The 16th Young Scientist Seminar

Yamaguchi, Japan

12th – 13th October 2019

Invitation

On behalf of the Organizing Committee, we are pleased to invite you to the 16th Young Scientist Seminar (YSS) in Yamaguchi, Japan. This seminar will be held on 12th – 13th Oct 2019. The YSS aims to establish international network among young researchers including students, to broaden their knowledge about recent developments in scientific field around the world.

Venue

The 16th YSS will be held at the Yamaguchi-ken Seminar Park, Yamaguchi, Japan. This is a prefectural facility to provide a wonderful environment to meet with colleagues in a relaxing atmosphere.

Yamaguchi prefecture is located in the westernmost tip of Honshu island, the 2nd most populous island in the world. Because of its geographical location and ocean current, it has long had cultural exchanges together ASEAN areas.

In addition, Yamaguchi Prefecture has become the stage of the turning point which makes a big change in the history of Japan many times. Yamaguchi city is situated in the center of the prefecture. It has been long called "Kyoto of the West" due to its cultural similarities with Kyoto, the capital of Japan in the 14th century.

The temperature in October ranges from 12°C in the morning to 23°C in the afternoon.

Organization Committe

General manager	Hideya Saeki
Financial manager	Shota Yuda, Sayaka Kanameda
Transportation	Riku Motohashi
Audio visual and placement	Masato Fukushima
Abstract and Registration	Hiroki Kikuta
Accounting Clerk	Satoshi Ebe, Shohei Noguchi
Public Relations	Takahiro Ono

Sessions

The scientific program is composed of plenary, parallel and discussion session

Scope

The scientific scope of the seminar follows most of the well received features of the previous events not only in the area of utilization of tropical bioresources but also in the biological field.

Advisory Committee

Dean, Prof. Dr. Jun KOBAYASHI
Prof. Dr. Mamoru YAMADA
Prof. Dr. Shinichi ITO
Prof. Dr. Masayoshi SHIGYO
Prof. Dr. Tsuyoshi IMAI
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Assist. Prof. Dr. Tomoyuki KOSAKA
Assist. Prof. Dr. Kazunori SASAKI
Assist. Prof. Dr. Naoya KATAOKA
Ms. Naoko MIYAJI

Language of the Seminar

The official language of the Seminar is English and no translation facilities are available.

Seminar Theme

Establishment of international research network for tropical bioresources and their utilization

Social Program

An icebreaker party will be taken place in the evening of the 12th Oct, 2019

Insurance

All delegates are advised to take out their own health and life insurance for the duration of the Seminar.

CONTENTS

Seminar Program

October 12th

7:30 Gather at Faculty of Agriculture, Yamaguchi University.
8:00 Move from Yamaguchi University by bus
8:30 Move from Shin-Yamaguchi Station by bus

9:00 Registration(Room: Seminar room 1)

Session I [Room:Audiorium] **Open Ceremony**

9:45 Brief Introduction
Mamoru Yamada

9:55 The 1st Keynote Lecture (Chairman: Hideya Saeki)
Assist Prof Dr. Sureewan Sittijunda
(Mahiidol University, Thailand)

"Development 1,3-propanediol production from crude glycerol derived from biodiesel production process"

11:00 Coffee break

11:15 Presentation of the 1st Invited speaker
(Chairman: Toshiharu Yakushi)
Dr. Nittaya Pitiwittayakul
(Rajamangala University of Technology, Thailand)
"Isolation and characterization of endophyticbacteria isolated from sugarcane and their plant growth promoting activities"

11:50 Lunch

Session II

13:30 **Group discussion** (Oral presentation of all young scientists)
Group 1(Room:201)
Group 2(Room:202)
Group 3(Room:203)
Group 4(Room:218)
Group 5(Room:219)
Group 6(Room:220)

17:30 **Dinner and Mixer**

October 13th

Breakfast (7:30-8:30)
Cloak room is open at 8:00-8:50 (Seminar room 1)

Session III

9:00 **Presentation of selected young scientist (I)**
(Chairman:)
3 young scientists, who are selected from 1-3 group
in group discussion session

9:50 Coffee break

10:10 **Presentation of selected young scientist (II)**
(Chairman:)
3 young scientists, who are selected from 4-6 group
in group discussion session

11:10 Presentation of the 2nd Invited speaker
(Chairman:)
Assist. Prof. Dr. Kazunori Sasaki
(Yamaguchi University, Japan)
" "

11:45 Lunch

Session IV

13:00 Presentation of the 3nd Invited speaker
(Chairman:)
Assoc. prof. Dr. Koichi Sugimoto
(Yamaguchi University, Japan)
"Natural variations in defense responses among
domesticated and wild tomatoes"

14:00 Coffee break

14:15 **Closing Ceremony**

14:35 Commemorative Photo

14:45 Move to Shin-Yamaguchi station and Yamaguchi University by bus
Move to individual final destination

Keynote

Invited speaker

Presentation

Group 1

Group 3

Group 4

Histone gene analysis with a plasmid that is able to use for gene expression in yeast and human culture cells

Takahiro Ono, Hisashi Hosida and Rinji Akada

Development 1,3-propanediol production from crude glycerol derived from biodiesel production process

Sureewan Sitijunda[1], Alissara Reungsang[2,3]

[1] Faculty of Environment and Resource Studies, Mahidol University, Nakhon Pathom 73170, Thailand
[2] Biotechnology program, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand
[3] Research Group of Development of Microbial Hydrogen Production Process from Biomass, Khon Kaen University, Khon Kaen 40002, Thailand

The ultimate goal of this research is to utilize crude glycerol derived from biodiesel production process to produce 1,3-propanediol (1,3-PD) by pure culture and mixed cultures. Firstly, 1,3-PD producing bacteria was isolated from up-flow anaerobic sludge blanket (UASB) granules and identified as *Enterobacter* sp. MU-01 by 16s rDNA gene sequence analysis. Response surface methodology with Box-Behnken design was applied to identify optimum culture conditions for 1,3-PD production from crude glycerol by strain MU-01. The optimum conditions were crude glycerol concentration of 10 g/L, yeast extract concentration of 1 g/L, and initial pH of 8. Under the optimum conditions, the maximum 1,3-PD production of 0.70 g/L was achieved. Secondly, the optimization of culture compositions for maximize 1,3-PD production from crude glycerol by anaerobic mixed cultures was investigated using RSM with Box-Behnken design. Endo-nutrient addition strongly influenced 1,3-PD production with a maximum 1,3-PD concentration of 33.72 g/L achieved at 60.18 g/L of crude glycerol, 2.73 g/L of yeast extract, 205.51 mg/L of Na_2HPO_4, and 0.60 ml/L of Endo-nutrient. A verification experiment determined maximum concentration of 1,3-PD and glycerol consumption at 30.30 g/L and 70.61%, respectively, confirming the validity of the predicted model. Microbial community analysis by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) revealed predominant 1,3-PD producing bacteria in the fermentation broth at optimum condition as *Enterobacter* sp. and *Citrobacter* sp. Despite the fact that 1,3-PD production using anaerobic mixed culture as the inoculum gave a higher than 1,3-PD production using pure cultures, therefore, the up-scale production of 1,3-PD was investigated using anaerobic mixed cultures as the inoculum. The optimal organic loading rate (OLR) for 1,3-PD production was 62.5 g/L d. Under the optimum OLR, a maximum 1,3-PD production, and 1,3-PD yield of 4.23 g/L and 0.30 mol/mol were achieved. Analysis of microbial community by PCR-DGGE indicated that the predominant 1,3-PD producers in the continuous system were *Enterobacter* sp., and *Klebsiella* sp.

Isolation and characterization of endophytic bacteria isolated from sugarcane and their plant growth promoting activities

Nittaya Pitiwittayakul[1], Toshiharu Yakushi[2], Gunjana Theeragool[3] and Somboon Tanasupawat[4]

[1]Department of Agricultural Technology and Environment, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima campus, Nakhon Ratchasima 30000, Thailand

[2]Department of Agriculture, Graduate School of Science and Technology for Innovation, Yamaguchi University, Yamaguchi 753-8515, Japan

[3]Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

[4]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

Endophytic bacteria are widely studied because of their plant growth promoting benefits. In this study, two hundred and thirty-eight endophytic bacteria were isolated from sugarcane roots and stems in Nakhon-Ratchasima province. Two kinds of medium; Tryptic Soy Agar (TSA) and LGI were used for endophytic bacterial isolation. One hundred and three isolates were enumerated by using TSA medium. Most of the cells were rod shape showing Gram-negative except thirty-six isolates were Gram-positive. Based on LGI medium isolation, all one hundred and thirty-five bacterial isolates were Gram-negative, short rod or regular rod shape. Endophytic bacteria were obtained from both root and stem interior of sugarcane. Among two hundred and thirty-eight isolates, ninety-seven isolates were evaluated the ability of IAA production. Only twenty-two isolates produced IAA more than 50 mg/L in the presence of the precursor 0.1% tryptophan. The maximum amount of IAA (187.33 mg/L) was produced by the isolate KSS 2. In addition, the nitrogen-fixing capacity of these endophytic bacteria were screened by analysis of ammonia synthesis with Nessler's reagent. From sixty-nine tested isolates, thirty-six isolates showed significantly color change from pale yellow to dark brown so they were able to fix nitrogen. Furthermore, nineteen endophytic bacterial isolates were screened for phosphate and zinc solubilizing ability. The tested bacteria were grown on Pikovskaya medium (PKV) for phosphate solubilization. Only eleven isolates showed the visible phosphate solubilizing ring and the isolate DKT 8 showed the highest phosphate solubilizing ability. Almost all of the tested isolates except K 5 were able to solubilize ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ (insoluble) in Tris-minimal medium. To understand the antagonistic potential, an *in vitro* antagonistic assay was performed to characterize and identify strains that were antagonistic effect to the plant pathogen *Fusarium moniliforme.* From the screening results, twenty-five endophytic bacterial isolates from one hundred and eighty-one tested isolates showed the antagonistic potential with more than 30% inhibition and ranged from 31.15-75.94% inhibition. The isolate CPK 24 showed the maximum mycelial inhibition. Some of endophytic bacteria were identified based on their 16S rRNA gene sequences, they were identified as *Nguyenibacter vanlangensis, Neoasaia chiangmaiensis*, *Mixta intestinalis*, *Enterobacter hormaechei*, *Klebsiella variicola*, *Asaia bogorensis, Paraburkholderia tropica, Bacillus zhangzhouensis* and *Staphylococcus gallinarum.* Finally, the potential isolates will be selected for the assay of growth promotion of sugarcane plantlets by tissue culture experiments. In conclusion, this research will provide the beneficial endophytic diazotrophic bacteria as the plant growth promoting bacteria that will be successfully used to control the phytopathogen and to promote the growth and yield of sugarcane.

Benefits of simultaneous saccharification and fermentation by thermotolerant yeast *Kluyveromyces marxianus*

Marino Hara[1], Pumin Nutaratat[2], Lertwattanasakul Noppon [2], Masayuki Murata[1], Tomoyuki Kosaka[1,3] and Mamoru Yamada[1,3]
[1]*Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan*
[2] *Faculty of Science, Kasetsart University, Thailand*
[3]*Faculty of Agriculture, Yamaguchi University, Japan*

High-temperature fermentation (HTF) with thermotolerant microbes is thought to have several advantages, such as reduction of cooling cost or saving water during the fermentation process (1, 2). It also allows us to save the cost of hydrolyzing enzymes of biomass when simultaneous saccharification and fermentation (SSF) is performed. Especially, cellulases for cellulosic biomass are expensive, and one of cellulases, cellobiohydrolase suffers from the end product inhibition. Thus, it is expected that HTF is suitable for conversion of cellulosic biomass to useful materials. However, the suitability or benefit of HTF for utilization of cellulosic biomass has not been examined. For efficient and stable HTF of ethanol, thermotolerant ethanologenic microbes are essential. We thus focused on yeast *Kluyveromyces marxianus*, which is able not only to produce ethanol at a high temperature and also to assimilate a wide variety of sugars including xylose (3). These characteristics are suitable for HTF with cellulosic biomass. In this study, in order to obtain fundamental data of SSF at high temperature, we performed with rice and regenerated pulp as a biomass.

First, to examine the effect of temperature on growth, *K. marxianus* was grown at different temperatures, 30 °C -45 °C, and the growth rate at the exponential phase was compared. The highest growth rate was found at 39 °C. SSF at high temperature was then carried out with rice as a biomass in a 10-L scale, and the fermentation efficiency was compared with that of separate hydrolysis and fermentation (SHF). Ethanol produced by both SSF and SHF was recovered and concentrated under a low pressure at high temperature. As a result, the ethanol recovery rate of SSF was almost the same as that of SHF, suggesting that SSF has the merit to reduce hydrolysis time in SHF. Moreover, comparison of ethanol productivity at different temperatures revealed that the productivity fell gradually down from 43 °C. It was found to take a long time for the saccharification of pulp compared to that of rice. Therefore, it is expected that the time of hydrolysis and fermentation can be reduced by SHF at high temperature.

References

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2) Kosaka T *et al*., Potential of thermotolerant ethanologenic yeasts isolated from ASEAN countries and their application in high-temperature fermentation. *In* Fuel Ethanol Production from Sugarcane. Thalita Peixoto Basso and Luiz Carlos Basso (eds) ISBN: 978-1-78984-937-0 (print) 978-1-78984-937-7 (online) IntechOpen, pp121-154 (Feb, 2019)
3) Lertwattanasakul N *et al*., Genetic basis of the highly efficient yeast *Kluyveromyces marxianus*: complete genome sequence and transcriptome analyses. *Biotechnol. Biofuels*, 8:47. doi: 10. 1186/s13068-015-0227-x (2015)

Investigation of thermotolerance in *Escherichia coli* growing by different catabolic metabolism

Hikaru Saito[1], Mamoru Yamada[2],and Tomoyuki kosaka[2]
[1]*Faculty of Agriculture, Yamaguchi University, Japan*
[2]*Graduate School of Sciences and Technology of Innovation, Yamaguchi University, Japan*

According to previous researchs on genes involved in thermotolerance in several mesophilic microorganisms, energy metabolism, membrane stabilization, transporters, DNA repair and modification, tRNA and rRNA modification, protein quality control, translation control, cell division, and transcriptional regulation have been shown to be important for microbial thermotolerance. On the other hand, in *Escherichia coli*, the heat shock gene GroEL, which is an essential gene at the high temperature limit, was revealed to help survival as other chaperons, DnaK and DnaJ. In consequence, the consumption of intracellular energy by the chaperone is probably happen in *E. coli* at high-temperature limit for survival, suggesting that energy metabolism is important for thermotolerance. However, the relationship between energy metabolism and thermotolerance has not been investigated in *E. coli*. To clarify how the thermotolerance of *E. coli* changes depending on the oxygen concentration of cultures, which relates to the energy metabolism, it was examined that the growth of *E. coli* BW25113 under aerobic and anaerobic conditions using M9 minimum medium with glucose as substrate at around 42°C to 47°C. No growth was observed over 45°C under a shaking condition, however, under an aerobic conditions, growth was observed at 45°C, and even at 46°C and 47°C. These results suggest that oxygen concentration may affect the high-temperature growth limit of *E. coli.*

Membrane-bound aldehyde dehydrogenase activity observation in *Frateuria* sp.

Miah Roni[1], Minenosuke Matsutani[1], Takeru Murate[1], Naoya Kataoka[1,2,3], Kazunobu Matsushita[1,2,3], Toshiharu Yakushi[1,2,3]

[1]Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Japan; [2]Faculty of Agriculture, Yamaguchi University, Japan; [3]Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Japan.

Acetic acid fermentation is carried out by two-step oxidation of ethanol to acetic acid with the membrane-bound alcohol and aldehyde dehydrogenases (ADH and ALDH) of acetic acid bacteria. Both the enzymes oxidize substrates by coupling reduction of membranous ubiquinone (Q) that connects to the respiratory chain. The genus *Frateuria* that is taxonomically distant from acetic acid bacteria has ADH. There is no detail report on structures and the chemical nature of ALDH enzymes of *F. aurantia*.

This study aimed at purification and characterization of ALDH of *Frateuria aurantia*. *Acetobacter* sp. has two different ALDH genes *aldFGH* and *aldSLC* that phylogenetically different from each other (Yakushi et al., 2018). We retrieved ALDH homologs from the genome data open to public and examined their phylogenetic relationships. According to the phylogenetic analyses, we proposed four subgroups for ALDH homologs: *aldFGH*, *aldSLC1*, *aldSLC2*, *aldXYZ*. *F. aurantia* have three different ALDH gene cluster (*aldFGH, aldXYZ and aldSLC*).

The growth of the *F. aurantia* strain was strongly inhibited by ethanol even at the concentrations of 0.06% (v/v). Specific activity of ALDH and ADH was 0.9 U/mg and 0.7 U/mg, respectively in the membrane suspension when cultured without ethanol. Addition of ethanol did not dramatically increase the both activities. ALDH was stable against heat treatment up to 50°C where ADH lost its activity in the membrane suspension. These data clearly indicate that the LMG1558 strain produces ALDH of independent molecular species from ADH. We attempted to purify ALDH to characterize the properties. ALDH was solubilized with Mydol-10 or Mydol-12 and 0.3 M KCl.

Yakushi et al. (2018), Appl Microbiol Biotechnol., 102: 4549.

Study of Physical, Chemical, and Mineral Properties of Tropical Red Soil from Quaternary and Tertiary Volcanic Parent Materials

Eka Widiawati Wijaya Kusuma[1,2], Djoko Mulyanto[2], and AZ Purwono Budi Santoso[2]
[1]Department of Soil Science, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia, [2]Study Program of Soil Science, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta, Indonesia

The warm and wet tropics climate accelerate the soil genesis process. According to Redness Rating, the redder soil color the more weathered soil, red soil can be one of the indicators of high soil weathering. This study aims to determine soil weathering stage of red soil developed from different volcanic rock age in tropical climate. We analyzed physical, chemical, and mineral properties of tropical red soils that developed from Quaternary and Tertiary volcanic parent material in Central Java and Special Region of Yogyakarta, Indonesia.

The soil profile was determinated by purposive method using the Indonesia Geological Map and the Indonesia Soil Map. Soil sampling location of the Quaternary red soil pedon was in Tugu Village, Jumantono Sub-district, Karanganyar Regency, Central Java, Indonesia and soil sampling location of the Tertiary red soil pedon was in Nglanggeran Village, Patuk Sub-district, Gunungkidul Regency, Special Region of Yogyakarta, Indonesia. Parameters include physical properties (soil color, texture 10 fractions, bulk density, particle density, and porosity), chemical properties (Al and Fe total, Fe dithionite, Fe oxalate, Fe pyrophosphate, C-Organic, pH H_2O, pH KCl, pH K_2SO_4, exchangeable bases, CEC, and bases saturation), and mineral properties (thin incision of rocks and sand fraction analysis).

Based on results, both red soil pedon have similar physical properties especially texture. The value of exchangeable bases, CEC, bases saturation, Al total and Fe total of the Tertiary volcanic red soil pedon is relatively higher than Quaternary volcanic red soil pedon. Both pedon are developed from andesitic volcanic rock with the Tertiary volcanic red soil pedon develops further. According to Soil Taxonomy USDA 2014, the Quaternary volcanic red soil is Typic Dystrudept subgroup and the Tertiary volcanic red soil is Typic Eutrudept subgroup.

Isolation and characterization of insoluble mineral solubilization fungi from soil in northern Thailand

Surapong Khuna[1,2], Nakarin Suwannarach[1,2], Jaturong Kumla[1,2], Kenji Matsui[3], Saisamorn Lumyong[1,2]
[1]Faculty of Science, Chiang Mai Univ., [2]Center of Excellence in Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai Univ., [3]Faculty of Agriculture, Yamaguchi Univ.

Mineral nutrients are important for the plant growth and crop productions. Only 1 to 5% mineral nutrients in soil are in a soluble and plant-available form. In nature, the mineral solubilizing microorganisms (e.g. bacteria, actinomycetes, yeasts and filamentous fungi) play an important role in supplying soluble mineral to plants by the solubilizing of insoluble minerals in soil. Moreover, they also promote plant growth by production of phytohormones, antibiotics, siderophores and plant disease control ability. The objectives of this study are to isolate and screen the mineral solubilizing fungi from rhizosphere soil in some agricultural areas in northern Thailand. Among three sampling sites in this study, the highest positive colony was found from the soil sample collected from site 1 as 55.00 CFU/g following by site 2 and 3 as 17.00 and 15.67 CFU/g, respectively. The obtained minerals solubilizing fungi were evaluated for their plant growth promoting and insoluble metal minerals solubilizing abilities. Four fungi, *Apophysomyces thailandensis* SDBR-CMUS26, *Aspergillus chiangmaiensis* SDBR-CMUI4, *Aspergillus pseudopiperis* SDBR-CMUI1 and *Aspergillus pseudotubingensis* SDBR-CMUO2 showed the highest solubilization ability on basal medium with addition of each insoluble metal minerals including $Ca_3(PO_4)_2$, $CaCO_3$, $CuCO_3$•$Cu(OH)_2$, CuO, $CoCO_3$, $FePO_4$, $MgCO_3$, MnO, $ZnCO_3$, ZnO, feldspar ($KAlSi_3O_8$), and kaolin ($Al_2Si_2O_5(OH)_4$). Only *As. pseudotubingensis* could produce 33.37 µg/ml indole-3-acetic acid. Moreover, *As. chiangmaiensis*, *As. pseudopiperis* and *As. pseudotubingensis* showed positive siderophore production on chrome azurol S agar.

Flood Susceptibility Mapping by Using GIS-Based Analytical Hierarchical Process (AHP) Technique: The Study Area of Chi River Basin, Mahasarakham and Roi-Et Provinces, Thailand

Kankanon Sakmongkoljit[1], and Nudthawud Homtong

[1]Department of Geotechnology, Khon Kaen University, Thailand
Email: S_kankanon@kkumail.com

Flood susceptibility map is used for flood prediction and prevention in many areas, including in the Chi River Basin of Thailand where flood is regularly occurred. The flood susceptibility map requires a good input data to enhance an accuracy of the map. An Analytical Hierarchical Process (AHP) technique was applied to manage parameters and create flood risk area map of the Chi River Basin in Mahasarakham and Roi-Et Provinces; in order to evaluate the flood risk area boundary in the basin. There are seven hydro-geomorpholgical parameters related to flood, which are distance from river, elevation, geology, land-use, soil type, drainage density, and geomorphology. The weighting and ranking of AHP were assigned via pairwise comparison method. Then, each parameter map was created, combined all maps together for generating a flood susceptibility map by using an overlay technique. As the result, the study area can be divided into five categories according to flood risk; which are very high flooding (16.9%), high flooding (17.7%), moderate flooding (22.4%), low flooding (29.08%), and very low flooding area (13.83%); with an accuracy of 77% as compared to compiled previous flood map. The map evaluation shows a good performance. Thus, this map is beneficial for water management in the basin, especially for flood warning system.

Keywords: Water Management; Flood Susceptibility Map; Analytical Hierarchical Processes Technique (AHP); Chi River Basin

Protein production using hyper-copy YHp plasmid vectors in the yeasts *Kluyveromyces lactis* and *Saccharomyces cerevisiae*

Shota Yuda[1], Hisashi Hoshida[1], Rinji Akada[1]
[1]Department of Applied Chemistry, Graduate School of Sciences and Technology for Innovation, Yamaguchi University,

The yeast *Saccharomyces cerevisiae* is used for bread making and brewery, and also for recombinant protein production. The yeast *Kluyveromyces lactis* is also known as a host for protein production. A common point of these two yeast species is that they possess endogenous plasmid, the 2μm circle plasmid in *S. cerevisiae* and pKD1 plasmid in *K. lactis*. These endogenous plasmids show high copy numbers and high stability, thus can be used for higher protein production. However, protein productions using these endogenous plasmids from these two yeasts have never been compared. Therefore, we compared the production of various therapeutic proteins from these two yeast species under various culture conditions.

For protein production, we selected human interleukins. The interleukins regulate immune response and will help pharmaceutical research. The interleukin IL36A, IL36B, IL36RN, IL1A, IL1B, and IL1RN were cloned in YHp vectors and expressed in *S. cerevisiae* and *K. lactis*. After protein extraction, protein electrophoresis was performed to compare protein production. In the case of IL36B, IL36G, and IL36RN, when culture was performed at 2% and 10% glucose, cell proliferation was observed and large amounts of the proteins were produced. However, at a concentration of 20% glucose, *K. lactis* grew poorly and produced only small amount, whereas *S. cerevisiae* grew well and produced protein. For the production of IL36A, when the glucose concentrations were 2% and 10%, *K. lactis* and *S. cerevisiae* grew well and produced protein but at a concentration of 20%, both yeasts grew poorly and did not produce protein. In the case of IL1A and IL1B, *K. lactis* and *S. cerevisiae* grew well when the glucose concentrations were 2% and 10%. However, both yeasts did not produced proteins. In contrast, IL1RN was produced at these glucose concentrations. The cell growth and the protein production of human IL proteins from these yeast species were similar. However, IL1A and IL1B were not produced from the two yeast hosts, suggesting that protein production is mainly depending on the protein sequence. It may be necessary to know why IL1A and IL1B could not be produced in yeasts.

Functionality of the insect food

Tomomi Osaki[1] and Yoshihito Iuchi[1]
[1]Faculty of Agriculture, Yamaguchi University, Japan

Insect is the final frontier for our food. In these years, importance of the insect food came to be discussed as a food. Furthermore, the United Nations Food and Agriculture Organization (FAO) announced the report about the insect food in 2013, and it attracts people's attention. It points out that the insect food can be the savior of the food crisis on the earth in near future, by the advantages with their ecological, economical and nutritional benefits. The insect food existed also in Japan from ancient time. Insect foods in Japan have linked deeply with culture in the past, however, that culture collapsed by change of eating habits. To actualize further recognition and popularization of insect food, we tried to find the added value contributing to our health and disease prevention into the insect food. To find the functionality in the edible insects on this study, we tried to do their ingredient analysis, and to realize their anti-inflammatory effect.

Construction of *AIGs*-knockout mutants by CRISPR/Cas9 system in *Arabidopsis thaliana*.

Yuto Takiyama[1], Wakana Tominaga[2], Ryota Matsui[2], Taishu Akiyama[1], Masayuki Murata[2], Tomoyuki Kosaka[1-3] and Mamoru Yamada[1-3]
[1]Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan
[2]Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan
[3]Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Japan

The immune-associated nucleotide-binding protein (IAN) family is functionally uncharacterized GTP-binding proteins (1) that are expressed in vertebrate immune cells during the T-cell development and plant cells during the antibacterial responses. In *Arabidopsis thaliana*, there are 14 proteins that show high homology with IAN1 of mouse. Of these, AIG1 is encoded by *AvrRpt2-induced Gene 1* that is expressed during infection with bacterial pathogens, causing hypersensitive response (2). In previous study, 14 IAN1 homologous genes were designated as *AIG1-1* to *AIG1-14* (3) and attempted to clarify their physiological functions. Furthermore, an *AIG1-4*-knockout mutant was constructed by using the CRISPR/Cas9 system, and detailed analysis of *AIG1-4* was performed by the GUS staining and Yeast Two-hybrid methods.

In this study, to understand the functions of other *AIGs* in *A. thaliana*, their *AIGs*-knockout mutants were attempted to be constructed by the CRISPR/Cas9 system.

References

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Improvement of thermotolerance in *Zymomonas mobilis* TISTR 548 by over-expression of genes for heat-shock proteins and reactive oxygen species-scavenging enzymes

Sakunda Anggarini[1], Masayuki Murata[1], Tomoyuki Kosaka[1-3], Mamoru Yamada[1-3]
[1]Life Science, Graduate School of Science and Technology for Innovation, Yamaguchi University, [2]Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, [3]Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Yamaguchi

The survival ability of microorganism at a critical high temperature (CHT) can be extended 2-3 degrees by adaptation to high temperatures (1). The presence of heat stress or reactive oxygen species (ROS) at such temperatures may have allowed microbial cells to acquire various protection mechanisms in which thermotolerant genes are involved (2). However, there are only a limited number of genes for heat shock protein (HSP) and reactive oxygen species (ROS)-scavenging enzyme (RSE) as a thermotolerant gene in *Escherichia coli*, *Acetobacter tropical* and *Zymomonous mobilis* (3-5). In this study, to understand the contribution of HSP and RSE genes to thermotolerance, we attempted to examine the effects of enhanced expression of these genes on survival at CHT in *Z. mobilis* TISTR 548.

For over-expression of genes for HSP and RSE, genes were connected to the *pdc* promoter by PCR and In-fusion cloning with pZA-22. Each recombinant plasmid was introduced into *Z. mobilis* TISTR 548 and the recombined strain was subjected to two-step cultivation to evaluate the improvement of CHT. As a result, the enhanced expression of some genes was able to improve CHT, about one degree, and to reduce ROS compared to the control. Moreover, the HSP and RSE genes-introduced strains were also subjected to growth experiments under various stresses. Increased expression of some genes was found to enhance tolerance to ethanol. These findings suggest that ROS or unfolded proteins that prevent cell growth are accumulated in cells at CHT.

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. Subtrate Recognition in the 5-keto-fluctose reductase of gluconobacter sp. revealed by amino acid substitution

Nguyen Minh Thuy[1], Masaru Goto[2], Shohei Noda[2], Naoya Kataoka[1,3,4], Osao Adachi[1], Kazunobu Matsushita[1,3,4], Toshiharu Yakushi[1,3,4]

[1] Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan
[2] Faculty of Science, Toho University, Japan
[3] Faculty of Agriculture, Yamaguchi University, Japan
[4] Research Center for Thermotolerant Microbial Resources, Yamaguchi University Japan

Acetic acid bacteria oxidize various sugars and alcohols incompletely to produce corresponding ketones and acids on the cell surface. *Gluconobacter frateurii* strain CHM43 oxidizes mannitol to fructose and does fructose to 5-keto-fructose (5KF). We identified *GLF_2050* as the gene for NADPH-dependent 5KF reductase (KFR). In this study, we attempted to characterize the structure and function of KFR.

We constructed over expression strain for KFR, which showed specific activity about 40-times higher than wild type. KFR activity in the mutant strain defective of the *GLF_2050* gene (Δ*GLF_2050*) was lower than that of wild type but still remained activity at approx. 29% of wild type. The results indicate that the *GLF_2050* gene encodes one of the KFR genes in the CHM43 genome. We purified the recombinant KFR. Molecular mass of the purified KFR in the native state was determined by gel filtration to be approx. 71 kDa, i.e. KFR is dimer of approx. 30-kDa protomer in solution. The crystal structure revealed that KFR is similar to $NADP^+$-dependent shikimate dehydrogenase (SDH), which catalyzes the reversible $NADP^+$-dependent oxidation of shikimate. We found that two amino acid residues are the major differences between the two enzymes; Asn^{21} and Pro^{227} in the putative substrate-binding site are Ser^{14} and Tyr^{207} in SDH. Additionally, the catalytic dyad Lys^{64} and Asp^{100} of SDH are also conserved in KFR as Lys^{72} and Asp^{108}. We constructed several KFR derivatives with amino acid substitution including the two residues N21S and P227Y in the subtrate-binding site. We also constructed the mutagenesis of K72N and D108N in the catalytic site . Interestingly, the mutant N21S showed the shikimate oxidation activity 8 times higher and less affinity for 5KF compared to wild-type KFR. The mutagenesis study suggested that Asn^{21} is important for the substrate binding

Optimal condition for cell entrapment with calcium alginate of ectomycorrhizal fungus *Astraeus odoratus*

Nicha Maneesilasan[1], Alisa Vangni[2] and Jittra Piapukiew[3]

[1]Program of Biotechnology, Faculty of Science, Chulalongkorn University, [2]Department of Biochemistry, Faculty of Science, Chulalongkorn University, [3]Department of Botany, Faculty of Science, Chulalongkorn University

Astraeus odoratus is an ectomycorrhizal (ECM) fungus associated with the trees in Diptercapaceae. It is important for inoculum for stimulating growth and survival of dipterocarp trees in tropical forests of Southeast Asia. Moreover, this fungal species is famous edible ECM mushroom with high economic value and high potential to be used as inoculum for the establishment of dipterocarp species in reforestation program. Thus, the aim of this study was to optimize condition for cell entrapment with calcium alginate of *A. odoratus*. The results showed that calcium alginate of *A. odoratus* supplemented with 0.5-2% glucose gave the highest germination rate with 100%. The tested protectant, 5% sorbitol had the highest germination rate with 96%. The variability test of *A. odoratus* mycelium entrapped with calcium alginate revealed that the calcium alginate beads stored in sterile distilled water at 25°C was the optimal condition for efficient preservation within 3 months.

Effect of electrolytes on oil-water separation using fine bubble

Takumi Nanba[1] and Tsuyoshi Imai[1]
[1]*Graduate School of Science and Technology for Innovation, Yamaguchi Univ.*

Oil-in-Water Emulsion wastewater which is discharged from oil storage base, food and oil factories is difficult to treat, so it is necessary to establish more useful oil-water separation process. As one of them, there is a floating using fine bubble, which is 100 μm or less diameter, and have the effect of efficiently adsorbing oil droplets and floating and separating them due to their slow rising speed. However, previous studies have shown that oil removal is not performed so much with low electrolyte concentration. The purpose of this study was to establish a method to apply this process with sufficient efficiency by using electrolyte to emulsified oil wastewater with different electrolyte concentration.

I conducted the experiment using a simulated emulsion prepared from an electrolyte solution and crude oil, and compared the time change of the oil removal rate in one hour. Five electrolytes were used: NaCl, $MgCl_2$, $MgSO_4$, $CaCl_2$, $Al_2(SO_4)_3 \cdot 14\sim 18H_2O$. In the case of using only single electrolyte, the oil removal rate increases in descending order of the electrolyte's cation valence and ascending order of the electrolyte's anion valence. In the case of using the two electrolytes in combination at the same concentration, the oil removal rate is lower than in case of using two different divalent cations or divalent and trivalent cations, as compared with using single cation. It was suggested that the oil removal rate decreased as the water temperature was higher.

Unique properties of anti-gravity muscle in primates

Sakura Okimune[1], Ryo Kawakubo[2] and Hirofumi Miyata[3]
Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, JAPAN

In our study on skeletal muscles of various mammals, a relationship was found between body weight and type I fiber ratio. However, it was also confirmed that some muscles of the primate have a different muscle fiber type composition as compared to other quadrupeds. Therefore, in this study, we investigated the composition of muscle fiber type and myosin heavy chain isoforms based on immunohistochemical stain and electrophoresis in whole body muscle of mammals including primates.
By autopsy or biopsy sampling methods, a part of several typical antigravity muscles (Soleus, Gastrocnemius, Vastus lateralis, Gluteus medius, Longissimus, etc.) were collected from some primates (squirrel monkeys, lemurs, brozza guenons, Japanese monkeys, baboons, chimpanzees, gibbon, black monkey) and other quadrupeds animals with body weights ranging from 0.005 kg (Gerbil) to 3000 kg (African elephant). Serial frozen sections were stained with myosin heavy chain antibodies (Fast, IIa, IIx) and rest of the muscle samples were used for Myosin heavy chain (MHC) isoforms separation by electrophoresis. Composition of muscle fiber types and MHC isoforms were calculated by an image processing system. For human, data of previous study (J Neurological Sci. 18: 111-129 (1973)) were cited except for the lateral vastus muscle.

The positive correlation coefficient was obtained between body weight and type I fiber ratio in all skeletal muscles, suggesting that the ratio of type I fibers tends to increase basically with larger animals. In the vastus lateralis muscle, a high ratio of type I fiber was observed in primate chimpanzees (46.9%) and humans (41.5%), and the high value was also observed in meerkats (44.8%) with frequent upright postures. In the gluteus medius muscle, a high type I fiber ratio was observed in squirrel monkeys (34.5%), lemurs (41.7%), baboons (40.2%) and humans (52.4%). In mammals with frequent upright postures, the ratio of type I muscle fibers of the vastus lateralis and longissimus muscles tended to be high.
Analysis of MHC isoforms is ongoing (early August 2019).

(This study were collaborated with faculty of veterinary medicine in Yamaguchi University)

Screening for the genes affecting intron-mediated enhancement using a *Saccharomyces cerevisiae* knockout strain collection.

Hiroki Kikuta[1], Rinji Akada[123] and Hisashi Hoshida[123]
[1]GraduateSchool of science and Technology for Innovation, Yamaguchi University
[2]Yamaguchi University Research Center for Thermotolerant Microbial Resources
[3]Yamaguchi University Biomedical Engineering Center

Introns are genetic elements on chromosomal DNAs of eukaryotes. Introns are transcribed together with coding sequences as a part of pre-mRNAs, but are removed by splicing to produce mature-mRNAs before translation. Therefore, they do not encode any amino acid sequence. However, introns have several important functions in eukaryotic cells. One of them is intron-mediated enhancement (IME), which is a phenomenon enhancing protein production from intron-containing genes. In previous study, we showed that expression of yCLuc, which encodes a secretory luciferase, by an intron-containing promoter was increased by about 40-fold compared with intron-less promoter. In order to elucidate the mechanism of IME, we screened for the genes affecting IME using a *S. cerevisiae* knockout collection.

In this study, we transformed a haploid knockout collection with a expression vector containing *RPS25A* intron between *TDH3* promoter and yCLuc. The collection consists of about 5000 knockout strains. Each strain has a disruption of a non-essential gene. Knockout strains were cultured in 30µl of YPD in 96-well plates for overnight. Twenty four micro-litter of the transformation solution containing 67.5% PEG600, 12.5% 1 M DTT and 12.5% 4 M sodium acetate, and one micro litter of plasmid solution to expres yCLuc by the intron promoter was mixed with the cultures. The mixture was incubated at 42 ℃ for 1 h and spottied on –U plate to produce transformant colonies. To measure secretory yCLuc activity, transformants were cultured in a buffered –U medium (pH7).

Luciferase activity of the transformants obtained was compared with that of wild type. The genes in the knockout strains showing decreased and enhanced expression will be involved in IME. Currently 2590 transformants were obtained, and 13 and 6 strains showed comparatively lower and higher activities, respectively. To select the strains which specifically affecting IME, yCLuc expression vector without intron was transformed into the selected strains. As a result, three disruptants decreased yCLuc expression with intron and other three disruptants increased yCLuc without intron over 20-fold. These genes may be involed in the IME and suppression yCLuc expression in *S. cerevisiae.*

Effects of mutations in thermo-adapted mutants from *Zymomonas mobilis* CP4 and improvement of thermotolerance by combination of mutations among these mutants

Takashi Kuga[1], Shun Kato[1], Yuki Shiromaru[2], Masayuki Murata[3], Tomoyuki Kosaka[1-3] and Mamoru Yamada[1-3]

[1] Division of Agricultural Sciences, Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan

[2] Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan

[3]Life Sciences, Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan

High-temperature fermentation has advantages such as suppression of contamination risk, improvement of enzyme reactivity, reduction of cooling cost, and simplification of operation. Therefore, high-temperature fermentation is expected as a cost-effective ethanol production technology, and superior strains having thermotolerance and tolerance to various stresses accumulated during fermentation are required for it. *Zymomonas mobilis*, gram-negative facultative anaerobic ethanol-producing bacterium, used in this study is known to show high ethanol productivity, using the Entner-Doudoroff pathway and incomplete TCA cycle, compared to *Saccharomyces cerevisiae* generally used for ethanol fermentation (1, 2). Thermo-adapted mutants Z4-80a, Z4-80b, Z4-80c and Z4-80d have been obtained after repeated cultivations of the wild-type strain CP4 under the condition of gradually raising temperature (3). Critical high temperature (CHT) of these mutants is 2 °C higher than that of the wild-type strain. They have 13 mutations in coding sequences in total. Single mutant strains have been constructed by introducing each mutation into CP4, and some of their physiological analyses have been performed.

In this study, mutant strains derived from the thermo-adapted mutants were examined in tolerance to various stresses including high temperature, high glucose concentration, ethanol, SDS, NaCl and KCl. Additionally, cell length at 30 °C and 38 °C were measured. In experiments of temperature stress, a-1646b (Z4-80a + 1646b) and ab (a-1646b + 0028M99I) in addition to single mutant strains were subjected to culture at 39, 39.5 and 40 °C. As a result, each mutant showed a distinct phenotype. Measurement of cell length showed that one of the two mutations that mainly contributed to the thermostability in each thermo-adapted strain also contributes to suppression of cell elongation. Strains of a-1646b and ab were found to grow at 39.5°C, which was a half degree higher than that of thermo-adapted mutants. These results suggest that the individual thermo-adapted mutant has 2-5 mutations that causes a different phenotype and that combinations of mutations among thermo-adapted mutants further improve thermotolerance.

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Total polyphenol content and antioxidant capacity of *Cayratia trifolia* (L) Domin berries in Ca Mau and Kien Giang provinces before and after fermentation using thermotolerant yeast *Saccharomyces cerevisiae* HG1.3

Doan Thi Kieu Tien, Huynh Thi Ngoc Mi, Trinh Gia Hung, Nguyen Duc Do, Ha Thanh Toan and Ngo Thi Phuong Dung

Biotechnology Research and Development Institute, Can Tho University

Cayratia trifolia (L) Domin berry is a source of high biological activity compounds, having the capacity of antioxidation, and is used as a medicinal ingredient as well as a good material source for alcoholic wine. This study focused on the wine fermentation from the raw materials of Cayratia trifolia (L) Domin berries collected in Ca Mau and Kien Giang provinces using thermotolerant yeast Saccharomyces cerevisiae HG1.3. The result showed that the pre-fermenting polyphenol content of Cayratia trifolia (L) in Ca Mau and Kien Giang provinces was 0.53 and 0.66 mg GAE / ml, respectively, not significantly different at 5% (p <0, 05) in comparison with the contents of the wine after fermentation (0.61 and 0.6 mg GAE / ml). The antioxidant capacity of Cayratia trifolia (L) wine products in Ca Mau and Kien Giang provinces were 51.4% and 57.3%, respectively, negligible change compared to the starting material 44.9% and 54.7%, showing that the antioxidant capacity and polyphenol content of the corneal fluid were not affected by the fermentation process. It can be indicated that Cayratia trifolia (L) is one of the good materials for wine production.

The Resistance of Chili Pepper Plants (*Capsicum frutescens* L.) to Fusarium Wilt Planted in Media AMB-P0K Quartz Sand With Mycorrhizal Inoculation

Nurhalimah[1] , Anton Muhibuddin[2] , and Antok Wahyu Sektiono[3]
[1]Faculty of Agriculture, Brawijaya Univ. [2,3]Departement of Plant Pest and Disease, Braaawijaya Univ.

Disease is one of the main obstacles in the cultivation of chili pepper. A common disease in chili plants is fusarium wilt caused by *Fusarium oxysporum f.sp. capsici* which can cause damage up to 50%. The level of resistance to *F. oxysporum* can be categorized rather resistant to very resistant. Appropriate information about the use of sand media and the administration of mycorrhizal which is needed for plant growth and its resistance to the intensity of disease attacks, especially fungi in plants is very necessary. The study aimed to determine the resistance of chili pepper pepper to fusarium wilt caused by the *pathogen Fusarium oxysporum f.sp. capsici* which was planted on quartz sand media AMB-POK through mycorrhizal inoculation

The research was conducted in the Greenhouse of the Biology Department, Faculty of Science and Technology, Maulana Malik Ibrahim Malang Islamic University, Laboratory of Plant Diseases Department of Plant Pests and Diseases, Faculty of Agriculture, Brawijaya University Malang and Pharmacy Laboratory, Ma Chung Malang University for 5 months from January - May 2019. The research stages were planting of test plants and mycorrhizal inoculation, inoculation of pathogenic fungi Fusarium oxysporum f.sp. capsici, and plant maintenance. The variables observed were the time of symptoms of illness, severity of disease, plant height, number of leaves, number of fruits, and saponin levels.

Based on the results of research on chili pepper pepper to the resistance caused by fusarium wilt by giving doses of 5, 10 and 15 g respectively. The results showed that administration of mycorrhizal at 15 g in AMB-P0K growing media showed good results compared to controls. It can be seen in the results of plant height, number of leaves, and the number of chili pepper fruit and the time the symptoms appeared and the severity of the disease due to fusarium wilt. It is known based on the results of the decrease in severity of the disease in the treatment of administration of 15 g mycorrhizal as big as (4.56%) which indicates that the resistance level of the plant increases and impacts on plant height, number of leaves, and number of fruits. Environmental factors namely temperature, soil moisture, pH, nutrients N, P, and K affect the development of fusarium wilt.

Comparing Phosphate Adsorption Performance of Biochar-Layered Double Hydroxides Composites Synthesized through Different Methods

Jittrera Buates [1] and Tsuyoshi Imai [1]
[1] Division of Environmental Engineering, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi 755-8611, Japan

Owing to environmental and economic benefits, biochar-based adsorbents have received substantial research attention for the remediation of phosphate from wastewater. However, the binding affinity of pristine biochar toward anionic contaminants is often hindered by its net negatively charged surface. This study has therefore been carried out to produce biochar-layered double hydroxides composites (BC-LDHs) via different approaches and to compare their phosphate adsorption capacities with unmodified biochar. The first preparation method was that the biomass was initially pyrolyzed at 600°C for 1 hour to obtain biochar and then functionalized with LDHs while the second technique was pyrolysis of LDHs preloaded rice straw at 475°C for 2 hours. Phosphate adsorption experiments revealed that all BC-LDHs had higher phosphate removal capacities than unmodified biochar indicating the efficiency improvement in adsorption ability of biochar by LDHs. Furthermore, due to the reconstruction process of heat-treated LDHs, the second method can provide the greater performance of BC-LDHs to uptake phosphate than the first method with the adsorption amounts of 21-25 mg/g. The maximum uptake capacity of 46 mg/g calculated by normalization on weight basis of LDHs can be achieved. It could be suggested that BC-LDHs from this study were promising materials for the removal of phosphate from aqueous solutions.

Effect of Sodium Chloride on *In Vitro* Protocorm-Like Bodies Multiplication of Two *Dendrobium* Cultivars

Petsupang Khamtae[1], Patchareeya Boonkorkaew[1] and Poonpipope Kasemsap[1]
[1]Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok

This study examined sodium chloride (NaCl) affects protocorm-like bodies (PLBs) multiplication of two *Dendrobium* cultivars (*Dendrobium* Sonia 'Earsakul' and *Dendrobium* 'Miss Orchid'), which were grown in vitro for 8 weeks on modified Vacin and Went medium containing five NaCl concentrations (0, 25, 50, 75 and 100 mM). The experimental design was a completely randomized design (CRD). The results showed that the survival rate of *Den.* Sonia 'Earsakul' PLBs was 90 and 80 percent in NaCl at 75 and 100 mM concentrations, respectively. The number of PLBs, fresh weight, and growth index decreased in the high NaCl concentrations. NaCl 0 mM produced the highest average number of PLBs (22.5 PLBs). The second highest was NaCl 50 mM, which produced 18.7 PLBs. Meanwhile; the other concentrations were not significantly different (6.7-12.1 PLBs). The survival rate of *Den.* 'Miss orchid' PLBs in NaCl 100 mM was the same, at 80 percent. The number of PLBs, fresh weight, and growth index decreased in the highest NaCl concentrations. NaCl 0 mM produced the highest average number of PLBs (36.5 PLBs). Meanwhile, NaCl 100 mM produced the lowest average number of PLBs (6.96 PLBs).

Functional analysis of membrane-bound alcohol dehydrogenase of *Gluconobacter oxydans*, a member of acetic acid bacteria

Kazuya Tanioka[1], Nittaya Pitiwittayakul[2], Naoya Kataoka[1,3,4], Minenosuke Matsutani[3],and Kazunobu Matsushita[1,3,4],and Toshiharu Yakushi[1,3,4]

[1] *Faculty of Agriculture, Yamaguchi University, Japan*
[2] *Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Thailand*
[3] *Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan*
[4] *Research Center for Thermotolerant Microbial Resources, Yamaguchi University Japan*

Acetic acid fermentation is a process in which ethanol is oxidized by acetic acid bacteria to produce acetic acid via acetaldehyde as an intermediate substance. This process is catalyzed by two enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) located on the outer surface of the cytoplasmic membrane. ADH of acetic acid bacteria oxidizes ethanol to acetaldehyde, coupling with reduction of ubiquinone. ADH of *Acetobacter* and *Gluconobacter* consists of three subunits: A subunit: a quinohemoprotein having pyrroloquinoline quinone (PQQ) and heme C as prosthetic groups, having the largest molecular size; B subunit that is cytochrome c performs ubiquinone reduction with having three heme C moieties as the prosthetic group; S subunit, of which the function is unknown. While, ADH of *Gluconacetobacter* and *Komagataeibacter* does not contain the S subunit but does the A and B subunits to perform acetic acid fermentation. It is expected that ADH can perform the function even without the S subunit. However, the Δ*adhS* mutant of *Acetobacter* that does not produce the S subunit looses ADH function, although the A and B subunits are produced. The data indicate that the S subunit is essential for the function. This study aims at the function of the S subunit of *Gluconobacter* ADH.

We constructed the gene deletion mutant strains (Δ*adhB*, Δ*adhS*, Δ*adhBS*) to compare the ADH activities with wild-type *Gluconobacter oxydans* NBRC 3293. The Δ*adhB* strain showed ferricyanide reducing activity at the acidic pH region but no ubiquinone reducing activity, suggesting the B subunit is responsible for the ubiquinone reduction. The Δ*adhS* strain showed ADH activity of lower than that of the parental strain, although the amounts of the A and B subunits seem similar to those of wild type, suggesting that the S subunit is not essential for ADH but has some function on ADH activity. The Δ*adhBS* strain did not show ferricyanide reductase activity and ubiquinone reductase activities, although the A subunit was detected by SDS-PAGE stained with heme-catalyzed peroxidase reaction. The Δ*BS* strain having only the A subunit has almost no activity, and it is considered that the function becomes unstable when the two subunits are lost.

The effect of injection of platelet-rich plasma on the gene expression and morphology of damaged skeletal muscle in Thoroughbred horses

Sakura Sasaki[1], Ryota Eguchi[1] and Hirofumi Miyata[1]
[1]Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, JAPAN

Platelet-rich plasma (PRP) is an autologous blood product containing concentrated platelets. From the platelet α-granules, various growth factors and cytokines are released, which are effective for wound healing and tissue regeneration. Although PRP is thought to be useful for the treatment of ligament and tendon injury, little is known about the effect on the repair of damaged skeletal muscle. In the present study, muscle regeneration by injection of PRP into damaged muscle was investigated in 12 Thoroughbred horses.

By needle biopsy technique, small samples were taken from gluteus medius muscles at pre (basal level), 1day (damaged level), 2 and 3 days after autologous PRP injection into the damaged area induced by bupivacaine injection. Serial 10 μm cross sections of the muscle were obtained on a cryostat and muscle fiber type, cross sectional area, and number of fibers with central nuclei were measured by immunehistochemical stain. Expression levels of mRNA of satellite cell-related factors, growth factors, and inflammation-related factors were quantified by real-time RT-PCR analysis.

The mRNA expression of satellite cell proliferation and differentiation marker (MyoD and myogenin) were significantly higher in PRP treated muscle than non-treated muscle. As a result of immunohistochemical procedure, appearance rate of central nuclear fiber tended to be higher in PRP treated muscle than non-treated muscle. The mRNA expression of inflammation-related factors (PGEs, COX-2, TNFα) was significantly higher in PRP treated muscle than non-treated muscle. These results suggested that injection of PRP into damaged skeletal muscle upregulate the expression of genes related to muscle regeneration.

(This study were collaborated with Equine Research Institute in Japan Racing Association)

Targeted gene disruption using CRISPR/Cas9 system in the yeast *Kluyveromyces marxianus*

Satoshi Ebe[1], Hisashi Hoshida[1,2,3] and Rinji Akada[1,2,3]
[1]Graduate School of Sciences and Technology for Innovation, Yamaguchi University,
[2]Yamaguchi University Research Center for Thermotolerant Microbial Resources
[3] Yamaguchi University Biomedical Engineering Center

Genome editing is a technology to modify target genes and is useful to develop organisms. However, correct editing was very difficult in most organisms. Recently, a reliable genome editing system called CRISPR/Cas9 was developed by using bacterial immune system. In the system, the end nuclease Cas9 is recruited to a target site by a guide RNA and produce a double strand break. It was joined by native repair systems, but an error during repair process generates a non-functional mutation.

The yeast *Kluyveromyces marxianus* is a thermotolerant yeast and grows at 50ºC. In previous study, we listed several genes which might be responsible for thermotolerance of *K. marxianus.* Gene disruption is most reliable to evaluate the candidate genes. In this study, CRISPR/Cas9 system was tested in *K. marxianus.* First, *ADE2* gene was targeted as a model, because the colonies of *ade2* mutants shows red color and easy to evaluate targeted disruption. The plasmid pUDP082 was obtained from Addgene. The plasmid expresses Cas9 and a self-processing guide RNA. The plasmid contains hygromycin resistant gene as a transformation marker, and a pan-autonomously replicating sequence but not centromere sequence of *K. marxianus.* For efficient transformant selection and plasmid removal after disruption, marker gene was changed to *URA3* and *K. marxianus* CenD sequence was added to the plasmid. In addition, the sequence required for plasmid manipulation in *E.coli* were also deleted. The plasmid, which was constructed in *Saccharomyces cerevisiae* through homologous recombination of PCR-amplified DNA fragments, was named YRp29937. To disrupt *ADE2* in *K. marxianus*, YRp29937 was amplified by PCR and directly introduced into a *K. marxianus ura3*$^{-}$ strain. In *K. marxianus,* the introduced DNA was self-ligated and produce a circular plasmid. Most of the colonies produced on-Ura selection plate did not show clear red. Twelve colonies were randomly selected, inoculated on a fresh selection plate. After incubation for four days, six colonies showed red color, suggesting that *ADE2* gene was disrupted. For further efficient disruption and easy plasmid manipulation, several types of DNA will be amplified by PCR and used for disruption in *K. marxianus*.

Effect of metal ions on the growth of thermotolerant *Zymomonas mobilis* TISTR 548 at critical high temperature

Kento Miura[1], Aya Nishioka[2], Kannikar Charoensuk[3], Tomoyuki Kosaka[1,2] and Mamoru Yamada[1,2]
[1] Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan
[2] Division of Agricultural Science, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Japan
[3]Rajamangala University of Technology Tawan-ok, Thailand

Biofuel production from renewable resources is becoming increasingly important due to the global crude oil crisis and resulting environmental concern about environmental issues (1). Ethanol production by high-temperature fermentation with thermotolerant microorganisms becomes attractive because it is expected to reduce the cost of production and prevent contamination from miscellaneous germs. *Zymomonas mobilis* is a Gram-negative facultatively anaerobic ethanologenic bacterium but the optimum temperature of general strains in the microbe is at around 30 °C. It has a strong Entner-Doudoroff pathway and an incomplete TCA cycle and thus can efficiently convert carbon sources to ethanol for acquisition of ATP (2). *Z. mobilis* TISTR 548 strain isolated from Thailand is thermotolerant and able to grow and produce ethanol at a relatively high temperature. Previous study showed that among metal ions, Mg^{2+} and K^{+} can improve the critical high temperature (CHT) of the strain. In this study, characteristics of improvement of both metals were examined.

First, reproducibility of previous study on the effects of Mg^{2+} and K^{+} on the growth of *Z. mobilis* TISTR 548 at a CHT was checked. Consistent with previous study, the two-step cultivation method (3) showed that the addition of 5 mM $MgCl_2$ or 30 mM KCl up-shifted the CHT by 1˚C and the strain became able to grow at 39˚C, but the effect of the former metal was stronger than that of the latter. In addition, only the former metal reduced the size of cells that became longer at CHT. Second, the effects of the metal-ions on intracellular ROS level at a CHT were examined. The accumulation of ROS at a cultivation time, which was equivalent to 12 h in the first culture of the two-step cultivation, was reduced by the addition of $MgCl_2$ or KCl. The effect of the former was further examined by the treatment of EDTA followed by the addition of metal ions, suggesting the possibility that Mg^{2+}stabilizes membrane structure. When reduced form of glutathione (GSH) as an antioxidant was added, the turbidity at the second culture was largely increased in the presence of KCl, but no increase in turbidity was observed without the addition of the metal. The addition of $MgCl_2$ or GSH, but not KCl, reduced cell size at 12 h in the first culture. These findings suggest that the function of Mg^{2+} is different from that of K^{+} at a CHT.

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Selection of a long-term adaptation thermotolerant *Kluyveromyces marxianus* strains for ethanol production at high temperature from cassava starch hydrolysate

Koudkeo Phommachan[1], Mochamad Nurcholis[1], Chansom Keo-oudone[2], Tomoyuki Kosaka[1] and Mamoru Yamada[1]
[1]*Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan*
[2]*Department of Biology, Faculty of Science, National University of Laos*

Cassava starch is considered as a potential source for the commercial production of bioethanol because of its availability and low market price. It can be used as a substrate to production of ethanol using thermotolerant microbes. Moreover, high-temperature fermentation and simultaneous saccharification and fermentation processes require thermotolerant microbes that able to utilize various substrates at high temperature to produce beneficial products. To achieve high temperature fermentation, the fermentation capability of yeasts and its ability to grow and produce ethanol under a variety of inhibitory at elevated temperatures and the accumulation of high concentration of ethanol. Therefore, in this study both of thermotolerance yeasts *Kluyveromyces marxianus* BUNL-21 and UB-5 isolated in Laos and Indonesia were subjected to long-term adaptation under a high temperature condition, and SSF was using cassava starch as a substrate, amylase and glucoamylase were used as an enzyme. Comparison with thermotolerant *K. marxianus* DMKU3-1042 which is one of the most tolerant yeasts that stronger ability for produce ethanol at high temperatures and tolerance to various stresses. The adaptation were found that BUNL-21.6.3 and UB-5.9 showed more tolerance to ethanol and slightly resistance to furfural than those of DMKU3-1042, BUNL-21 and UB5. However, glucose high concentration and acetic acid showed effect to adapted strains. The SSF activity to converted cassava starch to ethanol, BUNL-21.6.3 and UB-5.9 were found to have ethanol production slightly lower than DMKU3-1042, BUNL-21 and UB-5. The result suggested that adaptation yeast strains were enhance to stress resistance but not efficient to ethanol production.

Biological Phosphorus Removal Performance Using Salt-Tolerant Phosphorus Accumulating Organisms (PAOs) in Seawater

Rafitah Hasanah [1] and Tsuyoshi IMAI [1]

[1,1] Division of Environmental Engineering, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi 755-8611

Phosphate removal can be performed by enhanced biological phosphorus removal (EBPR) process. This method implements communities of microorganisms, some of these microorganisms are called polyphosphate accumulating organisms (PAOs). EBPR systems are sensitive to fluctuations in water quality and environmental conditions, such as salinity. The aim of this study was to investigate the correlation between phosphorus removal performance and PAOs that is involved in EBPR process under salinity condition same as sea water. The experiments were carried out as a batch experiment with biocarrier (sponges). These sponges were used as biocarrier to attach/keep microorganisms. The cultivation was conducted in 4 hours under anaerobic condition, and in 8 hours under aerobic with condition, phosphorus concentration from 0.401 mg/L to 3.312 mg/L (from day 0 until day 98). During anaerobic conditions, PAOs may have released stored phosphorus in their cells. In aerobic condition, the phosphorus concentration increase from 3.344 mg/L to 4.022 mg/L (from 0 until 98 days). This indicates PAOs can uptake phosphorus for energy storage occurred. In the future work, it needs to be done using a specific agar medium, PCR and gel electrophoresis to determine the correlation between the presence of PAOs and phosphorus removal (accumulation).

Growth of Gloxinia (*Sinningia speciosa*) under different electrical conductivity (EC) level of nutrients solution and carbon dioxide concentrations

Sunisa Sudthai[1] and Siriwat Sakonwasee[2]
[1]Master of Science students Department of Horticulture Faculty of Agriculture, Maejo University.,
[2]Assistant professor Department of Horticulture Faculty of Agriculture, Maejo University.

Gloxinia (*Sinningia speciosa*) is a flowering plant with beautiful flowers. Using a plant factory system that has adapted the environment to suit the growth, can be produced in every season and in large quantities. Within the plant factory, plants are planted vertically with controlled temperature, humidity, light intensity, light quality and the nutrient solution. Resulting in higher productivity per unit area. Currently, there is a widely studied study about increasing the concentration of Carbon dioxide (CO_2) in the atmosphere to accelerate plant growth.

In this study, we investigated growth of Gloxinia (*Sinningia speciosa*) response to nutrient solution concentration (1, 2 and 4 mS cm^{-1} EC, electrical conductivity) and the influence of Co_2 concentrations (400 and 1000 ppm). Gloxinia seeds were sown in peat moss placed in a growth chamber then 2 months after germination, each gloxinia plant was transferred to 6 inches diameter pot. Substrate in the pot consisted of coconut coir, sand and peat moss in 3 to 1 to 1 ratio
Modified hoagland fertilizer solution was used for regular fertigation.

The plants were received different nutrient solution concentration (EC) and carbon dioxide (CO_2) at the age of 90 days after sowing. It was found that in ambient conditions which received 400 ppm CO_2, plants with nutrient solution concentration at EC = 4 had canopy width, flower size, pod size and shoot fresh weight lower tendency than the other treatment. In the shoot and root dry weight plant were grown under ambient condition had lower tendency than CO_2 enrichment condition.

Effect of high temperature stress on resveratrol and oxyresveratrol accumulation and related gene expression in mulberry callus

Jittraporn Chusrisom[1] and Wannarat Phonphoem[1]

[1]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10903, Thailand

Resveratrol (res) and oxyresveratrol (oxyres) are the major stilbene phytoalexin that protect plant tissues from many environmental stresses. Res and oxyres have many of human-health benefits such as antioxidant, cancer-chemoprevention, cardioprotection as well as anti-aging. Previous study demonstrated that res and oxyres regulate mechanism of action of skin-whitening substances by inhibition of tyrosinase activity and reduction in melanocytes proliferation. Moreover, these compounds can stimulate the fibroblast proliferation and promoting collagen type III. Mulberry, one of native plants grown widely in Northeastern region of Thailand, has been exploited commercially for the silk industry. However, the accumulation of res and oxyres in this plant has not been well studied. Therefore, the aims of this study were to investigate the effect of high temperature on res and oxyres contents and determine the expression of *cinnamate-4-hydroxylase* (*C4H*), *4-coumarate-CoA ligase* (*4CL)* and *stilbene synthase* (*STS*) genes involved in resveratrol biosynthesis in mulberry. Callus induced from root and stem explants were used as a model system for stress treatment. Under high temperature stress condition, res and oxyres contents increased significantly at 12 h (20.74 and 91.70 $ug.g^{-1}$) when compared with control condition. Gene expression analysis revealed that *MaC4H* and *Ma4CL* pressions remain unchanged. In contrast, the expression of *MaSTS* increased signifiexcantly after 6 h incubation at 40 ºC and showed the highest at 12 h when compared with untreated callus. These results suggested that high temperature stress can induce res and oxyres contents in mulberry callus. The study of res and oxyres accumulation including biological synthesis will bring benefits for quality improvement and industrial exploration of mulberry in the future.

Expression levels of the different types of membrane-bound aldehyde dehydrogenases in several species of acetic acid bacteria

Takeru Murate[1], Seiya Fukunari[2], Naoya Kataoka[1,2,3], Kazunobu Matsushita[1,2,3] and Toshiharu Yakushi[1,2,3]

[1]Graduate School of Sciences and Technology for Innovation, [2]Faculty of Agriculture, and [3]RCTMR, Yamaguchi Uniiv.

Vinegar production is a consequence of oxidation of ethanol by two membrane-bound enzymes: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) of acetic acid bacteria. *Acetobacter pasteurianus* SKU1108 has two sets of ALDH gene clusters: *aldFGH* and *aldSLC*. It was shown that levels of the *aldH* transcripts under the acetic acid fermentation and non-fermentation conditions were similar to each other, but those of *aldL* under the fermentation conditions were much lower than those under non-fermentation conditions. Here, we examined whether such the transcriptional regulation and different expression of ALDH activities are found in other species of acetic acid bacteria.

We selected four strains, *Acetobacter pasteurianus*, *Acetobacter tropicalis*, *Komagataeibacter hansenii*, and *Komagataeibacter medellinensis*, having different repertories of the ALDH isogenes, and three strains, *Acetobacter aceti*, *Gluconacetobacter diazotrophicus*, and *Gluconacetobacter liquefaciens*, having the same ALDH repertories as *A. pasteurianus* to investigate expression of ALDH on acetic acid fermentation. In this study, the cultivation conditions were examined in order to establish stable protocols. We measured ADH and ALDH activities in the cells grown under acetic acid fermentation and non-fermentation conditions.

Optimization of glutamic acid fermentation with thermotolerant *Corynebacterium*

Ryo Koga[2], Saki Sogawa[1], Izumi Tomohiro[1],Minenosuke Matsutani[2], Naoya Kataoka[1,2,3], Toshiharu Yakushi[1,2,3]and Kazunobu Matsushita[1,2,3]

[1]*Department of Biological Chemistry, Faculty of Agriculture,* [2]*Division of Agricultural Science, Graduate School of Science and Technology for Innovation, and* [3]*Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Yamaguchi 753-8515, Japan.*

Corynebacterium glutamicum is a Gram-positive bacterium that produces amino acids such as glutamic acid and lysine. The cell growth and glutamic acid fermentation generate heat, which prevents growth and reduces its production efficiency. In order to overcome this problem, the temperature of the fermenter has been kept at 30 °C by a cooling system, but the system is problematic in that it takes a great deal of cost. Therefore, we aim to create strains that produce glutamic acid under various temperature environments. In this study, we used the thermo-tolerant strains obtained previously to compare the productivity of glutamic acid by adding penicillin under various temperature conditions, to search for useful thermo-tolerant strains, and to analyze their effectiveness.

Based on the results of flask culture, the production of glutamic acid was compared between the isolates PP80 and N24, which are thermo-tolerant strains, and the control strain KY9002, using the jar fermenter at 30, 37, and 39 °C. As a result, the production with KY9002 decreased with increasing temperature, whereas PP80 and N24, which are thermo-tolerant strains, showed high glutamic acid productivity even under high temperature conditions. In particular, PP80 showed the highest productivity of glutamic acid under any temperature conditions, and showed a productivity close to that at 30 °C even at 39 °C. In addition, we analyzed the electricity consumption by the glutamic acid production with the PP80 strain under various temperature conditions, and we will report it together.

Mild extraction of a red fluorescent protein from yeast cells

Shohei Noguchi[1], Hisashi Hoshida[1, 2, 3], and Rinji Akada[1, 2, 3]
[1]GraduateSchool of Science and Technology for Innovation, Yamaguchi University
[2]Yamaguchi University Research Center for Thermotolerant Microbial Resources
[3]Yamaguchi University Biomedical Engineering Center

Lipid bilayer of cell membrane divides intracellular environment from the outside. In general, small and non-polar molecules such as oxygen and carbon dioxide, and hydrophobic substances can transfer across membrane but polar molecules and proteins can not. These molecules that cannot penetrate the membrane utilize translocation proteins such as channels and transporters. However, there are proteins that are thought to be secreted without using channels, which is called non-conventional secretory pathway. We hypothesized that some proteins could directly translocate across cell membrane. To know such mechanism, we recently developed a protein extraction method from yeast cells without mechanical cell disruption process. However, after the extraction, yeast cells were dead due to the severe cell treatment. In order to find mild extraction conditions that allow extraction at lower temperatures, various extraction and cell culture conditions were examined by changing components for the protein extraction and culture conditions. Yeast cells expressing a red fluorescent protein (RFP) were cultured, and the red color migration from cells to the extracellular fluid was observed. First, extraction was performed by changing pH of the protein extraction solution. Extraction was possible at a pH of 8 or higher. Next, 10 mM or 100 mM of NaCl, KCl, $MgCl_2$, and $CaCl_2$ were mixed with the extraction solution. When extracted with 100 mM $MgCl_2$ or $CaCl_2$, the extraction was enhanced. Next, yeast cells were cultured with these ions, and then, the yeast cells were extracted with the extraction solutions. When the culture was performed with 100 mM $CaCl_2$, extraction was enhanced. When the extraction was performed to the cells cultured in medium containing 100 mM $CaCl_2$ with the extraction solution containing 100 mM $CaCl_2$, extraction was better than others. Then, using better conditions the extraction was performed at 30℃. RFP extraction was successful and also a part of yeast cells were alive. The developed mild extraction method might support the RFP transfer across the cell membrane of live yeast cells.

Search for amino acid residues involved in quinone binding in *Escherichia coli* membrane bound glucose dehydrogenase

Yumika Mizukami[1], Kenju Kobachi[2], Hanae Shinoda[2], Tomoyuki Kosaka[1] and Mamoru Yamada[1]
[1]Department of Biological Chemistry, Faculty of Agriculture and Life Science, Graduate School of Science and Technology for Innovation ,Yamaguchi University, Japan
[2]Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan

Membrane-bound glucose dehydrogenase (mGDH) in *Escherichia coli*, a monomeric apo-form of 87 kDa and a primary enzyme in the respiratory chain, performs a direct oxidation of D-glucose to D-gluconate and transfers electrons to ubiquinol oxidase via UQ (1). The mGDH requires pyrroloquinoline quinone (PQQ) as coenzyme and has two ubiquinone binding site, site Ⅰ for bound-UQ and site Ⅱ for bulk-UQ. Although site Ⅱ has been expected to locate near the surface of inner membrane (2) and the location of catalytic site and some amino acids involved in glucose oxidation have almost been characterized (3), the location of the UQ binding site and the amino acid residues interacting UQ have not been clarified yet. Since it has reported that arginine is high conserved in the quinoprotein around UQ in *Thermus thermophilus* of NADH dehydrogenase (4). In this study, several alanine substitution mutants (R432A, R503A, R560A) of mGDH were constructed by site specific mutagenesis and subjected to biochemical analysis. Of these mutant enzymes, the glucose dehydrogenase activity of R432A-mGDH was reduced to about 1% as compared with that of wild type. This suggest that Arg-432 is largely involved in the function of the enzyme. When the activity was measured by the addition of excess amount of PQQ to R432A-mGDH, the activity partially recovered. On the other hand, when the steric structure of mGDH was modeled based on the crystal structures of methanol dehydrogenase and alcohol dehydrogenase, Arg-432 was predicted to be located 19 Å away from PQQ. These results suggest that Arg-432 may not directly involved in the transfer of electrons in mGDH.

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Improvement of various stresses resistance in *Kluyveromyces marxianus* by long-term cultivation at high temperature

Sornsiri Pattanakitiivorakul[1], Tatsuya Tsuzuno[2], Tomoyuki Kosaka[1,2]and Mamoru Yamada[1,2]
[1]*Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan*
[2]*Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Japan*

To achieve the efficient ethanol fermentation at high temperature, yeast strains that tolerate not only high temperature but also other stresses present during fermentation, such as ethanol, acetic acid, osmotic and oxidative stresses, are essential. The objective of this study was to improve thermotolerant ethanol fermenting yeast to be stresses tolerance by long-term cultivation. The strain used was *Kluyeromyces marxianus* DMKU3-1042 (1, 2) as a one of thermotolerant yeast strain that can produce ethanol at high temperature and utilizes various sugars. In previous study, four adapted strains, ACT001, ACT002, ACT003 and TML001, were obtained after repeated long-term cultivations at high temperatures. All adapted strains were analysed by genome sequencing and found to have several mutations.

In this study, growth and ethanol production of adapted strains were determined by shaking flask cultivation in yeast extract peptone (YP) broth containing 16% glucose at 45 ˚C. The results showed that strain TML001 grew faster than the parental strain and all adapted strains produced ethanol concentrations higher than the parental strain. In addition, adapted strains were investigated for their tolerance to various stresses. Some adapted strains showed more tolerance to high ethanol concentration, acetic acid and formic acid than the parental strain at high temperature. Therefore, these results suggest that long-term cultivation is useful for improvement of ethanol production and of stresses tolerance of *K. marxianus* DMKU3-1042.

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Establishment of High Temperature Consolidated Fermentation Process by Thermotolerant Microbs

Md. Tareque Aziz[1], Udvashita Chakraborti[1] and Ali Azam Talukdar [1]

Department of Microbiology, Jahangirnagar University, Savar Dhaka-1342, Bangladesh.

Microorganisms that withstand high temperatures will continue to gain global significant prominence in consolidated bio-production of ethanol. Consolidated fermentation is an approach integrating enzymes production, saccharification and fermentation into a single process effective for ethanol production. It offers considerable advantages for the production of bioethanol. Main purpose of this study was to isolate strains capable of Xylose utilization and co-culture them with highly fermentative *Saccharomyces cerevisiae* in order to produce ecofriendly biofuels. So, microbial ethanol production via consolidated fermentation in recent years, has been consolidated as an alternative fuel in the near future.

We have collected samples from various natural fermented sources of Bangladesh for fuel ethanol production (1, 2). Samples were screened based on their capability of fermentation and saccharification by carbohydrate fermentation test with durham tube (1). Among them, *Saccharomyces cerevisiae, Kluveromyces marxianus* gave fermentation and *Bacillus licheniformis, Bacillus amyloliquefaciens* gave saccharification were performed (2). Various biochemical analysis like reducing sugar level and starch hydrolysis were carried out by Dinitrosalicylic acid (DNS) and starch hydrolysis were performed on above strains whether they can utilize sugar and starch. In order to standardize consolidated fermentation process, several physiological parameters for growth including various substrate concentrations, temperatures (25-45 ˚C), pHs (4.5-7.0), fermentation time (12-144 h) etc. were performed under the conditions we have employed here. Optimization of consolidated fermentation process-parameters revealed that the mono-culture of *Bacillus amyloliquefaciens* and *Bacillus licheniformis* gave the lowest concentration (less than 0.5 %) of ethanol except *Saccharomyces cereviciae* which gave 5.0 % at pH 6.0, 144 h at 37° C. The co-culture of *Saccharomyces cereviciae* and *Bacillus amyloliquefaciens* in glucose broth gave highest concentration of ethanol (7.5 %) at pH 6.0, 144 h in 37° C, respectively.

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Synthesis of amorphous-ZrO_2/Mg-Fe layered double hydroxide composite for phosphate adsorption

Atin Nuryadin[1] and Tsuyoshi Imai[1]
[1] Division of Environmental Engineering, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi 755-8611, Japan

Phosphorus is an essential nutrient in aquatic environments. The phosphorus usually exists as phosphate ions in water. Nevertheless, excessive release of this element into water bodies leads to eutrophication by stimulating algal bloom, disruption of aquatic animal populations, and deterioration of water quality. Great amounts of phosphorus have been discharged from various human activities, such as agricultural, industrial and domestic activities. Therefore, it is necessary to eliminate phosphate contamination in advance of wastewater discharge.

In this study, the composite of amorphous zirconia (am-ZrO_2) and magnesium-iron layered double hydroxide (Mg-Fe LDH) was synthesized. The ability of anion exchange of LDH and the porous with the highly hydrated structure of amorphous zirconia provide high adsorption capacity in both materials. Combination of nanosized amorphous zirconia with Mg-Fe LDH component was expected to provide a facile method to facilitate the high phosphate adsorption ability. Fresh prepared Mg-Fe LDH gel with Mg/Fe molar ratio of 3 was combined with different molar concentrations of $ZrOCl_2 \cdot 8H_2O$ (Zr/Fe molar ratio of 0.5, 1, 1.5, and 2) to get different Zr contents of the composite. The characteristics of uncalcined and calcined composites with different molar ratios of Zr were investigated by using X-ray diffraction (XRD) patterns, and its application on phosphate adsorption from aqueous solution was systematically examined by batch experiments.

The result showed that the uncalcined composites were the combination of LDH and am-ZrO_2, while the calcined samples were the combination of collapse-structured LDH and very small tetragonal ZrO_2 crystal. Uncalcined composites showed the highest phosphate adsorption capacity in 10 mg-P/l phosphate solution compared to individual uncalcined and calcined LDH and am-ZrO_2.

Production of recombinant beta-mannanase from *Escherichia coli* KMAN-3 using auto-induction system in 2-L bioreactor

Kanok-On Khongcharoen[1], Preuk Tangpromphan[2], Tanat Uan-On[3] and Kittipong Rattanaporn[4]

[1-4]Department of Biotechnology, Faculty of Agro-industry, Kasetsart Univ.

The beta-mannanase is an enzyme which hydrolyzes mannan and heteromannan such as glucomannan, galactomannan and glucogalactomannan to potentially produce health-promoting mannooligosaccharides (MOS) (Chauhan P.S., 2012). Recently, production of recombinant beta-mannanase has been developing to satisfy an industrial demand of enzyme-based production of MOS. In previous study, recombinant *Escherichia coli* KMAN-3 was developed to express high level of secreted beta-mannanase using auto-induction system of lactose (Piyanat, 2017). In this study, the effects of carbon source, the effects of alternative inducers and inducer concentration on the beta-mannanase production of *E. coli* KMAN-3 in shake flask were investigated. The results revealed that glucose was an optimal substrate to promote the cells growth while galactose was the appropriate inducer which provided high beta-mannanase activity at the similar level as isopropyl β-D-1-thiogalactoside (IPTG). The optimal galactose concentration for induction of recombinant beta-mannanase was 5.55 mM that could produce the maximum enzyme activity of 1.38×10^5 U/l at 36 h. In addition, we further studied on the production of beta-mannanase in 2-L bioreactor and determined the kinetic parameters of cell growth and recombinant enzyme production. The results showed that the production of beta-mannanase in 2-L bioreactor provided the maximum cell concentration of 1.535 g/l at 12 h and the maximum level of beta-mannanase activity of 1.49×10^5 U/l at 48 h. At the optimal glucose concentration, the kinetic parameters of cell growth and beta-mannanase production as specific growth rate (μ), substrate consumption rate (q_s), specific rate of product formation (q_p), the yield coefficient of cell ($Y_{x/s}$) and the yield coefficient of product ($Y_{p/s}$) were 0.168 h^{-1}, 0.013 g/l h, 3.1×10^3 U/l h, 2.02 $g_{cell}/g_{substrate}$ and 237.55 $U/g_{substrate}$, respectively.

Optimization of culture and physical conditions to produce of phytase from *Pholiota adiposa* through solid-state fermentation

Kritsana Jatuwong[1,2], Nakarin Suwannarach[1,2], Jaturong Kumla[1,2], Kenji Matsui[3] and Saisamorn Lamyong[1,2]
[1]Faculty of Science, Chiang Mai Univ. [2]Center of Excellence in Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai Univ. 2Faculty of Agriculture, Yamaguchi Univ.

Phytase is a group of enzymes that catalyze phytic acid, myo-inositol and inorganic phosphate. It is the most commonly enzyme used in feeding of monogastric animals such as poultry, pigs and fishes. It can reduce the antinutritional effect of phytate and improve the digestibility of phosphorus. Phytases could be produced from host sources including plants, animals, and microorganisms (bacteria, yeast and filamentous fungi). Several microorganisms such as *Aspergillus tubingensis, Schizophyllum commune* and *Ganoderma* sp. are considered as potential phytase producers when they grew with lignocellulose. In this study, among the twenty-seven different microorganisms are grown with five different types of lignocellulosic substrates including, sawdust, coffee parchment, oil palm empty fruit bunch, rice bran and water hyacinth. *Pholiota adiposa* was selected as the most phytase productive strain. Water hyacinth was selected as the suitable substrate for the optimum condition of increasing the phytase production though solid-state fermentation. The nutrient factors (carbon and nitrogen sources) and the physical parameters (inoculum size, weight of substrate, moisture content, pH temperature and profile of fermentation) were investigated. Our investigation revealed that optimal productively of phytase was achieved using water hyacinth supplemented with 0.2 % glucose and 0.01% ammonium nitrate. Additionally, optimal physical conditions were adjusted moisture to 85% (v/w) with pH 6.5 of culture media, incubated at 30 °C for 7 days. Phytase activity in optimum condition medium reached in 51.98±1.68 Units/gram of dry substrate.

Oxidization of disaccharides using membrane-bound glucose dehydrogenases of *Gluconobacter* spp.

Yuri Uchida[1], Toulaphone Keokene[2], Naoya Kataoka[1,3,4], Kazunobu Matsushita[1,3,4] and Toshiharu Yakushi[1,3,4]

[1]Graduate School of Sciences and Technology for Innovation, Yamaguchi Univ. [2]Faculty of Natural Science, National Univ. of Laos, [3]Faculty of Agriculture, Yamaguchi Univ. [4]RCTMR, Yamaguchi Univ.

Acetic acid bacteria have unique function that oxidizes various sugars and alcohols. The oxidation reactions are due to the action of membrane-bound enzymes of acetic acid bacteria. This study focuses on glucose dehydrogenase a pyrroloquinoline quinone (PQQ)-dependent enzyme as for the oxidization of the disaccharides such as lactose, cellobiose, maltose, and isomaltose.
We constructed *Gluconobacter oxydans* strain, NBRC3293 Δ*gdhM* harboring a plasmid carrying the $gdhM^{621H}$ gene, the *gdhM* gene from *G. oxydans* strain ATCC 621H, or $gdhM^{CHM43}$, that from *Gluconobacter* sp. CHM43. The membranes of the recombinant strains oxidize some sugars to sugar acids. GDHs showed the highest activity on glucose, and cellobiose and isomaltose were also good substrate on GDH. On the other hand, GDHs showed much lower activity on lactose and maltose than other disaccharides. The data suggest that lactose might have a much lower affinity to GDHs than glucose. Indeed the Km value of lactose on GDH was much larger than that of glucose. Furthermore, we found that the activity to maltose was significantly different from that to isomaltose. The difference between maltose and isomaltose is only the position of the bond between two molecules of glucose. i.e. α-1,4 bond on maltose and α-1,6 bond on isomaltose. This contribute to the structural difference between the two disaccharides. Because of the structural difference in the substrates, the binding force to GDH might change to affect the activity. It is remained as the future works to identify the structural features of GDH as for the difference in reactivity with these substrates. Currently, we atempt to reconstruct the expression system for GDH, because the first version of the recombinant strains cannot grow stably and the enzyme activities largely fluctuate.

Expression of bacterial proteins from *Bacillus subtilis* in the eukaryote *Saccharomyces cerevisiae*

Sayaka Kanameda[1], Rinji Akada[123]. and Hisashi Hoshida[123]
[1]GraduateSchool of science and Technology for Innovation, Yamaguchi University
[2]Yamaguchi University Research Center for Thermotolerant Microbial Resources
[3]Yamaguchi University Biomedical Engineering Center

The yeast *Saccharomyces cerevisiae* is used not only for food production but also for bioethanol production because of its high fermentation ability. Starch is one of major feed stocks for bioethanol production. For cost-effective bioethanol production from starch, it has been often attempted to express a bacterial enzymes for hydrolyzing starch and others. However, expression levels of bacterial proteins in the eukaryotic yeast sometimes lower. Therefore, in this study, proteins of *Bacillus subtilis* (Bs), which is known to secrete useful saccharification enzymes, were expressed in the yeast *S. cerevisiae*. At first, the proteins in the *Bacillus* secretory pathway were selected. Genes for BsSecY, BsSecA, BsSecG, BsTatE, BsTatAd, BsTatAc, BsTatAy, BsTatCd, and BsTatCy from *Bacillus subtilis* were amplified by PCR, and expressed as fusion proteins with yEGFP to observe expression and localization in *S. cerevisiae*. GFP signal of TatAc, TatAd and TatCd were not observed. BsSecY was expressed as large granulated form in the cell. In the case of BsSecG, localization was observed in cytoplasm when GFP was attached to the C-terminus. In the case of BsSecA, TatAy and TatCy, localizations were observed when GFP was attached to the N-terminus but granular localization was observed when attached to the C-terminus.

We are trying to express various bacterial proteins in yeast to obtain the functional form.

Heterologous expression in *Escherichia coli* of succinate dehydrogenases in *Pelotomaculum thermopropionicum* SI

Yusuke Shiota[1], Yuka Tsushima[1], Mamoru Yamada[2] and Tomoyuki Kosaka[2]
[1]*Division of Agricultural Sciences, Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan*
[2]*Department of Biological Chemistry, Faculty of Agriculture and Life Science, Graduate School of Science and Technology for Innovation, Yamaguchi University, Japan*

Succinate oxidation in syntrophic propionate oxidation, which is the most unfavorable reaction under a methanogenic condition, is catalyzed by succinate dehydrogenase (SDH). A thermophilic, propionate-oxidizing bacterium, *Pelotomaculum thermopropionicum* SI possesses two SDHs, membrane associated SDH1 and cytoplasmic SDH2. These SDHs have been predicted to play different roles as SDH1 working as a major succinate dehydrogenase in propionate oxidation and SDH2 presumably working as a fumarate reductase under a fumarate-utilizing condition. In this study, we tried to heterologously express these SDHs in *Escherichia coli* for elucidation of the more detail function of two SDHs. We constructed expression plasmids including SDH-coding gene clusters of *P. thermopropionicum* and these were transformed into *E. coli* BW25113Δ*sdh*Δ*frd*. However, no SDH activity was observed in these transformants. On the other hand, Δ*sdh*Δ*frd* strain harboring another plasmid, which includes the *E. coli sdh* operon but *sdhA* was exchanged by *sdh1A* of *P. thermopropionicum*, showed very weak activity. It was examined whether flavin adenine dinucleotide (FAD), which is the cofactor of SDH, bound to SdhAs expressed in *E. coli*. The observation of FAD binding indicated that the FAD fluorescence on the Sdh1A band of *P. thermopropionicum* expressed in *E. coli*, suggesting that there are other reasons for no or less SDH activity of SDHs of *P. thermopropionicum* expressed in *E. coli*.

Clarification of SulA-dependent cell lysis (SDCL) pathway in *Escherichia coli*

Natsuko Ota[1], Ayumi Osawa[1], Tomoyuki Kosaka[1] and Mamoru Yamada[1]
[1]*Graduate School of Sciences and Technology of Innovation, Yamaguchi University, Japan*

In *Escherichia coli*, the overexpression of *sulA*, which is one of SOS response genes (1, 2), lead to cell lysis. We designated this cell lysis as SulA-dependent cell lysis (SDCL). The pathway of SDCL is probably independent from the reported cell death pathways in *E.coli*, which are σ^E pathway, ALD pathway and *mazEF* pathway. However, the detail mechanism of SDCL has not been clarified yet. The previous studies suggested that SDCL occurs via the expression of *soxS*, which is a global transcriptional regulator responding to oxidative stress. In this study, to find out genes downstream from *soxS* in the SDCL pathway, we tried to compare the extent of SDCL among 19 candidate genes, which are activated or repressed by SoxS as a SoxS regulon (3). The experimental results suggested that LpxC, which is an essential gene related in the synthesis of lipopolysaccharide, was involved in SDCL downstream of SoxS because *lpxC*-overexpressed strain showed remarkable cell lysis. To clarify the involvement of *lpxC* in SDCL, we observed the effect of the LpxC inhibitor, CHIR090 (4), on SDCL. As a result, the level of SDCL was repressed by the increment of CHIR090 concentration. In conclusion, the main cascade of SDCL is in the order of SulA > SoxS > LpxC.

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Influence of Methyl salicylate(MeSA) applied to eggplant on the behavior of *Thrips palmi* Karny (Thysanoptera: Thripidae) and its natural enemy *Orius strigicollis* Poppius (Hemiptera: Anthocoridae)

Shan-Guan Syu [1] and Shaw-Yhi Hwang[1]
[1] Dept. of Entomology, National Chung Hsing University, Taichung 40227, Taiwan.

Eggplant [*Solanum melongena* L. (Solanaceae)] is one of the important vegetables in Taiwan. The most serious eggplant pest is the *Thrips palmi* Karny (Thysanoptera: Thripidae) that cause serious feeding damage to eggplants and also known to vector plant virus diseases, both of that always causing serious economic lost in Taiwan. Taiwan has a variety of native *Orius* spp (Hemiptera: Anthocoridae) that are good at preying on small pests such as thrips and spider mites. *Orius strigicollis* Poppius has the strongest activity and predation ability. There are many successful cases of release *O. strigicollis* applied to the field. But because in present the cost, technique and human resource that of rearing *O. strigicollis* still high, so need to the extend working efficiency or value of *O. strigicollis* in the open field. Methyl salicylate (MeSA) an herbivore-induced plant volatiles(HIPVs), has been reported to be released from crop plant species following attack by herbivores. MeSA is known that can elicit control of pests through attraction of beneficial arthropods. In this study will using Y-tube olfactometer in the laboratory to investigate *T. palmi* & *O. strigicollis* responses after direct contact with odor from eggplant treated with the MeSA. To investigate will eggplant amplifies the herbivore-induced volatile response or not. Will conduct Headspace Volatile Collection of eggplant to investigate the volatile variation when eggplant exposure to synthetic MeSA. The aim of this study was to investigate were MeSA have potential to be a tool that can use to improve or solve the strategy of using *O. strigicollis* in biological control.

NADH and NADPH oxidation in the mutant strain of *Gluconobacter oxydans* NBRC 3293 lost the membrane-bound glucose dehydrogenase grown on glucose

Feronika Heppy Sriherfyna[1], Kensuke Hirano[1], Minenosuke Matsutani[1], Naoya Kataoka[1,2,3], Tetsuo Yamashita[4], Eiko Nakamaru-Ogiso[5], Kazunobu Matsushita[1,2,3] and Toshiharu Yakushi[1,2,3]

[1]Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Japan; [2]Faculty of Agriculture, Yamaguchi University, Japan; [3]Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Japan; [4]School of Medicine, Kagawa University, Japan; [5]Perelman School of Medicine, University of Pennsylvania, USA

Glucose is mainly oxidized into gluconic acid in the periplasmic space by membrane-bound glucose dehydrogenase (the *gdhM* gene product) in *Gluconobacter oxydans*, followed by further oxidation to produce 2- or 5-ketogluconic acid. Most of gluconic acid and ketogluconic acids are remained in the culture medium. However, the mutant strain devoid of the *gdhM* gene drives glucose to pentose phosphate pathway or Entner–Doudoroff pathway due to an enzyme deficiency in Embdem-Myerehof-Parnas pathway. Interestingly, the mutant strain consumes most glucose to produce acetic acid the final metabolite. This central metabolism produces both NADH and NADPH. NADH is oxidized by membrane-bound type-II NADH dehydrogenase (NDH) transferring electron to ubiquinone and later to terminal oxidases to generate the proton motive force. *G. oxydans* strain NBRC 3293 has two phylogenetically different NDHs, c-NDH and s-NDH.

In order to figure out biochemical properties of the two NDHs, we designed plasmids for heterologous expression. The c-NDH and s-NDH were functionally expressed in an *E. coli* strain devoid of type-I and type-II NDHs. The two NDHs oxidized NADH, but s-NDH also did NADPH. Oxidation rate of NADPH with s-NDH was higher than that of NADH. It is concluded that c-NDH is an NADH-specific enzyme, but s-NDH oxidizes NADPH and NADH. We suggest that a role of s-NDH is oxidation of NADPH in *Gluconobacter* cells.

In order to perceive the physiological role of s-NDH, we constructed the *G. oxydans* NBRC 3293 derivatives defective of s-NDH and cultivated them in glucose medium. The Δ*gdhM* mutant strain that loss membrane-bound glucose dehydrogenase uptakes most of glucose directly to the cytoplasm without be converted to gluconic acid as in the wild-type case at the onset of catabolism. Therefore, it is expected that higher amount of NADPH would be produced than wild type. The result revealed that there is no significant growth difference in Δ*ndhS* strain to the wild-type strain, whereas the Δ*gdhM* Δ*ndhS* double mutant strain performed less growth rate than the reference Δ*gdhM* strain. Deficiency in s-NDH does not show prominent effect under the *gdhM*$^{+}$ background presumably due to less accumulation of NADPH the exertion of NADPH. In contrast, s-NDH deficiency under the Δ*gdhM* background affects the growth presumably due to NADPH accumulation.

Characterization of *Leafy Cotyledon1 (LEC1)* in oil palm (*Elaeis guineensis* Jacq.)

Mantira Suksirt[1], Bussarin Wachananawat[1], Kamolwan Khianchaikhan[1] and Chatchawan Jantasuriyarat[1]

[1]Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand

African oil palm (*Elaeis guineensis*) is an economic crop. Oil palm was used as a source in the production in several industries such as food, fuel and cosmetic product. Since the tissue culture is the best way to propagate the high yield oil palm, unfortunately the somatic embryogenesis during tissue culture takes about 7-8 months which is longer than the other economic plants. The molecular mechanism during somatic embryogenesis in oil palm remains largely unknown. Recent researches reported that Leafy Cotyledon1 (LEC1) is a key regulator of somatic embryogenesis. In this study, the promoter of *LEC1* was cloned and bioinformatics was used to analyze the sequence. Furthermore, oil palm LEC1 protein was expressed in *E. coli* to characterize its structure. Moreover, PHYTOCHROME-INTERACTING FACTOR (PIF4) was reported to be interacted with LEC1 protein in Arabidopsis during post embryogenic stage. It was reported to regulate the somatic embryogenesis by regulating the level of auxin hormone, which is an important phytohormone during somatic embryogenesis. Thus, *EgPIF4* gene was cloned. The gene structure was constructed and bioinformatics was used to analyze its amino acid sequence. RNA expression level of *EgPIF4* was examined using real-time PCR. Protein interaction between LEC1 and PIF4 was investigated using yeast two-hybrid assay. The results showed that *EgPIF4* was expressed in somatic embryo tissue specifically at globular stage, which it is co-expressed together with *EgLEC1*. Furthermore, LEC1 protein interacts with PIF4 protein in oil palm. The results from this study are the preliminary data that will help understanding the mechanism of somatic embryogenesis and will lead to shortening the tissue culture length in the future.

Effects of chitosan combined with chitosan-montmorillonite nanocomposites coating on postharvest quality of 'Hom Thong' banana fruit

Arisa Wantat[1], Pranee Rojsitthisak[2]and Kanogwan Seraypheap[1]

[1]Center of Excellence in Environment and Plant Physiology, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[2]Metallurgy and Materials Science Research Institute, Chulalongkorn University, Bangkok 10330, Thailand

Montmorillonite (MMT) has been widely used as nano-filler of polymer film coating to enhance their strength and barrier properties. In the present research, chitosan coating alone and distilled water were applied as control treatments. Chitosan combined with chitosan-montmorillonite nanocomposites (CTS/MMT); 1%, 2% and 4% (w/v) were applied as combination coating for 'Hom Thong' banana fruit which is the important fruit crop in Thailand. Fruits were dipped in the coating solution for 1 minute and then stored at 25 °C. The results exhibited that both of the application of chitosan coating alone and the combination coating could maintain banana fruit firmness while the combination treatment with 2% CTS/MMT coating showed the highest value of fruit firmness and also exhibited low total soluble solid content and retarded peel color change in terms of L value and hue angle throughout the storage period. Moreover, electrolyte leakage (%) value which indicated membrane leakage was reduced in the fruit coated with the combination solutions. Furthermore, based on DPPH inhibition (%) analysis, the combination treatment with 2% CTS/MMT coating also resulted in the highest antioxidant capacity among treatments. These results suggested that 2% (w/v) CTS/MMT might be the most appropriated material applying as nano-filler polymer to increase chitosan thin film stability and improve film barrier property thus leading to delay fruit softening and enhance antioxidant capacity after harvest. Therefore, the combination treatment with 2% (w/v) CTS/MMT could be the effective coating to prolong shelf life of 'Hom Thong' banana fruit.

Histone gene analysis with a plasmid that is able to use for gene expression in yeast and human culture cells

Takahiro Ono[1], Hisashi Hosida[1,2,3] and Rinji Akada[1,2,3]
[1]Dept. Appli. Chem., Grad. Sch. Sci. Tech. Innov., YamaguchiUniv., [2]YURC-TMR., [3]YUBEC

In order to express a favorite gene in various hosts such as yeast, *E. coli*, and human culture cells, it is necessary to construct a plasmid separately using host-specific promoters, host-specific origins of replication, and host-specific selectable markers. Even using a shuttle plasmid that can be handled in two host organisms, the host-specific promoters must be placed upstream of a target gene, so that the expression of a target gene is limited to one of two host organisms. If constructed plasmid could be used directly to multiple host organisms, it will be very useful. Therefore, in this study, we developed a single plasmid vecter which express a target gene directly in yeast, and also in *E. coli* and mammalian culture cells through PCR amplification.

Since the yeast *Saccharomyces cerevisiae* can perform homologous recombination correctly and efficiently, we considered that yeast plasmid should be the starting vector. In yeast, without any restriction enzymes and DNA ligases, transformation of PCR fragments can construct recombinant plasmids. The yeast plasmid vector was designed as follows. For yeast promoter, *TDH3*p was used, and a target gene was placed downstream of the *TDH3*p. The yeast replication origin (2μmOri) and *URA3* selectable marker were included in the vector. Downstream of the target gene, SV40 terminator sequence (SVter) for the expression in mammalian cells was placed. Then, downstream of the SVter, *E. coli* replication origin (EcOri) and ampicillin resistant gene (AmpR) were placed. Downstream of these *E. coli* sequences, the mammalian promoter CMVp and *E. coli* promoter *srlA*p were located. The direction of these promoters were same to the target gene. After all, the plasmid contains 2μmOri-*TDH3*p-GENE-SVter-EcOri-AmpR-CMVp-*srlA*p-*URA3* in order. To express a target gene in *E. coli*, PCR from *srlA*p to the target gene is designed to amplify using primers containing 12-base overlap sequences. Using this 12-base sequences, the target gene is joined with the *srlA*p by the homologous recombination in *E. coli*, resulting in plasmid construction that express the target gene in *E. coli*. In mammalian culture cells, PCR is performed from CMVp to the target gene without any overlaps. The target gene is joined with the CMVp by non-homologous end joining in mammalian culture cells, resulting in the expression in mammalian cells. Using the vector, human histone gene fused with a fluorescent protein was analyzed in yeast and human culture cells.

Free Wi-Fi

1. **DoSPOT-FREE (for MS only)**
2. **Yamaguchi_Free_Wi-Fi**

・Max　60 minutes/1 day
・You can use in auditorium, entrance hall, second floor lounge and training building first floor.

やまぐちFree Wi-Fi

How to use

1. Connect with “Yamaguchi Free Wi-Fi”

2. Start a browser

3. Please accept the user policy

4. Input of your e-mail address

Lunch Information (1)

We can eat lunch in restaurant of seminar-park. Please prepare the money to buy lunch.

Menu	food allergies or dietary restrictions	Price (yen)
Lunch set	※ 1	510
Tonkatsu-lunch set (deep-fried pork)	Pork	570
Oyako-don (A bowl of rice topped with chicken and eggs)	Chicken, Egg	470
Katu-don (A bowl of rice topped with slices of deep-fried pork)	Pork, Egg	580
Gyu-don (A bowl of rice topped with beef)	Beef	580
Beef-curry and rice	Beef, Animal oil	420
Katsu beef-curry and rice (deep-fried pork on the carry and rice)	Pork, Beef, Animal oil	530
Hash and rice	Beef, Animal oil	510
Kitsune-udon (Udon with deep-fried tofu)	-	320
Sansai-udon (Udon with edible wild plants)	-	370
Niku-udon (Udon with beef)	Beef	370
Kakiage-udon (Udon with deep-fried vegetable strips, shrimp, etc.)	-	370
Nikutama-udon (Udon with beef and egg)	Beef, Egg	400
Kitsune-soba (Soba with deep-fried tofu)	Soba	350
Sansai-soba (Soba with edible wild plants)	Soba	400
Niku-soba (Soba with beef)	Soba, Beef	400
Kakiage-soba (Soba with deep-fried vegetable strips, shrimp, etc.)	Soba	400
Nikuama-soba (Soba with beef and egg)	Soba, Beef, Egg	430
Ramen noodle※1	Pork, Animal oil	410
Rice with tsukemono	-	120
Rice ball (one) ※2	-	80
Inari (two) ※2 (a kind of sushi made of deep-fried tofu stuffed with rice)	-	100

There is also a seasonal menu.

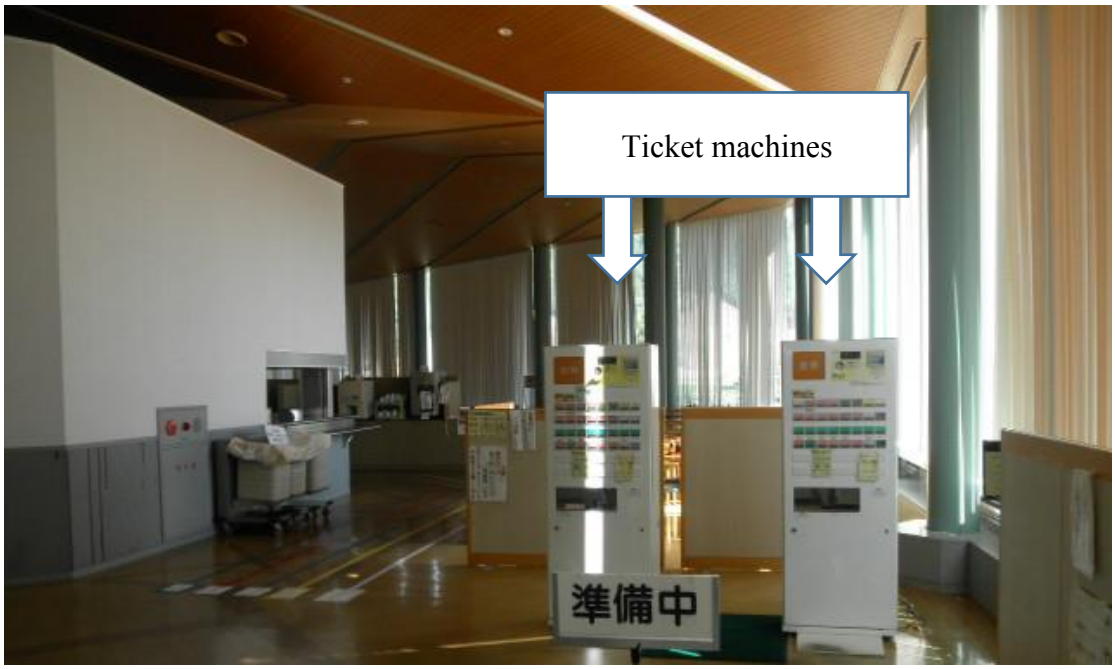
※1…Please ask staff, because menu changes depending on the day.

※2…It sells only on weekdays.

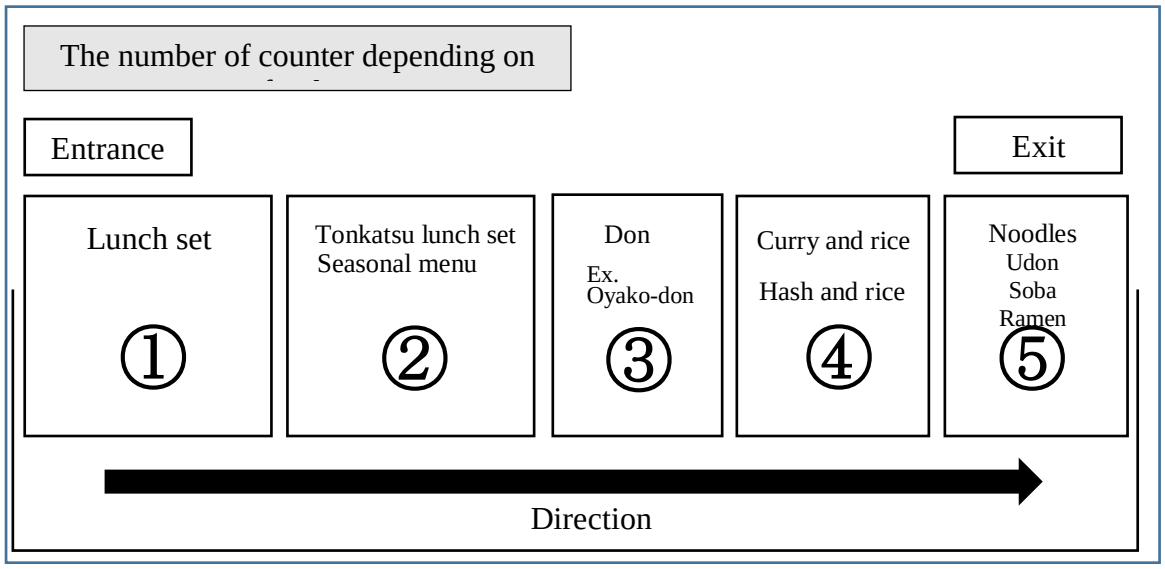
Lunch Information (2)

How to buy lunch

① Buy a ticket in ticket machine.



② Submit a ticket to the counter depending on foods, and receive foods at same counter.



Cautions

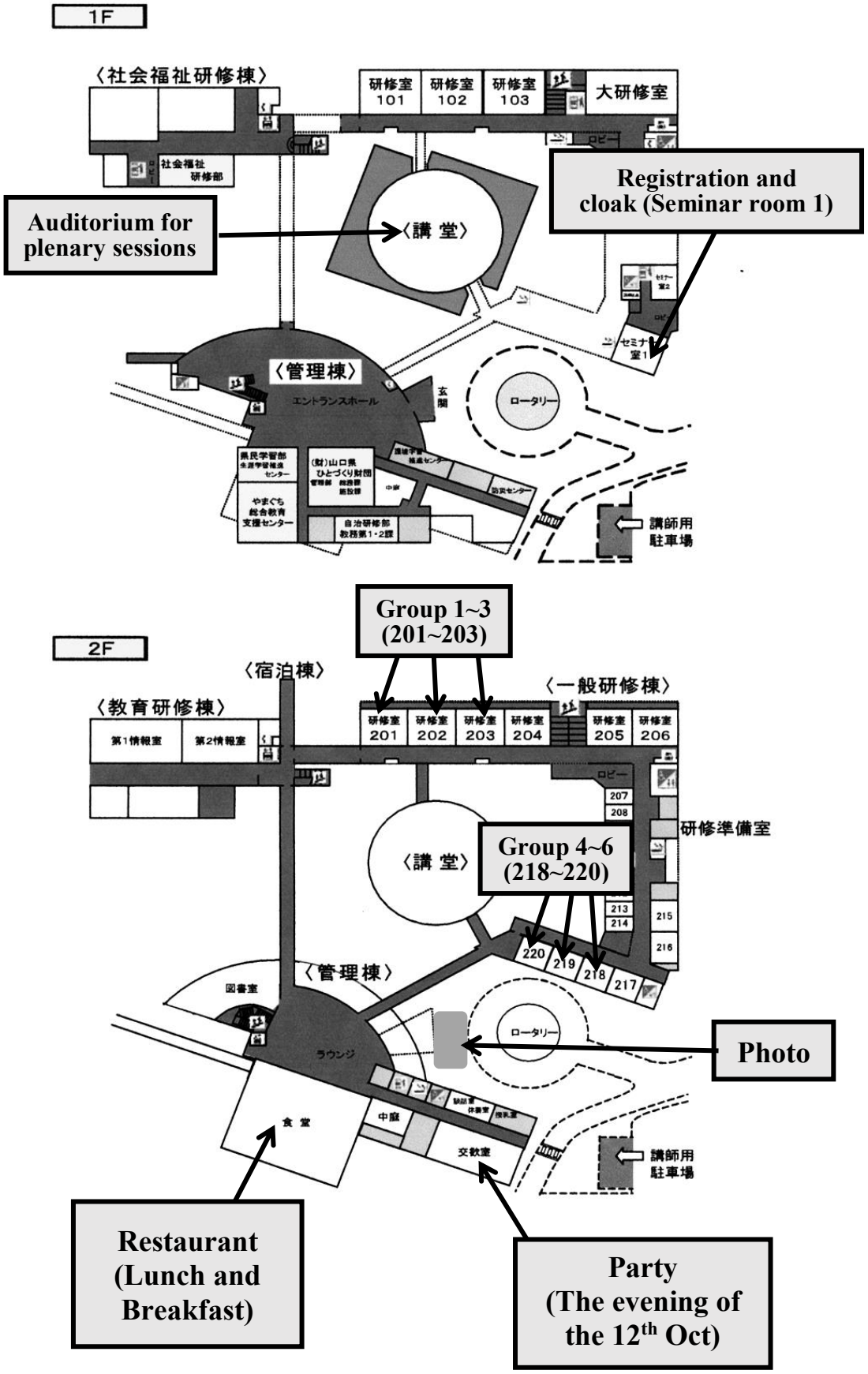
October 12th

- ◆ Take 2 sheets and 1 pillowcase from room where is beside the manager office
- ◆ Public bath room is open from 8:00 pm-10:00 pm
- ◆ Shower room (101-104) is open at 8:00 pm-10:30 pm and 5:30 am-7:30 am.
  (If you would like to use shower room, please make a reservation at the time for group discussion)
- ◆ The gate is close at 10:00 pm
  (Please come back to hotel by 10:00 pm)
- ◆ The light-out time is 11:00 pm, so you must not go outside your room from the time
- ◆ Don't have anything to eat and drink in your room
- ◆ Don't smoke outside the smoking room

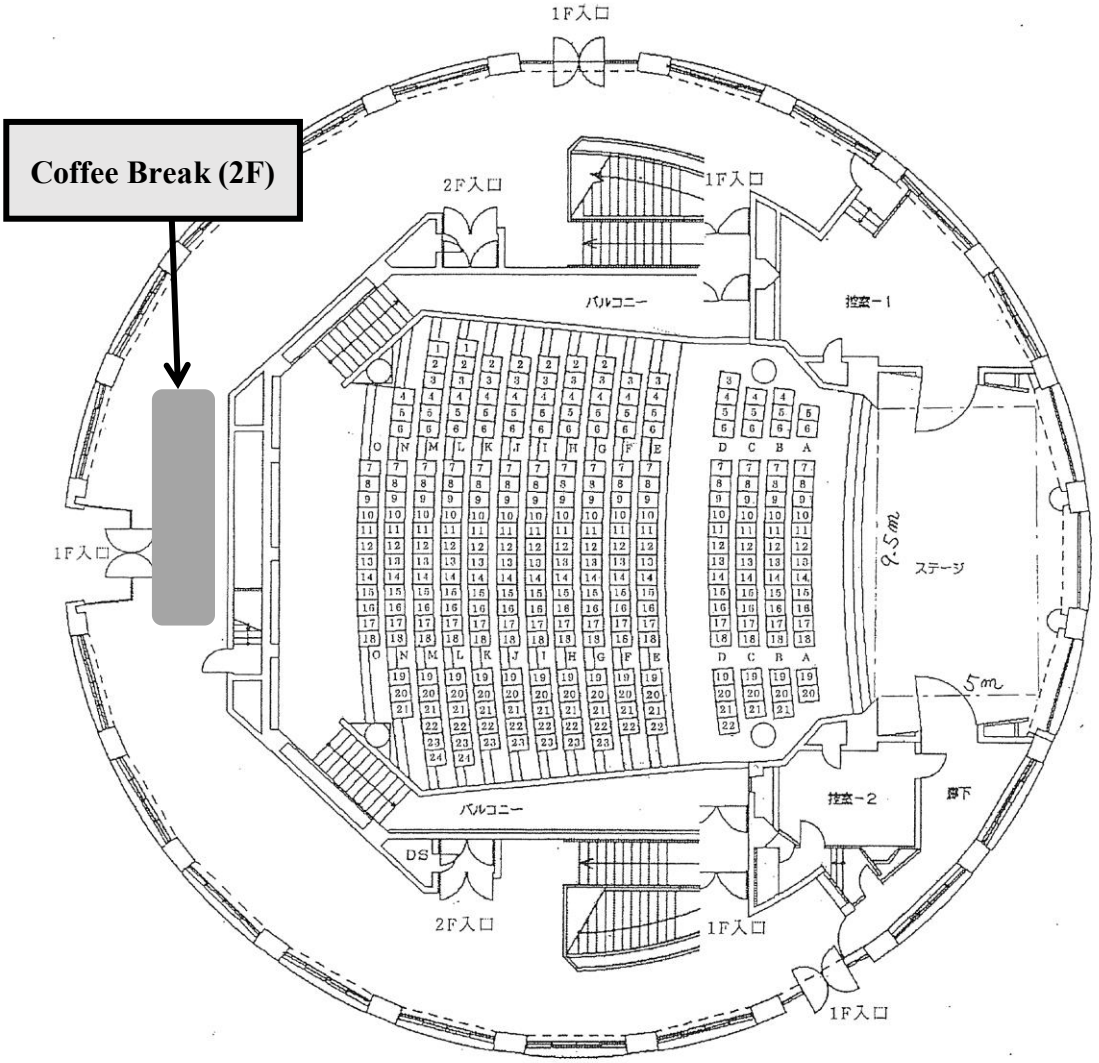
October 13th

- ◆ The gate is open at 7:30 am
- ◆ When you check out,
  1. Clean up your room
  2. Open the curtain
  3. Leave the door open
  4. Return the covers of blankets and pillow to the same place where you took those
- ◆ Please return the key to staff at the lobby by 8:30 am
- ◆ Breakfast time is during 7:30 am-8:30 am
- ◆ You can leave your baggage in the cloak room [**Seminar room 1**]

Map of Seminar Park



Map of Auditorium



Map of Accommodation building