

*e-ASIA Joint Research Program
Final Report*

1. Project title : Development of New Processes with Thermotolerant Microbes for Bio-refinery Including Biofuels, towards Utilization of ASEAN Biomass

2. Joint Research period : April 1, 2017 ~ March 31, 2020

3. Research Team :

■ **Japanese team** (up to 6 people including the Principal Investigator)

Funding period: April 1, 2017 - March 31, 2020

Total Funded Amount (in Local Currency): 37,260,000 JPY

	Name	Position	Affiliation	Role in the project
PI	Mamoru Yamada	Professor	Yamaguchi University	Breeding of ethanol-fermenting stress resistant strains
Collaborator	Toshiharu Yakushi	Professor	Yamaguchi University	High temperature acetic acid fermentation
Collaborator	Kenji Sakai	Professor	Kyushu University	Development of high-temperature lactate production process
Collaborator	Izumi Kumakiri	Professor	Yamaguchi University	Membrane separation
Collaborator	Hisashi Hoshida	Associate Professor	Yamaguchi University	Analysis of ethanol fermentation by CBP
Collaborator	Yukihiro Tashiro	Associate Professor	Kyushu University	Production of lactate by mixed culture
Total number of participants including students: 32				

■ **Thailand team** (up to 6 people including the Principal Investigator)

Funding period: Month, Day, Year - Month, Day, Year **In-kind**

Total Funded Amount (in Local Currency):

	Name	Position	Affiliation	Role in the project
PI	Savitree Limtong	Professor	Kasetsart University	Ethanol production with cellulose

				sis biomass
Co-PI	Gunjana Theerago ol	Associate Professo r	Kasetsart Universit y	Thermotoleranc e in thermotole rant acetic acid bacteria
Collaborator	Vichien Kitpreecha vanich	Associate Professo r	Kasetsart Universit y	Production of o ptically active L -lactate
Collaborator	Noppon Lertwattan asakul	Assistant Professo r	Kasetsart Universit y	Ethanol product ion with cellulo sic biomass
Collaborator				
Collaborator				
Total number of participants including students: 10				

■ **Indonesia team** (up to 6 people including the Principal Investigator)

Funding period: 2017 - 2019

Total Funded Amount (in Local Currency): 379,375,000 IDR

	Name	Position	Affiliation	Role in the pro ject
PI	Anton Muhibuddin	Professor	University of Br awijiya	Isolation of ther motolerant yeasts
Co-PI	Suprayogi	Lecturer	University of Br awijiya	Isolation of ther motolerant yeasts and fermentatio n with starchy bi omass
Collaborator	Jaya Mahar	Lecturer	University of Br awijiya	Isolation of ther motolerant yeasts
Collaborator	Sri Nurhatika	Lecturer	10 th Nopember Technology In stitute	Isolation of ther motolerant yeasts
Collaborator				
Collaborator				
Total number of participants including students: 7				

■ **Laos team** (up to 6 people including the Principal Investigator)

Funding period: Month, Day, Year - Month, Day, Year **In-kind**

Total Funded Amount (in Local Currency):

	Name	Position	Affiliation	Role in the pro ject
PI	Somchanh Bounp hanmy	Associate Professor	National Univer sity of Laos	Isolation of therm otolerant yeasts
Co-PI	Chansom Keo-Ou done	Lecturer	National Univer sity of Laos	Isolation of therm otolerant yeasts

				and fermentation with starchy bio mass
Collaborator	Toulaphone Keok ene	Lecturer	National University of Laos	Isolation of thermotolerant acetic acid bacteria
Collaborator	Pheng Phengsint ham	Lecturer	National University of Laos	Isolation of thermotolerant yeasts
Collaborator				
Collaborator				
Total number of participants including students: 8				

4. Summary of the joint research

Since the first half of 1990, we have been conducting joint research with Thailand and other ASEAN countries to "search for useful microbial resources inhabiting the tropical environment and develop their functions". In particular, we have developed many thermotolerant microorganisms that possess high fermentation capacity even at high temperatures, in Core University Program (1998-2007) and Asian Core Program (2008-2013). Furthermore, by utilizing the characteristics of thermotolerant microorganisms, we constructed a high-temperature fermentation technology using first-generation biomass in the MEXT-ARDA project (2011-2013). On the other hand, while there is a strong demand for the use of biomass that leads to CO₂ reduction against global warming, the utilization of cellulosic biomass as biofuels like ethanol has hardly been put to practical use due to problems including cellulose cost. In order to produce biofuels, which is expected to have about 10 times the demand in 2050, it is desirable to develop innovative technologies that reduce conversion costs.

In this research, to develop cost-saving fermentation technologies toward the utilization of agricultural residues containing cellulosic materials that are abundant in ASEAN countries, thermotolerant microorganisms were isolated from own countries, in anticipation of the application of the Convention on Biological Diversity, and bred by the robustization procedure developed by Japan team. Indonesia and Laos teams then established high-temperature fermentation with cassava starch as a raw material. Thailand team also established with sugarcane tops as the raw material for cellulosic biomass. On the other hand, based on the thermotolerant strains isolated in ASEAN countries, the Japan team introduced genes for thermotolerant and stress-resistant factors and saccharifying enzymes, and developed new fermentation processes and water- and acid-resistant separation membranes for concentration of fermentation products. We held a seminar every year to share information on problems and development technologies, and at the same time promoted the formation of a vital network including the training of young researchers.

WP1: Development of high-temperature ethanol fermentation technologies

WP1 developed thermotolerant yeasts in each country for high-temperature ethanol fermentation using starchy or cellulosic biomass as a raw material. The Japan team cooperated with the isolation of thermotolerant yeasts, and was in charge of developing a technology (robustization technology) to mutate a thermotolerant yeast into robustized strains that are more resistant to stress and supplying low-concentration ethanol to WP4.

1) Development of robustization technology

We developed a robustization technology using *Kluyveromyces marxianus* DMKU3-0142, which is highly heat-resistant and efficiently produces ethanol even at high temperatures. It was found that the robustization of yeasts can be made by repeating the culture for a long time while gradually increasing the temperature until the temperature reaches the limit of survival. Among the four robustized strains, ACT001, ACT002, and ACT003 were more resistant to acetic acid, formic acid, low pH, furfural, and 5-methoxyfurfural, and slightly resistant to ethanol, as compared with the parent strain. TM001 was more resistant to ethanol, furfural, and 5-methoxyfurfural. In addition to heat resistance, acid resistance can be used to prevent microbial contamination, so if the sterilization process of the fermentation medium can be omitted under high temperature and acid conditions, the equipment and running costs can be reduced.

The mutation sites of the robustized strains were determined by next-generation DNA sequencing. Additionally, the growth and ethanol productivity of these strains were examined in the presence of high glucose concentrations at 45°C. TM001 was found to grow faster than the parent strain, and all robustized strains produced more ethanol.

2) Cooperation with Thailand, Indonesia and Laos teams

The Indonesian and Laos teams collected samples of fruits, flowers and soil at many places and isolated a large number of thermotolerant yeasts. Growth at high temperature, sugar tolerance and ethanol tolerance were examined, and strains resistant to such

stresses and excellent in fermentation ability were selected. Species of strains with strong thermostability were identified. Of these, strains with particularly high fermentation ability were subjected to the robustization treatment developed by the Japan team. As a result, all robustized strains were resistant to ethanol and furfural. Flask level experiments showed high ethanol productivity at 44°C. In addition, in a 5-L scale experiments using cassava starch, high ethanol productivity was shown at 40°C.

Thailand team isolated 177 strains of xylose-utilizing yeast, but their ethanol productivity from xylose was found to be weak. The composition of sugarcane tops as a cellulosic biomass was analyzed. Fermentation conditions with DMKU 3-1042 were then examined using a solution in which sugarcane tops was treated with alkali and hydrolyzed with Accellerase 1500. On the other hand, about 100 newly isolated yeast strains were investigated for ethanol productivity from xylose. Two of them produced about 10 g/L ethanol from 30 g/L xylose. These two strains were identified as *Candida* sp. and achieved 36-38 g/L ethanol production from a solution (reducing sugar 90 g/L) prepared from sugarcane tops.

3) High-temperature ethanol fermentation with feed rice and cooperation with WP4

Using feed rice produced in Yamaguchi prefecture as a raw material, SHF (separate hydrolysis and fermentation) at high temperature was compared with SSF (simultaneous saccharification and fermentation). As a result, the ethanol production was almost the same, but it was shown that fermentation was rate-limiting and SSF saves time. Furthermore, it was found that high temperature fermentation was possible without lowering the efficiency up to around 43°C. In addition, ethanol was concentrated by vacuum distillation after fermentation. According to the research plan, a concentrated ethanol solution obtained by a single vacuum distillation was provided to WP4. In addition, we conducted a molecular biology analysis of *K. marxianus* on glucose suppression, which is one of problems in utilization of cellulosic biomass.

WP2: Development of acetic acid high temperature fermentation production technology

The WP2 conducted research related to acetic acid fermentation at high temperature using thermotolerant acetic acid bacteria. (1) Development of acetic acid bacteria and conditions for practical acetic acid fermentation. The Thailand team adapted the thermotolerant *Acetobacter pasteurianus* strain, which had been developed by the Japan team, under low-nutrient conditions to obtain a low-nutrition adapted strain. When "non-temperature controlled fermentation" was carried out using a practical acetic acid fermentation medium, electricity consumption was reduced by 63% compared to fermentation controlled at 30°C. (2) We worked on the molecular basis of thermal adaptation or malnutrition adaptation through the genome analysis of the developed strains. The Thailand team examined mutations in above-mentioned thermotolerant, low-nutrition adapted *Acetobacter* strain by the genome analysis to identify the gene responsible for low-nutrition adaptation with the Japan team. The gene for an oxidoreductase involved in fatty acid biosynthesis, one of the mutated gene in the adapted strain, of the parental strain was replaced to the mutated one. The constructed strain that has the mutation only in the oxidoreductase gene showed similar fermentation ability under low nutrition as the adapted strain. (3) The identification and characterization of acetic acid bacteria isolated from nature in Laos and the evaluation of high temperature acetic acid fermentation capacity were performed. Eight strains showing acetic acid-producing ability that can be used for acetic acid fermentation were isolated. (4) Acetic acid fermentation by co-culture with yeast or *Zymomonas* was examined. When the fermentation was done with inoculating equal amounts of yeasts and acetic acid bacteria, ethanol production occurred first, followed by consumption of ethanol and acetic acid production by acetic acid bacteria. As described above, at WP2, international collaborative research was able to proceed because of the functioning of the international research team.

WP3: Development of fermentation production technologies of L-lactic acid and n-butanol

We proposed a novel package for synergetic fermentation production of two useful chemicals, L-lactic acid and n-butanol. The former is important as a monomer building block of promising bio-plastic, poly L-lactic acid, and the latter is a candidate for prospective biofuel in next generation. By combining their production system plural synergetic effects are expected: to share different sugar sources including cassava residue starch, food waste, and lingo-cellulosic sugars in Thailand for the two different fermentations, and to carry energetically favorable downstream process could be constructed by introducing esterification-distillation process of raw butyl-lactate for simultaneous purification of the two products, bringing results in a cumulative increase in productivity and decrease in required energy.

Meta-fermentation production (controlled fermentation of L-lactic acid with mixed culture seed) of optically pure L-Lactic acid from non-sterilized food waste was optimized at 50 °C with pH swing control. The meta-fermentation system was reconstructed, with simply consisting of three thermotolerant *Bacillus* strains. It was found that they synergistically converted polysaccharides including starch, xylan, and cellulose in the food waste. It was found that constant pH control rather resulted higher productivity and final concentration in the reconstructed system.

Next, we aimed to overcome difficulties in bio-butanol production, such as low productivity catabolite repression by glucose, high cost of hydrolyzing enzyme. First, a new strategy of 'semi-hydrolysis' was proposed as well as new parameters of 'yield to enzyme loading' and 'overall productivity' and novel 'simultaneously repeated hydrolysis and fermentation (SRHF)' was developed from semi-hydrolyzed rice straw. Second, 'non-isothermal simultaneous saccharification and fermentation in-situ butanol recovery (NSSFR)' was designed from semi-hydrolyzed paper pulp without any pretreatments using immobilized cells and extractant. Finally, 'extractive fermentation process at large volume ratio of extractant to broth' was established using a new extractant of oleyl alcohol-tributyrin mixture and immobilized cells.

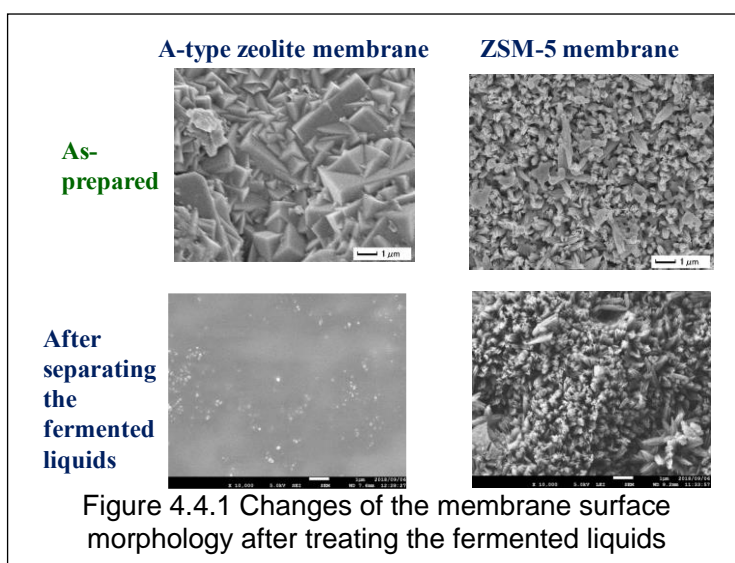
WP4: Membrane separation

Membrane separation is an energy-efficient alternative to the conventional distillation. In addition, membrane process has a simple configuration, allowing easy operation and maintenance.

In this project, three types of inorganic membranes were tested: A-type zeolite membranes (the same type as commercialized membranes for solvent drying), MOR zeolite membranes and ZSM-5 zeolite membranes.

The solution after a single distillation of fermented liquid produced in the WP1 contained some amount of organic acids and other contaminants. The pH of the distillate was about 4. A-type membranes deformed after soaking the membranes into the distillate as shown in Figure 4.4.1 (left images), and the dehydration performance dropped.

On the contrary, newly made MOR and ZSM-5 membranes were robust (right images). These new membranes were successfully applied to concentrate the ethanol in the distillate



from ca. 20% to over 90%.

WP5: Consolidated-bioprocessing for ethanol production

To establish consolidated-bioprocessing for ethanol production, a thermotolerant yeast *K. marxianus* and the conventional ethanol-producing yeast *Saccharomyces cerevisiae* was genetically engineered.

In this study, starch was used as a raw material for ethanol production. Therefore, glucoamylase genes were expressed in the yeasts. A glucoamylase gene SfGLU1, which was originated from *Sacchchromycopsis fibrigera*, showed better ethanol production efficiency compared with others, but ethanol yield was about 50% of the theoretical yield. To enhance heterologous gene expression, a new expression plasmid YHp was developed for *S. cerevisiae*. In the case of *K. marxianus*, promoters showing higher activity at high temperature were screened. However, enhanced glucoamylase did not increase ethanol yield.

To analyze starch degradation efficiency, several starch-degrading enzymes were tested. As a result, combination of glucoamylase, α -amylase, and pullulanase, which digests α -1,6 glucosidic bond, was effective for complete saccharification of starch. We have already expressed a secretory α -amylase by the yeasts. Therefore, to express an enzyme with digestion activity to α -1,6 glucosidic bond, several types of genes were cloned into an expression plasmid for *S. cerevisiae*. The activity of debranching enzymes and isomaltases was not obtained. A dextranase was successfully secretory expressed but did not increase ethanol production. A pullulanase was also expressed in an active form as a cytosolic enzyme by *S. cerevisiae*. CBP using starch will be realized by secretory production of the pullulanase in addition to a glucoamylase and an α -amylase in yeasts.

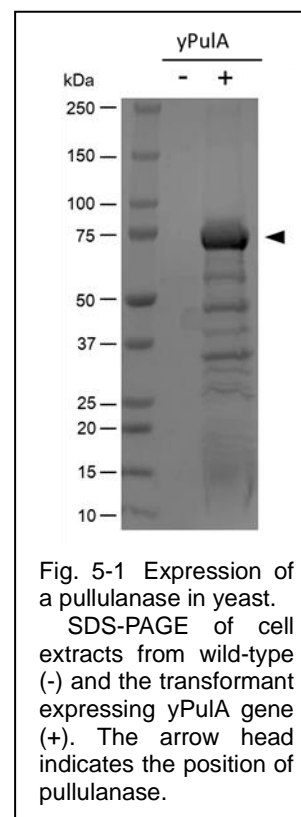


Fig. 5-1 Expression of a pullulanase in yeast.

SDS-PAGE of cell extracts from wild-type (-) and the transformant expressing yPulA gene (+). The arrow head indicates the position of pullulanase.

5. Outputs and Anticipated Outcomes of Joint Research

5-1 Scientific achievements and implemented activities of the joint research

With the researchers in Thailand, Indonesia, and Laos, we carried out various technological development researches leading to energy saving, focusing on the development of biofuel conversion process (fermentation production) technology with thermotolerant microorganisms, using agricultural residues. In Thailand, efficient yeasts that use cellulose-based biomass as the raw material and in Indonesia and Laos, thermotolerant yeasts that use starch-based biomass as the raw material were isolated, and their characteristic analysis and high-temperature fermentation ability were examined. On the other hand, the Japan team improved the capability of thermotolerant yeasts or bacteria by introduction of genes for thermotolerant and stress-tolerant factors or saccharifying enzymes, and developed new fermentation processes and water- and acid-resistant separation membranes for concentration of fermentation products. As research achievements, we published 13 international co-authored papers, 11 papers only by Japanese researchers, 2 papers not including Japanese research team and 2 other international co-authored publications in addition to many conference presentations including invited lectures.

5-2 Synergistic effects of the international joint research

In ASEAN countries, the amount of biomass such as agricultural residues is large, and there is a strong demand for utilization from the perspective of reducing CO₂ emissions and

promoting biorefinery. However, although it is possible to convert biomass into useful substances such as fuel, there is a cost problem. In this international collaborative research, we have developed technology that enables significant cost reduction of biomass conversion by combining the microbial resources, experiences and technologies of the participating countries. We exchanged opinions about research issues by e-mail and face-to-face, and held seminars of this project every year in participating countries other than Japan to share results and technical information. Almost every year, researchers from Laos, Indonesia, and Thailand conducted joint research in the Japanese laboratory, and because of their proximity, Laos researchers conducted research in the Thai laboratory. Also, every time there was an opportunity from the Japanese side as well, we visited the countries concerned and exchanged opinions directly.

5-3 Broader impacts including contribution to society

This international collaborative research project is aimed at biorefinery using biomass such as agricultural residue as a raw material, and all the technologies developed in this project are relevant to the Sustainable Development Goals (SDGs) and can contribute to society if can be put to practical use. In particular, the integration of the developed technologies for bioethanol is expected to become an innovative technology, as it is expected that the cost will be significantly reduced. In this project, seminars were held annually in participating countries to disseminate information on this initiative. In particular, information sharing with public funding institutions and companies is expected to lead to social implementation of the developed technologies in the future.

5-4 Development and sustainability of the cooperation

We have been conducting collaborative research for over 20 years with the Thai research team, over 10 years with the Laotian research team, and over 5 years with the Indonesian research team in international base projects and international joint projects. We have continued to interact with young researchers, including students, and have actively promoted the development of young researchers and network formation that will lead to the future. In this project as well, there were a total of 13 researcher exchanges, including stays of one month or more, and exchanges of students other than registered members were supported by the Japan Student Services Organization (JASSO). About 20 student exchanges were implemented during the project period as part of the “Human resource development program for international network formation of thermotolerant and useful microorganisms in tropical environments and their application”. We will continue to promote joint development of human resource to utilize thermotolerant microorganisms that live in tropical environments, and especially we aim to put the technology developed in this project into practical use in Japan and ASEAN countries.

6. Future Goals and Plan of Activities after the project period

For bioethanol, the integration of high temperature fermentation and membrane separation will provide a new technology for production of low cost transportation fuel. Using the strains isolated and improved in each country in this project, a small-scale production test will be conducted at the bioethanol plant in each country, and the scale will be expanded thereafter. Some companies in Thailand and Indonesia that are producing sugar and ethanol are considering joint research on application of the strains. Furthermore, when combined with a biofuel reforming power generator, it becomes a compact power generator that can be used in times of disaster or in depopulated areas. We are proceeding with joint research with a Japanese company that has already developed a biofuel reforming power generator.

On the other hand, L-lactic acid can be used as a raw material for biodegradable plastics. In addition, reinforced bioplastics can be produced in combination with high quality nanocellulose produced by acetic acid bacteria. The key to the social implementation of

these bioplastics is the consumer's awareness of fossil resource-derived plastics and increasing purchasing power of bioplastics. Since the number of food and drink companies that stop using plastic straws is increasing, the use of bioplastics can be expected to expand. Bacteria-derived nanocellulose and poly-L-lactic acid have high biocompatibility and can be used in the medical field.

7. Scientific Achievements and Implemented Activities (Publication, Research Exchange, Workshop, etc.)

**For this item, please fill in the attached Excel file.*

8. Recommendations and Comments to the Program

We would like to express our sincere gratitude to JST and RISTEKDIKTI for their kind supports in this joint research. On the other hand, the e-ASIA JRP member organizations in each country have different ways of thinking and support, and it took time and effort to make adjustments. Since the collaborators in the partner country also aim to return the results of this joint research to society, we would like you to make a program that leads to the next step.

9. Others (agenda of workshop, photos of research teams, meetings, and etc.)

e-ASIA JRP kick-off meeting
(Part of The Thailand Research EXPO 2017)
26th August 2017, 8.30 – 17.00 h
at The Centara Grand & Bangkok Convention Centre, Central World, Thailand

TIME	DESCRIPTION
26 August 2017	
07.00	Pick up Oversea participants at the Lobby of KU-Home
07.00 – 08.30	Go to Centara Grand by Car
08.30 – 09.00	Registration (<i>morning session</i>)
09.00 – 09.30	Opening Ceremony and Group Photo <ul style="list-style-type: none"> - Core to Core Program - e-ASIA JRP kick-off meeting - Group Photo
	Welcome Address by <ul style="list-style-type: none"> - Assoc. Prof. Dr. Gunjana Theeragool, <i>Thai Coordinator</i> - Prof. Dr. Makie Kokubun, <i>Professor Emeritus, Tohoku University</i> <i>Program Officer, e-ASIA JRP</i>
	Opening Address by <p>Prof. Dr. Makie Kokubun, <i>Prof. Emeritus, Tohoku University</i> <i>Program Officer, e-ASIA JRP</i></p> <p>Prof. Dr. Sirirung Songsivilai, <i>Secretary General, National Research Council of Thailand</i></p>
	Group Photo
14.30 - 14.45	* Coffee Break (box set)
e-ASIA JRP kick-off meeting	
14.45 - 15.00	Short speeches by <ul style="list-style-type: none"> - Agricultural Research Development Agency (Public Organization)

TIME	DESCRIPTION
	<ul style="list-style-type: none"> - Prof. Dr. Anton Muhibuddin, University of Brawijaya - Assoc. Prof. Dr. Somchanh Bounphanmy, National University of Laos
15.00 – 15.10	Introduction to e-ASIA JRP Activities by Mr. Masaki Sato, <i>Secretary-General, e-ASIA JRP Secretariat</i> <i>Director, Japan Science and Technology Agency Singapore Office</i>
Chairman: Prof. Dr. Savitree Limtong, <i>Kasetsart University</i> Co-Chairman: Prof. Dr. Mamoru Yamada, <i>Yamaguchi University</i>	
15.10 – 15.25	WP1: Development of ethanol fermentation technologies at high temperature by Prof. Dr. Mamoru Yamada, <i>Yamaguchi University</i>
15.25 – 15.40	WP2: High temperature acetic acid fermentation: Molecular basis on thermotolerance and application by Assoc. Prof. Dr. Toshiharu Yakushi, <i>Yamaguchi University</i>
15.40 – 15.55	WP3: Development of L-lactate and n-butanol fermentation technologies; Collaboration between Thailand and Japan - Construction of synergetic production process of the two chemicals - by Prof. Dr. Kenji Sakai, <i>Kyushu University</i>
15.55 – 16.10	WP4: Development of membrane separation technologies; Collaboration between Thailand and Japan by Assoc. Prof. Dr. Izumi Kumakiri, <i>Yamaguchi University</i>
16.10 – 16.25	WP5: What is the best process for ethanol fermentation from starchy and cellulosic materials? by Assoc. Prof. Dr. Hisashi Hoshida, <i>Yamaguchi University</i>
16.25 – 16.30	Closing Ceremony by Prof. Dr. Mamoru Yamada, <i>Yamaguchi University</i>

**The 2nd e-ASIA Joint Research Program Seminar
24th October 2018**

**Luangprabang City, Lao PDR
(National University of Laos)**

Time	Detail	Speaker
October 24, 2018		
08:00-09:00	Registration	
09:00-09:15	Opening Remarks	Assoc. Prof. Dr. Somchanh Bounphanmy
09:15-09:35	JST presentation	Prof. Makie Kokubun
9:35-10:15	Plenary Presentation	Most (Laos)
10:15-10:45	<ul style="list-style-type: none"> - Group photo - Coffee break 	All participants

Session 1	Chairman – Prof. Dr. Mamoru Yamada Co-Chairman - Assist. Prof. Dr. Vichai Leelavatcharamas	
10:45-11:15	WP1: Thai: Ethanol production from lignocellulose materials by thermotolerant yeast <i>Kluyveromyces marxianus</i> DMKU 3-1042 using simultaneous saccharification and fermentation process	Assist. Prof. Dr. Noppon Lertwattanasakul
11:15-11:35	WP1: Lao: Screening and Characterization of Thermotolerant Yeasts For Ethanol Fermentation From Casava Starch Hydrolyzed at High Temperature.	Ms. Chansom Keo-oudone
11:35-11:55	WP1: Indonesia: Isolation, identification and characterization of thermotolerant ethanol-fermenting yeast from Indonesia	Dr. Suprayogi
11:55-12:15	WP2: Thai: Cost-effective vinegar fermentation at high temperature by the adapted strain of <i>Acetobacter pasturianus</i> SKU1108	Assoc. Prof. Dr. Gunjana Theeragool
12:15-13:30	Lunch break	
Session 2	Chairman – Prof. Dr. Savitree Limtong Co-Chairman – Dr. Naoya Oku	
11:55-12:15	WP2: Lao: Characterization and Identification of Acetic acid bacteria Isolated from samples collected in Lao PDR	Mrs. Toulaphone Keokene
13:30 – 13:50	WP3: Japan: Synergetic fermentative production of a biochemical and biofuel: Efficient n-butanol production processes from renewable resources	Assoc. Prof. Dr. Yukihiro Tashiro
13:50-14:10	WP4: Japan: Membrane separation as an alternative downstream process for the recovery of fermentation products	Assoc. Prof. Dr. Izumi Kumakiri
14:10-14:30	WP5: Japan: Evaluation of yeast and gene expression systems for bioethanol production through consolidated bioprocessing of starch	Assoc. Prof. Dr. Hisashi Hoshida
14:30-14:50	Closing Remarks and ceremony	Assoc. Prof. Dr. Gunjana Theeragool
14:50-15:30	Tea break	
15:30-----	Free / Sightseeing	

The 3rd e-ASIA Joint Research Program Seminar
29th – 30th October 2019
Novotel Samator Surabaya Hotel, Surabaya

Time	Detail	Speaker

October 29, 2019		
08:00-09:00	Registration	
09:00-09:15	Opening Remarks	Prof. Anton & RISTEKDIKTI
09:15-09:35	Introduction of e-ASIA JRP	Prof. Mamoru Yamada
Session 1	Chairman – Prof. Dr. Kenji Sakai Co-Chairman – Dr. Mochamad Nurcholis	
09:35-09:55	WP1: Lao: Ethanol production from cassava starch at elevated temperatures by a stress-adapted strain of <i>Kluyveromyces marxianus</i>	Ms. Chansom Keo-oudone
09:55-10:15	WP1: Indonesia: Improvement of stress resistance by long-term cultivation at high temperature in <i>Kluyveromyces marxianus</i> UB5	Dr. Suprayogi
10:15-10:50	-Group photo -Coffee break	All participants
10:50-11:10	WP2: Japan: Involvement of amino acid or nitrogen metabolism in thermotolerance of <i>Acetobacter pasteurianus</i> , the acetic acid bacterium	Prof. Dr. Toshiharu Yakushi
11:10-11:30	WP3: Thai: Production and characterization of raw starch degrading enzyme by thermotolerant <i>Rhizopus microsporus</i> DMKU33 and its applications on simultaneous saccharification and fermentation of lactic acid and ethanol from raw cassava starch/chip	Assoc. Prof. Dr. Vichien Kitpreechavanich
11:30-13:00	Lunch Break	
Session 2	Chairman – Prof. Dr. Toshiharu Yakushi Co-Chairman – Assoc. Prof. Dr. Vichien Kitpreechavanich	
13:00-13:20	WP4: Japan: Membrane integrated process for concentrating bioethanol after fermentation	Assoc. Prof. Dr. Izumi Kumakiri
13:20-13:40	WP5: Japan: Searching the α-1,6-glycosidic-bond hydrolyzing enzymes capable of secretory production by yeasts	Assoc. Prof. Dr. Hisashi Hoshida
13:40-14:00	Indonesia: Mig1 as a global regulator in thermotolerant yeast <i>Kluyveromyces marxianus</i>	Dr. Mochamad Nurcholis
14:00-14:10	Closing Remarks and ceremony	Assoc. Prof. Dr. Gunjana Theeragool
14:10-14:40	Tea Break	All participants
14:40-18:30	Free / Sightseeing	All participants
18:30-21:00	Reception Dinner	Guests and all members

Lists of Achievements and Implemented Activities

1. Original Publication of Articles etc.

1. 1 Original Publications (Articles co-authored among Research Teams)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)
Srisakul Trakarnpaiboon, Nantana Srisuk, Kuakoon Piyachomkwan, Kenji Sakai, Vichien Kitpreechavanich, Enhanced production of raw starch degrading enzyme using agro-industrial waste mixtures by thermotolerant <i>Rhizopus microsporus</i> for raw cassava chip saccharification in ethanol production. <i>Prep. Biochem. Biotechnol.</i> ,	doi.org/10.1080/10826068.2017.1342264	published	Jul18,2017 Published
Yakushi T, Fukunari S, Kodama T, Matsutani M, Nina S, Kataoka N, Theeragool G, Matsushita K.Role of a membrane-bound aldehyde dehydrogenase complex AldFGH in acetic acid fermentation with <i>Acetobacter pasteurianus</i> SKU1108. <i>Appl Microbiol Biotechnol.</i> 102 (10): 4549–4561 (2018)	doi: 10.1007/s00253-018-8940-6.	published	Apr3,2018 Published
Kallayanee Naloka, Pattaraporn Yukphan, Kazunobu Matsushita and Gunjana Theeragool Molecular Taxonomy and Characterization of Thermotolerant <i>Komagataeibacter</i> Species for Bacterial Nanocellulose Production at High Temperatures <i>Chiang Mai J Sci.</i> 45 (4): 1610–1622 (2018)	none	published	Jul,2018 Published
Gunjana Theeragool, Nittaya Pitiwittayakul, Minenosuke Matsutani and Kazunobu Matsushita Disruption of the <i>groEL</i> Gene Revealed A Physiological Role for Chaperonin in the Thermotolerant Acetic Acid Bacterium, <i>Acetobacter pasteurianus</i> SKU1108 <i>Chiang Mai J Sci.</i> 45 (4): 1623–1633 (2018)	none	published	Jul,2018 Published
Mochamad Nurcholis, Sukanya Nitiyon, Suprayogi, Nadchanok Rodrussamee, Noppon Lertwattanasakul, Savitree Limtong, Tomoyuki Kosaka and Mamoru Yamada: Functional analysis of Mig1 and Rag5 as expressional regulators in thermotolerant yeast <i>Kluyveromyces marxianus</i> . <i>Appl. Microbiol. Biotechnol.</i> , 2018	DOI:10.1007/s00253-018-9462-y	published	Nov5,2018 Published
Tomoyuki Kosaka, Noppon Lertwattanasakul, Nadchanok Rodrussamee, Mochamad Nurcholis, Ngo Thi Phuong Dung, Chansom Keo-Oudone, Masayuki Murata, Peter Götz, Constantinos Theodoropoulos, Suprayogi, Jaya Mahar Maligan, Savitree Limtong and Mamoru Yamada: Potential of thermotolerant ethanologenic yeasts isolated from ASEAN countries and their application in high-temperature fermentation. In <i>Fuel Ethanol Production from Sugarcane</i> . Thalita Peixoto Basso and Luiz Carlos Basso (eds) ISBN: 978-1-78984-937-0 (print) 978-1-78984-937-7 (online) IntechOpen, pp121–154, 2018	DOI: 10.5772/intechopen	published	Nov5, 2018 Published
Sornsiri Pattanakittivorakul, Noppon Lertwattanasakul, Mamoru Yamada, Savitree Limtong: Selection of thermotolerant <i>Saccharomyces cerevisiae</i> for high temperature ethanol production from molasses and increasing ethanol production by strain improvement. <i>Antonie van Leeuwenhoek</i> , 2019	Doi: 10.1007/s10482-019-01230-6	published	Jan21,2019 Published
Konjanda, P., T. Yakushi, K. Matsushita and G. Theeragool. 2019. Enhanced growth and ethanol oxidation by overexpressed <i>caiA</i> gene encoding acyl-CoA dehydrogenase in <i>Komagataeibacter medellinensis</i> NBRC 3288. <i>Chiang Mai J. Sci.</i> 46 (2). 196 – 206 (2019)	none	published	Mar,2019 Published

1. 2 Original Publications (Articles by Single Team only)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)	Country name of the team
Srisakul Trakarnpaiboon, Nantana Srisuk, Kuakoon Piyachomkwan, Shang-Tian Yang, Vichien Kitpreechavanich, L-Lactic acid production from liquefied cassava starch by thermotolerant <i>Rhizopus microsporus</i> : Characterization and optimization. <i>Process Biochem.</i> , 63, 26–34, 2017	doi.org/10.1016/j.procbio.2017.08.019	published	Aug,2017 Published	Thailand
Srisakul Trakarnpaiboon, Suthasinee Praneetrattananon, Vichien Kitpreechavanich. Simultaneous Saccharification and Fermentation of L-(+)-Lactic Acid Production from Liquefied Cassava Starch by Immobilized <i>Rhizopus oryzae</i> in a 3 L Airlift Fermenter. <i>Chiang Mai J. Sci.</i> 45(1): 77–91, 2018	none	published	Jan,2018 Published	Thailand
Hirokuni Miyamoto, Hisashi Miyamoto, Yukihiro Tashiro, Kenji Sakai, Hiroaki Kodama, "Studies on highly functional fermented-products made from un-utilized biomass resources by thermophilic bacteria", <i>Journal of The Society for Biotechnology, Japan</i> , Vol.96 No.2 pp56–63, 2018	none	published	The 26th Technical Award of the Society for Biotechnology	Japan
Ming Gao, Yukihiro Tashiro, Qunhui Wang, Kenji Sakai, Kenji Sonomoto, "High acetone–butanol–ethanol production in pH–stat co–feeding of acetate and glucose ", <i>Journal of The Society for Biotechnology, Japan</i> , Vol.96 No.2 pp68, 2018	none	published	The 25th Excellent Paper Award of the Society for Biotechnology	Japan
Tao Zhao, Yukihiro Tashiro, Jin Zheng, Kenji Sakai, Kenji Sonomoto, Semi-hydrolysis with low enzyme loading leads to highly effective butanol fermentation . <i>Bioresour. Technol.</i> , 264, 335–342, 2018	doi.org/10.1016/j.biortech.2018.05.05	published	Sep,2018 Published	Japan
Matsutani M, Yakushi T. Pyrroloquinoline quinone-dependent dehydrogenases of acetic acid bacteria. <i>Appl Microbiol Biotechnol.</i> 102, pp 9531–9540, 2018	doi: 10.1007/s00253-018-9360-3.	published	Sep15,2018 Published	Japan
Rizki Fitria Darmayanti, Yukihiro Tashiro, Takuya Noguchi, Ming Gao, Kenji Sakai, Kenji Sonomoto. Novel biobutanol fermentation at a large extractant volume ratio using immobilized <i>Clostridium saccharoperbutylacetonicum</i> N1–4. <i>J. Biosci. Bioeng.</i> , 126(6) 750–757, 2018	doi.org/10.1016/j.jbiosc.2018.06.006	published	Dec,2018 Published	Japan
Misumi Y, Nishioka S, Fukuda A, Uemura T, Nakamura M, Hoshida H, Akada R., YHp as a highly stable, hyper-copy, hyper-expression plasmid constructed using a full 2– μ m circle sequence in <i>cir0</i> strains of <i>Saccharomyces cerevisiae</i> ., <i>Yeast</i> . 2018 Dec 7 [Epub ahead of print]	10.1002/yea.3371	published	Dec7,2018 Published	Japan
Tao Zhao, Kento Yasuda, Yukihiro Tashiro, Rizki Fitria Darmayanti, Kenji Sakai, Kenji Sonomoto. Semi-hydrolysate of paper pulp without pretreatment enables a consolidated fermentation system with in situ product recovery for the production of butanol. <i>Bioresour. Technol.</i> 278	doi.org/10.1016/j.biortech.2019.01.043	published	Mar,2019 Published	Japan

Lists of Achievements and Implemented Activities

2. presentations at Academic Conferences etc. (Seminars, Workshops, Symposia)

2. 1 Conference Presentations (Joint Presentations among Research Teams)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location, etc.
Aug26,2017	Oral Presentation	Toshiharu Yakushi, Naoya Kataoka, Gunjana Theeragool, and Kazunobu Matsushita, High temperature acetic acid fermentation: Molecular basis on thermotolerance and application, e-Asia JRP kick-off meeting, Bangkok
Sep4-5,2017	Guest/Invited Speaker	Gunjana Theeragool, Kallayanee Naloka, Pornchanok Taweecheep, Minenosuke Matsutani, Toshiharu Yakushi and Kazunobu Matsushita. "Improvement of Vinegar and Bacterial Nanocellulose Fermentation at High Temperatures by Adaptive Mutation."The 4th Sattelite Seminar in Core to Core Program, abstract, p16, Sep 4-5, 2017, Berlin, Germany
Sep4-5,2017	Oral Presentation	Sornsiri Pattanakittivorakul, Noppon Lertwattanasakul, Mamoru Yamada and Savitree Limtong: Effects of ethanol and sugar concentration on growth and ethanol fermentation of thermotolerant yeasts and increasing of ethanol fermentation by adaptation and mutagenesis. The 4th Sattelite Seminar in Core to Core Program, abstract, p20, Sep 4-5, 2017, Berlin, Germany
Mar17,2018	Oral Presentation	Seiya Fukunari, Minenosuke Matsutani, Toshiharu Yakushi, Naoya Kataoka, Gunjana Theeragool, Kazunobu Matsushita, "Expression of different molecular species of membrane-bound aldehyde dehydrogenases in acetic acid bacteria" The 2018 Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry, Nagoya, Japan
May26-27,2018	Oral Presentation	Megumi Ichiki, Shun Nina, Minenosuke Matsutani, Naoya Kataoka, Toshiharu Yakushi, Gunjana Theeragool, Kazunobu Matsushita, "Characterization of the membrane-bound aldehyde dehydrogenase responsible for acetic acid fermentation by Acetobacter sp." The Annual Meeting of the Japanese Biochemical science, Chu-shikoku Branch, Yonago, Japan
Sep20-21,2018	Oral Presentation	Toshiharu Yakushi, Hina Mukai, Nittaya Pitiwittayakul, Minenosuke Matsutani, Pornchanok Taweecheep, Naoya Kataoka, Gunjana Theeragool, Kazunobu Matsushita, "Characterization of the membrane-bound alcohol dehydrogenase of Komagataeibacter sp., a species of acetic acid bacteria" The 2018 Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry, Chu-shikoku Branch, Matsue, Japan
Oct23-24,2018	Oral Presentation	Suprayogi, F. K. Anggrarini Putri, Maleo, M. Nurcholis, A. Saputra, A. Muhibuddin, T. Kosaka and M. Yamada: Isolation of thermotolerant ethanol-fermenting yeasts from Indonesia and their identification and characterization. The 5 th Satellite Seminar and the 2 nd e-ASIA Joint Research Program, Program and Abstract, pp.28 Oct, 23-24, Luangprabang, Laos
Oct23-24,2018	Oral Presentation	Chansom Keo-Oudone, Mochamad Nurcholis, Noppon Lertwattanasakul, Somchanh Bounphanmy, Savitree Limtong and Mamoru Yamada: Screening and characterization of thermotolerant yeasts for ethanol fermentation with cassava starch at high temperatures. The 5th Satellite Seminar and the 2nd e-ASIA Joint Research Program, Program and Abstract, pp.27 Oct, 23-24, Luangprabang, Laos
Oct23-24,2018	Oral Presentation	Noppon Lertwattanasakul, Vipawee Najanthong, Tomoyuki Kosaka, Mamoru Yamada and Savitree Limtong: Ethanol production from sugarcane top by thermotolerant yeast Kluyveromyces marxianus DMKU 3-1042 using simultaneous saccharification and fermentation process. The 5th Satellite Seminar and the 2nd e-ASIA Joint Research Program, Program and Abstract, pp.26 Oct, 23-24, Luangprabang, Laos
Oct23-24,2018	Oral Presentation	Savitree Limtong, Thitinun Sumyai, Noppon Lertwattanasakul and Mamoru Yamada: Ethanol production from sugarcane top by newly selected xylose fermenting yeast strains. The 5th Satellite Seminar and the 2nd e-ASIA Joint Research Program, Program and Abstract, pp.13 Oct, 23-24, Luangprabang, Laos

Oct23-24,2018	Oral Presentation	Mamoru Yamada, Masayuki Murata, Tomoyuki Kosaka, Suprayogi, Sukanya Nitiyon, Chansom Keo-Oudone, Pornthap Thanonkeo, Nadchanok Rodrussamee, Noppon Lertwattanasakul and Savitree Limtong: Conversion of Biomass to ethanol by thermotolerant yeasts. The 5th Satellite Seminar and the 2nd e-ASIA Joint Research Program, Program and Abstract, pp.12 Oct, 23-24, Luangprabang, Laos
Oct23-24,2018	Oral Presentation	Toshiharu Yakushi, Nami Matsumoto, Hiromi Hattori, Minenosuke Matsutani, Chihiro Matayoshi, Hirohide Toyama, Naoya Kataoka, Kazunobu Matsushita: Thermotolerance acquired through metabolic change induced by inactivation of a transporter in <i>Gluconobacter</i> sp. strain CHM43. The 5th Satellite Seminar and the 2nd e-ASIA Joint Research Program, Program and Abstract, pp.18 Oct, 23-24, Luangprabang, Laos
Oct23-24,2018	Oral Presentation	Theeragool, G., T. Phathanathavorn, N. Naloka, T. Yakushi and K. Matsushita. Cost-effective Vinegar Fermentation at High Temperature by the Adapted Strains of <i>Acetobacter pasteurianus</i> SKU1108. The 5th Satellite Seminar and the 2nd e-ASIA Joint Research Program, Program and Abstract, pp. 29 Oct, 23-24, Luangprabang, Laos
Oct23-24,2018	Oral Presentation	Toulaphone Keokene, Chanhom Loinheuang, Toshiharu Yakushi, Gunjana Theeragool: Characterization and Identification of Acetic acid bacteria Isolated from samples collected in Lao PDR. The 5th Satellite Seminar and the 2nd e-ASIA Joint Research Program, Program and Abstract, pp. 30 Oct, 23-24, Luangprabang, Laos
Oct24,2018	Oral Presentation	Yukihiro Tashiro, Takeshi Zendo, Vichien Kitpreechavanich, Kenji Sonomoto, Kenji Sakai, Establish of efficient meta-fermentation process for optically pure lactic acid production from waste biomass using mixed culture system, The 2nd e-ASIA Joint Research Program Seminar, Louangprabang (Laos), Oct 24
Dec2-4,2018	Poster Session	Sornsiri Pattanakittivorakul, Noppon Lertwattanasakul, Mamoru Yamada and Savitree Limtong. Selection of thermotolerant strain of <i>Saccharomyces cerevisiae</i> for ethanol production from molasses at high temperature and strain improvement by adaptation and mutagenesis. Final Joint Seminar of CCP, Summary Book pp343-346, Dec Dec 2-4, 2018, Yamaguchi, Japan
Dec2-4,2018	Oral Presentation	Masayuki Murata, Isumi Kumakiri, Tomoyuki Kosaka, Pumin Nutaratat, Constantinos Theodoropoulos, Peter Gotz, Pornthap Thanonkeo, Savitree Limtong and Mamoru Yamada. Final Joint Seminar of CCP, Summary Book p335-338, Dec Dec 2-4, 2018, Yamaguchi, Japan
Dec2-4,2018	Poster Session	Suprayogi, Fika Ulan Anggrarini Putri, Maleo, Mochamad Nurcholis, Afriyagung Saputra, Anton Muhibuddin, Tomoyuki Kosaka and Mamoru Yamada: Isolation and characterization of thermotolerant yeasts isolated in Indonesia. Final Joint Seminar of CCP, Summary Book p73-76, Dec Dec 2-4, 2018, Yamaguchi, Japan
Dec2-4,2018	Oral Presentation	Mochamad Nurcholis, Suprayogi, Masayuki Murata, Minh T, Nguyen, Nadchanok Rodrussamee, Noppon Lertwattanasakul, Sukanya Nitiyon, Savitree Limtong, Tomoyuki Kosaka and Mamoru Yamada: Analysis of glucose repression mechanism in thermotolerant yeast <i>Kluyveromyces marxianus</i> , Final Joint Seminar of CCP, Summary Book p129-132, Dec 2-4, 2018, Yamaguchi, Japan
Dec2-4,2018	Oral Presentation	Noppon Lertwattanasakul, Tomoyuki Kosaka, Nadchanok Rodrussamee, Masayuki Murata, Suprayogi, Savitree Limtong and Mamoru Yamada: Complete genome sequence and transcriptome analyses of thermotolerant yeast <i>Kluyveromyces marxianus</i> DMKU 3-1042. Final Joint Seminar of CCP, Summary Book p113-116, Dec 2-4, 2018, Yamaguchi, Japan
Dec2-4,2018	Oral Presentation	Pitiwittayakul, N., P. Rattanawaree, S. Bureenok, T. Yakushi: Identification of acetic acid bacteria and assigned to the genus <i>Gluconobacter</i> by <i>groEL</i> gene sequence analysis. Final Joint Seminar of CCP, Summary Book p227-230, Dec 2-4, 2018, Yamaguchi, Japan
Dec2-4,2018	Oral Presentation	Theeragool, G., N. Naloka, P. Taweecheep, N. Pitiwittayakul, T. Keokene, T. Yakushi and K. Matsushita. Improvement of bacterial nanocellulose fermentation at high temperature by the adapted strains of thermotolerant <i>Komagataeiacet</i> . Final Joint Seminar of CCP, Summary Book p105-108, Dec 2-4, 2018, Yamaguchi, Japan
Mar24-27,2019	Oral Presentation	Nami Matsumoto, Naoki Osumi, Theerisara PHATHANATHAVORN, Riho Sueyoshi, Minenosuke Matsutani, Naoya Kataoka, Toshiharu Yakushi, Gunjana Theeragool, Yasushi Shiraishi, Kazunobu Matsushita, "Low-nutrition and thermal adaptation of <i>Acetobacter pasteurianus</i> for practical acetic acid fermentation" The 2019 Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry, Tokyo, Japan

23 Total

2. 2 Conference Presentations (by Single Team)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location etc.	Country name of the team
Jul25-28,2017	Guest/Invited Speaker	Mamoru Yamada (keynote lecture) Genomic aspects on thermotolerance, thermal adaptation and thermal stability, and application of thermotolerant microbes, the 7th FerVAAP & the 12th ABBS, Abstract p 4, July 25-28, 2017 Khon Kean, Thailand	Japan
Jul25-28,2017	Poster Session	Huynh Xuan Phong, Ngo Thi Phuong Dung, Preekamol Klanrit, Mamoru Yamada and Pornthap Thanonkeo: Ethanol production from pineapple waste by newly isolated thermotolerant yeasts, the 7th FerVAAP & the 12th ABBS, Abstract p 163, July 25-28, 2017 Khon Kean, Thailand	Japan
Jul26-27,2017	Oral Presentation	Toulaphone Keokene, Chansom Keo-oudone, Chanhom Loinheuang, Somchanh BOUNPHANMY, Manichanh SAYAVONG "Diversity of Ethanol Fermenting Yeast and InvertebratePathogenic Fungi Isolated from Lao PDR" The 7th International Conference on Fermentation Technology of Value Added Agricultural Products (Core to Core Session), Khon Kaen, Thailand, 26-27 July	Lao
Sep5,2017	Oral Presentation	Kenji Sonomoto, "Biobutanol Production with Designed Biomass on Biorefinery". The 4th Satellite Seminar, New Core to Core Program A. Advanced Research Networks on "Establishment of an international Research Core for New Bio-Research Fields with Microbes from Tropical Areas", Berlin (Germany), 2017/9/5	Japan
Sep5,2017	Oral Presentation	Yukihiro Tashiro, Kenji Sakai, "Establish of efficient meta-fermentation process for optically pure lactic acid production from waste biomass using mixed culture syste", The 4th Satellite Seminar, New Core to Core Program A. Advanced Research Networks on "Establishment of an international Research Core for New Bio-Research Fields with Microbes from Tropical Areas", Berlin (Germany), 2017/9/5	Japan
Sep11,2017	Guest/Invited Speaker	Hirokuni Miyamaoto, Hisashi Miyamoto. Yukihiro Tashiro, Kenji Sakai, Hiroaki Kodama, "Studies on highly functional fermented-products made from un-utilized biomass resources by thermophilic bacteria" The 69th Annual Meeting of the Socieity for Biotechnology, Japan, Tokyo, Japan	Japan
Sep12,2017	Poster Session	Toshiharu Mizoguchi, Shunya Kohara, Yukihiro Tashiro, Hirokuni Miyamoto, Kenji Sakai, "Reconstruction of mixed culture system for meta-fermentation production of highly-optical L-lactic acid" The 69th Annual Meeting of the Socieity for Biotechnology, Japan, Tokyo, Japan	Japan
Sep12,2017	Poster Session	Rizki Darmayanti, Takuya Noguchi, Ming Gao, Yukihiro Tashiro, Kenji Sakai, Kenji Sonomoto, 「High butanol production by immobilized extractive fermentation with large extractant volume」, The 69th Annual Meeting of the Socieity for Biotechnology, Japan, Tokyo, Japan	Japan
Sep22,2017	Oral Presentation	Rina Nakanishi, Minenosuke Matsutani, Naoya Kataoka, Toshiharu Yakushi, Kazunobu Matsushita, "Elevation of thermotolerance by supplementation with C4-dicarboxylates and amino acids in <i>Acetobacter</i> sp.", The 2017 Joint Meeting of the Japan Society for Biosceience, Biotechnology and Agrochemistry, Kansai. Chu-shikoku and Nishi-Nihon branches, Osaka, Japan	Japan
Nov9,2017	Oral Presentation	Rizki Fitria Darmayanti, Takuya Noguchi, Ming Gao, Yukihiro Tashiro, Kenji Sakai, Kenji Sonomoto, "High Butanol Production on Extractive Fermentation with Large Extractant Volume by Immobilized Cells" International Symposium on Agricultural, Food, Environmental and Life Sciences in Asia, 2017, Kyushu University, 2017/11/9	Japan
Nov9,2017	Oral Presentation	Tao Zhao, Yukihiro Tashiro, Kenji Sakai, Kenji Sonomoto, "Repeated Enzymatic Hydrolysis and Acetone-Butanol-Ethanol Fermentation of Rice Straw Using Free and Immobilized Cells of <i>Clostridium saccharoperbutylacetonicum</i> N1-4". International Symposium on Agricultural, Food, Environmental and Life Sciences in Asia, 2017, Kyushu University, 2017/11/9	Japan
Nov9,2017	Poster Session	Kento Yasuda, Tao Zhao, Yukihiro Tashiro, Kenji Sakai and Kenji Sonomoto, "Butanol Fermentation with Designed Pulp Hydrolysates by <i>Clostridium saccharoperbutylacetonicum</i> N1-4". International Symposium on Agricultural, Food, Environmental and Life Sciences in Asia, 2017, Kyushu University, 2017/11/9	Japan
Nov24,2017	Oral Presentation	Chisato Kawasaki, Hisashi Hoshida, Rinji Akada, "Development of consolidated bioprocessing using the thermotolerant yeast <i>Kluyveromyces marxianus</i> for efficient ethanol production". The 35th YEAST WORKSHOP, Takamatsu, Japan	Japan
Dec6-9,2017	Poster Session	Tatsuya Tsuzuno, Seiki Nishida, Tomoyuki Kosaka, Isamu Miyakawa, Mamoru Yamada, " Metabolic change at high temperature in thermotolerant yeast <i>Kluyveromyces marxianus</i> " The 40th the Molecular Biology Society of Japan and the 90th the Japanese Biochemical Society Joint Annual Meeting, Kobe, Japan	Japan

Dec6-9,2017	Poster Session	Mamoru Yamada, Kannikar Charoensuk, Tomoko Sakurada, Amina Tokiyama, Masayuki Murata, Tomoyuki Kosaka, "Analysis of thermotolerant genes in thermotolerant <i>Zymomonas mobilis</i> and comparison of thermotolerant genes with those of other microbes" The 40th The Molecular Biology Society of Japan and The 90th The Japanese Biochemical Society Joint Annual Meeting, Kobe, Japan	Japan
Dec8,2017	Oral Presentation	Toshiharu Yakushi, Soemphol Wichai, Phong Huynh, Naoya Kataoka, Hirohide Toyama, Kazunobu Matsushita, "Molecular maturation process of the membrane-bound heterotrimeric sorbitol dehydrogenase of <i>Gluconobacter</i> sp.: Role of the small subunit SldS" The 40th The Molecular Biology Society of Japan and The 90th the Japanese Biochemical Society Joint Annual Meeting, Kobe, Japan	Japan
Mar3,2018	Oral Presentation	Naoyuki Aso, Yasuhiro Kajimura, Nunes Catarina, Izumi Kumakiri, Kazuhiro Tanaka, Hidetoshi Kita, "Effect of operating conditions on the dehydration performance of MOR-zeolite membranes " The20th Annual Meeting of the Society of Chemical Engineers, Japan, Hiroshima, Japan	Japan
Mar3,2018	Oral Presentation	Ryutaro Kishibe, Nunes Catarina, Izumi Kumakiri, Kazuhiro Tanaka, Hidetoshi Kita, Mamoru Yamada, "Application of hydrophilic zeolite membrane for concentrating fermentation products", The20th Annual Meeting of the Society of Chemical Engineers, Japan, Hiroshima, Japan	Japan
Mar16,2018	Oral Presentation	Yukihiro Tashiro, Yuki Ishida, Tomohiro Kimura, Yuki Okugawa, Hirokuni Miyamoto, Kenji Sakai, "Meta-fermentation production of high quality chemicals using various kinds of mixed culture seeds", The 2018 Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry, Nagoya, Japan	Japan
May18,2018	Oral Presentation	Rinji Akada, Yukie Misumi, Satoko Nishioka, Mako Enokida, Natsadaphon Saeliu, Shahira S. Aly, Sanom Nonklang, Yoshinori Sekiguchi, Akira Fukuda, Takeshi Uemura, Mikiko Nakamura, Hisashi Hoshida, Red fluorescent protein as a marker for protein production and genetic engineering in yeasts, Non-conventional yeasts: from basic research to application, University of Rzeszów, Poland, 2018,5/18	Japan
Jul16,2018	Oral Presentation	Mochamad Nurcholis, Tomoyuki Kosaka, Mamoru Yamada " : Functional roles of MIG1 and RAG5 in thermotolerant yeast <i>Kluyveromyces marxianus</i> ", The 51th Lecture of the Japan Society for Bioscience, Biotechnology and Agrochemistry Chu-shikoku Branch, Yamaguchi, Japan	Japan
Sep4-7,2018	Oral Presentation	Masutani M, Matsumoto N, Yamashita T, Tatsuno M, Furukawa A, Sueyoshi R, Kataoka N, Yakushi T, and Matsushita K Natural mutation could alter the thermotolerance among closely related <i>Acetobacter pasteurianus</i> strains The 5th International Conference on Acetic Acid Bacteria, p33, Sep 4-7, 2018, Freising, Germany	Japan
Sep5,2018	Oral Presentation	Takuya Abe, Junya Hirota, Hisashi Hoshida, Rinji Akada, "Characterization of the thermotolerant-yeast <i>Kluyveromyces marxianus</i> strains for bioethanol production " The 70th Annual Meeting of the Society for Biotechnology, Japan, Osaka, Japan	Japan
Sep7,2018	Guest/Invited Speaker	Yukihiro Tashiro, Kenji Sakai, Hirokuni Miyamoto, " Fermentation production system studying on mixed culture system" The 71th Annual Meeting of the Society for Biotechnology, Japan, Osaka, Japan	Japan
Sep7,2018	Guest/Invited Speaker	Kenji Sonomoto, "Fermentation production system studying on medium, substrate, and downstream process" The 71th Annual Meeting of the Society for Biotechnology, Japan, Osaka, Japan	Japan
Sep18-20,2018	Poster Session	Ryutaro Kishibe, Izumi Kumakiri, Kazuhiro Tanaka, Hidetoshi Kita, Marino Hara, Tomoyuki Kosaka, Mamoru Yamada, " Application of zeolite membranes for a high temperature fermentation process" The 50th Autumn Meeting of the Society of Chemical Engineers, Japan, Kagoshima, Japan	Japan
Sep18-20,2018	Poster Session	Aso Naoyuki, Izumi Kumakiri, Kazuhiro Tanaka, Hidetoshi Kita, "Effect of acids on the permeation property of MOR-zeolite membranes" The 50th Autumn Meeting of the Society of Chemical Engineers, Japan, Kagoshima, Japan	Japan
Oct5-8,2018	Oral Presentation	Izumi Kumakiri, Mamoru Yamada, Javier Herguido and Miguel Menendez, "Micro-porous inorganic membranes – their unique dehydration properties and potential applications", 10th KIFEE, Norway, 2018/10/05-08	Japan
Nov17,2018	Poster Session	Takaharu Mizoguchi, Yukihiro Tashiro, Kenji Sakai, Reconstruction of a complex microbial system for producing optically pure L-lactic acid, JSBBA West 1st Student Forum, Fukuoka (Japan), 2018/11/17	Japan
Nov18-19,2018	Oral Presentation	Ryutaro Kishibe, Izumi Kumakiri, Kazuhiro Tanaka, Hidetoshi Kita, Marino Hara, Tomonori Kosaka and Mamoru Yamada, "Dehydration zeolite membranes applied to concentration ethanol produced by a high temperature fermentation", The 13th Young Scientist Seminar, Yamaguchi, Japan 2018/11/18-19	Japan
Dec1,2018	Oral Presentation	Toshiharu Mizoguchi, Yukihiro Tashiro, Kenji Sakai, "Reconstruction of meta-fermentation process for production of optically active L-lactic acid and functional analysis of bacteria " The 25th Annual Meeting of the Society for Biotechnology, Japan, Kyushu Branch, Kagoshima, Japan	Japan

Dec13,2018	Guest/Invited Speaker	Mamoru Yamada, "Bioethanol production by next-generation fermentation technology-Ethanol conversion of raw garbage and paper waste using thermotolerant microorganisms-, "Biomass / waste clothes / technology for utilization of ethanol derived from waste materials and power generation" Seminar, Tokyo, Japan	Japan
Dec18-20,2018	Poster Session	Yusuke Maruo, Yasuhiro Kajimura, Izumi, Kazuhiro Tanaka, Hidetoshi Kita, " Influence of synthesis conditions on the dehydration performance of MOR membranes", The 28th Annual Meeting of MRS-J, Kokura, Japan	Japan
Dec18-20,2018	Poster Session	Naoyuki Aso, Izumi, Kazuhiro Tanaka, Hidetoshi Kita, "Application of MOR-zeolite membranes in separation of acid solutions", The 28th Annual Meeting of MRS-J, Kokura, Japan	Japan
Dec18-20,2018	Poster Session	Takashi Myojin, Nunes Catarina, Izumi Kumakiri, Kazuhiro Tanaka, Hidetoshi Kita, "Influence of acids on the permeation property of MFI membranes" The 28th Annual Meeting of MRS-J, Kokura, Japan	Japan
Mar24-27,2019	Oral Presentation	Hiroki Agari, Rina Nakanishi, Nami Matsumoto, Naoya Kataoka, Toshiharu Yakushi, Kazunobu Matsushita, "Thermotolerant mechanism in experimentally evolved Acetobacter pasteurianus TH-3: relation with the cell surface and amino acid metabolism" The 2019 Annual Meeting of the Society for Bioscience, Biotechnology and Agrochemistry, Tokyo, Japan	Japan
Mar24-27,2019	Oral Presentation	Yukihiro Tashiro, Takaharu Mizoguchi, Shunya Kohara, Hirokuni Miyamoto, Kenji Sakai, "Reconstruction of meta-fermentation process using isolated strains for production of optically active L-lactic acid" The 2019 Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry, Tokyo, Japan	Japan

37	Total
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Lists of Achievements and Implemented Activities

3. Workshops, Seminars, Symposia and Other Events (Organized by the Project)

Event duration	Name of Organizer	Title of the Event	Location (Country, City, Venue)	Number of Participants (Including Team Members)	Overview
August 26, 2017	Gunjana Theeragool, Mamoru Yamada	e-ASIA kick off meeting	Thailand, Bangkok, Centara Grand Hotel	8	In the first meeting of this program, representatives of each WP gave a presentation focusing on the research plan. It was held by the NRCT support as a session of Thailand Research EXPO2017.
October 30, 2017	Toshiharu Yakushi	The 4th Seminar of Priority Universities for Cooperation in Japan	Yamaguchi, Yamaguchi University	40	A symposium was held at Yamaguchi Univ to have presentations including collaborative researches. Six researchers from Kasetsart Univ and Chulalongkorn Univ that Yamaguchi Univ has set as a priority university were invited.
Nov 18–19, 2017	Toshiharu Yakushi	The 13th Young Scientist Seminar Seminar	Yamaguchi, Yamaguchi Prefectural Seminar Park	100	This seminar was organized by graduate course students, including foreign students from Yamaguchi Univ. Students from Southeast Asia and young researchers (half of the participants were foreigners) gathered and reported on research results. In addition, there were several lectures by experienced researchers as invited talks.
March 6, 2018	Mamoru Yamada	The 14th Young Scientist Seminar Seminar	Yamaguchi, Yamaguchi University	40	This seminar was organized by graduate course students, including foreign students from Yamaguchi Univ. Students from Southeast Asia and young researchers (half of the participants were foreigners) gathered and reported on research results. In addition, there were two lectures by experienced researchers as invited speakers.
October 24, 2018	Somchanh Bounphanmy, Mamoru Yamada	e-ASIA JRP Seminar	Lao, Luangprabang	40	In the second meeting of this program, there were presentations mainly on the research results by foreign researchers. In addition, Satellite Seminar of the Core-to-Core Program was held the day before, and related researches were presented there as well.
Nov 13–14, 2018	Mamoru Yamada	The 15th Young Scientist Seminar Seminar	Yamaguchi, Yamaguchi Prefectural Seminar Park	100	This seminar was organized by graduate course students, including foreign students from Yamaguchi Univ. Students from Southeast Asia and young researchers gathered and reported on research results. In addition, there were several lectures by experienced researchers as invited talks.
Dec 2–4, 2018	Mamoru Yamada	Final Joint Seminar of Core-to-Core Program	Yamaguchi, Yamaguchi University	150	Yamaguchi Univ and universities in 5 countries as core universities held the final joint seminar of the international core program, in which the research results of this program were presented as oral and poster.
March 4, 2019	Hisashi Hoshida	10th YU-RCTMR symposium and ALCA/JST Workshop The International Symposium on Cellular Responses, Adaptation and Fermentation in Stress Environments	Yamaguchi, Yamaguchi University	50	The international symposium was held with a financial support by JST-ALCA. The symposium was organized by Thermotolerant Microbial resources Center, Yamaguchi University. Three invited speakers from Belgium, China and RIKEN, and two speakers from the center gave talks about thermotolerance, stress tolerance, high-temperature fermentation and strain development with plant and microorganisms including algae.

8 Total

Lists of Achievements and Implemented Activities

4. Record of Research Exchanges

Date of Departure	Date of Return	Last Name & First Name	Country of Affiliation	Affiliation	Position	Exchange Destination	Description of Exchange Content/Purpose	Duration of Exchange
2017/7/25	2017/7/29	Mamoru Yamada	Japan	Yamaguchi University	Professor	Thailand, Khon Kaen University	Conference	5
2017/8/22	2017/8/27	Mamoru Yamada	Japan	Yamaguchi University	Professor	Thailand, Bangkok, NRCT	Symposium, Meeting	6
2017/8/25	2017/8/27	Toshiharu Yakushi	Japan	Yamaguchi University	AssociateProfessor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2017/8/25	2017/8/27	Kenji Sakai	Japan	Kyushu University	Professor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2017/8/25	2017/8/27	Izumi Kumakiri	Japan	Yamaguchi University	AssociateProfessor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2017/8/25	2017/8/27	Hisashi Hoshida	Japan	Yamaguchi University	AssociateProfessor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2017/9/3	2017/9/7	Mamoru Yamada	Japan	Yamaguchi University	Professor	Germany, Berlin, Beuth University of Applied Sciences	Symposium, Meeting	5
2017/9/3	2017/9/7	Naoya Kataoka	Japan	Yamaguchi University	Assistant Professor	Germany, Berlin, Beuth University of Applied Sciences	Symposium, Meeting	5
2017/9/3	2017/9/7	Kenji Sonomoto	Japan	Kyushu University	Professor	Germany, Berlin, Beuth University of Applied Sciences	Symposium, Meeting	5
2017/9/3	2017/9/7	Yukihiro Tashiro	Japan	Kyushu University	AssociateProfessor	Germany, Berlin, Beuth University of Applied Sciences	Symposium, Meeting	5
2018/2/26	2018/3/2	Izumi Kumakiri	Japan	Yamaguchi University	AssociateProfessor	Thailand, Nakhon Pathom, Silpakorn University	Meeting, Lecture	5
2018/2/26	2018/3/2	Naoyuki Aso	Japan	Yamaguchi University	Student	Thailand, Nakhon Pathom, Silpakorn University	Meeting	5
2018/2/26	2018/3/2	Ryutaro Kishibe	Japan	Yamaguchi University	Student	Thailand, Nakhon Pathom, Silpakorn University	Meeting	5
2018/9/25	2018/10/13	Takuya Abe	Japan	Yamaguchi University	Student	Thailand, Ubon Ratchathani University	Joint Research	19
2018/10/22	2018/10/26	Mamoru Yamada	Japan	Yamaguchi University	Professor	Lao, Luang Prabang	Symposium, Meeting	5
2018/10/22	2018/10/26	Toshiharu Yakushi	Japan	Yamaguchi University	Professor	Lao, Luang Prabang	Symposium, Meeting	5

2018/10/22	2018/10/26	Yukihiro Tahisro	Japan	Kyushu University	AssociateProfessor	Lao, Luang Prabang	Symposium, Meeting	5
2018/10/22	2018/10/26	Izumi Kumakiri	Japan	Yamaguchi University	AssociateProfessor	Lao, Luang Prabang	Symposium, Meeting	5
2018/10/22	2018/10/26	Hisashi Hoshida	Japan	Yamaguchi University	AssociateProfessor	Lao, Luang Prabang	Symposium, Meeting	5
2019/3/17	2019/3/19	Mamoru Yamada	Japan	Yamaguchi University	Professor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2019/3/17	2019/3/19	Toshiharu Yakushi	Japan	Yamaguchi University	Professor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2017/6/1	2017/7/31	Noppon Lertwattanasakul	Thailand	Kastsart University	Assistant Professor	Japan,Yamaguchi University	Joint Research	61
2017/6/26	2017/8/25	Keo-Oudone Chansom	Lao	National University of Laos	Lecturer	Japan,Yamaguchi University	Joint Research	61
2017/8/1	2017/8/30	Toulaphone Keokene	Lao	National University of Laos	Lecturer	Japan,Yamaguchi University	Joint Research	30
2017/8/25	2017/8/27	Anton Muhibuddin	Indonesia	Brawijaya University	Assistant Professor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2017/8/25	2017/8/27	Somchanh Bounphanmy	Lao	National University of Laos	AssociateProfessor	Thailand, Bangkok, NRCT	Symposium, Meeting	3
2017/9/3	2017/9/7	Gunjana Theeragool	Thailand	Kastsart University	AssociateProfessor	Germany, Berlin, Beuth University of Applied Sciences	Symposium, Meeting	5
2017/9/3	2017/9/7	Savitree Limtong	Thailand	Kastsart University	Professor	Germany, Berlin, Beuth University of Applied Sciences	Symposium, Meeting	5
2017/10/16	2017/12/15	Suprayogi	Indonesia	Brawijaya University	Lecturer	Japan,Yamaguchi University	Joint Research	61
2017/10/16	2017/12/15	Jaya Mahar	Indonesia	Brawijaya University	Lecturer	Japan,Yamaguchi University	Joint Research	61
2017/10/29	2017/10/31	Gunjana Theeragool	Thailand	Kastsart University	AssociateProfessor	Japan,Yamaguchi University	Meeting	3
2017/12/5	2017/12/8	Anton Muhibuddin	Indonesia	Brawijaya University	Assistant Professor	Japan,Yamaguchi University	Meeting	4
2018/2/11	2018/3/12	Toulaphone Keokene	Lao	National University of Laos	Lecturer	Thailand, Bangkok, Kasetsart University	Joint Research	30
2018/3/1	2018/3/31	Keo-Oudone Chansom	Lao	National University of Laos	Lecturer	Japan,Yamaguchi University	Joint Research	31
2018/4/1	2018/5/28	Keo-Oudone Chansom	Lao	National University of Laos	Lecturer	Japan,Yamaguchi University	Joint Research	58

2018/7/24	2018/9/20	Toulaphone Keokene	Lao	National University of Laos	Lecturer	Japan,Yamaguchi University	Joint Research	59
2018/10/22	2018/10/25	Savitree Limtong	Thailand	Kastsart University	Professor	Lao, Luang Prabang	Seminar, Meeting	4
2018/10/22	2018/10/25	Gunjana Theeragool	Thailand	Kastsart University	AssociateProfessor	Lao, Luang Prabang	Seminar, Meeting	4
2018/10/22	2018/10/25	Suprayogi	Indonesia	Brawijaya University	Lecturer	Lao, Luang Prabang	Seminar, Meeting	4
2018/11/6	2018/12/7	Suprayogi	Indonesia	Brawijaya University	Lecturer	Japan,Yamaguchi University	Seminar, Meeting	32
2018/11/30	2018/12/5	Somchanh Bounphanmy	Lao	National University of Laos	AssociateProfessor	Japan,Yamaguchi University	Seminar, Meeting	6
2018/12/1	2019/1/3	Noppon Lertwattanasakul	Thailand	Kastsart University	Assistant Professor	Japan,Yamaguchi University	Seminar, Meeting	34
2018/12/1	2018/12/5	Savitree Limtong	Thailand	Kastsart University	Professor	Japan,Yamaguchi University	Seminar, Meeting	5
2018/12/1	2018/12/5	Gunjana Theeragool	Thailand	Kastsart University	AssociateProfessor	Japan,Yamaguchi University	Seminar, Meeting	5
2018/12/6	2018/12/10	Anton Muhibuddin	Indonesia	Brawijaya University	Professor	Japan,Yamaguchi University	Meeting	5
2019/3/4	2019/3/31	Keo-Oudone Chansom	Lao	National University of Laos	Lecturer	Japan,Yamaguchi University	Joint Research	28
2019/3/24	2019/3/30	Kiatkittipong Worapon	Thailand	Silpakorn University	Assistant Professor	Japan, Yamaguchi university	Symposium, Joint Research	7
Total (Person)					47	Total (Person-day)		717

Lists of Achievements and Implemented Activities

5. Patent Applications

5. 1 Independent Applications by Single Team

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)	Country Name of the Team
2020-37036	Method for producing ethanol-producing yeast having heat resistance and resistance to culture stress	2020.3.4	Yamaguchi University		Mamoru Yamada, Tomoyuki Kosaka	Japan		

1

Total (Number of Application)

0

Total (Number of Registration)

5. 2 Joint Applications

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)

0

Total (Number of Application)

0

Total (Number of Registration)

Lists of Achievements and Implemented Activities

6. Awards

Date of Award	Name of Award	Recipient	Remarks	Country Name of the Team
June 2, 2017	Research Encouragement Award, Amano Enzyme	Toshiharu Yakushi		Japan
September 11, 2017	The 26th Technical Award of the Society for Biotechnology	Kenji Sakai, Yukihiro Tashiro and others		Japan
September 11, 2017	The 25th Excellent Paper Award of the Society for Biotechnology	Kenji Sonomoto, Kenji Sakai, Yukihiro Tashiro and others		Japan
September 12, 2017	The 69th Topics Award of the Society for Biotechnology	Naoya Kataoka		Japan
October 4, 2017	Kasetsart University Award for International Publication	Gunjana Theeragool		Thailand
March 9, 2018	Kasetsart University, Faculty of Science Award for International Publication	Gunjana Theeragool		Thailand

6	Total
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