have been increasingly considered and developed in recent years. They are able to be potentially and popularly utilized in many different aspects of fermentation technology, especially processing at high temperature as well as reducing cooling cost.

To study geographical distribution of thermotolerant AAB, AAB were isolated from different kinds of sources, especially fruits in PDR, and then identified and characterized.

There were 113 isolates of thermotolerant AAB isolated from 5 sampling sites in Lao PDR, Xiengkhuang Province, Luangprabang Province, Vientiane Province, Bolikhamxay Province and Vientiane Capital. These thermotolerant AAB were isolated by the enrichment culture, which were performed at 37°C in the presence of 1% acetic acid or 1%, 2%, 3% and 4% ethanol, from various kinds of fruit samples collected in Lao PDR. On YPGD (1% glucose, 1% glycerol, 1% yeast extract, 1% peptone) agar plates containing 0.5% calcium carbonate or 0.004% bromocresol purple, AAB were detected as colonies having clear zone or yellow zone, respectively, after cultivation at 3rd to 7th days. Thus, 113 isolates were obtained and characterized. The 59 isolates were classified into Acetobacter sp. and the other 54 isolates were Gluconobacter sp., according to their acetate-oxidizing ability. The 77 isolates could grow at 37°C on YPGD agar containing 1% acetic acid and 4% ethanol, 22 isolates could grow on YPGD agar containing 4% ethanol and 34 isolates could grow on YPGD agar containing 1% acetic acid. Of these isolates, 13 isolates (SNL81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 52 and SNL65) were selected and examined for their growth at higher temperature because all of them could grow at 41°C after 48 hrs (Table 1).

Acetic acid production was examined with the 13 isolates (SNL81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 52 and 65), all of which produced 3.0-3.5% of acetic acid from 4% ethanol after 72 hrs incubation at 37°C, whereas Acetobacter pasterianus SKU1108 could produce 3.2% acetic acid (Fig. 3). Of these 13 strains, SNL90 exhibited the highest productivity (~3.5%) and SNL81 produced acetic acid more rapidly.

The 13 isolates were identified based on the 16S rDNA analysis. As shown in Table 1, SNL65 and 87 were most closely to Acetobacter cibinongenis and Acetobacter pasteurianus, respectively, while all others seemed to be Acetobacter tropicalis. Another 4 isolates (SN06, SN21, SN57, and SN66) were also identified with 16S rDNA to be Acetobater pasteurianus (99% identity), Acetobacter pasteurianus (100%), Acetobacter malorum (99%), and Gluconobacter oxydans (99%), respectively.

References:

Saeki, A., G. Theeragool, K. Matsushita, H. Toyama, N. Lotong, and O. Adachi, 1997. Development of thermotolerant acetic acid bacteria useful for vinegar fermentation at high temperatures. *Biosci Biotech Biochem* 61 (1).

(Presentation 10)

Isolation and Characterization of Xylose Utilizing Yeast for Ethanol Fermentation

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Abstract

Nineteen xylose-utilizing yeasts were isolated from different samples collected in Lao PDR by an enrichment technique at 30°C for 24-48 h in YNB medium containing 2% xylose. Cell morphology of strains isolated was observed after growing in YPD medium at 30°C for 24 h. These strains exhibited globose to ellipsoidal shapes and reproduced by budding or fission. The cell growth was tested on plates of YPD, YPX and YNB containing xylose at 35°C, 37°C and 40°C. The results showed that most strains grew well on YPD and YPX plates at 35C°and 37°C, but less grew at 40°C. On plates of YNB containing xylose, 11, 14 and 12 strains moderately grew at 35C°, 37°C and 40°C, respectively. All isolated strains grew well and 4 strains showed the ability of ethanol fermentation in YP liquid medium containing 4% xylose at 30°C. Their production range of ethanol, however, was low (0.06-0.14%) during 96 h while *Pichia stipitis* was 1.15%. Analysis of the D1/D2 domain of the large subunit rRNA gene revealed that some of them are *Candida tropicalis* and *Kluyveromyces marxianus*.

(Presentation 11)

Surveying the Invertebrate Pathogenic Fungi Diversity in The Huay Yang Forest Reserve, Vientiane Capital

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Abstract

A total of 59 entomopathogenic fungal samples were collected from Huay Yang Forest Reserve Vientiane Capital. These included 6 genera and 9 identified species: Beauveria sp., Akanthomyces sp., Akanthomyces pistillariiformis, Nomuraea sp, Ophiocordyceps nutans, Ophiocordyceps sp. Cordyceps sp., Isaria tenuipes, Aschersonia sp. and 6 unidentified species. While 7 strains of entomopathogenic fungi were kept at Biology Department, Faculty of Science, and National University of Laos for further research.

(Poster presentation)

Multi-cell wall degrading enzyme from *Aspergillus aculeatus* for viscosity reduction in very high gravity cassava fermentation

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Cassava is one of the most important starch feedstock used as a raw material for many industrial, such as bioethanol production; however, fermentation of cassava in very high gravity (VHG) process is limited by high mash viscosity. In this study, the viscosity of fresh cassava root mash, chips, and pulp, that differed in starch-fiber component and physical properties were reduced by multi-cell wall degrading enzyme prepared from solid state fermentation of *Aspergillus aculeatus* BCC17849. Proteomic analysis using LC-MS/MS showed that the enzyme was a complex mixture of starch and non-starch degrading enzymes comprising endo- and exo-acting cellulases, hemicellulases, and pectinases in various glycosyl hydrolase families. Treatment of cassava substrates with multi-enzyme containing 0.4-21.0 IU/g endoglucanase; 0.007-0.4 IU/g FPase; 0.2-10.0 IU/g xylanase; 1.0-56.0 IU/g polygalacturonase; 1.6-80.0 IU/g β-glucanase; and 0.6-30.0 IU/g mannanase at 45°C, pH 5.0 for 2 h led to reduction in viscosity to the operating level of < 500 cPs, equivalent to the final viscosity of 3.0-51.3% of the initial levels for different substrate. The final ethanol concentration of 17.54% v/v was achieved under simultaneous saccharification and fermentation of the pretreated root mash (32% initial solid) by *Saccharomyces cerevisiae* with a raw starch degrading amylase at 32°C for 96 h, which was slightly lower than the yield obtained using a conventional high-temperature liquefaction process at 19.65% v/v. The results demonstrated the potential application of the multi-enzyme for viscosity reduction in very high gravity fermentation allowing improvement in process efficiency and eliminate the expensive step in bioethanol process.

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