1st Satellite Seminar
of
New Core to Core Program

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)

Organized By
University of Brawijaya (UB)

In association with

Japan Society for the Promotion of Science (JSPS)
National Research Council of Thailand (NRCT)
Vietnam Ministry of Science and Technology (MOST)
Yamaguchi University, Kasetsart University, Can Tho University, National University of Laos, Beuth University of Applied Science, The University of Manchester

8th August 2014
5th Floor of East Java Bank, Surabaya

Abstract Book of The 1st Satellite Seminar of CCP, 8 August 2014, Venue: 5th Floor, East Java Bank, Surabaya-Indonesia

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Message from Indonesia Coordinator

Dr. Anton Muhibuddin

Dear All,

Firstly, I introduce Indonesia as a new member of Core to Core Program (CCP). Thank you very much for Prof. Mamoru Yamada, as a coordinator of CCP for Japan side who has facilitated the participation of Indonesian scientist in this program. I hope, participation of Indonesian scientist can support CCP programs better. As a new member of CCP, it is not easy to run this program as well as Thailand and Japan which has run since 1987. This is due to the schedule of academic and research funding are different in Indonesia. An example is that the budget submission for the program in Indonesia is limited by time up to the end of March every year. However, of course Indonesia will try best to support this program. As the country with the third largest population in the world, the number of scientists in Indonesia is also very big and potential to support development of science in the world. Moreover, the quality and quantity of our natural resources is very great to support the development of world's science.

Overall, I am delighted to welcome all of the speakers and participants to the 1st Satellite Seminar of the New 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)".

On behalf of Indonesia Coordinator, I would like to express my sincere appreciation to all participants for contributing their research work to this seminar. Thanks also to the Japanese, Thailand, Vietnamese, Laotian, Germany and UK coordinators for their cooperation in arranging this seminar. I also would like to express sincere gratitude to East Java Bank, University of Brawijaya, DIKTI, University of KH. Abd. Wahab Hasbullah, JSPS, NRCT, Vietnam Ministry of Science & Technology (MOST), the National University of Laos, Beuth University of Applied Sciences (Germany) and The University of Manchester (England) for their financial support.

Kind regards,

Anton Muhibuddin
Indonesia coordinator

Message from Japanese Coordinator
Prof. Mamoru Yamada

It is our great pleasure to hold the 1st 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)". I would like to take this opportunity to acknowledge the enormous effort of the organizing committee members of this seminar at the Indonesia side, especially Dr. Anton Muhibuddin and his colleagues, and the financial support of University of Brawijaya.

We have launched the five-year Core-to-Core Program, in which many scientists participate from seven countries of Thai, Vietnam, Laos, Germany, Indonesia, United Kingdom and Japan. For the approval of this program, we would like to appreciate tremendous activities and highly valuable achievements of the members in the ten-year JSPS-NRCT Core University Program entitled "Development of Thermotolerant Microbial Resources and Their Application" and in the five-year JSPS-NRCT Asian Core Program entitled "Capacity Building and Development of Microbial Potential and Fermentation Technology towards New Era". The Core-to-Core Program is designed to create top world-class research centers that partner over the long term with other core research institutions around the world in advancing research in leading-edge fields, on issues of high international priority. Thus, we should challenge the new bio-research fields with microbes from tropical areas.

In our Core-to-Core Program, there are the following five projects, which will be performed by internationally collaborative researches among seven countries. I thus hope members at Indonesia side to find out good counterparts to work together 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.

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

After this seminar, the 1st Joint Seminar in Thailand, which is organized by Associate Professor Dr. Gunjana Theeragool and members at the Thai side, and the 10th Young Scientist Seminar in Japan, which is organized by young scientists, are scheduled on Aug 10-11 and Nov 16-17 this year, respectively. This new Core Program is thus expected to contribute not only to microbial sciences but also to education to foster our successors.

Finally, I would like to appreciate all attendants and their contributions to this seminar, and the financial supports of University of Brawijaya, National Research Council of Thailand (NRCT) and 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 1st Satellite Seminar of the New 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 from 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, Beuth University of Applied Sciences (Germany) and The University of Manchester (England).

This 1st Satellite Seminar of the Core to Core Program is the second academic activity arranged after the successful JST workshop on Advanced Low Carbon Biotechnology which was held at Kasetsart University during the period July 3-4, 2014. This seminar will provide a good opportunity for all of 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. With our hard work and contributions, I am sure that this Core to Core Program will be another successful project, similar to our previous Core University Program and Asian Core Program.

On behalf of Thai Coordinator, I would like to express my sincere appreciation to The University of Brawijaya especially Dr. Ir. Anton Muhibuddin, Indonesian Coordinator, for organizing the 1st Satellite Seminar. My thanks also go out to the invited speakers and all of the oral and poster presenters for contributing their research work to this seminar. Thanks also to the Japanese, Vietnamese, Laotian, Indonesian, German and English coordinators for their cooperation in arranging this 1st Satellite Seminar. Last, but not least, I would like to express sincere gratitude to JSPS and NRCT for their continuing financial support.

Gunjana Theeragool
Thai coordinator and Chairperson of the Organizing Committee

Message from Vietnamese Coordinator

Assoc. Prof. Dr. Ngo Thi Phuong Dung

With all pleasure, I am very delighted to welcome all of the distinguished participants to the 1st Satellite Seminar of the Core to Core Program on "Establishment of an international research core for new bio-research fields with microbes from tropical areas", hosted by University of Brawijaya, held on 8th August 2014 at University of Brawijaya, Malang-Indonesia.

The year 2014 has officially marked the great success to start a new Core to Core Program (April 2014- March 2019) as our wish to further develop our collaboration on science and education after the Asian Core Program (April 2008- March 2013) on "Capacity building and development of microbial potential and fermentation technology towards New Era", which was financially supported by Japan Society for the Promotion of Science and National Research Council of Thailand.

It is a great honor and pleasure for the Vietnamese team to be available to continue our participation in this new program of Advanced Research Networks on "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. Our team is also very happy to have a good opportunity to join with more counterparts from Japan, Thailand, Laos, Germany, Indonesia, United Kingdom and Vietnam.

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

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

Message from UK Coordinator

Prof. Dr. Colin Webb

It has been, for me, a great pleasure to join the Core to Core Programme led by Prof. Dr. Mamoru Yamada of Yamaguchi University in Japan and to participate in the 1st Joint Seminar on "Capacity Building and Development of Microbial Potential and Fermentation Technology towards New Era" being held in Bangkok, Thailand.

Unfortunately, I am unable to participate in the 1st Satellite Seminar of 2014, organized by the University of Brawijaya (UB) to be held in Malang-Indonesia on 8th August. This seminar promises to present a wide ranging programme discussing all topics of the core-to-core project. I would like to pass on my sincerest best wishes for a successful and productive day. I hope also that I, or members of my team, will be able to participate in future Satellite Seminars.

Colin Webb
UK Coordinator
Professor, University of Manchester

Schedule and Program of The 1st Satellite Seminar
New Core-to-Core Program
8th August 2014 at 5th Floor of East Java Bank Surabaya – Indonesia

Time	Detail	Speaker
August 6, 2014		
09:40 – 13:05 16:30 - 17:50	Thai members leave for Suvarnabhumi Airport to Singapore Airport by SQ973 then connect to Juanda Airport (Surabaya) by **MI226**	
	Check-in at IBIS Hotel in Surabaya. Location: Jalan Basuki Rahmat, Surabaya, Jawa Timur 60271, (031) 5358885	
August 7, 2014		
(23:00) – 00:35	Japanese Members arrive at Sukarno Hatta International Airport (Jakarta), then depart to Juanda Airport (Surabaya) by GA332	
01:30 - 02:00	Depart for IBIS Hotel in Surabaya and check in. Location: Jalan Basuki Rahmat, Surabaya, Jawa Timur 60271, phone: (031) 5358885	
	Late Breakfast	
12:00 – 13:00	Lunch	
13:00 -15:00	To meet President of Brawijaya University (in Malang or Surabaya)	
August 8, 2014		
08:00 – 08:30	Depart for 5th Floor of East Java Bank Surabaya for Satellite Seminar	
08:30 – 09:00	Registration	
09:00 – 09:30	Opening ceremony	
09:30 – 10:00	Introduction of Brawijaya University Indonesia	Dr. Anton Muhibuddin
10:00 – 10:30	Group Photo and coffee break	
10:30 – 11:00	Introduction of Core to Core Program	Prof. Dr. Mamoru Yamada
Seminar Session I	***Chairman : Prof. Dr. Poonsuk Prasertsan***	
	Co-Chairman : Prof. Dr. Shinichi Ito	
	Project I: Explorational Research of Useful Microbes	
11:00 – 11:25	**Oral I-1**: Biomass-degrading enzymes and value-added products from tropical strains of *Aureobasidium pullulans*	Assist. Prof. Dr. Sehanat Prasongsuk *Chulalongkorn University*
11:25 – 12:30	Lunch	
12:30 – 12:55	**Oral I-2** Isolation and identification of nitrogen fixing non-simbiotic bacteria on restoration land with legume cover crop (lcc) in the area of Pasirian, Lumajang, East Java	Ir. Tutik Nurhidayati, MS. *Institute Technology of 10th Nopember Surabaya*

Time	Detail	Speaker
	Project II: Genome-based Research on Thermotolerat Microbes	
12:55 – 13:20	**Oral II-1**: Analysis of thermotolerant genes in thermotolerant *Zymomonas mobilis*	Assist. Prof. Dr. Tomoyuki Kosaka *Yamaguchi University*
13:20 – 13:45	**Oral II-2**: Essentiality of respiratory activity for pentose utilization in thermotolerant yeast *Kluyveromyces marxianus*	Dr. Noppon Lertwattasanakul *Katsetsart University*
Seminar Session II	***Chairman : Prof. Dr. Kenji Matsui***	
	Co-Chairman : Dr. Ir. Marjuki	
	Project III: **Research on Environmental Microbes Sustaining Tropical Ecosystem**	
13:45 – 14:10	**Oral III-1**: Newly recognized food-borne diseases associated with ingestion of myxosporean spores in marine fish	Prof. Dr. Hiroshi Sato *Yamaguchi University*
14:10 – 14:35	**Oral III-2** The potency of phylloplane saprophytic fungi on shallot as antagonists against purple blotch disease(*Alternariaporri*) in East Java, Indonesia	Dr. Hery Nirwanto University of Veteran Surabaya
14:35 – 14:50	Coffee Break	
14:50 - 15:15	**Oral III-3** Maximizing the essential roles of rumen microbes in supplying nutrients for ruminants	Dr. Ir. Marjuki University of Brawijaya
	Project IV: Research on Microbes Useful for Food, Food Preservation, Health, and Ecosystem	
15:15 – 15:40	**Oral IV-1**: Development of effective bioremediation technology utilizing beneficial biofilms	Prof. Dr. Masaaki Morikawa *Hokkaido University*
Seminar Session III	***Chairman :* Dr. Ir. Hery Nirwanto**	
	Co-Chairman : Assist. Prof. Dr. Sehanat Prasongsuk	
15:40 – 16:05	**Oral IV-2**: Microbial diversities in the chemical and organic agricultural soils	Prof. Dr. Motoki Kubo *Ritsumeikan University*
16:05 – 16:30	**Oral IV-3**: Exploration of marine bacteria as an alternative source of enzyme production for industry and biodegradation	Assist. Prof. Dr. Jittima Charoenpanich *Burapha University*
16:30 – 16:45	Coffee Break	
	Project V: Development of Next-generation Fermentation Technology for New Wave Industry	

Time	Detail	Speaker
16:45 – 17:10	**Oral V-1**: Effects of inoculum size and reactor type on biohydrogen production from Palm Oil Mill effluent under thermophilic condition	Prof. Dr. Poonsuk Prasertsan *Prince of Songkhla University*
17:10 – 17:35	**Oral V-2**: *In Vitro* thermal adaptation useful for the development of high-temperature fermentation	Emeritus Prof. Kazunobu Matsushita *Yamaguchi University*
17:35 – 18:35	**Group Discussion** Project 1 Leaders: Dr. Shinichi Ito Dr. Sehanat Prasongsuk Project 2 Leaders: Dr. Mamoru Yamada Dr. Noppon Lertwattasanakul Project 3 Leaders: Dr. Anton Muhibuddin Dr. Tomoyuki Kosaka Project 4 Leaders: Dr. Kenji Matsui Dr. Jittima Charoenpanich Project 5 Leaders: Dr. Poonsuk Prasertsan Dr. Naoya Kataoka	
18:35 – 19:00	**Closing Ceremony**	
19:00 – 21:00	**Dinner**	
August 9, 2014		
08.00	Thai member check out and depart for Juanda Airport Surabaya	
08:30 – 10:30	Check in ticket and immigration office in Juanda Airport Surabaya	
10:30 – 13:40	Depart for Singapore Airport by SQ931	
16:00 – 17:25	Depart for Suvarnabhumi Airport Bangkok by SQ976	
10:00 - 10:30	Japanese members check out and depart for Juanda Airport Surabaya	
10:30 – 11:30	Lunch	
11:30 – 13:30	Check in ticket and immigration office in Juanda Airport Surabaya	
13:30 – 15:05	Depart for Sukarno Hatta Airport Jakarta by GA317	
16:40 – 20:10	Depart for Suvarnabhumi Airport Bangkok by GA864	

Note: Oral presentation for 20 min and discussion for 5 min

Biomass-degrading enzymes and value-added products from tropical strains of *Aureobasidium pullulans*

Sehanat Prasongsuk[1], Wichanee Bankeeree[1], Benjawan Yanwisetpakdee[1], Rinji Akada[2], Pongtharin Lotrakul[1], and Hunsa Punnapayak[1]

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A number of *Aureobasidium pullulans* strains were obtained from various habitats in Thailand and were identified using morphological observation and molecular characterization. These strains were evaluated for their production of biomass-degrading enzymes, mainly β-xylanase and β-xylosidase, and value added products including exopolysaccharide (pullulan and β-glucan), antifungal agent and siderophores. Some of these tropical isolates were able to produce cellulase-free xylanase which as applicable in pulp bleaching. The produced xylanase was also used for degrading xylan, extracted from various tropical weeds, in order to produce a functional prebiotic, and xylooligosaccharides. The full-length gene of β-xylosidase, an enzyme responsible for xylobiose degradation from a tropical strain of *A. pullulans* was revealed. Moreover, the abilities of these *A. pullulans* strains for the production of pullulan, β-glucan, antifungal agent and siderophores were also investigated.

ISOLATION AND IDENTIFICATION OF NITROGEN FIXING NON SIMBIOTIC BACTERIA ON RESTORATION LAND WITH LEGUME COVER CROP (LCC) IN THE AREA OF PASIRIAN, LUMAJANG, EAST JAVA

Tutik Nurhidayati, Nur Hidayatul Alami, and Amik Agisti
Institute Technology of 10th Nopember Surabaya, Indonesia

Abstrack

Application of LCC using peanut (*Arachis hypogaea*) on critical agricultural land is able to improve soil fertility. This system produces a good rizosfer that support the bacteria growth. This research was conducted to determine the abundance and identify bacteria genus of nitrogen fixing non symbiotic bacteria in land restoration with LCC in Pasirian, Lumajang, East Java.

Bacterial abundance was calculated using Total Plate Count (TPC) method. Charateristic was to know are bacterial mophological in cultivated medium and biochemistry test. Determination the bacterial genus was use *Bergey's manual of determinative Bacteriology 9th edition*.

The research results show population of Nitrogen fixing non-symbiotic bacteria on agricultural land Pasirian before LCC is 3x102 CFU/g and after LCC is 2x103 CFU/g, and show three bacterial genus of Nitrogen fixing non-symbiotic bacteria found in land restoration with LCC *Azotobacter Beijerinckia, Derxia. Azotobacter* are gram-negative and coccus bacterium, *Beijerinckia* are gram-negative bacilli, with positive catalase test, and *Derxia* is a gram-negative bacilli with negative catalase test.

Keywords: LCC, nitrogen fixing bacteria, non symbiotic.

Analysis of thermotolerant genes in thermotolerant *Zymomonas mobilis*

Tomoyuki Kosaka[1], Tomoko Sakurada[2], Amina Tokiyama[1], Kannikar Charoesuk[1,3], Keisuke Hisano[2], Masayuki Murata[2], Mamoru Yamada[1,2]

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To develop thermotolerant and highly efficient ethanol-producing microbes, the elucidation of the thermotolerant mechanism is indispensable. Thermotolerant microorganisms may intrinsically possess the mechanism to prevent the cell damage by heat stress at a critical high temperature (CHT). In *Escherichia coli*, 51 thermotolerant genes and 26 semi-thermotolerant genes, which are required for growth at CHT, have been identified (1). These genes can be classified into energy metabolism, amino acid metabolism, vitamin metabolism, outer membrane stabilization, DNA repair, tRNA modification, and cell division. In this study, identification of the thermotolerant genes in a relatively thermotolerant ethanol-producing bacterium, *Z. mobilis* TISTR 548 was performed to understand the functional commonality and difference of thermotolerant genes among different microorganisms.

In TISTR 548, 67 thermosensitive mutants at 39.5 °C were screened from ca. 8,000 transposon-inserted mutants. They were then subjected to TAIL-PCR followed by nucleotide sequencing to determine the corresponding thermotolerant gene. In addition, the polar effect of transposon insertion on the expression of down-stream genes was examined by RT-PCR. As a result, 31 thermotolerant genes were identified in TISTR 548. The identified thermotolerant genes were classified into 9 groups as energy and general metabolism, membrane stabilization, DNA repair, tRNA modification, chaperon/protease, translation and translation control, cell division, transcription regulation and others.

1. Murata M, Fujimoto H, Nishimura K, Charoensuk K, Nagamitsu H, Raina S, Kosaka T, Oshima T, Ogasawara N, Yamada M (2011) Molecular strategy for survival at a critical high temperature in *Escherichia coli*. *PLoS ONE*, **6**: e20063.

Essentiality of respiratory activity for pentose utilization in thermotolerant yeast *Kluyveromyces marxianus*

Noppon Lertwattanasakul[1], Nadchanok Rodrussamee[2], Masayuki Murata[3], Sukanya Nitiyon[3], Suprayogi[3], Savitree Limtong[1], Tomoyuki Kosaka[4] and Mamoru Yamada[3,4]
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The development of sustainable and renewable biofuels has attracted growing interests with concerns on increase oil demands and a cleaner environment worldwide. The economy of fermentation-based bioprocess including bioethanol production relies extensively on the performance of fermentative microbes.

Kluyveromyces marxianus has been adopted by industries for a relatively broad range of applications from biomass production to bioremediation due to advantages of its traits such as rapid growth, thermotolerance, secretion of the enzyme inulinase and production of ethanol from various carbon sources including glucose, mannose, galactose, xylose and arabinose. A particularly attractive application of this yeast is high-temperature fermentation and bioconversion of hemicellulose. The negative effect by coexisting glucose in the substrates is critical for utilization of biomass containing mixed sugars. In *K. marxianus*, glucose repression effect was found to be weaker than that of *Saccharomyces cerevisiae*[1), 2)]. As a disadvantage, the respiratory activity in *K. marxianus* becomes strong when it was grown on pentose sugar, resulting in low production of ethanol. In order to identify key factors involved in pentose metabolism, experiment on *K. marxianus* by a random *kan*MX4-insertion mutagenesis was performed[3)]. We obtained three mutants of *COX15*, *ATP25* and *CYC3* encoding a cytochrome oxidase assembly factor (singleton), a transcription factor required for assembly of the Atp9p subunit of mitochondrial ATP synthase and cytochrome *c* heme lyase, respectively, as mutants lacking growth capability on xylose and/or arabinose. The three mutants were thermosensitive and their biomass formation in glucose medium was reduced, but ethanol yields were increased compared to those of the parental strain. Experiments on the mutants with respiratory inhibitors and uncoupling agent revealed that the respiratory activity and ATP are essential for utilization of pentoses. Taken together, this knowledge will be useful to further improve the strain for high-temperature ethanol fermentation.

References:

1) Rodrussamee N., Lertwattanasakul, N., Hirata, K., Suprayogi, Limtong, S., Kosaka, T. & M. Yamada. Growth and ethanol fermentation ability on hexose and pentose sugars and glucose effect under various conditions in thermotolerant yeast *Kluyveromyces marxianus. Appl. Microbiol. Biotechnol.* **90**, 1573–1586 (2011).
2) Lertwattanasakul N., Rodrussamee, N., Suprayogi, Limtong, S., Thanonkeo, P., Kosaka, T. & M. Yamada. Utilization capability of sucrose, raffinose and inulin and its less-sensitiveness to glucose repression in thermotolerant yeast *Kluyveromyces marxianus*. *AMB Express.* **1**, 20 (2011).
3) Lertwattanasakul N., Suprayogi, Murata, M., Rodrussamee, N., Limtong, S., Kosaka, T. & Yamada, M. Essentiality of respiratory activity for pentose utilization in thermotolerant yeast *Kluyveromyces marxianus* DMKU 3-1042. *Antonie van Leeuwenhoek*. **103**, 933–945 (2013).

Newly recognized food-borne diseases associated with ingestion of myxosporean spores in marine fish

Hiroshi Sato[1], Tetsuya Yanagida[1], Takahiro Ohnishi[2], Yoichi Kamata[2,3] and Yoshiko Sugita-Konishi[2,4]
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Food borne diseases due to unknown causes, manifested as diarrhea and vomiting after an incubation time from 3.4 to 16.3 hours after consumption of raw marine fish slices (sashimi), have been noticed since 1999 in the western part of Japan. In 2011, a novel multivalvulid species parasitic to olive flounder, *Kudoa septempunctata* Matsukane et al., 2010 (Myxozoa: Myxosporea), was identified as one of the causative agents of "the unidentified food borne disease associated with consumption of raw fresh fish" by a research group supported by the Ministry of Health, Labour and Welfare of Japan (Kawai et al., 2012). *Kudoa septempunctata* is morphologically characterized by spores with 6—7 shell valves and polar capsules, localized in the myofiber of trunk muscles of olive flounder by forming pseudocysts (Matsukane et al., 2010). Based on an epidemiological analysis, the threshold for the onset of symptom is estimated to be approximately 7.2×10^7 *K. septempunctata* spores/person. Pathogenetic mechanisms of *K. septempunctata* spores were explored using rodent models (suckling mice and house musk shrews) and a culture model using human intestinal cell (Caco-2) monolayer.

In additon to *K. septempunctata* in olive flounder, multiple new myxosporean species in edible marine fish around Japan have been described by us to list up all possible causative agents of "Kudoa food borne disease" emerging recently along with the development of marine fish culture.

2. Kawai T, Sekizuka T, Yahata Y, Kuroda M, Kumeda Y, Iijima Y, Kamata Y, Sugita-Konishi Y and Ohnishi T (2012) Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in raw fish. Clin Infect Dis **54**: 1046-1052
3. Li Y-C, Sato H, Kamata Y, Ohnishi T and Sugita-Konishi Y (2012) Three novel myxobolid species of genera *Henneguya* and *Myxobolus* (Myxosporea: Bivalvulida) from marine fish in Japan. Parasitol Res 111: 819-826
4. Li Y-C, Sato H, Tanaka S, Ohnishi T, Kamata Y, Sugita-Konishi Y (2013) Characterization of the ribosomal RNA gene of *Kudoa neothunni* (Myxosporea: Multivalvulida) in tunas (*Thunnus* spp.) and *Kudoa scomberi* n. sp. in a chub mackerel (*Scomber japonicus*). Parasitol Res **112**: 1991-2003
5. Matsukane Y, Sato H, Tanaka S, Kamata Y and Sugita-Konishi Y (2011): *Kudoa iwatai* and two novel *Kudoa* spp., *K. trachuri* n. sp. and *K. thunni* n. sp. (Myxosporea:

Multivalvulida), from daily consumed marine fish in western Japan. Parasitol Res **108:** 913-926
6. Matsukane Y, Sato H, Tanaka S, Kamata Y and Sugita-Konishi Y (2010) *Kudoa septempunctata* n. sp. (Myxosporea: Multivalvulida) from an aquacultured olive flounder (*Paralichthys olivaceus*) imported from Korea. Parasitol Res**107**: 865-872

THE POTENCY OF PHYLLOPLANE SAPROPHYTIC FUNGI ON SHALLOT AS ANTAGONISTS AGAINST PURPLE BLOTCH DISEASE (*Alternaria porri*) in EAST JAVA, INDONESIA

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ABSTRACT

The purple blotch disease caused by *Alternaria porri* (Ell.) Cif.is known as one of a mayor disease at shallot growing area and is responsible for a great loss.
The obyective of the research is to explore various type of phylloplane and phyllosphere fungi on shallot crops which have potency as microbial antagonist to *A. porri* that cause purple blotch disease, and also to analyse its community.
The Research was conducted at Plant Pest and Disease laboratory, UPN " Veteran" East Java and in rainy season. The methods used in this research is to conduct survey on shallot crops which applied pesticide. Sample were taken by purposive sampling to healthy shallot plant among diseased plants. The areas of survey lied on District of Probolinggo, Malang, Nganjuk and of Kediri at height between 150 - 600 m above sea level. Antagonism experiment was done by breading pathogen isolate and antagonist fungi isolate in Potato Dextrose Agar media.
.Results of the research showed that diversity index of saprophytic fungy on shallot crops of Malang isolate equal to 2,99 and of Probolinggo is, 3,54. The research also found that the isolate of saprophytic fungi of shallot crop which have potency as antagonist is *Trichoderma sp*. and *Penicillium sp*

Key words:, phyllosphere and phylloplane fungi, antagonism, *Alternaria porri*, saprophytic, shallot

MAXIMIZING THE ESSENTIAL ROLES OF RUMEN MICROBES IN SUPPLYING NUTRIENTS FOR RUMINANTS

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Fibers or forages are the most abundant biomass available in the world. By ruminant animals, these low quality biomass are potentially to be converted into high quality foods for human being. Ruminants are very well known to have capability in digesting and utilizing the low quality biomass for their live and production and convert the biomass into power, meat, milk, and other products. Indeed, ruminants by themselves are just like other animals, even they can not digest and utilize fibers or forages that are as their main own feed, without any essential help of microbes present in their rumen. There is no any digestive enzymes that can digest fibers or forages, but rumen microbes digest and utilize the low quality biomass for their live and growth producing high quality microbial cells and fermentation products volatile fatty acids (VFA) including acetic, propionic, and butyric acids. These fatty acids are absorbed from the rumen and utilized by ruminant's body as the main energy source. In addition, rumen microbial cells flowing to the small intestine are ready for enzymatic digestion and release their nutrients, especially protein, vitamin and minerals, which are then ready for absorption to supply the nutrients requirement of ruminant animals. Thus, rumen microbes play an essential role in helping ruminant to convert low quality feeds into high quality nutrients to supply most of nutrients required by ruminants. There is a saying in ruminant feeding that *"Feed the rumen microbes first, then let the microbes feed the ruminants for their live and production"*. The first priority in ruminants feeding is to maximize the rumen microbe's activity and growth from which ruminants get their most nutrients supply for their live and production. Rumen microbes require appropriate supply of substrates and rumen conditions for their maximum activity and growth. Hence, both requirement of rumen microbial for maximum activity and growth are directly affected by feeds consumed by ruminants. The feeds do not only function as nutrients source for rumen microbes, but also creating appropriate rumen conditions for rumen microbial activity and growth. Thus, feed and its feeding strategy and manipulation are important to ensure maximum rumen microbial activity and growth, which supply nutrients for maximum ruminant's productivity.

Key words: forages, energy, microbes, nutrients, protein, rumen

Development of effective bioremediation technology utilizing beneficial biofilms

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In natural environments, bacteria often exist in close association with surfaces and interfaces. There they form "biofilms", multicellular community structures held together by extracellular matrices. The biofilms confer on the constituent cells high resistance to environmental stresses and diverse microenvironments that help generate cellular heterogeneity. Biofilm-associated cells exhibit specific gene expression, many times controlled by quorum sensing systems, or dormancy, to allow their increases in resistance. Thus, forming biofilms is considered a natural strategy of microorganisms to construct and maintain a favorable niche in stressful environments. Application of biofilms to bio-production and bio-augmentation process is challenging but it could be a simple and rational choice (Morikawa, 2006).

Biofilm-associated cells of *Pseudomonas stutzeri* T102, as compared with that of planktonic cells, degraded aromatic contaminants naphthalene and survive for longer time in petroleum-contaminated soils (Shimada et al., 2012). When the fitness of T102 biofilm-associated cells was tested in natural petroleum-contaminated soils, they were capable of surviving for 10 weeks; by then T102 planktonic cells were mostly extinct. Naphthalene degradation activity in the soils that had been inoculated with T102 biofilms was indeed higher than that observed in soils inoculated with T102 planktonic cells. These results suggest that inoculation of contaminated soils with *P. stutzeri* T102 biofilms should enable bio-augmentation to be a more durable and effective bioremediation technology than inoculation with planktonic cells.

Biofilms are formed not only on abiotic but on biotic surfaces including plant roots. A phenol degrading *Acinetobacter* sp. P23 was isolated from the rhizosphere of duckweed (Yamaga et al., 2010). P23 rapidly colonized on the surface of sterilized duckweed roots and formed biofilms, indicating that the conditions provided by the root system of duckweed are favorable to P23. A long-term performance test using duckweed-P23 system showed that continuous removal of phenol can be attributed to the beneficial symbiotic interaction between duckweed and P23. The results in this study suggest the potential usefulness of colonizing a particular bacterium in the rhizosphere of duckweeds to achieve efficient and sustainable bioremediation of polluted water.

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Microbial diversities in the chemical and organic agricultural soils

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Environmental microorganisms play important roles for material circulation in agricultural soil. When total carbon (TC) and total nitrogen (TN) in the agricultural soils were controlled at TC≧30,000 and TN≧3,000, and C/N:8-18, the nitrogen circulation activity was enhanced. Subsequently, inorganic materials such as NH_4^+ and NO_3^- were also gradually increased in the soil. Based on these results, soil fertile index (SOFIX) was constructed (suitable nitrogen and phosphate circulations etc.) for improvement of the organic agricultural soil condition.

In order to improve the agricultural soil conditions, the bacterial diversities in the chemical and organic agricultural soils were analyzed by environmental DNA and PCR-DGGE. The microbial number in the organic agricultural soil was clearly higher than that in the chemical soil, and the bacterial number was enhanced when TC, TN, and C/N ratio were controlled (TC≧30,000 and TN≧3,000, and C/N:8-18).

Bacterial diversities in the chemical and organic agricultural soils were different on the PCR-DGGE gel, and the bacterial species were increased in the improved organic agricultural field by SOFIX. These results indicate that the bacterial diversity in the agricultural soil was influence by the contents of chemical and organic materials.

In this meeting, we will show you a creation of suitable organic agricultural soil condition by controlling microbial diversity with biomass resources. The microbial diversity in the continuous cropping soil will also be introduced.

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Exploration of marine bacteria as an alternative source of enzyme production for industry and biodegradation

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Marine environment is a source of unique microorganisms with great potential for biotechnological exploitation. Very few studies concerning the isolation and characterization of marine bacteria have been carried out, and investigations in this field may lead to many new discoveries. Our group attempts to find the marine bacteria produce unique characteristic hydrolase for application in industry and biodegradation.

Among 12 marine bacteria isolated from sea sediments of Koh Chan, Samaesan (9 and 24 meters depth), three were found secreting high protease activity. The strains were identified as *Bacillus megaterium*, *B. subtilis*, and *Staphylococcus warneri*. All enzymes were stable at alkali pH and active at a broad temperature range 10-80 °C. Metal ions did not affect the activities of *B. megaterium* and *S. warneri* proteases in contrast improve their activities. Three enzymes were stable in surfactants and hydrophobic solvents. The high temperature stability, alkaliphilic and ability to work in metal ions, solvents and surfactants support the potential of these proteases as vigorous biocatalysts for industrial applications.

Microorganisms living in the sea have specially adapted features that allow them to live and grow in the extreme environment. Many organic compounds have been utilized by these microorganisms however no report could be found for toxicant removal. We discover a new benzonitrile-degrader, *Staphylococcus sciuri* from sea sediments after an oil spill disaster in Ao Phrao beach, Samet Island. The strain could completely remove benzonitrile at alkali pH and mesophilic temperature. Moreover, two novel acrylamide-degrading bacteria (*Serratia liquefacian* and *B. cereus*) were also isolated from the sea sediments of Koh Chan, Samaesan (9 meters depth). Both strains grew well in the presence of acrylamide as 0.5% (w/v) which is higher concentration than published documents and prefer acidic pH. Our findings render marine bacteria attractive as an alternative source of enzyme production for industry and biodegradation.

Effects of inoculum size and reactor type on biohydrogen production from Palm Oil Mill effluent under thermophilic condition

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Biohydrogen production from palm oil mill effluent (POME) in anaerobic sequencing batch reactor (ASBR) and continuous stirred tank reactor (CSTR) at thermophilic condition was investigated. The inoculum size of *Thermoanaerobacterium thermosaccharolyticum* PSU-2 was tested in the range of 0-30%. The maximum hydrogen yield of 296 mL H_2/g-COD was achieved at 30% inoculums in both ASBR and CSTR, with the COD removal efficiency of 23%. The COD removal could be increased at continuous production of hydrogen at the hydraulic retention time (HRT) of 4 days, corresponding to the organic loading rate (OLR) of 11.3 g COD/ L·day. The COD removal efficiencies of ASBR and CSTR were 37.7% and 44.8%, respectively.

In Vitro thermal adaptation useful for the development of high-temperature fermentation

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Stable and successful industrial fermentations, in which mesophilic microorganisms used to be used, could be achieved by consuming a large amount of energy, especially for cooling and/or sterilization, and also by requiring expensive facilities and/or laborious labor. Recent global climate change makes more difficult to keep such a stable fermentation, and thus it is requested to develop a high-temperature fermentation system with thermally adapted and robust microbes, by which the energy consumption could be reduced and more efficient productivity provided.

We have isolated a large numbers of thermotolerant useful fermentative microbes such as acetic acid bacteria and glutamate-producing *Corynebacterium glutamicum* by collaborating with Thai groups. In addition, we have also tried to acquire strains adapted to higher temperature conditions in a laboratory. Thus, we have successfully obtained several thermo-adapted strains from mesophilic or thermotolerant acetic acid bacteria and also from *C. glutamicum*. Of these thermo-adapted strains, TH-3 obtained from *Acetobacter pasteurianus* SKU1108[1)], ITO-3 from *Gluconacetobacter xylinus* IFO3288[2)], CHM43AD from *Gluconobacter frateurii* CHM43[3)], and FT-1 from *C. glutamicum* N24[4)] have been shown to have a high potential to make high-temperature fermentation or non-temperature control-fermentation possible. The former two strains are used for vinegar (acetic acid) fermentation, and the latter two strains for sorbose and glutamic acid fermentations, respectively.

Aiming at development of the high-temperature fermentation systems, now we have tried to understand molecular mechanism of their thermotolerance by understanding their genome modification during the adaptation process[1)], and also to compare their fermentation ability at different temperature conditions.

In this seminar, I would like to summarize and show their fermentation abilities at high-temperature condition and also in fementor condition, and to discuss on their ability for the application to the high temperature fermentations.

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