Welcome Address

Prof. Dr. Masaaki OKA

On behalf of Yamaguchi University (YU), I would like to express our sincere appreciation to the Japan Society for the Promotion of Science (JSPS), the National Research Council of Thailand (NRCT), the Vietnam Ministry of Science & Technology (MOST) and Ministry of Research, Technology and Higher Education (RISTEKDIKTI) in Indonesia, Kasetsart University (KU), Can Tho University, the National University of Laos, the University of Brawijaya, Beuth University of Applied Sciences, University of Manchester and other organizations which have entrusted and honoured Yamaguchi University to be the host of the Final Joint Seminar of the Core-to-Core Program 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 wish to convey my sincerest gratitude to all coordinators of 7 countries and the professors involved in promoting the above mentioned research fields. I would also like to express my appreciation for Japanese committee members for their efforts in organizing this seminar.

YU and KU have a long history of collaboration and have been conducting the International Core Programs since 1998, supported by JSPS and NRCT. We have also been developing the Core-to-Core Program with Japan, Thailand, Germany, Indonesia, Laos, the United Kingdom and Vietnam since 2014, in which over 70 universities and over 250 scientists are involved. The purpose of these programs is the investigation and application of ‘Environmental Microbes Sustaining Tropical Ecosystem,’ and the program has been very successful in exploring new research fields and developing various technologies. Of these, several technologies have been verified at the semi-pilot or pilot scale by the Asia Science & Technology Strategic Cooperation Promotion Program, which was financially supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) and the Agricultural Research and Development Agency, Thailand (ARDA), thus the name MEXT-ARDA Project. Furthermore, a series of projects has produced many great achievements such as ripple effects on the industrial world as well as academic accomplishments. I would like to emphasize and admire the excellent outcomes of the past 20 years which have developed through the efforts of many researchers.

In this seminar, new data from the following five projects will be presented and discussed; “Explorational Research of Useful Microbes,” “Genome-based Research on Thermotolerant Microbes,” “Research on Environmental Microbes Sustaining Tropical Ecosystem,” “Research on Microbes Useful for Food, Food Preservation, Health, and Ecosystem Preservation,” and “Development of Next-generation Fermentation Technology for New Wave Industry.” I believe the outcomes through the above mentioned projects will prove prosperous when put to practical use. I sincerely hope that these five projects will lead to significant achievements and contribute to solving global issues related to energy, environment, medicine, hygiene, and food by cooperating with private companies.

Once again, I wish to express my gratitude to all sponsors such as JSPS, NRCT, Vietnam MOST, RISTEKDIKTI, Kasetsart University, Can Tho University, National University of Laos, Brawijaya University, Beuth University of Applied Sciences and the University of Manchester for organizing this wonderful seminar, and to everyone who has gotten involved in the seminar. I wish you all a fulfilling and successful experience and look forward to working with you again in the future.

Masaaki Oka
President, Yamaguchi University
Opening Remarks

Dr. Chongrak Wachrinrat

Masaaki Oka, President of Yamaguchi University,
Distinguished Guests,
Ladies and Gentlemen,

Good morning! On behalf of Kasetsart University, it gives me a great pleasure to deliver this opening message on the occasion of the Final Joint Seminar in Core-to-Core Program here at Yamaguchi University, Japan.

Kasetsart University has a long relationship with Yamaguchi University, especially in terms of collaboration in training programs, research projects, both student and academic exchanges.

Opportunities to pursue advanced studies, research, and training programs in Asia are considered efficient ways to improve not only our faculty staff and students but also the higher education system for our universities.

On this occasion, please allow me to express my sincere appreciation for Yamaguchi University, for kindly inviting me to this joint seminar. I believe that this seminar will mark a great step of our collaboration and help strengthen our relationship, I also hope that we can initiate new collaborative activities in the future.

Finally, I would like to thank all distinguished guests for joining this seminar and the organizers of this event for their hard work, I am most grateful to be here with you all, and I wish everybody the most enjoyable and productive experience, and the success seminar.

Thank you.

Dr. Chongrak Wachrinrat
Acting President, Kasetsart University
Opening Remarks

Prof. Dr. Sirirurg Songsivilai

It is a great honor for me to deliver the remarks at the Final Joint Seminar of the Core-to-Core Program (CCP), which brings together members from seven countries who participate in this important program. This seminar will disclose new results and technologies by scientists and researchers under this Core-to-Core Program.

The Core-to-Core Program in advance research network entitled “Establishment of an international research core for new bio-research fields with microbes from tropical areas” was launched by the cooperation from seven countries with the aims to investigate and apply useful substances from biomass using thermotolerant microbes from tropical areas. The program has been successful in expanding new research fields and developing various technologies. Specifically, the program strives for the resolution of common problems and thereby the advancement of science and technology across the region. These challenges are being realized through the provision of support to multilateral joint research and the exchange of human resources. Hence, I believe that it is very valuable to have this CCP as a medium for sharing experience and lessons learned among seven country members.

The National Research Council of Thailand (NRCT) is proud to participate in this Program from the beginning. The key features of the CCP are knowledge sharing and technology transfer within their network and fields. The outcomes of the CCP have been extended to wider range of users who can further develop and exploit the technology in the new products, processes, materials or services. We would like you to utilize this seminar as an opportunity to share new results and technologies and combine the expertise of your experts with other fields and create collaborative networks. This kind of combination will lead to creative and higher-impact research. It is of the utmost importance that all of us gathering here will come forth with innovative ideas, more effective, more applicable and flexible, in adapting useful thermotolerant microbes from tropical areas. It is also my wish that this seminar could enhance closer and fruitful relationships among our countries and our people, to work collaboratively both within and beyond boundary.

Taking this opportunity, let me on behalf of NRCT, express my sincere appreciation to all researchers who have concerted effort in driving the collaborative program towards the foreseeable success. Please accept my strong encouragement to you all to reach your common goal.

Professor Sirirurg Songsivilai, M.D. Ph.D.
Secretary-General of National Research Council of Thailand
Opening Address

Mr. Rikutarou Hamada

On behalf of Japan Society for the Promotion of Science (JSPS), I am very honored to make a greeting on the occasion of the final joint seminar of Core-to-Core program “Establishment of an International Research Core for New Bio-research Fields with Microbes from Tropical Areas” coordinated by Prof. Mamoru YAMADA, Yamaguchi University.

JSPS is Japan’s sole independent funding agency with a mission of advancing science, which it undertakes through a wide spectrum of programs. These include funding scientific research, fostering talented researchers, promoting science-related international exchange, and supporting the reform and globalization of universities. Core-to-Core program is one of its principal programs.

The faculty members of Yamaguchi University are currently conducting a world-class research on microbes from tropical areas grounded in their excellent results of international collaborative research projects in Core University Program (JFY1998-2007) and Asian CORE program (JFY2008-2012). Five years since JFY2014, JSPS Core-to-Core Program has provided support for their research project. It is a top class research among the projects adopted in Core-to-Core Program that has carried out more than 300 international researcher exchanges over the four years.

Particularly, it excels in researcher exchanges with Thailand, Vietnam, Indonesia, Laos and Germany. We closely follow the research outcomes from their project as the one that uniquely focuses on Southeast Asia.

I would like to pay tribute to their great effort devoted to the research activities for five years. Finally, I extend my best wishes to each of you for the utmost success in this final joint seminar.

At this auspicious moment, I declare the seminar open.

Rikutarou HAMADA

Head of the Research Cooperation Division I in the International Program
Department of the Japan Society for the Promotion of Science
Message from Japanese Coordinator

Prof. Dr. Mamoru Yamada

It is our great pleasure to open the final (3rd) Joint Seminar in the Core-to-Core Program (CCP) as Advanced Research Network (2014-2019) on “Establishment of an International Research Core for New Bio-research Fields with Microbes from Tropical Areas”. At this opportunity, I would like to acknowledge the Japan Society for the Promotion of Science (JSPS), the National Research Council of Thailand (NRCT), Vietnam Ministry of Science & Technology (MOST), Ministry of Research, Technology and Higher Education (RISTEKDIKTI) and related universities for their financial supports and Kasetsart University, Beuth University of Applied Sciences, Can Tho University, Brawijaya University, National University of Laos and Yamaguchi University for their contribution as a Core University. I also wish to express my gratitude to all CCP members for their active contribution to this program and all coordinators and general coordinators for their supporting activities.

After the successful achievements of international collaboration between Thai and Japan in JSPS-NRCT Core University Program (1998-2008) entitled “Development of Thermotolerant Microbial Resources and Their Application” and among Thai, Japan, Vietnam and Laos in Asian Core Program (2008-2013) entitled “Capacity Building and Development of Microbial Potential and Fermentation Technology towards New Era”, we have started the CCP in association with six countries of Thai, Germany, Vietnam, Laos, Indonesia and United Kingdom. About 300 scientists from more than 70 universities from seven countries participated in this CCP. These three programs have created the new field of “thermotolerant microbes” in the scientific world. Along with the spirit of the former two Core Programs, the CCP has been carried out 5 projects: 1) Explorational Research of Useful Microbes, 2) Genome-based Research on Thermotolerant Microbes, 3) Research on Environmental Microbes Sustaining Tropical Ecosystem, 4) Research on Microbes Useful for Food, Packaging, Health, and Ecosystem and 5) Development of Next-generation Fermentation Technology for New Wave Industry. Most of their achievements are summarized in the Summary Book, and you can also find out our activities in this seminar.

For stimulation of our collaboration and research activity, we held Joint Seminars including this one: 2014 at Bangkok and 2016 at Chonburi, and Satellite Seminars: 2014 at Surabaya, 2015 at Fukuoka, 2016 at Can Tho University, 2017 at Beuth University of Applied Sciences and 2018 at Luang Prabang. For fostering young researchers, Young Scientist Seminars were held every year at Yamaguchi. I would like to acknowledge all organizers of these Seminars including Dr. Gunjana Theeragool, Prof. Anton Muhibuddin, Prof. Kenji Sakai, Dr. Ngo Thi Phuong Dung, Prof. Peter Götz and Dr. Somchanh Bounphanmy with their colleagues. Moreover, for advanced researches and innovation of next-generation technologies, our members have been carried out specific projects related to CCP like ALCA or e-ASIA JRP, which were supported by several public agencies including Japan Science and Technology Agency (JST), RISTEKDIKTI and Agricultural and Research Development Agency (ARDA). I thus acknowledge these agencies. Altogether, this CCP has been proceeded very successfully and fruitfully. Its extension to the next Core Program is thus highly expected.

Finally, I would like to thank keynote and special lecturers, oral speakers and poster presenters for their contributions to this seminar. I also wish to acknowledge members of organizing committee at Japan side and members in Yamaguchi University for their enormous efforts, and Faculty of Agriculture, Yamaguchi University and Yamaguchi University for their financial supports for this Seminar.

Mamoru Yamada
Japan 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 Final Joint 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 Final Joint Seminar is the fourth academic activity in 2018 arranged after the successful International Joint Seminar in Thailand Research EXPO (August 13, 2018) in Bangkok, the 5th Satellite Seminar (October 23-24, 2018) in Luang Prabang and ALCA-JST workshop (November 24, 2018) in Bangkok. This seminar will provide a good opportunity for the participants to discuss and summarize their output/outcome of collaboration in order to obtain the most fruitful results. In addition, I hope that the presentation and discussion which take place during this seminar will spur the participants towards the development of new research opportunities and productive collaboration for the next Core-to-Core Program.

On behalf of Thai Coordinator, I would like to express my sincere appreciation to Yamaguchi University especially Prof. Dr. Mamoru Yamada, Japanese Coordinator, for organizing the Final Joint Seminar. My thanks also go out to the keynote speakers and all of the 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 Final Joint 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
Associate Professor, Kasetsart University
Message from German Coordinator

Prof. Dr.-Ing. Peter Götz

The final joint seminar of the Core-to-Core Program on “Establishment of an international research core for new biological fields with microbes from tropical area” closes the current funding period but is also the beginning for new activities towards future cooperation. For me personally, participation in the Asian Core-to-Core Program was and is a great privilege which allowed me to be part of a well-established international scientific community. Therefore I want to thank all partners and colleagues and especially my colleague Professor Yamada for making this possible.

Under the Core-to-Core Program we implemented a large network over 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 and scientist 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 should be in our interest to continue and intensify our activities.

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 final joint seminar at Yamaguchi University in Japan. 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 Yamaguchi University for being our host for the final joint seminar.

Peter Götz
German Coordinator
Professor, Beuth University of Applied Sciences, Berlin
Message from Vietnamese Coordinator

Assoc. Prof. Dr. Ngo Thi Phuong Dung

As the life cycle, our Core-to-Core Program comes to an end soon. With all pleasure and it is a great honor for me to preside over the message of the Vietnamese side on the occasion of the Final Joint Seminar of the Core-to-Core Program, that will be held on 2nd to 4th December 2018 at the University Hall, Yamaguchi University and kindly hosted by the Japanese counterpart of CCP.

As we grow, during the years of CCP, the Vietnamese team has actively implemented and participated in all program activities. We are very happy to be available to continue our active participation in this 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 delighted to have a good opportunity to join and work with many more counterparts from Japan, Thailand, Laos, Germany, Indonesia, United Kingdom and Vietnam. Most significantly, thanks to the collaboration of our experienced scientists from these countries, we have been able to advance our research and development of thermo-tolerant micro-organisms leading to a number of publications including international and national scientific papers and proceedings of oral and poster presentations, as well as the database of culture collection of newly thermo-tolerant micro-organisms isolated from Vietnam. We are also following up our research findings on the processing feasibility of fermented products by using the selected cultures and it has been indicated the promising application of such newly functional thermo-tolerant micro-organisms in region.

May I take this occasion to express a sincere thanks to the support institutions of all partner countries, and we would like to acknowledge the excellent effort of the Japanese organizing committee and team, especially Prof. Mamoru Yamada - the CCP Japanese Coordinator.

We are also grateful to the keynote lecturers, the oral speakers and the poster presenters as well as all participants who significantly contribute to the success of this Final Joint Seminar.

We believe this event will be very informative and highly useful for a summary of all our project outputs, and also for a discussion on the next coming project, strengthening and contributing toward the further success of our research collaboration program.

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

Assoc. Prof. Dr. Somchanh Bounphanmy

On behalf of the National University of Laos I would like to express our great pleasure, once again, to join the Final Joint Seminar of The Core-to-Core Program in Advanced Research Network on the Topic “Establishment of an International Research Core for New Bio-Research Fields with Microbes from Tropical Areas” hosted by Yamaguchi University, on December 1-5, 2018.

I would like to take this opportunity to express our sincere appreciation to the success of the CCP Program. The Final Joint Seminar will summary the achievement of our collaborative 5 research projects which were implemented by members from 7 countries such as Japan, Thailand, Vietnam, Indonesia, Germany, United Kingdom and Laos.

As for Laos side, through the activities of the CCP Program such as trainings, the exchanges of scientist, workshops, satellite seminars and joint seminars we gained greatly beneficial to the development of higher education, research, and particularly to the discoverability of useful thermotolerant microbes from our home land for fermentation technology. National University of Laos was very pleased to host the 5th which is last Satellite Seminar of the Program in Luang Prabang City on October 23-24, 2018.

I would like to express my deepest gratitude to the CCP committee members, the Japan’s Society for the Promotion of Science (JSPS), The National Research Council of Thailand (NRCT) for kind support and cooperation. My sincere appreciation to all coordinators and research participants from 7 countries for kind cooperation and for kind sharing research results through the events of CCP Program.

Finally, may I wish the Final Joint Seminar of CCP a great success and result of the Seminar will be a great source of inspiration for our future scientific investigation.

Somchanh Bounphanmy
Laos Coordinator
Associate Professor, National University of Laos
It is my great pleasure to participate at the Final Joint Seminar of the Core-to-Core Programme in Yamaguchi University, Japan.

It is a sad occasion to see the final event of this great network for collaboration between Institutions in Japan, Thailand, United Kingdom, Vietnam, Laos, Germany and Indonesia, but also a great opportunity for a new bigger and better endeavour, which we are looking forward to join.

The Core-to-Core Programme has offered a strong and dynamic platform for exchange of ideas and researchers and has created opportunities for advances in industrial biotechnology. It is my fourth time participating in the seminar series: first in Japan three years ago, in Thailand two years ago and in Germany last year. The strong participation form all the partners and happy the quality of presentations made all 3 of them great events.

I am looking forward to continue the fruitful discussions with a number of members of the programme, which produced a number of successful visits from program partners to my lab in Manchester, as well as to establish new dynamic interactions with other Core-to-Core network members. The UK side can offer a number of contributions in the area of Integrated Fermentation technology using a combination of state-of-the-art experimental and computational techniques.

Finally, I would like to thank the local organisers of this Final Joint Seminar as well as the JSPS and NRCT for their financial and academic support of the Core-to-Core Program.

Constantinos Theodoropoulos  
UK Coordinator  
Professor, University of Manchester
COMMITTEES

Advisory Committee
President, Japan Society for the Promotion of Science (JSPS)
Secretary General, National Research Council of Thailand (NRCT)
Minister, Vietnam Ministry of Science & Technology (MOST)
Director General of Resources for Science, Technology and Higher Education (RISTEKDIKTI)
President, Yamaguchi University
President, Kasetsart University
Rector, Can tho University
President, The National University of Laos
Rector, University of Brawijaya
President, Beuth University of Applied Sciences
Emeritus Prof. Dr. Osao Adachi

Program Steering Committee

Japan Side
Prof. Dr. Kazunobu Matsushita General Coordinator
Prof. Dr. Mamoru Yamada Coordinator
Prof. Dr. Kenji Matsui Vice-coordinator
Prof. Dr. Shinich Ito, Leader of Project I Committee
Assoc. Prof. Dr. Toshiharu Yakushi, Leader of Project II Committee
Prof. Dr. Ken Maeda, Leader of Project III Committee
Prof. Dr. Kenji Matsui, Leader of Project IV Committee
Assoc. Prof. Dr. Hisashi Hoshida, Leader of Project V Committee
Assist. Prof. Dr. Tomoyuki Kosaka, Sub-leader of Project I Committee
Prof. Dr. Rinji Akada, Sub-leader of Project II Committee
Assoc. Prof. Dr. Osami Misumi, Sub-leader of Project III Committee
Prof. Dr. Tsuyoshi Imai, Sub-leader of Project IV Committee
Assist. Prof. Dr. Naoya Kataoka, Sub-leader of Project V Committee
Ms. Naoko Miyaji Committee and Secretariat

International Side
Assoc. Prof. Dr. Napavarn Noparatnaraporn General Coordinator
Prof. Dr. Vo-Tong Xuan General Coordinator
Assoc. Prof. Dr. Gunjana Theeragool Coordinator
Prof. Dr. Ing. Peter Goetz Coordinator
Assoc. Prof. Dr. Ngo Thi Phuong Dung Coordinator
Prof. Dr. Ir. Anton Muhibuddin Coordinator
Assoc. Prof. Dr. Somchanh Bounphanmy Coordinator
Prof. Dr. Constantinos Theodoropoulos Coordinator
Assist. Prof. Dr. Vichai Leelavatcharamas Vice-coordinator
Dr. Phong Huynh Xuan Vice-coordinator
Prof. Dr. Yanuar Vice-coordinator
Assoc. Prof. Manichanch Sayavong Vice-coordinator
Prof. Dr. Piamsook Pongsawasdi, Leader of Project I Committee
Assoc. Prof. Dr. Pornthap Thanonkeo, Leader of Project II Committee
Assoc. Prof. Dr. Sunee Nitisiprasert, Leader of Project III Committee
Prof. Dr. Kosum Chansiri, Leader of Project IV Committee
Prof. Dr. Savitree Limtong, Leader of Project V Committee
Dr. Kaewta Sootsuwan, Sub-leader of Project I Committee
Assist. Prof. Dr. Noppon Lertwattanasak, Sub-leader of Project II Committee
Assoc. Prof. Dr. Alisa Vangnai, Sub-leader of Project III Committee
Assist. Prof. Dr. Chartchai Khonongnuch, Sub-leader of Project IV Committee
Prof. Dr. Poonsuk Prasertsan, Sub-leader of Project V Committee
Ms. Ratchada Khadat Committee and Secretariat
Organizing Committee (Yamaguchi University)

Prof. Dr. Mamoru Yamada  
Prof. Dr. Kenji Matsui  
Assoc. Prof. Dr. Toshiharu Yakushi  
Prof. Dr. Shinichi Ito  
Prof. Dr. Ken Maeda  
Assoc. Prof. Dr. Hisashi Hoshida  
Prof. Dr. Rinji Akada  
Prof. Dr. Tsuyoshi Imai  
Assoc. Prof. Dr. Osami Misumi  
Assist. Prof. Dr. Naoya Kataoka  
Assist. Prof. Dr. Tomoyuki Kosaka  
Ms. Naoko Miyaji  
Mr. Hiroyuki Fukuoka  
Ms. Yoshiko Yamamoto

Chairman  
Vice-Chairman  
Committee (General Affairs)  
Committee (Program)  
Committee (Program)  
Committee (Program)  
Committee (Hall)  
Committee (Hall)  
Committee (Hall)  
Committee (Hall)  
Committee (Reception)  
Committee and the Secretary  
Chief of the Secretariat  
Assistant to the Secretariat

Secretariat

Japanese Side  
Ms. Naoko Miyaji  
Faculty of Agriculture, Yamaguchi University,  
1677-1 Yoshida, Yamaguchi 753-8515, Japan  
Tel: +81-83-933-5868, Fax: +81-83-933-5820, E-mail: jsp@yamaguchi-u.ac.jp

Thai Side  
Ms. Ratchada Khadat  
Kasetsart University Research and Development Institute, Kasetsart University  
P.O. Box 1077 KURDI, Kasetsart University, Chatuchak, Bangkok, Thailand  
Tel/Fax: +66-02-5795547 ext.12, E-mail: rdirdk@ku.ac.th
## Schedule of the Final Joint Seminar of CCP

### 2nd Dec. 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:30-09:00</td>
<td>Registration</td>
<td></td>
</tr>
<tr>
<td>09:00-09:20</td>
<td>Opening Ceremony</td>
<td>Welcome Address and Opening Remarks by Presidents of YU and KU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Opening Remarks and Opening Address by NRCT and JSPS</td>
</tr>
<tr>
<td></td>
<td><strong>Chair person: Prof. Hiroshi Matsuno</strong></td>
<td></td>
</tr>
<tr>
<td>09:20-10:10</td>
<td>Keynote (PB, 35p)</td>
<td>Synthetic Biology Approach towards Creation of an Organism with a new Genetic Code by Prof. Barry Wanner</td>
</tr>
<tr>
<td>10:10-10:35</td>
<td>Group Photo and Coffee Break</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Chair person: Prof. Piamsook Pongsawasdi</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Co-Chair person: Prof. Shin-ichi Ito</strong></td>
<td></td>
</tr>
<tr>
<td>10:35-10:50</td>
<td>Oral 1 (SB, 1-)</td>
<td>Exploration of marine bacteria with the potential for biological reduction of hexavalent chromium by Dr. Jittima Charoenpanich</td>
</tr>
<tr>
<td>10:50-11:05</td>
<td>Oral 2 (SB, 5-)</td>
<td>Antifungal activity of <em>Streptomyces hygroscopicus</em> subsp. <em>angustmyceticus</em> NR8-2 against leaf spot fungi of <em>Brassica rapa</em> subsp. <em>pekinesis</em> by Dr. Anurag Sunpapao</td>
</tr>
<tr>
<td>11:05-11:20</td>
<td>Oral 3 (SB, 9-)</td>
<td>Production of heat shock metabolites (HSM) in thermotolerant streptomyces by Dr. Etsu Tashiro</td>
</tr>
<tr>
<td>11:20-11:35</td>
<td>Oral 4 (SB, 13-)</td>
<td>Culture collection of newly thermotolerant ethanologenic yeasts isolated in the Mekong Delta - Vietnam by Dr. Dung Ngo Thi Phuong</td>
</tr>
<tr>
<td>11:35-11:50</td>
<td>Oral 5 (SB, 17-)</td>
<td>Ethanol production from xylose without glucose repression in <em>Spathaspora passalidarum</em> and evaluation of stress tolerance in acetic acid-tolerant yeasts by Dr. Nadchanok Rodrussamee</td>
</tr>
<tr>
<td>11:50-13:00</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Chair person: Prof. Yoichi Honda</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Co-Chair person: Prof. Savitree Limtong</strong></td>
<td></td>
</tr>
<tr>
<td>13:00-13:15</td>
<td>Oral 6 (SB, 21-)</td>
<td>Characterization and expression of thermostable β-xylosidase from <em>Aureobasidium pullulans</em> for xylan saccharification and alkyl xylolides synthesis by Dr. Sehanat Prasongsuk</td>
</tr>
<tr>
<td>13:15-13:30</td>
<td>Oral 7 (SB, 25-)</td>
<td>Identification and characterization of GH62 bacterial α-L-arabinofuranosidase from thermotolerant <em>Streptomyces</em> sp. SWU10 by Dr. Wasana Sukhumsirichart</td>
</tr>
<tr>
<td>13:30-13:45</td>
<td>Oral 8 (SB, 29-)</td>
<td>A Novel malathion degrading thermoactive carboxylesterase from plastics eating <em>Thermobifida alba</em> AHK119 by Dr. Uschara Thumarat</td>
</tr>
<tr>
<td>13:45-14:00</td>
<td>Oral 9 (SB, 33-)</td>
<td><em>Candida easanensis</em> JK8 β-glucosidase: Purification, characterization and potential wine aroma precursors hydrolysis by Dr. Jantaporn Thongekkaew</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Title</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>14:00-14:15</td>
<td>Oral 10</td>
<td>Bioconversion of lipid from industrial wastes by thermotolerant yeast <em>Pichia</em> sp. Scj 01</td>
</tr>
<tr>
<td>14:15-14:30</td>
<td>Oral 11</td>
<td>Engineering and improvement of carbohydrate-modifying enzymes for synthesis of functional oligosaccharides</td>
</tr>
<tr>
<td>14:30-14:45</td>
<td>Oral 12</td>
<td>Identification of crucial amino acid residues involved in large-ring cyclodextrin synthesis by amylomaltase from <em>Corynebacterium glutamicum</em></td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>Oral 13</td>
<td>Isolation and genome sequence analyses of dibenzofuran degrading bacteria from Vietnamese soil</td>
</tr>
<tr>
<td>15:00-15:15</td>
<td>Oral 14</td>
<td>Dynamics of bacterial flora in traditional fermented foods</td>
</tr>
<tr>
<td>15:15-15:30</td>
<td></td>
<td>Coffee Break</td>
</tr>
<tr>
<td>15:30-16:15</td>
<td></td>
<td><em>Poster Session I</em> (presentation of odd-numbered posters)</td>
</tr>
<tr>
<td></td>
<td><strong>Chair person: Assoc. Prof. Somchanh Bounphanmy</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Co-Chair person: Prof. Hitoshi Iwahashi</strong></td>
<td></td>
</tr>
<tr>
<td>16:15-16:30</td>
<td>Oral 20</td>
<td>Analyses of thermotolerance of thermotolerant acetic acid bacteria and their applications</td>
</tr>
<tr>
<td>16:30-16:45</td>
<td>Oral 16</td>
<td>Efficient fruit fly trapping by modified rice vinegars</td>
</tr>
<tr>
<td>16:45-17:00</td>
<td>Oral 17</td>
<td>Improvement of bacterial nanocellulose fermentation at high temperature by the adapted strains of thermotolerant <em>Komagataeibacter</em></td>
</tr>
<tr>
<td></td>
<td><strong>3rd Dec. 2018</strong></td>
<td></td>
</tr>
<tr>
<td>09:00-09:20</td>
<td></td>
<td>Invitation to Next CCP</td>
</tr>
<tr>
<td></td>
<td><strong>Chair person: Prof. Makoto Kawamukai</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Co-Chair person: Prof. Kosum Chansiri</strong></td>
<td></td>
</tr>
<tr>
<td>09:20-09:35</td>
<td>Oral 15</td>
<td>Development of Glutamate Fermentation by Using Thermotolerant <em>Corynebacterium glutamicum</em></td>
</tr>
<tr>
<td>09:35-09:50</td>
<td>Oral 19</td>
<td>Complete genome sequence and transcriptome analyses of thermotolerant yeast <em>Kluyveromyces marxianus</em> DMKU 3-1042</td>
</tr>
<tr>
<td>09:50-10:05</td>
<td>Oral 23</td>
<td>Analysis of glucose repression mechanism in thermotolerant yeast <em>Kluyveromyces marxianus</em></td>
</tr>
<tr>
<td>10:05-10:20</td>
<td>Oral 21</td>
<td>Construction and analysis of temperature-sensitive mutants by chromosomal site-directed mutagenesis in <em>Saccharomyces cerevisiae</em></td>
</tr>
</tbody>
</table>
## Schedule

### 10:20-10:35

**Coffee Break**

### Chair person: Prof. Constantinos Theodoropoulos  
**Co-Chair person: Prof. Masaya Imoto**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:35-10:50</td>
<td>Oral 22</td>
<td>Homemade thermostable DNA polymerases and its application for micro- to milliliter-scale PCR</td>
<td>by Prof. Rinji Akada</td>
</tr>
<tr>
<td>10:50-11:05</td>
<td>Oral 18</td>
<td>Understanding the thermotolerant mechanisms in mesophilic bacteria: essential genes for survival at critical high temperature and physiological and genetical characteristics of thermo-adapted mutants from ethanologenic bacterium</td>
<td>by Dr. Tomoyuki Kosaka</td>
</tr>
<tr>
<td>11:05-11:20</td>
<td>Oral 24</td>
<td>Effect of modernized/globalized diets on Thai gut microbiota</td>
<td>by Dr. Jiro Nakayama</td>
</tr>
<tr>
<td>11:20-11:35</td>
<td>Oral 25</td>
<td>The impact of <em>Lactobacillus reuteri</em> KUB-AC5 on chicken gut microbiota</td>
<td>by Dr. Massalin Nakphaichit</td>
</tr>
<tr>
<td>11:35-11:50</td>
<td>Oral 26</td>
<td>Dietary probiotics improved growth performance and disease resistance in cultured fish</td>
<td>by Dr. Saowanit Tongpim</td>
</tr>
</tbody>
</table>

### 11:50-13:00

**Lunch**

### Chair person: Prof. Fusako Kawai

### 13:00-13:30

**Special Lecture I**  
*(PB, 36p)*

**Title:** Fundamental to Translational Research in Environmental Biotechnology  
**Presenter:** by Dr. Alisa Vangnai

### Chair person: Prof. Masaharu Ishii  
**Co-Chair person: Assoc. Prof. Ngo Thi Phuong Dung**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:30-13:45</td>
<td>Oral 27</td>
<td>Model-supported process development for heterologous expression of Superoxide Dismutase A from <em>Deinococcus radiodurans:</em> From shaking flask to fed-batch fermentation</td>
<td>by Dr. Johannes Klinger</td>
</tr>
<tr>
<td>13:45-14:00</td>
<td>Oral 28</td>
<td>Characterization and mutation analysis of a halotolerant serine protease from <em>Bacillus subtilis</em> isolated from Thai traditional fermented shrimp paste</td>
<td>by Prof. Shinji Takenaka</td>
</tr>
<tr>
<td>14:00-14:15</td>
<td>Oral 29</td>
<td>Purification, characterization and structure of alcohol acyltransferases from plant</td>
<td>by Prof. Yasuhisa Asano</td>
</tr>
<tr>
<td>14:15-14:30</td>
<td>Oral 30</td>
<td>Expression and purification of Acylaminoacyl-Peptidase 3 from hyperthermophilic <em>Sulfolobus solfataricus</em> in <em>E. coli</em> BI21</td>
<td>by Dr. Magdalena John</td>
</tr>
<tr>
<td>14:30-14:45</td>
<td>Oral 31</td>
<td>Production of α-1,3-glucanase and chitinase for biotechnological applications</td>
<td>by Prof. Mamoru Wakayama</td>
</tr>
<tr>
<td>14:45-15:00</td>
<td>Oral 32</td>
<td>Production of <em>Streptomyces</em> inoculum by Solid State Cultivation for Biocontrol Agent of Chinese Kale Disease</td>
<td>by Dr. Vichien Kitpreechavanich</td>
</tr>
</tbody>
</table>

### 15:00-15:15

**Coffee Break**
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:15-16:00</td>
<td>Poster Session II (presentation of even-numbered posters)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chair person: Prof. Jun-ichi Kato</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Co-Chair person: Assoc. Prof. Worapot Suntornsuk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00-16:15</td>
<td>Oral 33 (SB, 157-)</td>
<td>Surveillance of mosquitoes-borne infectious diseases in Asian countries</td>
<td>by Prof. Ken Maeda</td>
</tr>
<tr>
<td>16:15-16:30</td>
<td>Oral 34 (SB, 161-)</td>
<td>Isolation and characterization of Fipronil degrading bacteria in paddy soils of Tra Vinh province, Vietnam</td>
<td>by Dr. Nguyen Huu Hiep</td>
</tr>
<tr>
<td>16:30-16:45</td>
<td>Oral 35 (SB, 165-)</td>
<td>Bioremediation of paraquat (1,1′-dimethyl-4,4′-dipyridinium dichloride) by the effective microorganisms</td>
<td>by Dr. Pairote Wongputtisin</td>
</tr>
<tr>
<td>16:45-17:00</td>
<td>Oral 36 (SB, 169-)</td>
<td>Efficiency of dual inoculation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth and yield of Jerusalem artichoke (Helianthus tuberosus L.)</td>
<td>by Dr. Sophon Boonlue</td>
</tr>
<tr>
<td>17:00-17:15</td>
<td>Oral 37 (SB, 217-)</td>
<td>Isolation and Characterization of Exopolysaccharide Indigenic Bacteria (EPS) and Quality Improvement of Liquid Biofertiliser NFB-PSB (Nitrogen Fixing-Phosphate Solubilizing Bacteria) with Viability Control</td>
<td>by Dr. Novi Arfarita</td>
</tr>
<tr>
<td>17:15-17:30</td>
<td>Oral 38 (SB, 221-)</td>
<td>Analysis of soil fertility in Thailand, Indonesia, and Japan</td>
<td>by Prof. Motoki Kubo</td>
</tr>
<tr>
<td></td>
<td><strong>4th Dec. 2018</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chair person: Prof. Saisamorn Lumyong</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Co-Chair person: Prof. Kazuhide Kimbara</td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:00-09:15</td>
<td>Oral 39 (SB, 173-)</td>
<td>Isolation and identification of potential novel polyketide producing endophytic Sphaerimonospora mesophila GKU 363</td>
<td>by Dr. Arinthip Thamchaipenet</td>
</tr>
<tr>
<td>09:15-09:30</td>
<td>Oral 40 (SB, 177-)</td>
<td>Identification of biosynthetic pathway and the optimal condition for indole-3-acetic acid production by an endophytic fungus, Colletotrichum fructicola CMU-A109</td>
<td>by Prof. Saisamorn Lumyong</td>
</tr>
<tr>
<td>09:30-09:45</td>
<td>Oral 41 (SB, 225-)</td>
<td>Antioxidant activity of alcoholic beverages made from various cereal grains using koji made with Amylomyces rouxii YTH3 as saccharifying agent</td>
<td>by Prof. Yuji Teramoto</td>
</tr>
<tr>
<td>09:45-10:00</td>
<td>Oral 42 (SB, 227-)</td>
<td>Identification of acetic acid bacteria and assigned to the genus Gluconobacter by GroEL gene sequence analysis</td>
<td>by Dr. Nittaya Pitiwittayakul</td>
</tr>
<tr>
<td></td>
<td>Chair person: Prof. Kenji Sonomoto</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00-10:30</td>
<td>Special Lecture II (PB, 37p)</td>
<td>Micro-porous inorganic membranes in bio-refinery</td>
<td>by Dr. Izumi Kumakiri</td>
</tr>
<tr>
<td>10:30-10:45</td>
<td></td>
<td>Coffee Break</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Title</td>
<td>Presenter(s)</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>10:45-11:00</td>
<td>Oral 43</td>
<td>Thermostable Pigment Production form Hot spring Cyanobacteria: from Laboratory to Commercial production</td>
<td>Dr. Chayakorn Pumas</td>
</tr>
<tr>
<td>11:00-11:15</td>
<td>Oral 44</td>
<td>Development of beneficial biofilms and biosurfactants from Auerobasidium pullulans YTP6-14</td>
<td>Prof. Masaaki Morikawa</td>
</tr>
<tr>
<td>11:15-11:30</td>
<td>Oral 45</td>
<td>Roles of antimicrobial substance producing lactic acid bacteria in agro-industry and characterization of novel bacteriocin produced by lactobacilli species</td>
<td>Dr. Sunee Nitisinprasert</td>
</tr>
<tr>
<td>11:30-11:45</td>
<td>Oral 46</td>
<td>Screening, characterization and applications of lactic acid bacteria producing bacteriocins</td>
<td>Dr. Takeshi Zendo</td>
</tr>
<tr>
<td>11:45-12:00</td>
<td>Oral 47</td>
<td>Comparative studies on bioactive peptide activities of ASEAN traditional Monascus -fermented foods and products</td>
<td>Dr. Shinjiro Tachibana</td>
</tr>
<tr>
<td>12:00-13:00</td>
<td></td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>13:00-13:15</td>
<td>Oral 48</td>
<td>Model-based Algal Cultivation Strategies for Enhanced Starch and Lipid Production</td>
<td>Prof. Constantinos Theodoropoulos</td>
</tr>
<tr>
<td>13:15-13:30</td>
<td>Oral 49</td>
<td>Metabolic engineering of Escherichia coli for the production of short-chain alcohols and fatty acid</td>
<td>Dr. Naoya Kataoka</td>
</tr>
<tr>
<td>13:30-13:45</td>
<td>Oral 50</td>
<td>Manno-oligosaccharides production by lipase defective mutant strain of Bacillus sp. MR10 using copra meal as substrate</td>
<td>Dr. Chartchai Khanongnuch</td>
</tr>
<tr>
<td>13:45-14:00</td>
<td>Oral 51</td>
<td>Bioprocess development for mucic acid production by Aspergillus sp. TPG-01 and Gluconobacter oxydans NBRC 12528</td>
<td>Dr. Apilak Salakkam</td>
</tr>
<tr>
<td>14:00-14:15</td>
<td>Oral 52</td>
<td>Bioconversion of sugarcane juice to hydrogen and methane by two stage fermentation process</td>
<td>Prof. Tsuyoshi Imai</td>
</tr>
<tr>
<td>14:15-14:30</td>
<td>Oral 53</td>
<td>Thermal Chemical Pretreatment of Rice Straw and Bagasse for Reducing Sugar and Hydrogen Production</td>
<td>Dr. Prapaipid Chairattananamokorn</td>
</tr>
<tr>
<td>14:30-14:45</td>
<td>Oral 54</td>
<td>Development of two-stage and solid-state thermophilic fermentation of palm oil mill effluent and oil palm biomass for biohythane and biogas production</td>
<td>Dr. Sompong O-Thong</td>
</tr>
<tr>
<td>14:45-15:00</td>
<td></td>
<td>Coffee Break</td>
<td></td>
</tr>
<tr>
<td>15:00-15:40</td>
<td></td>
<td>Conclusion</td>
<td></td>
</tr>
<tr>
<td>15:40-15:50</td>
<td></td>
<td>Closing Ceremony</td>
<td></td>
</tr>
</tbody>
</table>
### Project 1: Explorational Research of Useful Microbes

**Thermotolerant advantageous microbes, Biopolymer degradation, Functional materials, Bioactive compounds**

<table>
<thead>
<tr>
<th>PI-01</th>
<th>Production of heat shock metabolites (HSM) in thermotolerant streptomyces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shun Saito¹, Wataru Kato¹, Etsu Tashiro¹, Ramida Watanapokasin² and Masaya Imoto¹</td>
</tr>
<tr>
<td></td>
<td>¹Department of Biosciences and Informatics Faculty of Science and Technology Keio University, Japan, ²Department of Biochemistry Faculty of Medicine, Srinakharinwirot University, Thailand</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PI-02</th>
<th>Isolation and genome sequence analyses of dibenzofuran degrading bacteria from Vietnamese soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lê Thị Hà Thanh¹,², Trần Vũ Ngọc Thị¹, Masaki Shintani³, Ryota Moriuchi³, Hideo Dohra³, Pichakon Sriyapai⁴, Nguyễn Hoàng Lộc⁵ and Kazuhide Kimbara¹</td>
</tr>
<tr>
<td></td>
<td>¹Department of Environment and Energy System, Graduate School of Science and Technology, Shizuoka University, Japan, ²Department of Bioscience, Graduate School of Science and Technology, Shizuoka University, Japan, ³Research Institute of Green Science and Technology, Shizuoka University, Japan, ⁴Department of Microbiology, Faculty of Science, Srinakharinwirot University, Thailand, ⁵Department of Biology, College of Sciences, Hue University, Vietnam</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PI-03</th>
<th>Screening of manganese-tolerant mutants from thermotolerant yeast Kluyveromyces marxianus and comparison from a similar mutant of brewing yeast Saccharomyces cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kazuki Honda¹, Naoki Matsumoto¹, Tu Tang², Masakazu Furuta², Vichai Leeravatcharamas³ and Masao Kishida¹</td>
</tr>
<tr>
<td></td>
<td>¹Department of Applied Life Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Japan, ²Department of Quantum and Radiation Engineering, Graduate School of Engineering, Osaka Prefecture University, Japan, ³Fermentation research center for value added agricultural products, Faculty of Technology, Khon Kaen University, Thailand</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PI-04</th>
<th>Dynamics of bacterial flora in traditional fermented foods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Takashi Koyanagi¹, Thida Chaiwangsri², Chayaphon Sripunpatakul¹, Chiaki Matsuzaki², Shin Kurihara², Toshihiko Katoh³, Hisashi Ashida³, Hisanori Tamaki⁴, Kenji Yamamoto⁵, Takane Katayama⁶</td>
</tr>
<tr>
<td></td>
<td>¹Faculty of Bioresources and Environmental Sciences, Ishikawa Prefectural University, Japan, ²School of Medi5cal Science, University of Phayao, Thailand, ³Department of Biochemistry, Faculty of Medical Science, Naresuan University, Thailand, ⁴Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Japan, ⁵Host-Microbe Interaction Research Laboratory, Ishikawa Prefectural University, Japan, ⁶Graduate School of Biostudies, Kyoto University, Japan, ⁷Faculty of Biology-Oriented Science and Technology, Kinki University, Japan, ⁸Faculty of Agriculture, Kagoshima University, Japan</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PI-05</th>
<th>Identification and characterization of GH62 bacterial α-L-arabinofuranosidase from thermotolerant Streptomyces sp. SWU10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pornpimol Phuengmaung¹, Yuika Kunishige², Wasana Sukhumsirichard⁴ and Tatsuji Sakamoto²</td>
</tr>
<tr>
<td></td>
<td>¹Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Thailand, ²Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Japan</td>
</tr>
</tbody>
</table>
| PI-06 | Antifungal activity of *Streptomyces hygroscopicus* subsp. *angustmyceticus* NR8-2 against leaf spot fungi of *Brassica rapa* subsp. *pekinensis*  
*Anurag Sunpapao*¹, Prisana Wonglom¹ and Shin-ichi Ito³  
¹Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Thailand; ²Faculty of Technology and Community Development, Thaksin University, Thailand; ³Department of Biological and Environmental Science, Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan |
| PI-07 | *Candida easanensis* JK8 β-glucosidase: Purification, characterization and potential wine aroma precursors hydrolysis  
*Jantaporn Thongekkaew*¹, Tsutomu Fujii²,³ and Kazuo Masaki²,³  
¹Department of Biological Science, Faculty of Science, Ubon-Ratchathani University, Thailand; ²Graduate School of Biosphere Sciences, Hiroshima University, Japan; ³National Research Institute of Brewing, Japan |
| PI-08 | Exploration of marine bacteria with the potential for biological reduction of hexavalent chromium  
*Jittima Charoepanich*¹, Jutamas Pantab², Jariya Supakit¹, Sriruda Nithetham³, Dang Saeba³ and Akio Tani³  
¹Department of Biochemistry, Faculty of Science, Burapha University, Thailand; ²Bioengineering Program, Faculty of Engineering, Burapha University, Thailand; ³Department of Chemical Engineering, Faculty of Engineering, Burapha University, Thailand, ³Group of Plant-Microbe Interactions, Institute of Plant Science and Resources, Okayama University, Japan |
| PI-09 | Isolation and screening of microorganisms with cellulase activity from rice straw  
*Kaewta Sootsuwan*¹, Arpaporn Punpad¹, Tomoyuki Kosaka² and Mamoru Yamada²,³  
¹Department of Biotechnology, Faculty of Agro-Industrial Technology, Kalasin University, Thailand; ²Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan; ³Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Japan |
| PI-10 | Preparation, characterization and release study of astaxanthin encapsulated-levan nanoparticles  
*Kamontip Kittiyawong*¹, Santhana Nakapong², Kuakarun Krasong³, Jarinee Kauliboon⁴, Prakarn Rudeekulthamrong⁵, Shuichiro Murakami⁶, Kazuo Ito⁷ and Piamsook Pongsawasdi³  
¹Department of Chemistry, Faculty of Liberal Arts and Science, Kasetsart U, Kamphaeng Saen Campus, Nakhon Pathom, Thailand; ²Department of Chemistry, Faculty of Science, Ramkhamhaeng U, Bangkok, Thailand; ³Department of Biochemistry, Faculty of Science, Chulalongkorn U, Bangkok, Thailand; ⁴Department of Preclinical Science (Biochemistry), Faculty of Medicine, Thammasat U, Pathumthani, Thailand; ⁵Department of Biochemistry, Phramongkutkla College of Medicine, Bangkok, Thailand; ⁶Department of Agricultural Chemistry, Graduate School of Agriculture, Meiji U, Kawasaki, Japan; ⁷Department of Biology, Graduate School of Science, Osaka City U, Osaka, Japan |
| PI-11 | Thermotolerant genes essential for survival at a critical high temperature in thermotolerant ethanologenic *Zymomonas mobilis* TISTR 548  
*Kannikar Charoenzuk*¹, Tomoko Sakurada¹, Amina Tokiyama¹, Masayuki Murata², Tomoyuki Kosaka²,³,⁴, Pornthap Thanonkeo and Mamoru Yamada²,³,⁴  
¹Division of Product Development and Management Technology, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus, Thailand; ²Life Science, Graduate School of Science and Technology for Innovation, Yamaguchi University, Ube, Japan; ³Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan; ⁴Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Yamaguchi, Japan, ²Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen., Thailand |
| PI-12 | Identification of crucial amino acid residues involved in large-ring
cycloextrin synthesis by amylomaltase from Corynebacterium glutamicum

Kuakarun Krusong1,2, Sirikul Ngawiset3, Shuichiro Murakami3, Thanyada Rungrommongkol2 and Piamsook Pongsawasdi1

1Starch and Cyclodextrin Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 2Structural and Computational Biology Research Group, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 3Department of Agricultural Chemistry, Graduate School of Agriculture, Meiji University, Kawasaki, Japan.

Engineering and improvement of carbohydrate-modifying enzymes for synthesis of functional oligosaccharides

Jarunee Kaulpiboon1, Prakarn Rudeekulthamrong2, Kuakarun Krusong3, Kamontip Kutiyaewong4, Santhana Nakapong1, Shuichiro Murakami3, Kazuo Ito1 and Piamsook Pongsawasdi1

1Department of Preclinical Science (Biochemistry), Faculty of Medicine, Thammasat U, Pathumthani, Thailand, 2Department of Biochemistry, Phramongkutkla College of Medicine, Bangkok, Thailand, 3Department of Biochemistry, Faculty of Science, Chulalongkorn U, Bangkok, Thailand, 4Department of Chemistry, Faculty of Liberal Arts and Science, Kasetsart U, Kamphaeng Saen Campus, Nakhon Pathom, Thailand.

Molecular and Biochemical characterizations of Levanase from Bacillus amylo liquefaciens

Santhana Nakapong1, Kamoltip Kutiyaewong2, Kazuo Ito1 and Piamsook Pongsawasdi4

1Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand, 2Department of Chemistry, Faculty of Liberal Arts and Science, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, Thailand, 3Laboratory of Enzyme Chemistry, Faculty of Science, Osaka City University, Osaka, Japan, 4Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

Ethanol production from xylose without glucose repression in Spathaspora passalidarum and evaluation of stress tolerance in acetic acid-tolerant yeasts

Nadchanok Rodruessamee1, Pachara Sattayawat1 and Mamoru Yamada2

1Department of Biology, Faculty of Science, Chiang Mai University, Thailand, 2Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan.

Characterization and expression of thermostable β-xylosidase from Aureobasidium pullulans for xylan saccharification and alkyl xylosides synthesis

Wichanee Bankeeree1, Pongtharin Lotrakul1, Hunsa Punnapayak1, Rinji Akada2, Hisashi Hoshida1 and Sehanat Prasongsuk1,2

1Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand, 2Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Yamaguchi University, Japan.

Diversity of lactic acid bacteria from Thai fermented fish (Plasom) and evaluation on antibacterial activity

Thida Chaiwangsri1, Takashi Koyanagi2, Chiaki Matsuzaki3 and Takane Katayama3,4

1School of Medical Science, University of Phayao, Thailand, 2Department of Food Science, Ishikawa Prefectural University, Japan, 3Host-Microbe Interaction Research Laboratory, Ishikawa Prefectural University, Japan, 4Graduate School of Biostudies, Kyoto University, Japan.

A Novel malathion degrading thermoactive carboxylesterase from plastics eating Thermobifida alba AHK119

Uschara Thumarat1*, Apipat Saleepot1, Masayuki Oda2, Fusako Kawai1
<table>
<thead>
<tr>
<th>PI</th>
<th>Title</th>
<th>Authors</th>
<th>Location/Institute</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI-19</td>
<td>Bioconversion of lipid from industrial wastes by thermotolerant yeast</td>
<td>Kamon Sritongon¹, Apilak Salakkam² Massao Kishida³ and Vichai Leelavatcharamas*¹,²</td>
<td>¹Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Thailand, ²Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan, ³Center for Fiber and Textile Science, Kyoto Institute of Technology, Japan</td>
</tr>
<tr>
<td>PI-20</td>
<td>Bacterial community diversity and antimicrobial activity of lactic acid bacterial from pla-jom, a Thai traditional fermented fish</td>
<td>Chayaphon Sriphannam¹, Aksarakorn Kummasook² and Takashi Koyanagi³</td>
<td>¹Department of Biochemistry, Faculty of Medical Science, Naresuan University, Thailand, ²Division of Clinical Microbiology, Department of Medical Technology, School of Allies Health Sciences, University of Phayao, Thailand, ³Department of Food Science, Ishikawa Prefectural University, Japan</td>
</tr>
<tr>
<td>PI-21</td>
<td>Culture collection of newly thermotolerant ethanologenic yeasts isolated in the Mekong Delta - Vietnam</td>
<td>Ngo Thi Phuong Dung¹, Huynh Xuan Phong¹, Pornthap Thanonkeo² and Mamoru Yamada³</td>
<td>¹Biotechnology Research and Development Institute, Can Tho University, Can Tho, Vietnam, ²Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand, ³Faculty of Agriculture and Graduate School of Science and Technology for Innovation, Yamaguchi University, Yamaguchi, Japan</td>
</tr>
<tr>
<td>PI-22</td>
<td>Study on application of thermotolerant yeasts for ethanol fermentation and fruit wine production</td>
<td>Ngo Thi Phuong Dung¹, Huynh Xuan Phong¹, Mamoru Yamada² and Pornthap Thanonkeo³</td>
<td>¹Biotechnology Research and Development Institute, Can Tho University, Can Tho, Vietnam, ²Faculty of Agriculture and Graduate School of Science and Technology for Innovation, Yamaguchi University, Yamaguchi, Japan, ³Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand</td>
</tr>
<tr>
<td>PI-23</td>
<td>Isolation and characterization of thermotolerant yeasts isolated in Indonesia</td>
<td>Suprayogi¹, Fika Ulan Anggrarini Putri¹, Maleo¹, Mochammad Nurcholis², Affiyagung Saputra¹, Anton Muhibuddin¹, Tomoyuki Kosaka², Mamoru Yamada²⁴</td>
<td>¹Agricultural Technology Faculty, Brawijaya University, Indonesia, ²Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Japan, ³Indonesia Ministry of Agriculture, Indonesia, ⁴Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan</td>
</tr>
<tr>
<td>PI-24</td>
<td>Thermotolerant Yeast from Sugarcane Wastes and Their Potential as Bioremediator of Heavy Metals Lead (Pb)</td>
<td>Anton Muhibuddin¹, Moh. Saifudin Afandi¹, Antok Wahyu Sektiono¹, Syamsuddin Djausher¹, Budi Prasetya¹ and Tjuk Eko Hari Basuki²</td>
<td>¹Agricultural Faculty, Brawijaya University, Indonesia, ²Indonesia Ministry of Agriculture</td>
</tr>
<tr>
<td>Not attend</td>
<td>Characterization of thermotolerant ethanologenic yeasts isolated in Lao PDR</td>
<td>Chansom Keo-oudone¹, Sukanya Nitiyön², Phoneasith Sotitham¹, Mochammad Nurcholis¹, Khonesavanh Milavong¹, May Thammany¹, Bouachanh Seeyakeo¹, Maniphone Phoudphong¹, Hiem Seulo¹, Khamlan Sisoungvong¹, Vannapha Chanthavongs¹, Khamaeng Sayaket¹, Bouapha Ngothachack¹, Akio Tani², Noppon Lertwattanasakul¹, Somchanh Bouphanmy¹, Savitree Limtong⁵ and Mamoru Yamada²³</td>
<td></td>
</tr>
</tbody>
</table>
Project 2: Genome-based Research on Thermotolerant Microbes

**Distribution of thermotolerant microbes, genome modification deepsequencing**

**PII-01**

High temperature acetic acid fermentation with thermotolerant acetic acid bacteria and some other application with engineered acetic acid bacteria

Toulaphone Keokene\(^1\), Toshiharu Yashiki\(^2,3\), Gunjana Theeragool\(^4,5\), Watchara Kanchanaratch\(^6\), Uraivan Tippayak\(^7\), Wilawan Sintuprapa\(^8\), Kannipa Tasanapak\(^9\), Pattaraporrn Rattanawaree\(^7\), Shinsuke Fujiwara\(^10\), and Kazunobu Matsushita\(^2,3\)

\(^1\) Fac of Natural Sci, National Univ of Laos, \(^2\) Grad Sch of Sci & Technol for Innovation, Yamaguchi Univ, \(^3\) Research Center for Thermotolerant Microbial Resources, Yamaguchi Univ, \(^4\) Dept of Microbiol, Fac of Sci, Kasetsart Univ, \(^5\) Interdisciplinary Grad Program in Genetic Engineer, The Grad Sch, Kasetsart Univ, \(^6\) Fac of Sci, Mahasarakham Univ, \(^7\) Fac of Agroindustry, Kasetsart Univ, \(^8\) Fac of Med Sci, Naresuan Univ, \(^9\) National Center for Genetic Engineer and Biotechnol, \(^10\) Grad Sch of Sci and Technol, Kwansei-Gakuin Univ

**PII-02**

Analyses of thermotolerance of thermotolerant acetic acid bacteria and their applications

Wichai Soemphol\(^1\) and Hirohide Toyama\(^2\)

\(^1\) Faculty of Applied Science and Engineering, Khon Kaen University (Nong Khai Campus), Thailand, \(^2\) Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa Japan

**PII-03**

Understanding the thermotolerant mechanisms in mesophilic bacteria: essential genes for survival at critical high temperature and physiological and genetical characteristics of thermo-adapted mutants from ethanologenic bacterium

Tomoyuki Kosaka\(^1,2\), Shun Kato\(^3\), Maiko Yamashita\(^4\), Tomoko Sakurada\(^5\), Yuki Shioromaru\(^6\), Ayana Ishii\(^7\), Kannikar Charoensuk\(^8\), Masayuki Murata\(^9\), Minenosuke Matsutani\(^1\), Pornthap Thanonkeo\(^2\), Mamoru Yamada\(^1,2\)

\(^1\) Department of Biological Chemistry, Faculty of Agriculture and Life Science, Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan, \(^2\) RCTMR, Yamaguchi University, Japan, \(^3\) Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Japan, \(^4\) Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan, \(^5\) Division of Product Development and Management Technology, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-ok, Thailand, \(^6\) Department of Biotechnology, Faculty of Engineering, Khon Kaen University, Thailand

**PII-04**

Analysis of glucose repression mechanism in thermotolerant yeast Kluyveromyces marxianus

Mochamad Nurcholis\(^1,2\), Suprayogi\(^3\), Masayuki Murata\(^4\), Minh T. Nguyen\(^5\), Nadchanok Rodrussamee\(^6\), Noppon Lertwattanasakul\(^7\), Sukanya Nityyon\(^1\), Savitree Limtong\(^2\), Tomoyuki Kosaka\(^4,8,9\) and Mamoru Yamada\(^4,8,9\)

\(^1\) Graduate School of Medicine, Yamaguchi University, Japan, \(^2\) Faculty of Agriculture Technology, Brawijaya University, Indonesia, \(^3\) Faculty of Agriculture Technology, Brawijaya University, Indonesia, \(^4\) Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan, \(^5\) Faculty of Environment, Vietnam National University of Agriculture, Vietnam, \(^6\) Faculty of Science, Chiangmai University, Thailand, \(^7\) Faculty of Science, Kasetsart University, Thailand, \(^8\) Faculty of Agriculture, Yamaguchi University, Japan, \(^9\) Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Japan

**PII-05**

Homemade thermostable DNA polymerases and its application for micromo to milliliter-scale PCR

Rinji Akada\(^1,2,3\), Yuko Okada\(^1\), Yukie Misumi\(^1\), Junya Ai-hara\(^1\), Sorawit Na Nongkhai\(^4\)

\(^1\) Department of Biology, Faculty of Natural Science, National University of Laos, Lao PDR, \(^2\) Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Japan, \(^3\) Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan, \(^4\) Institute of Plant Science and Resources, Okayama University, Japan, \(^5\) Department of Microbiology, Faculty of Science, Kasetsart University, Thailand
PII-06  Development of Glutamate Fermentation by Using Thermotolerant Corynebacterium glutamicum

Nawarat Namtapong1, Savit Trakulnauleumsai2, Minenouke Matsutani1, 4, Toshiharu Yakushi1, 4, 5, Naoya Kataoka1, 4, 5 and Kazunobu Matsushita1, 4, 5

1Institute of Science, Suranaree University of Technology, Thailand 2Faculty of Science, Kasetsart University, Thailand 3Faculty of Agriculture, Yamaguchi University, Japan 4Graduate School of Science and Technology for Innovation, Yamaguchi University, 5Research Center for Thermotolerant Microbial Resources, Yamaguchi University

PII-07  Complete genome sequence and transcriptome analyses of thermotolerant yeast Kluyveromyces marxianus DMKU 3-1042

Nopporn Lertwattanasakul1, Tomoyuki Kosaka2, Nadchanok Rodrussamee3, Masayuki Murata4, Suprayog5, Savitree Limtong6 and Mamoru Yamada7

1Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand 2Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan 3Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand 4Department of Agro-Industrial Technology, Faculty of Agricultural Technology, Malang, Brawijaya University, Indonesia

PII-08  Construction and analysis of temperature-sensitive mutants by chromosomal site-directed mutagenesis in Saccharomyces cerevisiae

Kamponchai Cha-aim1, Hisashi Hoshida2 and Rinji Akada3

1Division of Biotechnology and Agricultural Products, Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Ongkharak Campus, Nakhon Nayok, Thailand 2Department of Applied Chemistry, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Ube, Japan

PII-09  Improvement of bacterial nanocellulose fermentation at high temperature by the adapted strains of thermotolerant Komagataeibacter

Gunjana Theeragool12, Kallayanee Naloka1, Pornchanok Taweecheep2, Nittaya Pitiwittayakul3, Touaphone Keokene4, Toshiharu Yakushi5, 6 and Kazunobu Matsushita5, 6

1Dept. of Microbiology, Fac. of Science, Kasetsart Univ., 2Interdisciplinary Graduate Program in Genetic Engineering, The Graduate School, Kasetsart Univ., 3Dept. of Agricultural Technology and Environment, Fac. of Sciences and Liberal Arts, Rajamangala Univ., 4Fac. of Natural Science, National Univ. of Laos, 5Dept. of Biological Chemistry, Fac. of Agriculture, Yamaguchi Univ., 6Research Center for Thermotolerant Microbial Resources, Yamaguchi Univ.

PII-10  Diversification of thermotolerant acetic acid bacteria from Vietnam and their application in high-temperature acetic acid and vinegar production

Huynh Xuan Phong1, Ngo Thi Phuong Dung1, Toshiharu Yakushi2, Kazunobu Matsushita2

1Biotechnology Research and Development Institute, Can Tho University, Can Tho, Vietnam 2Biological Chemistry Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan

Not attend

Efficiency improvement of bacterial cellulose production from acetic acid bacteria by stimulants

Wilawan Sintuprapa1, Nuttika Chaleiart1, Pattaraporn Yakphan2, Gunjana Theeragool3 and Toshiharu Yakushi4

1Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand, 2BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency

23
**Project 3: Research on Environmental Microbes Sustaining Tropical Ecosystem**

**Symbiosis, plant- or animal (insect)-microbe interaction, microbial consortia in fermented foods & sewages, metagenome analysis**

<table>
<thead>
<tr>
<th>PIII-01</th>
<th>Investigation of microbial mechanisms involving bioremediation of hazardous chemicals and microbe-plant interaction for improvement of ecosystem and environmental reclamation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Junichi Kato¹, Takahisa Tajima¹, Shota Oku¹, Akiko Hida¹, Gunpant Mahipant², Merry Sipahutar², Sivagnanam Silambarasan³⁴, Jittra Piapukiew³⁵, and Alisa S. Vangnai³⁴⁶</td>
<td>¹Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan, ²Biological Sciences Program, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, ³Biocatalyst and Environmental Biotechnology Research unit, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, ⁴Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, ⁵Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, ⁶Center of Excellence in Hazardous Substance Management, Chulalongkorn University, Bangkok, Thailand</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PIII-02</th>
<th>Isolation of green algal strains accumulating arachidonic acid-containing lipids from plant materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yasuo Kato¹, Taiji Nomura¹, Naoki Kitaoka¹, Wichien Yongmanitchai² and Duenrut Chonudomkul²</td>
<td>¹Biotechnology Research Center, Toyama Prefectural University, Toyama, Japan, ²Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PIII-03</th>
<th>Effect of modernized/globalized diets on Thai gut microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiro Nakayama¹, Juma Kissuse¹, Orawan La-ongkham², Phathanaphong Therditatha¹, Massalin Nakphaichit¹⁴, Sunee Nitisinprasert¹²</td>
<td>¹Division of Systems Bio-engineering, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan, ²Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand, ³Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Chatuchak, Bangkok, Thailand, ⁴Specialized Research Unit: Probiotics and Prebiotics for Health, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PIII-04</th>
<th>Isolation and identification of potential novel polyketide producing endophytic Sphaerimonaspora mesophile GKU 363</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arinthip Thamchaiyapong¹, Chantra Indananda² and Yasuhiro Igarashi³</td>
<td>¹Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand, ²Department of Biology, Faculty of Science, Burapha University, Chonburi, Thailand, ³Biotechnology Research Center, Toyama Prefectural University, Toyama, Japan</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PIII-05</th>
<th>Surveillance of mosquitoes-borne infectious diseases in Asian countries</th>
</tr>
</thead>
</table>
PIII-06  The impact of *Lactobacillus reuteri* KUB-AC5 on chicken gut microbiota  
*Massalin Nakphaichit*1,2, *Suparat Siemuang*3, *Wanwipa Vongsangnak*4,5, *Jiro Nakayama*6 and *Sunee Nitisinprasert*1,2  
1Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Chatuchak, Bangkok, Thailand.  
2Specialized Research Unit: Probiotics and Prebiotics for Health, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand  
3Research and development center, Betagro group, Klong Luang Pathumthani, Thailand  
4Department of Zoology, Faculty of Science, Kasetsart University, Chatuchak, Bangkok, Thailand  
5Computational Biomodelling Laboratory for Agricultural Science and Technology (CBLAST), Faculty of Science, Kasetsart University, Bangkok, Thailand  
6Division of Systems Bio-engineering, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

PIII-07  Bioremediation of paraquat (1,1′-dimethyl-4,4′-dipyridinidium dichloride) by the effective microorganisms  
*Pairote Wongputtisin*1, *Yoichi Honda*2, *Chantana Supo*1, *Takehito Nakazawa*2, *Nakarin Suwannarach*2, *Saisamorn Lumyong*1 and *Charuchai Khanongnuch*1  
1Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai, Thailand.  
2Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

PIII-08  Efficiency of dual inoculation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth and yield of Jerusalem artichoke (*Helianthus tuberosus* L.)  
*Sophon Boonlue*1,2, *Sabaiporn Nacoon*1, *Sanan Jokloy*2 and *Kenji Matsui*3  
1Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand.  
2Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand.  
3Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi, Japan  
* Corresponding author: bsopho@kku.ac.th

PIII-09  Identification of biosynthetic pathway and the optimal condition for indole-3-acetic acid production by an endophytic fungus, *Colletotrichum fructicola* CMU-A109  
*Saisamorn Lumyong*1, *Tosapon Numponsak*1, *Jaturong Kumla*1, *Nakarin Suwannarach*1 and *Kenji Matsui*2  
1Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.  
2Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi, Japan

PIII-10  Isolation and characterization of Fipronil degrading bacteria in paddy soils of Tra Vinh province, Vietnam  
*Nguyen Hua Hiep*1* and Pham Khanh Doan*1  
1Biotechnology Research and Development Institute, Cantho University, Vietnam.  
*Corresponding author: nhhiep@ctu.edu.vn

PIII-11  Research on treatment of aquaculture environment by probiotic product  
*Minh Thi Nguyen*1, *Giang Thi Huong Vu*2 and *Huyen Thi Khanh Nguyen*1  
1Faculty of Environment, Vietnam National University of Agriculture. Trau Quy town, Gia Lam district, Hanoi, Viet Nam

Not attend  
Analysis of plant-microbe interaction at the phyllosphere and production of useful compounds by phyllosphere microorganisms.  
Project 4: Research on Microbes Useful for Food, Food Preservation, Health, and Ecosystem Preservation

Preservation, food and beverage, bio-packaging, chemicals, biopreservation, probiotics, bioremediation, biocontrol

PIV-01
Analysis of soil fertility in Thailand, Indonesia and Japan
Motoki Kubo1, Kiwako Araki1, Sirilak Sanpa2, Andi Kurniawan3, Wasana Suyotha4, Shigekazu Yano5, and Mamoru Wakayama1
1Department of Biotechnology, Faculty of Life Sciences, Ritsumeikan University, Japan, 2School of Medical Science, University of Phayao, Thailand, 3Fishery and Marine Science Faculty, Brawijaya University, Indonesia, 4Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Thailand, 5Department of Biochemical Engineering, Faculty of Engineering, Yamagata University, Japan

PIV-02
Comparative studies on bioactive peptide activities of ASEAN traditional Monascus-fermented foods and products
Shinjiro Tachibana1, Indika Pradeep Wanninaika1,2, Hiroki Nakama1, Arina Matsumoto1, Hitomi Yara1, Hiroko Teshima1, Masaaki Yasuda1, Worapot Suntornsk1, Hanifah Lioe4
1Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan, 2The United Graduate School of Agriculture Sciences, Kagoshima University, Kagoshima, Japan, 3Department of Microbiology, Faculty of Science, King Mongkut’s University of Technology Thonburi, Bangkok, Thailand, 4Department of Food Science and Technology, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, Bogor, Indonesia

PIV-03
Antioxidant activity of alcoholic beverages made from various cereal grains using koji made with Amylomyces rouxii YTH3 as saccharifying agent
Yuji Teramoto1, Thalisa Yuwa-amornpitak2, Ngo Thi Phuong Dung1 and Somchanh Bounphanmy3
1Department of Applied Microbial Technology, Faculty of Biotechnology and Life Science, Sojo University, Japan, 2Department of Biotechnology, Faculty of Technology, Mahasarakham University, Thailand, 3Department of Biotechnology, Can Tho University, Vietnam, 4Department of Biology, Faculty of Science, National University of Laos, Lao PDR

PIV-04
Screening, characterization and applications of lactic acid bacteria producing bacteriocins
Takeshi Zendo1, Amonlaya Tosukhowong2, Rodney Honrada Perez1, Siriphan Sobanbua1, Adisorn Swetiwiwathana4, Pairat Sorpong6, Nghi Ngo Lan V9, Long Hoang Dang Bui7, Duong Thi Bich4, Nguyen Van Ba4, Ngo Thi Phuong Dung7, Wonnop Vissessanguan2, Sunee Nitisinprasert1 and Kenji Sonomoto1
1Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan, 2National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Khlong Luang, Thailand, 3Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand, 4Department of Food Safety Management, Faculty of Agro-industry, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, 5Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand, 6Department of Medical Microbiology, Faculty of Pharmacy and Nursing, Tay Do University, Can Tho City, Vietnam, 7Biotechnology Research and Development Institute, Can Tho University, Can Tho City, Vietnam

PIV-05
Roles of antimicrobial substance producing lactic acid bacteria in agro-industry and characterization of novel bacteriocin produced by lactobacilli species

221

265

225

239

235
PIV-06 Selection and application of thermotolerant lactic acid bacteria isolated in the Mekong Delta - Vietnam
Bui Hoang Dang Long¹, Ngo Thi Phuong Dung¹, Takeshi Zendo² and Kenji Sonomoto³
¹Biotechnology Research and Development Institute, Can Tho University, Can Tho, Vietnam, ²Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

PIV-07 Production of α-1,3-glucanase and chitinase for biotechnological applications
Wasana Suyotha¹, Shigezaku Yano², Junji Hayashi³, Motoki Kubo³, Kiwako Araki³, Asep A. Prihanto⁴, Andi Kurniawan⁴ and Mamoru Wakayama⁴
¹Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Thailand, ²Graduate School of Sciences and Engineering, Yamagata University, Jonan, Yonezawa, Yamagata, Japan, ³Department of Biotechnology, College of Life Sciences, Ritsumeikan University, Shiga, Japan, ⁴Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Indonesia

PIV-08 Improvement of productivity of α-1,3-glucanase in Escherichia coli by deletion of uncharacterized domain
Shigezaku Yano¹, Wasana Suyotha¹, Motoki Kubo³, Kiwako Araki³, Sakunnee Bovonsonbuth⁴, Wasu Pathom-aree⁴, Yingmanee Tragoolpua⁴ and Mamoru Wakayama⁴
¹Graduate School of Sciences and Engineering, Yamagata University, Japan, ²Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Thailand, ⁴Department of Biotechnology, College of Life Sciences, Ritsumeikan University, Japan, ⁴Department of Biology, Faculty of Science, Chiang Mai University, Thailand

PIV-09 Exploration of Monascus spp. for ethanol bioprocess from algal resources
Kangsadon Boonprab¹, Kenji Matsui² and Naoya Kataoka²
¹Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand, ²Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan

PIV-10 Development of beneficial biofilms and biosurfactants from Auerobasidium pullulans YTP6-14
Jiraporn Thaniyavarn¹, Natwara Amatyakul¹, Sudarat Luepongpatana¹ and Masaaki Morikawa⁴
¹Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, ²Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan

PIV-11 Manno-oligosaccharides production by lipase defective mutant strain of Bacillus sp. MR10 using copra meal as substrate
Chartchai Khanongnuch¹, Siriporn Chaikaew⁴, Apinun Kanpiengjai², Kridsada Unban¹, Keiko Uechi³ and Goro Takata⁴
¹Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand, ²Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ³Department of Bioscience and Biotechnology, Faculty of Agriculture, Ryukyu University, Okinawa, Japan, ⁴Department of Applied Biological Industry, Kasetsart University, Chatuchak, Bangkok, Thailand

AB 42p
Thermostable Pigment Production form Hot spring Cyanobacteria: from Laboratory to Commercial production

Chayakorn Pumas\textsuperscript{1*}, Metinee Khanniwat\textsuperscript{1}, Kanoknate Supasri\textsuperscript{1}, Oranit Kraseasinsira\textsuperscript{1}, Kanjana Mahanil\textsuperscript{2} and Masaharu Ishii\textsuperscript{2}

\textsuperscript{1}Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
\textsuperscript{2}Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Identification of acetic acid bacteria and assigned to the genus Gluconobacter by groEL gene sequence analysis

Nittaya Pitiwittayakul\textsuperscript{1}, Pattaraporn Rattanawaree\textsuperscript{2}, Smerjai Bureenok\textsuperscript{1} and Toshiharu Yakushi\textsuperscript{3}

\textsuperscript{1}Department of Agricultural Technology and Environment, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand, \textsuperscript{2}BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand, \textsuperscript{3}Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan

Effects of reduced graphene oxide aerogel on Saccharomyces cerevisiae

Patcharaporn Siwayaprahm\textsuperscript{1}, Phonphan Watthanarat\textsuperscript{1}, Chaimongkol Kongphakdee\textsuperscript{1}, Monthree Sawangphruk\textsuperscript{1}, Akihiro Moriyama\textsuperscript{3} and Hitoshi Iwahashi\textsuperscript{3}

\textsuperscript{1}Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand, \textsuperscript{2}Department of Chemical and Biomolecular Engineering, School of Energy Science and Technology, Vidyasirimedhi Institute of Science and Technology, Rayong, Thailand, \textsuperscript{3}Department of Applied Life Science, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan

Volatile organic compound and non-volatile bioactive compound producing fungi isolated from Japan and northern Thailand

Pattana Kakumyan\textsuperscript{1}, Surat Laphookhieo\textsuperscript{1}, Natsaran Saichana\textsuperscript{1}, Nakarin Suwannarach\textsuperscript{2}, Saisamorn Lunyong\textsuperscript{2} and Kenji Matsui\textsuperscript{3}

\textsuperscript{1}School of Science, Mae Fah Luang University, Chiang Rai, Thailand, \textsuperscript{2}Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, \textsuperscript{3}Graduate School of Sciences and Technology for Innovation, Faculty of Agriculture, Yamaguchi University, Yoshida, Yamaguchi, Japan

Isolation and biocontrol of antagonistic bacteria against some plant pathogenic fungi

Sirilak Sanpa\textsuperscript{1}, Patikorn Inongkarn\textsuperscript{1}, Motoki Kubo\textsuperscript{2}, Narikazu Yano\textsuperscript{1}, and Mamoru Wakayama\textsuperscript{2}

\textsuperscript{1}School of Medical Science, University of Phayao, Thailand, \textsuperscript{2}Department of Biotechnology, Faculty of Life Sciences, Ritsumeikan University, Japan, \textsuperscript{3}Department of Biochemical Engineering, Faculty of Engineering, Yamagata University, Japan

Fermentation process of L(+)-lactic acid and raw starch degrading enzyme production by Rhizopus spp.

Srisakul Trakarnpaiboon\textsuperscript{1}, Yukihiro Tashiro\textsuperscript{2}, Kenji Sakai\textsuperscript{2}, and Vichien Kitpreechavanich\textsuperscript{1}

\textsuperscript{1}Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand, \textsuperscript{2}Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Hakoza-Ku, Fukuoka, Japan

Production of Streptomyces inoculum by Solid State Cultivation for Biocontrol Agent of Chinese Kale Disease

Sathit Wongbuchasin, Shinji Tokuyama\textsuperscript{2} and Vichien Kitpreechavanich\textsuperscript{1}

\textsuperscript{1}Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand, \textsuperscript{2}Department of Applied Biological Chemistry Faculty of Agriculture, Shizuoka University, Shizuoka, Japan

Application of plant growth promoting actinobacteria for green...
agriculture: Evidence from *Streptomyces* on mungbean and Thai jasmine rice
Wasu Pathom-aree¹, Krisna Lasudee¹ and Shinji Tokuyama²
¹Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, ²Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka, Japan

### PIV-20

Characterization of Thai traditional fermented meat products
*P. Jarueesontornsakul*¹, *S. Kittisakulnam*¹, *T. Kleekayai*¹, *Shinjiro Tachibana*² and *Worapot Suntornskul*¹
¹Department of Microbiology, Faculty of Science, King Mongkut’s University of Technology Thonburi, Bangkok, Thailand, ²Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan

### PIV-21

Dietary probiotics improved growth performance and disease resistance in cultured fish
*Saowanit Tongpim*¹, *Ratchanu Meidong*², *Kulwadee Khotchanalekha*³, *Yukihiro Tashiro*⁴ and *Kenji Sakai*⁴
¹Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand, ²Department of Microbiology, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok, Thailand, ³Graduate School of Science and Engineering, Faculty of Engineering, Yamaguchi University, Yamaguchi, Japan, ⁴Department of Biosciences and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan

### PIV-22

Isolation and Characterization of Exopolysaccharide Indigenic Bacteria (EPS) and Quality Improvement of Liquid Biofertiliser NFB-PSB (Nitrogen Fixing-Phosphate Solubilizing Bacteria) with Viability Control
*Novi Arfarita*¹, *Mahayu Woro Lestari*¹, *Siti Mustikah*¹, *Indiyah Murwani*¹, *Tsuyoshi Imai*²
¹Faculty of Agriculture, Universitas Islam Malang, Jl. MT. Haryono, Malang, Indonesia, ²Graduate School of Science and Engineering, Faculty of Engineering, Yamaguchi University, Yamaguchi, Japan
*correspondence author, e-mail: arfarita@unisma.ac.id

### PIV-23

Expression and purification of Acylaminoacyl-Peptidase 3 from hyperthermophilic *Sulfolobus solfataricus* in *E. coli* Bl21
*Magdalena John*, *Johannes Klinger*, *Johannes Bader*, *Peter Götz*
Beuth University of Applied Sciences Berlin, Department of Bioprocess Engineering, Berlin, Germany

### PIV-24

Model-supported process development for heterologous expression of Superoxide Dismutase A from *Deinococcus radiodurans*: From shaking flask to fed-batch fermentation
*Johannes Klinger*, *Magdalena John*, *Johannes Bader*, *Peter Götz*
Beuth University of Applied Sciences Berlin, Department of Bioprocess Engineering, Berlin, Germany

### Not attend

Characterization and genetic analysis of bile salt hydrolase from *Lactobacillus salivarius* L61
*Pairat Sornplang*¹, * Takeshi Zendo*², *Naoki Ishibashi*² and *Kenji Sonomoto*²
¹Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand, ²Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

### Not attend

Isolation and characterization of *Lactobacillus paracasei* lytic phage ΦT25 from fermented milk in Thailand
*Sirinathorn Suntornthummas*¹, *Onanong Pringsulaka*¹, *Yasuhiro Fujino*², and *Katsumi Doi*²
¹Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand, ²Laboratory of Microbial Genetic Resources, Department of Bioscience and Biotechnology, Graduate School of Agriculture, Kyushu University, Fukuoka, Japan
**Not attend**

Purification of putative novel bacteriocin produced by *Lactobacillus brevis* BCC 26343

Amonlaya Tosakhowong¹, Takeshi Zendo¹, Sittirak Roytrakul¹, Janthima Jaresitthikunchai¹, Phisinee Jaikaew¹, Pullop Tungtrakoolsub², Wonnop Vissessanguan¹ and Kenji Sonomoto³

¹National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand, ²Tubkwang Research Station, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, ³Laboratory of Microbial Technology, Division of System Bioengineering, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Japan.

---

**Project 5: Development of Next-generation Fermentation Technology for New Wave Industry**

**High-temperature fermentation, pilot scale fermentation, renewable energy**

**PV-01** Characterization and mutation analysis of a halotolerant serine protease from *Bacillus subtilis* isolated from Thai traditional fermented shrimp paste

Shinji Takenaka¹, Airi Takada¹, Charin Techapun², Noppol Leksawasdith, Phisit Seesuriyachan², Thanongsak Chaiyasso³, Masanori Watanabe¹, Ampin Kuniya²

¹Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, Kobe, Japan, ²Division of Biotechnology, Faculty of Agro-industry, Chiang Mai Univ., ³Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University, Japan

**PV-02** Characteristics of fermentative L-(+)-lactic acid production from non-sterilized by-product of rice by LAB’s and its relationship with formation of microbial consortia in SSF

Masanori Watanabe¹, Yuta Yamamura¹, Charin Techapun², Noppol Leksawasdith³, Thanongsak Chaiyasso² Thanongsak Chaiyasso², Phisit Seesuriyachan², Ampin Kuniya² and Shinji Takenaka¹

¹Department of Food, Life and Environmental Science, Faculty of Agriculture, Yamagata University, Tsuruoka, Japan, ²Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Thailand, ³Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, Japan

**PV-03** Purification, characterization and structure of alcohol acyltransferases from plants

Yasuhsa Asano¹,², Fumihiro Motojima², Chiaki Yoshikawa¹, Toshiaki Mori¹, Ryoitaro Ohshina¹, Yasuo Kato¹, Sayaka Shichida¹, Yuko Ishida¹, and Kimiyasu Isobe²

¹Biotechnology Research Center, Toyama Prefectural University, Imizu, Japan, ²ERATO Asano Active Enzyme Molecule Project, Toyama Prefectural University; JST, ERATO, Imizu, Japan

**PV-04** High-temperature ethanol fermentation in a demonstrative plant and consolidated bioprocessing using starchy materials as feedstock

Hisashi Hoshida¹,²,³, ChisatoKawasaki⁴, Sayaka Kanamed¹, Takuya Abe¹, Jidapa Sangswan⁵, and Rinji Akada¹,²,³

¹Division of Applied Chemistry, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Ube, Japan, ²Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Yamaguchi, Japan, ³Yamaguchi University Biomedical Engineering Center, Ube, Japan, ⁴Department of Applied Chemistry, Faculty of Engineering, Ube, Japan, ⁵Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani, Thailand

**PV-05** New technologies for energy-saving ethanol production

Masayuki Murata¹, Izumi Kumakiri¹, Tomoyuki Kosaka¹,², Pumin Nutaratat¹, Constantinos Theodoropoulos², Peter Götz³, Pornthap Thanonkeo⁴, Savitree Limtong⁵, and Mamoru Yamada¹,²

¹Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan, ²Faculty of Agriculture, Yamaguchi University, Japan, ³Faculty of Science, Kasetsart University, Thailand, ⁴School of Chemical Engineering and Analytical
PV-06 Recombinant expression and characterization of two mannan metabolic enzymes from Enterococcus phoeniculicola.
Goro Takata1, Kohei Mino1, Tae Hasegawa1, Akkharapimon Yotsombat1, Chartchai Khanongnu1
1Faculty of Agriculture, Kagawa University, Kagawa, Japan, 2Faculty of Agro-industry, Chiang Mai University, Chiang Mai, Thailand

PV-07 Metabolic engineering of Escherichia coli for the production of short-chain alcohols and fatty acid
Naoya Kataoka1,2, Alisa S. Vangnai3,4, Thunyarat Pongtharangkul5, Toshiharu Yakushi3,5, Masaru Wada3, Atsushi Yokota5, and Kazunobu Matsushita1,2
1Division of Agricultural Sciences, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi, Japan, 2Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Yamaguchi, Japan, 3Biocatalyst and Environmental Biotechnology Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 4Center of Excellence in Hazardous Substance Management (HSM), Chulalongkorn University, Bangkok Thailand, 5Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand, 6Laboratory of Microbial Physiology, Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

PV-08 Bioprocess development for mucic acid production by Aspergillus sp. TPG-01 and Gluconobacter oxydans NBRC 12528
Apilak Salakkam1 and Yoshitaka Ano2
1Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand, 2Department of Bioscience, Graduate School of Agriculture, Ehime University, Matsuyama, Japan

PV-09 Bioconversion of sugarcane juice to hydrogen and methane by two stage fermentation process
Chatchawin Nualsri1, Alissara Reungsang2 and Tsuyoshi Imai3
1Faculty of Food and Agricultural Technology, Piabulsongkram Rajabhat University, Phitsanulok, Thailand, 2Research Group for Development of Microbial Hydrogen Production Process from Biomass, Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand, 3Department of Civil and Environmental Engineering, Faculty of Engineering, Yamaguchi University, Tokiwadai, Ube, Japan

PV-10 Thermal Chemical Pretreatment of Rice Straw and Bagasse for Reducing Sugar and Hydrogen Production
Prapaipid Chairattanamanokorn1, Chanoknan Kongsamoot1, Kantika Nonthamit1 and Tsuyoshi Imai2
1Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok, Thailand, 2Division of Environmental Science and Engineering, Graduate School of Science and Engineering, Yamaguchi University, Yamaguchi, Japan,

PV-11 Development of two-stage and solid-state thermophilic fermentation of palm oil mill effluent and oil palm biomass for biohythane and biogas production
Sompong O-Thong1, Poonsuk Prasertsan2 and Tsuyoshi Imai3
1Biotechnology Program, Department of Biology, Faculty of Science, Thaksin University, Phatthalung, Thailand, 2Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla, Thailand, 3Division of Environmental Science and Engineering, Graduated School of Science and Engineering, Yamaguchi University, Yamaguchi, Japan

PV-12 Purification, characterization of thermostable alkaline serine protease from Bacillus halodurans SES and its application on bio-bleaching of yellow cocoon
Thanongsak Chaiyaso1*, Kamon Yakul1, Charin Techapun1, Noppol Leksawasdi1,
PV-13  Optimal microalgal cultivation strategies for biofuels production during nitrogen and phosphorus limitation

Gonzalo M. Figueroa-Torres a, Jon K. Pittman b and Constantinos Theodoropoulos a,*

a School of Chemical Engineering and Analytical Science, Biochemical and Bioprocess Engineering Group, The University of Manchester, Manchester, M13 9PL
b School of Earth and Environmental Sciences, The University of Manchester, Manchester, M13 9PL
* Corresponding author: Constantinos Theodoropoulos
E-mail: k.theodoropoulos@manchester.ac.uk

-----  Ethanol production from sugarcane tops by thermotolerant yeast Kluuyveromyces marxianus DMKU 3-1042 and two newly selected xylose-fermenting yeasts Candida sp. ST-958 and ST-2914

Noppon Lertwattanasakul1, Thitinun Sumyai1, Vipawee Najanthong1, Tomoyuki Kosaka2, Mamoru Yamada2 and Savitree Limtong1

1Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand, 2Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Japan

-----  Selection of thermotolerant strain of Saccharomyces cerevisiae for ethanol production from molasses at high temperature and strain improvement by adaptation and mutagenesis

Sornsiri Pattanakittivorakul1, Noppon Lertwattanasakul1, Mamoru Yamada2 and Savitree Limtong1

1Department of Microbiology, Faculty of Science, Kasetsart University, Chatuchak, Bangkok, Thailand, 2Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Ube, Yamaguchi, Japan

Program Book

Keynote Lecture

Synthetic Biology Approach towards Creation of an Organism with a new Genetic Code

Barry L. Wanner

Microbiology, Harvard Medical School, Boston, MA 02115

Special Lecture I

Fundamental to Translational Research in Environmental Biotechnology

Alisa S. Vangnai1,2,3, Junichi Kato4, Jittra Piapukiew1,2, Borimas Krutsakorn4, Suwat Soonglerdsongpha5

1Biocatalyst and Environmental Biotechnology Research unit, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 2Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 3Center of Excellence in Hazardous Substance Management, Chulalongkorn University, Bangkok, Thailand, 4Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan, 5Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 6Environmental Technology Research Department, PTT Research and Technology Institute, PTT Public Company Limited, Thailand.

Special Lecture II

Micro-porous inorganic membranes in bio-refinery

Izumi Kumakiri

Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Ube, Japan

PB-01 Efficient fruit fly trapping by modified rice vinegars

Shinsuke Fujiwara

School of Science and Technology, Kwansei-Gakuin University
Application of endoglycosidases responsible for the release of N-glycans from the glycoproteins in basidiomycetes and posttranslational processing of the enzymes by a novel protease

Kazuo Ito1, Piamsook Pongsawasdi2, Jarunee Kaulpiboon1, Kuakrun Kruong3, Sanathana Nakapong4, Kamontip Kutiyawong5 and Prakarn Rudeekulthamrong6
1Graduate School of Science, Osaka City Univ., 2Department of Biochemistry, Faculty of Science, Chulalongkorn Univ., 3Biochemistry and Molecular Biology Program, Faculty of Medicine, Thammasat Univ., 4Department of Chemistry, Faculty of Science, Ramkhamhaeng Univ., 5Department of Chemistry, Faculty of Liberal Arts and Science, Kasetsart Univ., 6Department of Biochemistry, Phramongkutklao College of Medicine.

PB-02 Study on Unique Polysaccharides produced by Bacillus paralicheniformis strain 43-1
Shuichiro Murakami1, Kohei Abe1, Kuakrun Kruong2, Jarunee Kaulpiboon3, Prakarn Rudeekulthamrong4, and Piamsook Pongsawasdi2
1 School of Agriculture, Meiji University, Kawasaki, Japan, 2 Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 3 Faculty of Medicine, Thammasat University, Pathumtani, Thailand, 4 Phramongkutkla Collage of Medicine, Bangkok, Thailand

PB-03 Screening of novel compounds from marine organisms-associated bacteria
Enjiro Harunari and Yasuhiro Igarashi
Biotechnology Research Center, Toyama Prefectural University, Japan

PB-04 Study on the novel polyketide linfuranones from Sphaerimonospora mesophilna GMKU363
Hiromami Akiyama1, Chandra Indananda2, Arinhip Thamchaipenet1, Hisayuki Komaki2, Akira Hosoyama3, Akane Kimura4, Naoya Oku5, Yasuhiro Igarashi6
1Toyama Prefectural University, Japan, 2Burapha University, Thailand, 3Kasetsart University, Thailand, 4NBRC

PB-05 Screening, some properties and gene expression of fabrick PLA-degrading enzyme from Actinomadula sp. T16-4
Choko Hara1, Kota Anzai1, Sukhumaporn Krajangsang2, Vichien Kitpreechavanich3 and Shinji Tokuyama1
1Faculty of Agriculture, Shizuoka University, Shizuoka, Japan, 2Department of Microbiology, Faculty of Science, Srinakharinwirot University, Watthana, Bangkok, Thailand, 3Department of Microbiology, Faculty of Sciences, Kasetsart University, Bangkok, Thailand.

Pedioicin PA-1 producing Pediococcus pentosaceus TISTR 536 : a starter to improve microbiological safety during Nham production
Adisorn Swetiwiwathana1, Aphacha Jindaprasert1, Takeshi Zendo1, Jiro Nakayama2 and Kenji Sonomoto1
1Department of Food Safety Management, Faculty of Agro-industry, King Mongkut's Institute of Technology Ladkrabang (KMITU), Chalong-kroong rd., Ladkrabang, Bangkok, Thailand, 2Lab. of Microbial Technol., Div. of Systems Bioeng., Dep. of Biosci. & Biotechnol., Fac. of Agr. Graduate School, Kyushu University, Hakozaki, Higashi-ku, Fukuoka, Japan

Isolation of a novel bacteriocin-producing Lactobacillus plantarum Ski2 from Sai-krog Isan (Thai traditional fermented meat-rice sausage
Adisorn Swetiwiwathana1, Aphacha Jindaprasert1, Takeshi Zendo1, Jiro Nakayama2 and Kenji Sonomoto1
1Department of Food Safety Management, Faculty of Agro-industry, King Mongkut’s Institute of Technology Ladkrabang (KMITU), Chalong-kroong rd., Ladkrabang, Bangkok, Thailand, 2Lab. of Microbial Technol., Div. of Systems Bioeng., Dep. of Biosci. & Biotechnol., Fac. of Agr. Graduate School, Kyushu University, Hakozaki, Higashi-ku, Fukuoka, Japan

Production of α-D-glucosyl-(1,4)-α-D-alloside by immobilized maltose phosphorlyase.
Tae Hasegawa, Akkharapimon Yotsombat, Yuji Terami and Goro Takata

PB-05

PB 39p
PB 41p
PB 43p
PB 44p
PB 45p
47-50p
Cloning and functional expression of the D-glucoside 3-dehydrogenase from Rhizobium sp.

Akkharapimon Yotsombat¹, Kohei Mino¹, Tae Hasegawa², Goro Takata²

¹Department of Applied Bioresource Science, The United Graduate School of Agricultural Sciences, Ehime University, Ehime, Japan. ²Department of Applied Life Science, Faculty of Agriculture, Kagawa University, Kagawa, Japan
Synthetic Biology Approach towards Creation of an Organism with a new Genetic Code

Barry L. Wanner
Microbiology, Harvard Medical School, Boston, MA 02115

Recoding—the repurposing of genetic codons—is a powerful strategy for enhancing genomes with functions not commonly found in nature. I will report on the computational design, synthesis, and progress toward assembly of a 3.97-megabase, 57-codon Escherichia coli genome in which all 62,214 instances of seven codons were replaced with synonymous alternatives across all protein-coding genes. We validated 63% of recoded genes by individually testing 55 segments of 50-kilobases each. We observed that 91% of tested essential genes retained functionality with limited fitness effect. We demonstrate identification and correction of lethal design exceptions, only 13 of which were found in 2229 genes. This work underscores the feasibility of rewriting genomes and establishes a framework for large-scale design, assembly, troubleshooting, and phenotypic analysis of synthetic organisms. An initial report has been published: Ostrov, N., Landon, M., Guell, M., Kuznetsov, G., Teramoto, J., Norville, J. E, Cervantes, N., Zhou, M., Napolitano, M. G., Moosburner, M., Pruitt, B., Conway, N., Goodman, D., Singh, K., Gardner, C. L., Wanner, B.L., Lajoie, M., Church, G. M. 2016. Design, Synthesis and Testing of a 57-Codon Genome Science 353: 819-822 DOI: 10.1126/science.aaf3639. A more technical report is Norville, J. E., Gardner, C. L., Aponte, E., Complisson, C. K., Gonzales, A., Barclay, D. K., Turner, K. A., Mincheva, M., Teramoto, J., Tominaga, K., Sugimoto, R., DiCarlo, J. E., Guell, M., Hysolli, E., Aach, J., Gregg, C. J., Wanner, B. L., Church, G. M. 2016. Assembly of Radically Recoded E. coli Genome Segments BioRxiv 1-25 DOI: http://dx.doi.org/10.1101/070417. My seminar will also include a progress report.
Fundamental to Translational Research in Environmental Biotechnology

Alisa S. Vangnai1,2,3, Junichi Kato4, Jittra Piapukiew1,5, Borimas Krutsakorn6, Suwat Soonglerdsongpha6
1Biocatalyst and Environmental Biotechnology Research unit, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330, 2Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330, 3Center of Excellence in Hazardous Substance Management, Chulalongkorn University, Bangkok, Thailand 10330, 4Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8530, Japan, 5Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330, 6Environmental Technology Research Department, PTT Research and Technology Institute, PTT Public Company Limited, Thailand.

Environmental biotechnology is a branch of biotechnology that harnesses biological processes to mitigate environmental problems such as pollution treatment, and to exploit natural resources for sustainability such as biomass conversion, and renewable energy production. Basic science research has played a key role for discoveries and understanding biological systems in environmental biotechnology. Nevertheless, since “translational research” has been introduced and emphasized in a medical research world, it has rapidly expanded into several fields of research. By gathering many definitions, it can be described as an effort to convert basic science knowledge into practical applications essentially with stakeholder participation or collaboration1–3.

Our research unit has so far focused on fundamental research in bioremediation and bioconversion investigating microbial tolerance and responses to toxic substances, their biochemical and genetic systems relating to pollutant biodegradation, and all with a touch of their potential applications. With directions of and collaboration with the stakeholders, laboratory-bench results have been translated to actionable uses. Here we show how basic knowledge of bacterial benzene biodegradation system was turned into uses including: 1) a benzene-degrading, biofilm-forming formula used for biotricking filter in petrochemical industries, 2) a bioreporter as a commercial ecotoxicity test kit, and 3) a prototype system for benzene bioconversion for a value-added chemical production.

References
Micro-porous inorganic membranes in bio-refinery

Izumi Kumakiri
Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Ube, Japan

Bio-fuels and bio-chemicals have increasing attention as alternatives to the fossil-fuel based economy. Large efforts have been made on developing technologies to convert non-edible biomass to fuels and chemicals. Separation and purification processes are required after the conversion, for the targeted fuel/chemical is often obtained as a mixture fluid. Various separation processes are industrially employed that includes adsorption, absorption, distillation, stripping, extraction and membrane separation. Among these processes, membrane separation is a rather new method with unique characteristics. For example, membranes can significantly reduce the energy required to separate close-boiling mixtures compared to conventional distillation. In addition, membrane process is in a simple and compact design, making the operation and the maintenance easier. The wide range of working temperatures of membranes is another advantage to concentrate thermosensitive chemicals.

Different types of polymeric membranes are commercially available and have been applied to separate gaseous mixtures, liquid mixtures and particles from liquid. Recent developments of inorganic membranes\(^1\) expanded the application fields of membranes to e.g. solvent separations\(^2\), separations at higher temperature\(^3\) and pressure\(^4\), and other harsh conditions where polymeric membranes cannot be applied due to their insufficient stability. Membrane reactor is another field where huge efforts have been made on the research and development. For example, applying a membrane to water-gas shift reaction (\(\text{CO} + \text{H}_2\text{O} \rightleftharpoons \text{CO}_2 + \text{H}_2\)) and removing the formed hydrogen \textit{in situ} enhances the conversion. Integrating fermentation or other biomass conversion technologies with membranes have a potential to realize novel energy-efficient, compact and easy operation/maintenance bio-refinery processes. Recent developments and application examples of micro-porous inorganic membranes will be discussed in this presentation.

References
Efficient fruit fly trapping by modified rice vinegars

Shinsuke Fujiwara
School of Science and Technology, Kwansei-Gakuin University

Acetoin in vinegar is known as an attractant to fruit flies when combined with acetic acid. To make vinegar more effective in attracting fruit flies with increased acetoin production, *Komagataeibacter europaeus* KGMA0119 was modified by specific gene disruption of the acetohydroxyacid isomeroreductase gene (*ilvC*). A previously constructed mutant KGMA 7203 lacking the putative ligand-sensing region in the leucine responsive regulatory protein (*KeLrp*) was also used. The *ilvC* and *Kelrp* disruptants (KGMA5511 and KGMA7203, respectively) produced greater amounts of acetoin (KGMA5511, 0.11%; KGMA7203, 0.13%) than the wild-type KGMA0119 (0.069%). KGMA7203 produced a trace amount of isobutyric acid (0.007%), but the other strains didn’t. These strains produced approximately equal amounts of acetic acid (0.7%). The efficiency of fruit fly attraction was investigated with cultured *Drosophila melanogaster*. *D. melanogaster* (approximately 1,500) were released inside a cage (2.5m×2.5m×1.5m) and were trapped with a device containing vinegar and a sticky sheet. The flies trapped on the sticky sheet were counted. The cell-free supernatant from KGMA7203 culture captured significantly more flies (19.36–36.96% of released flies) than those from KGMA0119 (3.25–11.40%) and KGMA5511 (6.87–21.50%) cultures. Contrastingly, 0.7% acetic acid solution containing acetoin (0.13%) and isobutyric acid (0.007%), which mimicked the KGMA7203 supernatant, captured significantly fewer flies (0.88–4.57%). Furthermore, the KGMA0119 supernatant with additional acetoin (0.13%) and isobutyric acid (0.007%) captured slightly more flies than the original KGMA0119 supernatant, but fewer than the KGMA7203 supernatant, suggesting that the synergistic effects of acetic acid, acetoin, isobutyric acid, and unidentified metabolites achieved the efficient fly trapping of the KGMA7203 supernatant(1). In order to detect critical genes involved in attractant synthesis by identifying genes under control of *KeLrp*, a null mutant strain KGMA7110 (*ΔKelrp*) which lacks complete *Kelrp* was constructed. KGMA7110 cells were cultivated in a series of minimal mediums containing various amino acids. KGMA7110 cell growth was restored only in the presence of methionine (Met) except for branched chain amino acids (BCAAs). Besides Met, growth was restored in the presence of S-adenosylmethionine (SAM) and spermidine (SPD) which are the major downstream metabolites of Met. Based on those results, pathway for SPD synthesis was speculated to be controlled by *KeLrp*. In fact, intracellular amount of SPD was reduced in KGMA7110. Quantitative mRNA analysis of genes involved in the biosynthesis of Met, SAM and SPD showed that only the expression of *metK* encoding SAM synthetase was specifically reduced in KGMA7110. Gel mobility shift assay is carried out, indicating that *KeLrp* binds to the upstream region of *metK*. *KeLrp* is considered to act as an activator for a *metK* and to play an important role to maintain the intracellular levels of SPD (2).

To examine whether the high SPD contents contribute to fruit fly (*D. melanogaster* and *D. suzukii*) attraction, we evaluated the attractiveness of acetic acid, isobutylic acid, acetoin and SPD (3). SPD showed attractiveness as well as acetic acid, isobutylic acid, and acetoin. *D. melanogaster* possesses several odorant receptors with different affinities to a single olfactory ligand. *D. melanogaster* and *D. suzukii* might express specific receptors that have high affinity for polyamines and induce modest attraction behaviors.

References
Application of endoglycosidases responsible for the release of N-glycans from the glycoproteins in basidiomycetes and posttranslational processing of the enzymes by a novel protease

Kazuo Ito1, Piamsook Pongsawasdi2, Jarunee Kaulpiboon3, Kuakarun Krusong2, Santhana Nakapong4, Kamontip Kutiyawong5 and Prakarn Rudeekulthamrong6

1Graduate School of Science, Osaka City Univ., 2Department of Biochemistry, Faculty of Science, Chulalongkorn Univ., 3Biochemistry and Molecular Biology Program, Faculty of Medicine, Thammasat Univ., 4Department of Chemistry, Faculty of Science, Ramkhamhaeng Univ., 5Department of Chemistry, Faculty of Liberal Arts and Science, Kasetsart Univ., 6Department of Biochemistry, Phramongkutklao College of Medicine.

N-Glycans attached to glycoproteins have important roles for their structures, functions and biological recognitions. N-glycans are structurally classified into the types of high mannose, hybrid and complex. Their structures change during cell proliferation or under pathological condition. Glycan manipulation is indispensable for the utilization of the functions of N-glycans. Endo-β-N-acetylglucosaminidases (ENGases) hydrolyze diacetylchitobiose moiety in N-glycans to release them from protein moieties. In the previous study, we revealed that ENGases are widely distributed in various basidiomycetes, mushrooms. ENGases from basidiomycetes are likely to be classified into 2 groups in specificity for N-glycans, one group including Endo FV from F. velutipes is specific for high mannose type and another group including Endo AB from A. bisporus can act on N-glycans of complex type along with high mannose type. Furthermore, Endo FV and Endo AB belong to different GH family, 18 and 85, respectively.

We constructed the functional expression system of rEndo FV and rEndo AB, respectively. rEndo FV and rEndo AB transferred N-glycans of high mannose and complex type to other agents, respectively, indicating that both recombinant enzymes can be applicable for the synthesis of neoglycoconjugates bearing N-glycans. Furthermore, they released N-glycans from native glycoproteins. We succeeded to release N-glycans from glycoproteins in native human plasma by using rEndo FV and rEndo AB, indicating that they can be useful tools for enzymatic analysis of N-glycans in biospecimens for medical diagnosis.

On the other hand, we found that rEndo FV and rEndo AB post-translationally underwent limited proteolysis by a common endogenous protease to be a mature form by using specific antibodies for them. The protease was commonly expressed in all basidiomycetes that we examined. Analysis of the cation requirement and the gene of the protease indicate that it is a Ca2+-dependent Zn2+-metalloprotease classified into metalloprotease family M43B. These results suggest that structure and function of ENGases may be controlled by the specific protease in basidiomycetes.
Study on Unique Polysaccharides produced by *Bcillus paralicheniformis* strain 43-1

Shuichiro Murakami¹, Kohei Abe¹, Kuakarun Krusong², Jarunee Kauliboon³, Prakarn Rudeekulthamrong⁴, and Piamsook Pongsawadi²

¹ School of Agriculture, Meiji University, Kawasaki 244-8571, Japan
² Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
³ Faculty of Medicine, Thammasat University, Pathumtani 12121, Thailand
⁴ Phramongkutkloa College of Medicine, Bangkok 10400, Thailand

Cyclodextrins are cyclic oligosaccharides consisting of 6-8 glucose units and utilized in various industrial fields because they are able to include a guest molecule. To overcome the limitation of sizes of a guest molecule depending on the sizes of their inside, we tried to isolate microorganisms producing saccharides to be able to include bigger guest molecules than those included by cyclodextrins.

To isolate microorganisms producing unique oligosaccharides with inclusion complex-forming ability, bromochresol green (BCG) was selected as an indicator emerging in inclusion complex-forming reaction and strain 43-1, excellent halo-forming bacterium around a colony, was isolated on a plate containing BCG. The strain was identified as *Bacillus paralicheniformis* on the basis of morphological and physiological properties, and sequence analysis of 16S rDNA.

Amylase-like enzymes secreted from strain 43-1 cells were amalayled by DEAE cullmn chromatography. The strain 43-1 was cultivated in 1% soluble starch medium (pH 9.0) at 45°C with shaking. After 36-h cultivation, the cultural supernatant was collected by centrifuge, and dealyzed. The sample was applied to a DEAE cartridge cullmn and eluted by a linear gradient of 0 – 0.2 M of NaCl. Strach degrading activities (increase of reducing sugar and decolorization of Iodo – starch reaction) and protein cincentrations were assayed in collected fractions. As results, we found a big and some small active peaks, and some protein peaks in an elution profile.

Each fraction was used as an enzyme source in reaction containing soluble starch as a substrate and a reaction mixture was treated with glycoamylase to degrade non-cyclic remaining sugars. The reaction mixture was dialyzed using a membrane with a pore size corresponding to molecular weight of 1000 to remove materials with low molecular weights and remaining materials were analyzed by high performance anion exchange chromatography (HPAEC). HPAEC analysis revealed that any intereqsing sugers weren’t produced when fractions showing soluble starch-degrading activities were used as enzyme sources. However, polysaccharides with higher molecular weight than large cyclodextins were produced in a reaction mixture where a fraction with one protein peak but without starch-degrading activity was added as an enzyme source. These results suggest that enzymes producing the polysaccharides isn’t typical amylase-like enzyme.
Screening of novel compounds from marine organisms-associated bacteria

Enjuro Harunari and Yasuhiro Igarashi
Biotechnology Research Center, Toyama Prefectural Univ.

Actinomycetes are the high GC Gram-positive filamentous bacteria. They have been a major source for drug discovery. However, the number of novel compounds from terrestrial actinomycetes are considerably decreasing. For this reason, marine-inhabiting actinomycetes are attracting attention as a new alternative source of drug discovery.

On the other hand, a number of pharmaceutically useful natural products were isolated from marine organisms such as sponges and tunicates. Such marine invertebrates are the important source of drug screening; however, the most significant concern is the supply of producing organisms. Recent investigations showed that bacteria are the true producers of natural products isolated from marine invertebrates as exemplified by bryostatin 1 and didemnin B. For this reason, bacteria residing in marine organisms are attracting much attention as a new source of drug discovery. Especially, there are few reports for secondary metabolites isolated from hard corals. To the best of our knowledge, no new compounds isolated from bacterial species associated with hard corals are reported.

The objective of this study is to understand the potential of secondary metabolites production in marine organisms-associated bacteria. Our currently ongoing study suggests that some bacterial isolates from cultured hard corals are producing structurally new compounds. We report taxonomy, screening, and structure analysis of the isolates.
Roles of antimicrobial substance producing lactic acid bacteria in agro-industry and characterization of novel bacteriocin produced by lactobacilli species

Sunee Nitisinprasert 1, Siriphan Sobanbua 1, Phattanapong Thedtratha 1, Putthapong Phumsombat 1, Massalin Nakphaichit 1, Komkhae Pilasombut 2, Chaowarit mapato 5, Sunthorn Rungruang 2, Rutjawate Taharnklaew 2, Orapin Sukpiriyagul 3, Takeshi Zendo 5, Jiro Nakayama 5 and Kenji Sonomoto 5

1Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Lat Yao, Chatuchak, Bangkok 10900, Thailand
2Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut’s Institute of Technology Ladkrabang, Bangkok, 10520, Thailand
3Pakthongchai Dairy Farm, CPF, Thailand
4Betagro Agro-Group Public Co. Ltd, Thailand
5Laboratory of Microbial Technology, Division of Applied Molecular Microbiology and Biomass Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Higashi-ku, Fukuoka, Japan

Five lactic acid bacteria (LAB) species exerting antimicrobial substance (AMS) or owning probiotics were previously screened from fermented food and feed based on AMS production. Three strains of Lb. reuteri KUB-AC5, Lb. plantarum KUB-SP1-3 and Pd. acidilactici KUB-M6 were previously characterized. The strain KUB-AC5 was able to produce bacteriocin named KAC5 against both G+ and G- bacteria and exhibited bactericidal mode of action. This strain tolerated at wide pH range, bile salt and high temperature as well as exhibited high adherence activity which was proposed as probiotics and further applied as antibiotic growth promoter for chicken growth. While the strain KUB-SP1-3 and KUB-M6 were able to grow at wide pH and temperature range, and produced high yields of lactic acid from both hexose and pentose under aerobic and anaerobic condition. A combination of both named KUB-G2 was further applied as a starter to improve silage quality.

To examine protection activity of KUB-AC5, high )10^7 CFU/bird( and low dosage )10^5 CFU/bird( were tested and analysed for chicken microbiota of S. Enteritidis challenged chicken. Four treatments of the negative control group )without Salmonella challenging(, the positive control group )Salmonella challenged chick( and the 5 and 7 log CFU probiotic supplementation to Salmonella infected chicks were performed and resulted that their microbial diversities at growing and finisher stage were not significantly different )p>0.05(. However, high dosage of KUB-AC5 supplement could maintain its microbial diversity similar to the negative control at early stage and remove both S. Enteritidis and Clostridium perfringens at the finishing step. This suggested for its potential probiotics application to the poultry industry.

To improve silage quality by starter supplementation technology, the quality of 20 tons silage using the inoculants containing KUB-G2 was compared to the one with the commercial product consisting of Lb. plantarum and Lb. buchneri. The results showed that no significant difference was obtained by their biochemical and nutritional characteristics. However, KUB-G2 treated silage had different microbiota, higher Lactobacilli and better silage characteristics of color and smell.

In addition, novel bacteriocins produced by both Lb. plantarum KL-1 and Lc. lactis KA-FF1-4 were characterized. Both exhibited inhibitory activity against food borne pathogens especially enterohemorrhagic Escherichia coli O157: H7 while a commercial agent of nisin did not. They would be a promising AMS to apply for food safety in the future.
Screening, some properties and gene expression of fabric PLA-degrading enzyme from Actinomadula sp. T16-4

Choko Hara 1, Kota Anzai 1, Sukhumaporn Krajangsang 2, Vichien Kitpreechavanich 3 and Shinji Tokuyama 1

1 Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan, 2 Department of Microbiology, Faculty of Science, Srinakharinwiro University, Watthana, Bangkok 10110, Thailand, 3 Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

PLA is biodegradable and non-toxic polyester which can be produced from renewable resources such as cassava, corn, rice and potatoes. Thermophilic PLA-degrading actinomycetes were isolated from forests soils on emulsified PLA medium at 50 ºC. Actinomadiura sp. 16-4 were selected based on the clear zone formation and their PLA-degrading activity. The purification and characterization of PLA-degrading enzymes from thermophilic actinomycetes were limited. The aims of this work were to purify the enzymes from thermophilic PLA-degrading actinomycetes and show some characters. In addition, gene cloning and gene expression in Streptomyces lividans TK21 were studied to improve the PLA-degrading activity of S. lividans transformants.

Purified PLA-degrading enzymes from these strains show high specific activity. (Actinomadiura sp: 358 U/mg). This enzymes hydrolyse casein, gelatin and BSA as well as PLA and are inhibited by PMSF. From these results, this enzymes is classed as a serine protease. PLA-degrading enzymes produce lactic acid and lactide as main degradation product in the reaction mixture. PLA-degrading enzyme (20 U/ml) from Actinomadiura sp. 16-4 decomposes PLA files in 2 hours and PLA nonwoven fabric in 24 hours at 50 ºC clearly. The genes for this enzyme have been cloned and expressed in S. lividans TK21. The enzyme from Actinomadiura sp. 16-4 shows 51% and 52% of similarity with Aqualysin I from Thermus aquaticus and PLA-degrading enzyme from Micromonospora sp. B12-1, respectively. These results show that this PLA-degrading enzymes from thermophilic actinomycetes are new type of serine protease.

S. lividans transformants carrying the gene from Actinomadiura sp. 16-4 produce 32.4 U/ml of this enzymes, which is 6.6 times production of the original strain.
Pediocin PA-1 producing *Pediococcus pentosaceus* TISTR 536: a starter to improve microbiological safety during Nham production

Adisorn Swetwiwathana¹, Aphacha Jindaprasert¹, Takeshi Zendo², Jiro Nakayama² and Kenji Sonomoto²

¹ – Department of Food Safety Management, Faculty of Agro-industry, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Chalong-krung rd., Ladkrabang, Bangkok, Thailand. 10520

² - Lab. of Microbial Technol., Div. of Systems Bioeng., Dep. of Biosci. & Biotechnol., Fac. of Agr. Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

Nham, a traditional Thai fermented meat product, is normally consumed without cooking and considered to be a ready-to-eat food after 3–4 days of spontaneous fermentation. The reports on salmonellae contamination in this product, such as *Salmonella Anatum*, are therefore a serious public health concern. Since the advantage of using lactic acid bacteria (LAB) as starter cultures, especially the bacteriocin-producing ones, was reported to exert a positive effect on the microbiological quality and safety of various fermented meat products, thus, the selection of bacteriocin-producing LAB isolated from Nham and the use of the potent bacteriocin-producing LAB as starter cultures to control the growth of *S. Anatum* in Nham were studied under Core University Program (CUP), Asian Core Program (ACP) and Core to Core Program (CCP) supported by JSPS –NRCT program. Among the selected strains of bacteriocin-producing LAB, LAB strain TISTR 536, which was isolated from Nham and collected in Thailand Institute of Scientific and Technological Research (TISTR) since 1986, was identified as *Pediococcus pentosaceus* and its bacteriocin was later confirmed as pediocin PA-1. This strain was known as generally recognized as safe (GRAS) for using as starter culture in various fermented foods and was confirmed in our study that the strain was not produced biogenic amines during fermentation. The strain was later studied for using as starter culture to control the growth of *S. Anatum* in the simulated Nham model broth (NMB) compared to the broth with nonbacteriocin-producing *P. pentosaceus* (JCM 5890). The results revealed that the presence of both cultures alone in NMB without nitrite and fresh garlic exhibited no effect on the growth of *S. Anatum* during the early stage of NMB fermentation. The reduction of *S. Anatum* was shown after a day of NMB fermentation due to the high decrease in pH in NMB by each starter strain. Moreover, we found that the reduction of this pathogenic bacteria concerned to the synergistic effect of lowering the pH to 4.5 and the additional of crude pediocin PA-1 from *P. pentosaceus* TISTR 536 according to the sublethal injured of *S. Anatum* cells after surviving in a high concentration of weak acid which led the injured cells sensitive to pediocin PA-1. The treatment of *S. Anatum* (8-10 and 80-100 cfu/g) with 10⁶ cfu/g starter cultures of both pediocin PA-1 producer and non bacteriocin producer of *P. pentosaceus* strains was performed in real Nham product and compared to those of naturally fermented samples (control without starters) and left to ferment for 6 days. The results revealed that the advantage of using both starter cultures in the production of salmonellae free Nham was reported when compared to those control samples. Among the use of these 2 strains of LAB as starter cultures, pediocin PA-1 producer was found to be the most inhibitory on *S. Anatum*. Thus, this pediocin PA-1 producer of *P. pentosaceus* TISTR536 is the possible strain for using as starter culture for safety Nham production and related traditional fermented meat products.
Isolation of a novel bacteriocin-producing *Lactobacillus plantarum* Ski2 from Sai-krog Isan (Thai traditional fermented meat-rice sausage)

Adisorn Sweethiwatana¹, Aphacha Jindaprasert¹, Takeshi Zendo², Jiro Nakayama² and Kenji Sonomoto²

¹ – Department of Food Safety Management, Faculty of Agro-industry, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Chalong-kroeng Rd., Ladkrabang, Bangkok, Thailand. 10520
² – Lab. of Microbial Technol., Div. of Systems Bioeng., Dep. of Biosci. & Biotechnol., Fac. of Agr. Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

This study is to report a novel bacteriocin-producing lactic acid bacteria (LAB) which coded as Ski2 associated in ready-to-cook Sai-krog Isan (Thai traditional fermented meat-rice sausage) product produced from one local fermented meat factory located in Bangkok. The preliminary antagonistic results from colony spot-on-lawn on bacteriocin-screening medium (BSM) implied that the colony of Ski2 showed the widely inhibition zone against *Staphylococcus aureus*. Thus, this strain was brought to further study of its antagonistic produced and strain identification under CCP program in the Lab of Microbial Technology, Kyushu University during FT 2017. The results of cell free supernatant from this strain after cultured in MRS broth for 20 h under 30 °C were confirmed for its inhibitory products against 8 indicators pattern to estimate the inhibitory spectrum and the type of bacteriocin compared to the known bacteriocin producers (*Lactococcus lactis* subsp. *lactis* NCDO- nisin A producer, *Lc. lactis* subsp. *lactis* IO1 – nisin Z producer and *Pediococcus pentosaceus* TISTR 536 – pediocin PA-1 producer) using spot-on-lawn technique. The results revealed Ski2 showed the inhibitory effect on 2 of 8 indicator strains used in the indicators pattern (*Lactobacillus dextranicus* and *Bacillus coagulans*). The inhibitory spectrum of Ski2 was different from both of nisin and pediocin group. The identification using partial 16S rDNA sequence under the NCBI blast program revealed that this strain was related to *Lb. plantarum* with 100% identity. Thus, this potent strain was named as *Lb. plantarum* Ski2. The purified peptides of bacteriocin from Ski2 implied to be 2 peptides bacteriocins which showed the molecular weight (MW) about 3,390 dalton and 3,102 dalton. These two MW results have not been reported from any bacteriocin-producing *Lb. plantarum* strains. Hence, the bacteriocin from this *Lb. plantarum* Ski2, which might be a novel bacteriocin, is in our interest for further study. Besides, this strain might be possible to use as starter culture for improving the good quality and safety production of traditional Thai fermented meat products for the next JSPS-NRCT program.
Study on the novel polyketide linfuranones from *Sphaerimonospora mesophila* GMKU363

Hirofumi Akiyama¹, Chantra Indananda², Arinthip Thamchaipenet³, Hisayuki Komaki⁴, Akira Hosoyama⁴, Akane Kimura⁴, Naoya Oku¹, Yasuhiro Igarashi¹

¹ Toyama Prefectural Univ., ² Burapha Univ., ³ Kasetsart Univ., ⁴ NBRC

The actinomycete strain *Sphaerimonospora mesophila* GMKU 363 was isolated from a root tissue of the Thai medicinal herb “Lin Ngu Hoa” (*Clinacanthus siamensis* Bremek.) collected in Chachoengsao Province, Thailand. We previously reported the isolation of a furanone-containing polyketide, linfuranone A (1), and then found its acylated congener linfuranone B (2), which will be reported in this paper. Accordingly, the draft genome of strain GMKU 363 was sequenced to assess its biosynthetic potential in secondary metabolism as well as to identify the biosynthetic genes for 1 and 2. A deduced biosynthetic gene cluster contains five PKS genes in which a total of 11 modules are present along with the genes of tailoring enzymes for furanone construction. The PKS module organization predicted that the intact PKS product is assembled from six methylmalonates and five malonates. However, contrary to this prediction, the main carbon chain of 1 and 2 is five carbons shorter than that of the predicted structure. This inconsistency between the predicted structure and the actually isolated structures was suggestive of the presence of linfuranone congeners other than 1 and 2, prompting us to reinvestigate the metabolites of this strain.

To search for a metabolite that has the aforementioned predicted skeleton, culture conditions were examined and the optimal growth temperature of strain GMKU 363 was found to be around 37 to 40 °C, higher than the temperature commonly used for secondary metabolite production by actinomycetes as well as for the production of 1. The strain was thus precultured and cultured at 37 °C for 4 days and 7 days, respectively, and metabolites were analyzed in comparison with those produced at 30 °C. In the culture extract obtained by fermentation at 37 °C, a new intense peak that showed a similar UV–vis spectrum to the furanone chromophore was observed near the peak of 2. This new peak was not detectable when the strain was cultured at 30 °C. UV-guided purification from the extract led to the isolation of another furanone-type polyketide, linfuranone C (3), that has the same carbon skeleton as the predicted structure. Herein, we describe the isolation, structure elucidation, biological property, and biosynthesis of 2 and 3.

In conclusion, two new furanone-containing polyketides, 2 and 3, were isolated from an actinomycete of the genus *Sphaerimonospora*. Compound 3 was discovered through *in silico* analysis of biosynthetic genes and temperature optimization for production fermentation. Compound 2 is generated from 3 by oxidative cleavage of the polyketide backbone by Baeyer–Villiger oxidation. This type of decoration of the polyketide main carbon chain is not common. The absolute configurations of the hydroxylated carbons C7 and C15 were determined by applying the chiral anisotropy method. The absolute configuration of C14 was established by NOESY and *J*-based analysis. C18 chiral centers in 2 and 3 were determined to have *R*-configuration by chemical degradation and HPLC analysis.
Production of $\alpha$-D-glucosyl-(1,4)-$\alpha$-D-alloside by immobilized maltose phosphorylase.

Tae Hasegawa, Akkharapimon Yotsombat, Yuji Terami and Goro Takata
Department of Applied Life Science, Faculty of Agriculture, Kagawa University, Kagawa, 7610795 JAPAN

We found that maltose phosphorylase from Enterococcus sp. showed activity against D-allose as an acceptor, which is C-3 epimer of D-glucose. Maltose was transformed into novel disaccharide with D-allose using immobilized maltose phosphorylase. The structure of product was analyzed by HPLC to determine sugar composition and by X-ray crystallography to determine the structure and the glucosidic linkage. Finally, the product was identified as $\alpha$-D-glucosyl-(1,4)-$\alpha$-D-alloside.

Introduction
Rare sugars are defined as monosaccharides and their derivatives occurring in small quantities in nature. D-allose, a rare sugar, has several physiological features such as antioxidant and antitumor were reported\(^1\)\(^-\)\(^2\) and has attractive attention as starting material for other unnatural compound such as rare disaccharide. It might be expected that novel rare disaccharide with D-allose would have better functionality than the rare sugar itself, because oligosaccharides also show various functionalities such as prebiotics, immunity enhancement and mineral adsorption.

In previous study, synthesis of $\alpha$-D-glucosyl-(1,2)-$\beta$-D-allululose with sucrose phosphorylase was reported\(^3\). Maltose phosphorylase serve as a catalyst of conversion reaction from maltose to $\beta$-D-glucose 1-phosphate and D-glucose and vice versa. This enzyme acts as inverting anomeric configuration mechanism of $\beta$-D-glucose 1-phosphate. This enzyme is known to be region-selective enzyme except at $\beta$-OH configuration of C-2.

We recently found that a maltose phosphorylase derived from Enterococcus sp. showed slight enzyme activity toward D-allose, which is C-3 epimer of D-glucose, and recognized as an acceptor. In this study, we describe enzymatic synthesis of novel disaccharide, $\alpha$-D-glucosyl-(1,4)-$\alpha$-D-alloside from maltose. Figure 1 shows strategy of $\alpha$-D-glucosyl-(1,4)-$\alpha$-D-alloside production by combination of maltose phosphorylase and chemical hydrogenation, which is a new method for the production of rare disaccharide.

Materials and Methods
Maltose phosphorylase activity was measured by glucose oxidase method. The enzyme reaction was stopped by boiling for 3 minutes. One unit (U) is defined as the amount of 1 μmol of D-glucose formation per min at pH 7.0 and 30°C. Maltose phosphorylase dissolved in Na-phosphate buffer (pH 7.0) was immobilized onto IRA958CL anion-exchange resin. The resin was added 1 ml per 10 U of enzyme activity and leave it for 2 days. The reaction mixture contained 250U per 100 ml of immobilized maltose phosphorylase, 10% maltose and 10% D-allose. The enzyme reaction was performed with Na-phosphate buffer (pH 7.0) at 30°C. The product formation was analyzed by HPLC using a GL-C611 column at 60°C eluted with 0.1 mM NaOH at a flow rate of 1.0 ml/min. After enzyme reaction, D-glucose was removed by a resting cells reaction of E. coli. The reaction mixture was deionized and concentrated. For separation of products, Preparative HPLC was carried out using ligand exchange chromatography with GL-P2611 column. The fraction containing the product was pooled. The purified product was concentrated until high concentration. Finally, concentrate product was leaved to stand at room temperature before crystal formation. The formed crystal was analyzed by X-ray crystallography.
Results and Discussion
The reaction of maltose with D-allose was done using immobilized maltose phosphorylase. The reaction was accomplished within 58 hrs. The reaction development was analyzed by HPLC (Figure 2); the retention time (RT) of first peak (RT14.0 min) was maltose, second peak (RT15.7 min) was D-glucose, and fourth peak (RT22.5 min) was D-allose. Another one peak (RT17.6 min) was supposed to be novel disaccharide, glucosyl alloside. After finish the reaction, in order to remove D-glucose, the reaction mixture was treat with *E coli* cells. As the result of HPLC, D-glucose could remove completely (Figure 3). Finally, the product was separated by ligand-exchange chromatography. The products was crystallized (Figure 4a) and identified as α-D-glucosyl-(1,4)-α-D-alloside by X-ray crystallography (Figure 4b).
In this study, We succeeded α-D-glucosyl-(1,4)-α-D-alloside production by enzymatic reaction using immobilized maltose phosphorylase. The reaction mixture contained any by-products other than the objective product, maltose, D-glucose, and D-allose. To purify the product from the reaction mixture, we tried to remove D-glucose first by biochemical method, because the product might be difficult to separate with D-glucose by column chromatography. After separation of ligand exchange chromatography, we succeeded to purify and crystallize the product. To know the structure and the glucosidic linkage, X-ray crystallography was done with obtained crystal. This disaccharide consisted of two monosaccharide and these monosaccharide were linked with α-1, 4 bond, which result showed this disaccharide is α-D-glucosyl-(1,4)-α-D-alloside.
Furthermore, we found that this disaccharide included water molecules, which structure may cause instability of this disaccharide. This is first report on the crystal structure of rare disaccharide as we know.

Acknowledgment
This study was supported in part by KAKENHI number 26450097, 22580088 (Grant-in-Aid for Scientific Research (C)). Authors also acknowledge JSPS-NRCT (Core-to-Core Program A. Advanced Research Networks “Establishment of an International Research Core for New Bio-research Fields with Microbes from Tropical Areas”).

References
Figure 1  Strategy for the production of α-D-glucosyl-(1,4)-α-D-alloside using maltose phosphorylase

Figure 2  HPLC chromatogram of the reaction mixture with maltose phosphorylase. Retention time: maltose (14.0 min) and D-glucose (15.7 min), product (17.5 min), D-allose (22.5 min).
**Figure 3** HPLC chromatogram after *E. coli* cells treatment. Retention time: maltose (13.6 min), product (17.2 min), and D-allose (22.3 min).

**Figure 4** Crystal of the product (a), and crystal structure of novel disaccharide, α-D-glucosyl-(1,4)-α-D-alloside (b).
Cloning and functional expression of the d-glucoside 3-dehydrogenase from *Rhizobium* sp.

Akkharapimon Yotsombat¹, Kohei Mino¹, Tae Hasegawa², Goro Takata²

¹Department of Applied Bioresource Science, The United Graduate School of Agricultural Sciences, Ehime University, Ehime 790-8566, Japan
²Department of Applied Life Science, Faculty of Agriculture, Kagawa University, Kagawa 761-0795, Japan

**Introduction**

Several flavine adenine dinucleotide (FAD) related dehydrogenase complexes have been reported, for example, Formate dehydrogenase (FDH), succinate dehydrogenase (SDH), and D-glucoside 3-dehydrogenase (G3DH). These dehydrogenase complexes are hetero-oligomeric enzymes composed of three subunits; a catalytic subunit harboring FAD, a cytochrome C subunit responsible for electron transportation, and a small subunit acting like a chaperon. Among these enzymes, G3DH is distinguished because of its regioselectivity of oxidizing various glucosides at the C-3 position to the corresponding 3-ketoglucosides, as well as its broad substrate specificity. Moreover, G3DH can react with many artificial electron acceptors, for instance, 2,6-dichlorophenolindophenol (DCPIP), phenazine methosulfate (PMS), and ferric cyanide. The catabolism of 3-ketoglucoside is very interesting for industrial applications therefore G3DH has lately become a topic of intensive research.

D-Glucoside 3-dehydrogenase (EC 1.1.99.13) was first discovered in *Agrobacterium tumefaciens* and later found in many strains such as *Cytophaga marinoflava*, *Flavobacterium saccharophilum*, *Halomonas* (Deleya) sp. α-15, *Sphingobacterium faecium* ZIF-D6 CCTCC M 2013251, and in *Agaricus bisporus*, a fungus, as an extracellular enzyme. There were reports about the attempt to clone G3DHs and overexpress in *E. coli*. However, G3DH gene from *Agrobacterium tumefaciens* was inactive. While the recombinant G3DHs from *Halomonas* (Deleya) sp. α-15 and *Sphingobacterium faecium* ZIF-D6 CCTCC M 2013251 were less active than the wild type. Although the cloning difficulties, the recombinant G3DH could gain an advantage over the wild type for 3-ketocompounds producing because there was no α-3-keto glucosidase in *E. coli*.

**Objectives**

1. To screen for the novel G3DH producing microbe
2. To create the recombinant G3DH to further study on the fundamental and biochemical properties

**Results and Discussion**

**Screening for G3DH producing microbes**

The collected soil samples were initially screened using yeast extract agar with 1% lactitol as a carbon source. The fully grown microbes were transferred to a new liquid medium and incubated, then the keto-sugar producing ability was examined using cysteine-carbazole method. The following screening was observing G3DH activity by following the reduction rate of DCPIP. From the screening methods, a microbe with the highest lactitol oxidation activity was selected. Its 16s rDNA was analyzed, and the genome was identical to *Rhizobium* sp. The optimal condition for G3DH production was found to be using YE medium containing 1% maltitol and incubate at 30°C for 24 h.

**Cloning of the G3DH gene**

To create a G3DH gene fragment of 2700 bp with restriction sites for cloning, the primers contain BamHI and HindIII restriction site were designed. The primers used are 5'-GAGAGGATCCGCAATTCATGATGTGGATCCCAGCATGGATGAGG-3' and 5'-GAGAAAGCTTTCACTCAGCCGGCTTGGAAG-3'. The PCR product was digested by appropriate restriction enzymes. Finally, the amplified G3DH gene was ligated into the expression vector pQE30 and was then transformed into *E. coli* JM109.

**Overexpression of the recombinant G3DH**

The recombinant *E. coli* JM109 carrying pQE30-G3DH was cultured in SB medium and incubated at 37°C until the culture reached an optical density at 600 nm of 0.4-0.6. The protein expression was then induced by adding 1 mM IPTG. After inducing, the culture was incubated at 23°C for another 16 hours.

**Characterization of the recombinant G3DH**

The recombinant G3DH was successfully
expressed in E. coli and was purified through two chromatography columns; Hi-Trap Q HP column and His-Trap column. The purified recombinant G3DH showed a single band with an approximate molecular weight of 66 kDa on SDS-PAGE (Figure 1) and was analyzed to be about 63 kDa by MALDI-TOF mass spectrometry, which is consistent with the predicted molecular mass based on the protein sequence. Interestingly, most of the G3DHs have higher molecular weight than 66 kDa, whereas that of S. faecium was about 62 kDa. The enzyme recovery was 42.23%, with a 10.64-fold enrichment (Table 1). The G3DH activity was measured by following the reduction rate of DCPIP at the wavelength of 600 nm when 20 µl of enzyme solution was added to the 180 µl of reaction mixture containing 1% cellobiose and 20 µM DCPIP in 50 mM Tris-HCl buffer pH 7.5. One unit (U) of the G3DH activity is an amount of enzyme that reduce 1 µmol of DCPIP per minute. Protein concentration was measured by Bradford method. To characterize the purified recombinant G3DH, the effect of temperature on enzyme activity was determined in the temperature range from 0 to 60°C. For thermal stability, the enzyme was incubated at 30, 40, 50, and 60°C for 90 mins. The optimal temperature for enzyme activity was determined to be 40°C, and G3DH was relatively stable under 40°C for over 90 mins (Figure 2A, B). The effect of pH on activity and stability were observed in various buffers. The result showed that the optimum pH for enzyme activity was 7.0, and the enzyme was stable between pH 5.5 and 9.0 (Figure 2C, D).

Identification of the enzyme reaction product
The ability to produce 3-ketoglucoisides was investigated using PMS as an artificial electron acceptor against cellobiose, a substrate with highest specificity. The chromatogram of the cellobiose (Figure 3A) and 3-ketocellobiose, enzyme product, (Figure 3B) indicated that retention times (Rt) were corresponding to those of cellobiose (8.03 min) and 3-ketocellobiose (8.52 min). Therefore, the recombinant G3DH from E. coli was confirmed with an ability to produce 3-ketocellobiose.

**Conclusion**
G3DH was first discovered and characterized in Agrobacterium tumefaciens during 1960s and later found in other organisms. In this study, G3DH from Rhizobium sp. was isolated, and the enzyme was cloned into E. coli. The recombinant G3DH was functional expressed in the soluble fraction with specific activity higher than 20 U/mg. Further research on genetic information is necessary to clarify the native structure of G3DH and its substrate binding mechanism.

**Acknowledgment**
This study was supported in part by KAKENHI number 26450097, 22580088 (Grant-in-Aid for Scientific Research (C)). Authors also acknowledge JSPS-NRCT (Core-to-Core Program A. “Establishment of an International Research Core for New Bio-research Fields with Microbes from Tropical Areas”).

**References**
**Table 1** purification of recombinant G3DH from *E. coli* pQE30-G3DH

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Activity (U)</th>
<th>Specific activity (U/mg)</th>
<th>Purification (fold)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude enzyme</td>
<td>592.5</td>
<td>250</td>
<td>0.42</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>HiTrap Q HP</td>
<td>25.2</td>
<td>48.28</td>
<td>1.92</td>
<td>4.54</td>
<td>19.31</td>
</tr>
<tr>
<td>HisTrap(^\text{TM})</td>
<td>1.0</td>
<td>20.39</td>
<td>20.39</td>
<td>10.64</td>
<td>42.23</td>
</tr>
</tbody>
</table>

**Figure 1** Overexpression and purification of recombinant G3DH protein. *Lane 1*: insoluble fraction, *lane 2*: soluble fraction, *lane 3*: partial purified recombinant G3DH, *lane 4*: purified recombinant G3DH, and *lane M*: standard molecular weight markers.
Figure 2  Characterization of the purified recombinant G3DH. Effects of temperature on G3DH activity (A) and stability (B). Effects of pH on G3DH activity C) and stability (D). The effect of pH on recombinant G3DH activity was examined with acetate buffer (pH 3.0–6.0), sodium phosphate buffer (pH 6.0–8.0), Tris-HCl buffer (pH 7.0–10.0), MES buffer (pH 6.0–7.0), and sodium bicarbonate buffer (pH 10.0–11.0).

Figure 3  HPLC chromatogram of the conversion of cellobiose to 3-ketocellobiose by purified recombinant G3DH at 0 hour (A) and 10 hour (B). Retention time; cellobiose (8.03 min) and 3-ketocellobiose (8.52 min).