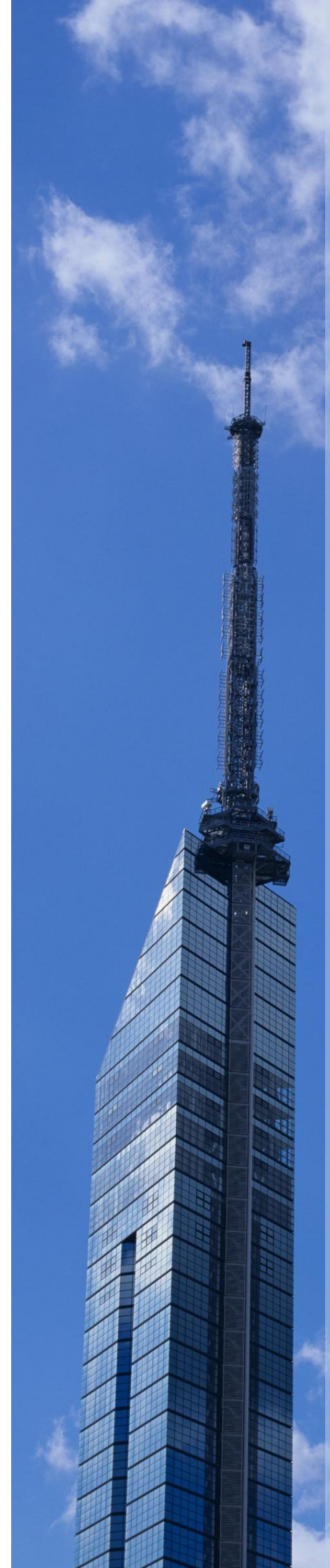

Japan Society for Bioscience,
Biotechnology and Agrochemistry (JSBBA)

Proceedings of the

JSBBA West 3rd Student Forum

December 19th, 2020

The day of issue:

in **Zoom Webinar**

Organizer:
JSBBA WEST Student Committee
Sponsors:

JSBBA WEST
Kyushu University

Preface

This third student forum could only be held with the cooperation of everyone. The present forum is held online to prevent the spread of COVID-19. We would like to sincerely thank everyone who participated in this forum, especially all the professors and staff of Kyushu University who supported the preparation of this forum. This organization was established as a subordinate organization of the West Japan Branch of the Japan Society for Bioscience, Biotechnology and Agrochemistry for the purpose of international exchange among students. We hope that your experience here will prove useful in the future as demand for globalization continue to rise. While this event might be considered a success, we recognize that there is much room for improvement. We apologize for any inconveniences we may have caused or failed to foresee. We hope to be able to address these problems and organize a better conference next year. We look forward to seeing you again in the next forum.

Chairperson Hayato NYUNOYA

Vice-chairperson Kota WATANABE

ご案内

（1）参加者のみなさまへ

形式：オンライン（Zoom Webinar）

開催時間：2020年12月19日（土）9:30〜17:00

（入室は9時10分開始となっております。）

1. オンライン参加方法

Zoomウェビナーを用いてのオンライン参加となります。**オンラインによる講演会場へのアクセスは、別の添付ファイルにあるID・パスコードまたはリンクより会場に入室ください。**

Zoomを初めて使用される場合は、Zoomのアプリケーションをインストールください。

（以下のURL からミーティング用 Zoom クライアントをインストールしてください）

https://zoom.us/download#client_4meeting

正しくインストールされているかを確認する場合は、以下のURLでお試しください。

https://zoom.us/test

※サインアップは必要ありません。

2. 質疑応答

講演に対する質疑応答は、Q&Aの「チャット」に書き込んでください。座長が代読します。質疑応答の選択は座長に一任しておりますので、ご理解のほどよろしくお願いします。

3. 参加に当たっての注意事項

講演を写真撮影、録画、録音等することは一切お断りします。

（2）発表者のみなさまへ

- 発表はすべて英語で執り行います
- 発表者の中から優秀発表賞を数名選出します
- 発表時間は9分、質疑応答は2分とし、発表前に1分間の接続時間を設けます
- スライドは標準サイズ（4:3）で準備してください

4. Zoom接続テストについて

前日の12/18金曜日15:00~16:00にZoom接続テストを予定しています。

別に添付されているファイルを参考に参加してください。

Guidance
(1) To all participants
STYLE: Online (Zoom Webinar)
TIME: 19th December 2020 (Sat) 9:30 AM ~ 5:00 PM
(The room in opening at 9:10 AM)

1. How to Enter the room
You can participate online using the Zoom webinar. **To access the room online, please enter the room from the ID / passcode or link in the separately attached file.**

If it is the first time for you to use Zoom, please install the Zoom application.
(Install the Zoom Client for Meetings from the URL below)
https://zoom.us/download#client_4meeting
If you want to check if it is installed correctly, please try at the following URL.
https://zoom.us/test
* No sign-up required.

2. Questions and answers
Please write the question for the presentation in "Chat" of Q&A. The chair will read it for you. The choice of questions is left to the chairperson, so we appreciate your understanding.

3. Precautions for participation
Photographing, recording, recording, etc. of the presentations is strictly prohibited.

3. To all presenters
- All presentations will be in English
- We will select several presentation awards from the presenters
- The presentation time will be 9 minutes, the question and answer session will be 2 minutes, and there will be a 1-minute connection time before the presentation.
- Prepare slides in standard size (4: 3)

4. Zoom connection test

We are going to hold a Zoom connection test at Dec. 18th 3:00~4:00 PM. Please refer to the separately attached file and participate in it.

Program プログラム

09:30 ~ **Opening speech　開会挨拶**

10:00 ~ 12:20 **Best presenters session　優秀発表者賞講演**

10:00 ~ 11:00 **A01 ~ A05 session　(演題番号A01 ~ A05)**

A01　10:00 ~ 10:11
Isolation of main bacteria on human scalp hair
〇NISHI Y, WATANABE K, TASHIRO Y, SAKAI K
（Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ.）

A02　10:12 ~ 10:23
Identification and characterization of a key component involved in mulberry leaves for preventing vasospasm
〇TSURUDOME N, MINAMI Y, KAJIYA K
（Bioscience and Biotechnology, Kagoshima Univ.）

A03　10:24 ~ 10:35
Effect of different buffers on predatory activity of *Bdellovibrio bacteriovorus* 109J
〇MORIYA T, HOSHIKO Y, MAEDA T
（Kyutech・Department of Biological Functions Engineering）

A04　10:36 ~ 10:47
Metagenomic analysis of gut microbiome of Indonesian and Japanese infants
〇SEINO T, NAKAYAMA J
（Bioscience and Biotechnology, Kyushu Univ.）

A05　10:48 ~ 10:59
Comparison of Growth Media for Diverse Colony Formation
〇YAMAMOTO K, TOYA S, SABIDI S, HOSHIKO Y, MAEDA T
（Kyutech・Department of Biological Functions Engineering）

11:00 ~ 11:20 **Short break　休憩**

11:20 ~ 12:20 **A06 ~ A10 session　(演題番号A06 ~ A10)**

A06　11:20 ~ 11:31
Association analysis between early endosome dynamics and kojic acid secretory production in *Aspergillus oryzae*
〇HIRAKAWA Y, TAKEGAWA K, HIGUCHI Y
（Kyushu Univ.・Applied Microbiology）

A07　11:32 ~ 11:43
Comprehensive Analysis of Gut Bacterial Metabolites Involved in Neurodevelopmental Disorders
〇KAWASAKI R[1], MAEDA N[1], MIYAMOTO K[2], OTSUKA H[1]
YOSHIDA S[2], YAMAZAKI T[1], KOSHIMURA M[1]
（1:NIT, Sasebo College, 2:Toyohashi University of Technology）

A08　　11:44 ~ 11:55

Potential use of sulfur-responsive region of sulfate transporter *SULTR2;1* for control of recombinant gene expression in plants

〇NGUYEN HT, SUYAMA A, OHTAKI C, MARUYAMA-NAKASHITA A

（Kyushu Univ.・Bioresource and Bioenvironmental Sciences）

A09　　11:56 ~ 12:07

Immobilization of 1,6-α-L-fucosidase and endo-β-*N*-acetylglucosaminidase for defucosylation of N-glycan.

〇HONDA A, TAKEGAWA K

（Applied Microbiology, Kyushu Univ.）

A10　　12:08 ~ 12:19

Identification and characterization of a bacterial α-galactosylceramide synthase

〇QU Q, ISHIBASHI Y, OKINO N

（Kyushu Univ.・Bioscience and Biotechnology）

12:20 ~ 13:20　　**Lunch break　昼休憩**

13:20 ~ 16:30　　**General presenters session　一般講演**

13:20 ~ 14:08　　**B01 ~ B04 session　(演題番号B01 ~ B04)**

B01　　13:20 ~ 13:31

Integration of foreign genes into transposon-like gene sequences in fission yeast.

〇MITSUTA Y, FUJIKI M, MAEKAWA H, HIGUCHI Y, TAKEGAWA K

（Applied Microbiology, Kyushu Univ.）

B02　　13:32 ~ 13:43

Comparative genome analysis of predominant bacterial strains in meta-fermentation

〇HIRANO K[1], YUKIHIRO T[1], HIROAKI K[2], HIROKUNI M[2], WATARU S[3], MASAHIRA H[3], KENJI S[1]

（[1]Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ., [2]Chiba Univ., [3]RIKEN）

B03　　13:44 ~ 13:55

Elucidation of gene expression mechanism for the pellicle biofilm formation in agrBD-knockout mutant of Clostridium perfringens

〇ITO R, HONDA K, ADACHI K, NAKAYAMA J

（Kyushu Univ.・Bioscience and Biotechnology）

B04　　13:56 ~ 14:07

(Cancelled)

14:08 ~ 14:30　　**Short break　休憩**

14:30 ~ 15:18 **B05 ~ B08 session (演題番号B05 ~ B08)**

B05 14:30 ~ 14:41
Isolation of uncultured major bacteria in the ATAD process by FISH observation and micromanipulation
〇TSUNEYOSHI K, ARAI T, SAKAI K, TASHIRO Y
（Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ.）

B06 14:42 ~ 14:53
Analysis of outcrossing and inbreeding in *Ogataea polymorpha*
〇KAI N, TAKEGAWA K, MAEKAWA H
（Kyushu Univ.・Bioscience and Biotechnology）

B07 14:54 ~ 15:05
Elucidation of secretory mechanism of the multiple bacteriocin transporter, EnkT
〇TAKEUCHI A, WADA , SUSHIDA H, NAKAYAMA J, ZENDO T
（Kyushu Univ.・Bioscience and Biotechnology）

B08 15:06 ~ 15:17
Analysis of glycolipids of *Pseudomonas aeruginosa* under phosphate starvation conditions
〇ZHU H, ISHIBASHI Y, OKINO N
（Kyushu Univ.・Bioscience and Biotechnology）

15:18 ~ 15:40 **Short break 休憩**

15:40 ~ 16:28 **B09 ~ B12 session (演題番号B09 ~ B12)**

B09 15:40 ~ 15:51
Elucidation of mechanism of antifungal bacteria Anti-G isolated from palm co-compost.
〇KONDO Y, NAKANO T, ASAHARA R, TASHIRO Y, SAKAI K
（Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ.）

B10 15:52 ~ 16:03
Development of a new gene expression vector for *Thermus thermophilus* using a silica-inducible promoter
〇IIDA T, FUJINO Y, SUEMATSU Y, DOI K
（Kyushu Univ.・Microbial generic resource ）

B11 16:04 ~ 16:15
Structural identification and characterization of novel bacteriocins
〇YOSHIDA H[1], NOMIYAMA T[1], KUWAHARA M[1], FUKAMI K[2], NAKAYAMA J[1], ZENDO T[1, 2]
（[1]Faculty of Agriculture, [2]Material Manager Center, Kyushu Univ.）

B12 16:16 ~ 16:27
Analysis of unconventional protein secretion for AoSod1 in *Aspergillus oryzae*
〇KUBOTA K, TAKEGAWA K, HIGUCHI Y
（Kyushu Univ.・Applied Microbiology）

16:50 ~ **Closing speech 閉会挨拶**

A01

Isolation of main bacteria on human scalp hair

○NISHI Y , WATANABE K , TASHIRO Y , SAKAI K
（Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ.）

【Introduction】It is known that many bacteria inhabit the human epidermis. In previous studies, it has been reported that high-density bacteria adhered to human scalp hair, and *Pseudomonas*, *Cutibacterium*, *Lawsonella*, *Staphylococcus* spp. are the main bacteria by bacterial community structure analysis. Meanwhile, the growth behaviors and the relationship of bacteria with hair have not been clarified. Therefore, in this study, to investigate the interaction between hair and major bacteria on human scalp hair, we isolated and identified hair-attached bacteria and investigated whether they are the major bacteria or not.

【Methods and Results】The samples were the subject's hairs and cotton swabs rubbed on the subject's scalp. The hair was placed directly on several solid media (5% sheep blood media, NA media, NA diluted media, LB diluted media), and the cotton swab was drawn on the solid media. After culturing at 30°C for colony formation and twice purifications, bacterial DNA extraction, amplification of 16S rRNA gene by PCR, and sequence analysis were performed. The obtained sequence data were used to identify bacterial species by EZ taxon. The sequence analysis of total 289 strains revealed that 51 species including major species of *Cutibacterium acnes* subsp. *defendens* and *Staphylococcus* spp. were isolated.

A02

Identification and characterization of a key component involved in mulberry leaves for preventing vasospasm

○TSURUDOME N , MINAMI Y , KAJIYA K
（Bioscience and Biotechnology, Kagoshima Univ.）

【Introduction】 There has been a sharp increase in sudden cardiac death due to vasospasm, one of the major factors involved in vascular disease, and currently there is no preventive measure or first line drug. Therefore, we identified active components that could prevent abnormal contraction, and investigated the structure-activity relationship.

【Methods and Results】We found that mulberry leaves are the most effective to prevent vasospasm, and identified fisetin as the active component. Fisetin is a type of flavonoid, but there are no reports on its effect on vasospasm and inclusion in mulberry leaves (Japanese Patent Application No. 2020-170315). Moreover, in order to clarify that the presence of hydroxyl group of fisetin has the preventive effect on vasospasm, the structure-activity relationship of related compounds were studied. We clarified the importance of the hydroxyl group located at the C-3 position of the flavonoid skeleton.

A03

Effect of different buffers on predatory activity of *Bdellovibrio bacteriovorus* 109J

○MORIYA T , HOSHIKO Y , MAEDA T
（Kyutech・Department of Biological Functions Engineering）

【Introduction】*Bdellovibrio* and Like Organisms (BALOs) are present in a wide range of environment such as river, sea, and soil. This group of bacteria invades other Gram-negative bacteria and finally lyses host bacteria. Due to this unique lifecycle, BALOs are expected to be applied to various fields in recent years. However, several environmental factors must be considered to utilize this strain in the future. In previous research, some chemical compounds such as a surfactant affect predatory activity, but there are no studies to prove the effects of compounds used in buffers on predatory activity, so we investigated the effect of different buffer solutions on the predation of *Bdellovibrio bacteriovorus* 109J.

【Methods and Results】Co-cultivation of *B. bacteriovorus* 109J and *E. coli* BW25113 as a host in various buffer solutions such as HEPES, MOPS, Tris, and phosphate buffer. Cell turbidity and buffer pH were checked during the experiments. As a result, except phosphate buffer, all buffer used in this study were observed the decrease of cell turbidity. On the other hand, 25 mM phosphate buffer inhibited predatory activity, but not inhibited under 5 mM phosphate buffer. Furthermore, 5 mM phosphate buffer showed a slower decrease of cell turbidity than 25 mM HEPES, so phosphate buffer affects the activity of *B. bacteriovorus* 109J.

A04

Metagenomic analysis of gut microbiome of Indonesian and Japanese infants

○SEINO T , NAKAYAMA J
（Bioscience and Biotechnology, Kyushu Univ.）

【Introduction】Asian Microbiome Project (AMP) aims to gain insights in gut microbial community of Asians which is expected to be deeply involved in the health of Asian. In the past AMP study focusing primarily on adults and school-age children, we found two enterotype-like clusters, each driven by high abundance of *Prevotella* or *Bifidobacterium*/*Bacteroides* and dominated in Indonesian and Japanese, respectively. The aim of this study is to identify the differences in the gut microbiota of Indonesian and Japanese infants as a developing process of these enterotypes and explore their driving factors.

【Methods and Results】

We collected 84 fecal samples from Japanese (n=48) and Indonesian (n=36) infants. The metagenomic DNA sequences were analyzed by shot-gun and paired-end sequencing using Illumina Hiseq genome sequencer. As a result, some bacterial species belonging to *Firmicutes*, such as *Veillonella* and *Clostridium*, were found to be significantly more abundant in Japanese infants. Next, *Bacteroides* was found to be significantly more abundant in Indonesian pre-weaning infants. Moreover, we found galactose metabolism genes significantly more abundant in Indonesian pre-weaning infants. These results may reflect to differences in the intestinal environment of infants between these two countries.

A05	**Comparison of Growth Media for Diverse Colony Formation** ○YAMAMOTO K , TOYA S , SABIDI S , HOSHIKO Y , MAEDA T （Kyutech・Department of Biological Functions Engineering）

【Introduction】
Colony formation is essential to accurately observe bacterial functions. However, there are many uncultivable bacteria in environmental samples, which are not able or hard to form any colony. Therefore, the diversification of colony formation for culturable bacteria is a big challenge to seek a unique bacterial function.

【Methods and Results】
In this study, the best concentration of diluted LB medium and waste sewage sludge (WSS) was determined by counting the number of colonies and by analyzing biodiversity of colonies at the different dilution by using Illumina next-generation sequencer. As a result, a particular concentration of LB (10%) or WSS (1%) as a growth medium showed the best number of the operational taxonomic units of colonies. In addition, the results of β-biodiversity indicate that the bacterial composition was different between agar plates and liquid culture. This study demonstrates the optimization of dilution of LB medium and WSS on colony formation. Our results show a certain potential to isolate a unique bacterial strain through a method of colony formation.

A06	**Association analysis between early endosome dynamics and kojic acid secretory production in *Aspergillus oryzae*** ○HIRAKAWA Y , TAKEGAWA K , HIGUCHI Y （Kyushu Univ.・Applied Microbiology）

【Introduction】*Aspergillus oryzae* has been used in Japanese fermentation and brewing industries since it has high secretion ability of useful enzymes. In membrane trafficking, it has been suggested that early endosome (EE), an organelle of endocytic pathway, is connected with protein secretory pathway. We have previously identified AoHok1, the linker protein between EEs and motor proteins, and demonstrated that α-amylase production is reduced in *Aohok1* disruptant. In this study, we revealed an unknown function of EE dynamics by focusing on kojic acid (KA) production, which is a useful secondary metabolite produced in *A. oryzae*.

【Methods and Results】First, we quantified the production of KA for 9 days, and found that it was reduced in *Aohok1* disruptant. Next, we analyzed an effect of EE dynamics on the localization of the proteins encoded by *kojA, kojR* and *kojT* which are involved in the KA biosynthesis. As a result, we found that there was no association between EE dynamics and KA proteins localization. Moreover, we investigated the expression levels of KA biosynthesis-related genes when cultured on agar plate, and revealed that these were reduced in the *Aohok1* disrupted. This result suggests that EE dynamics may regulate transcription of KA biosynthesis-related genes through signal transduction to the nucleus.

A07	**Comprehensive Analysis of Gut Bacterial Metabolites Involved in Neurodevelopmental Disorders** ○KAWASAKI R[1], MAEDA N[1], MIYAMOTO K[2], OTSUKA H[1] YOSHIDA S[2], YAMAZAKI T[1], KOSHIMURA M[1] （1:NIT, Sasebo College, 2:Toyohashi University of Technology）

【Introduction】

・Objective

In this present study, we compared short-chain fatty acids (SCFAs) (target) and other metabolites (non-target) in the gut flora of offspring born to glyphosate-exposed and non-exposed maternal rats using metabolomics to determine the effect of glyphosate exposure on the offspring born to the mother rats.

By comparing individuals with different forms of glyphosate exposure (acute and chronic), we will determine how the different forms of exposure are affected.

【Methods and Results】

・Results

SCFA: For all SCFAs (butyric acid, acetic acid, and propionic acid), there was a tendency for each concentration to increase in the order of control > acute exposure > chronic exposure.

Other Metabolites: Reproducibility could not be confirmed except for acute exposure, which may be due to large individual differences.

A08	**Potential use of sulfur-responsive region of sulfate transporter *SULTR2;1* for control of recombinant gene expression in plants** ○NGUYEN H.T., SUYAMA A, OHTAKI C, MARUYAMA-NAKASHITA A （Kyushu Univ.・Bioresource and Bioenvironmental Sciences）

【Introduction】

In most high-level expression systems in plants, the upstream region of genes is modified. However, it sometimes results in problems such as loss of expression characteristics defined by the upstream region or inhibition of plant growth due to overexpression. In a previous study, we found that the downstream region of a sulfate transporter *SULTR2;1* is responsible for the increased gene expression under sulfur deficient (−S) conditions, regardless of the upstream region. Here, we aimed to establish an expression regulation system by sulfur concentration using this downstream region.

【Methods and Results】

We selected three genes, CPC, a positive regulator of root hair development; GAMT1, an enzyme that inactivates gibberellic acid; PAP1, a positive regulator of anthocyanin synthesis.Their upstream region and coding region were fused to the downstream region of *SULTR2;1*. Then the constructs were introduced into *Arabidopsis*. The transgenic plants were grown under +S and −S conditions. The expression levels of all tested genes were increased compared to the wild-type plants under −S conditions. Although *GAMT1* and *CPC* expressions did not influence the plant phenotypes, *PAP1* expression significantly changed the root color into purple due to the over-production of anthocyanin. This result indicated the usefulness of *SULTR2;1* downstream region for tentative gene expression technology.

A09	**Immobilization of 1,6-α-L-fucosidase and endo-b-*N*-acetylglucosaminidase for defucosylation of N-glycan.**
	○HONDA A , TAKEGAWA K （Applied Microbiology, Kyushu Univ.）

【Introduction】

The importance of therapeutic antibodies has greatly expanded, and much research has been conducted on the modification of glycan to improve their functionality. It has been found that core fucosylation of N-glycans in the Fc-region of IgGs, which are used as therapeutic antibodies, is crucial for their activity, and removal of fucose residue from N-glycans significantly increases effector function. To increase throughput and enzyme reusability, this work evaluated several immobilization methods for microbial fucosidase and endo-b-*N*-acetylglucosaminidase.

【Methods and Results】

The 1,6-a-L-Fucosidase from *Bifidobacterium longum* subsp. *infantis* was immobilized on Ni sepharose gel, and Endo-CoM from *Cordyceps militaris* was immobilized on cyanogen bromide-activated-Sepharose gel. As a result of measuring their activities, we found that the activity of both immobilized-enzymes was not much different from that of the original enzymes, suggesting that these immobilized enzymes could be used for deglycosylation of glycoproteins. We also examined whether the thermal stability and optimum pH of these enzymes were changed due to immobilization.

A10	**Identification and characterization of a bacterial α-galactosylceramide synthase**
	○QU Q , ISHIBASHI Y , OKINO N （Kyushu Univ.・Bioscience and Biotechnology）

【Introduction】

α-galactosylceramide (GalCer) was first found in a marine sponge *Agelas mauritianus*, collected in the Okinawan sea, as an antitumor agent. Later, α-GalCer was identified as the first CD1d-presented lipid antigen for invariant natural killer T (*i*NKT) cells, although its synthase has not been reported. Recently, α-GalCer was isolated from an intestinal symbiotic bacterium, *Bacteroides fragilis*, and was found to be a specific molecule that can regulate the homeostasis of the host's intestinal immune system. Very recently, we have succeeded to identify the glucuronosylceramide synthase of *Zymomonas mobilis and found the homologous sequence in the genome of B. fragilis. In this study, we report the identification and characterization of* α-GalCer synthase from *B. fragilis.*

【Methods and Results】

We first cloned the gene from the genome DNA of *B. fragilis* and expressed it in *Escherichia coli as a fusion protein with maltose binding protein*. Using the purified enzyme, we revealed that it has high glycosyltransferase activity with UDP-galactose and ceramide as sugar donor and acceptor substrates, respectively. Furthermore, hydrophilic interaction liquid chromatography-phase LC-MS analysis defined the synthesized GalCer as the α-GalCer.

B01	**Integration of foreign genes into transposon-like gene sequences in fission yeast.** ○MITSUTA Y , FUJIKI M , MAEKAWA H , HIGUCHI Y , TAKEGAWA K （Applied Microbiology, Kyushu Univ.）

【Introduction】
Recombinant technology to allow stable maintenance of genes in the cell is critical for stable production of heterologous proteins in the recombinant yeast cells. We have developed multicopy gene integration technology targeting 13 copies of the *Tf2* transposon-like sequence present on three chromosomes of fission yeast *Schizosaccharomyces pombe*. This method increases the copy number and helps stable maintenance of target genes.

【Methods and Results】
A linearized DNA fragment with a *Tf2* region sequence, *ura4* marker, and model protein EGFP region was transformed into a uracil-requiring wild-type strain of *S. pombe*. The genome of each transformant was extracted, and the *Tf2* locus was analyzed by PCR. As a result, we were able to obtain strains in which EGFP was incorporated into multiple locations in 13 *Tf2* loci. Furthermore, we investigated the application of the CRISPR/Cas9 system, which induces double-strand breaks at the *Tf2* locus, and then integrates a foreign gene (*Hyg*-, hygromycin resistant gene). We were able to obtain multiple strains with hygromycin resistance, but we could not identify which *Tf2* locus was replaced with *Hyg* gene.

B02	**Comparative genome analysis of predominant bacterial strains in meta-fermentation** ○HIRANO K[1], TASHIRO Y[1], KODAMA H[2], MIYAMOTO H[2], SUDA W[3], HATTORI M[3], SAKAI K[1] （[1]Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ., [2]Chiba Univ., [3]RIKEN）

【Introduction】We have proposed "meta-fermentation" as a method by complex microorganism producing valuables from renewable resources (Tashiro et al., Bioresour. Technol., 2016). In meta-L lactic acid fermentation, *B. coagulans* MN-07, *B. thermomylovorans* OM-556, and *B. hisahii* N-11 were the major strains. Meanwhile, *B. hisahii* N-11 was reported to have beneficial effects as probiotics. Therefore, the L-lactic acid fermentation residue containing *B. hisahii* N-11 is expected to be used as functional feed. In this study, the aim is to analyze draft genomes of 3 strains, and to compare functional genes for characterization.

【Methods and Results】Draft genome analysis of *B. coagulans* MN-07 and *B. thermomylovorans* OM-556 by Illumina MiSeq, and comparative analysis with *B. hisashii* N-11 were performed. Functional gene analysis suggested that several specific genes for *B. coagulans* MN-07 are involved in producing ability of high L-lactic acid. It was suggested that several specific genes for *B. hisashii* N-11 strain encoded polysaccharide hydrolysis enzymes including pullulanase, which would contribute to L-lactic acid production in the late phase of meta-fermentation. In addition, antibacterial substance-producing genes were confirmed from 3 strains, which suggested that 3 strains have antibacterial activity against animal-pathogen.

B03	**Elucidation of gene expression mechanism for the pellicle biofilm formation in agrBD-knockout mutant of *Clostridium perfringens***
	○ITO R , HONDA K , ADACHI K , NAKAYAMA J （Kyushu Univ.・Bioscience and Biotechnology）

【Introduction】

Quorum sensing (QS) is the bacterial cell density-dependent mechanism for regulating gene expression using signal molecules. *Clostridium perfringens* secretes autoinducing peptide (AIP_{Cp}) as the QS signal that triggers the expression of toxin genes when its cell density reaches a certain level, resulting in the coordination of its pathogenicity. *C. perfringens* TS230, lacking *agrBD,* is unable to produce AIP_{Cp}, thus cannot express toxin genes but instead, pellicle biofilm formation was observed. This study aims to address the molecular mechanism of gene expression involved in the biofilm formation apparently regulated by the *agr* system.

【Methods and Results】

C. perfringens str. 13 (wild type) and TS230 (the AIP_{Cp}-negative strain) was cultured with 500 nM of AIP_{Cp} added every three hours. The expression level of *pfoA* and gene related to biofilm was quantified by RT-qPCR every hour during culture. As a result, the *pfoA* expression level of str. TS230 has recovered to the level equal to str. 13. However, biofilm phenotype of TS230 was not changed despite the presence of AIP. Namely, its biofilm formation was independent of the *agr* system, suggesting the presence of a novel gene regulatory system that controls the expression of genes involved in biofilm formation.

B04	(Cancelled)

B05	**Isolation of uncultured major bacteria in the ATAD process by FISH observation and micromanipulation** ○TSUNEYOSHI K ,ARAI T , SAKAI K , TASHIRO Y （Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ.）

【Introduction】In Chikujo Town, human excreta is converted to liquid fertilizer by Autothermal Thermophilic Aerobic Digestion (ATAD). Previous study revealed that this process is divided into 3 unique phases, and that uncultured bacterium related to *Longimicrobium terrae* ^{T} (phylum: *Gemmatimonadetes*) with 85% similarity would be predominant at the final phases (Tashiro et al., AEM, 2018). In order to improve the efficiency of this process, it is necessary to elucidate the functions of this bacterium. Therefor, this study aimed to isolate this bacterium by fluorescence in situ hybridization (FISH) and micromanipulation.

【Methods and Results】First, FITC probe was designed based on 16S rRNA gene of targeted major bacterium to confirm if the target bacterium was present in the sample of the final phase by the FISH. By this observation, it was suggested that it was present in single cell and flock. Base on the results of FISH observation, single cells were isolated by micromanipulation and cultured for 2 weeks at 50ºC in autoclaved ATAD sample at the final phase with the pH adjustment at 9.4. 157 bacteria were isolated in total, and proliferations of 13 cells were observed. These bacteria were identified as four species of *Anoxybacillus mongoliensis*, *Bacillus thermolactis*, *Ureibacillus thermosphaericus*, *Anoxybacillus flavithermus* subsp. *yunnanensis*. These results suggested that the isolation method would be improved.

B06	**Analysis of outcrossing and inbreeding in *Ogataea polymorpha*** ○KAI N , TAKEGAWA K , MAEKAWA H （Kyushu Univ.・Bioscience and Biotechnology）

【Introduction】*Ogataea polymorpha* can grow using methanol as the only carbon source, and can grow up to about 50 ° C, so its usefulness in heterologous protein production is drawing attention. There are two types of haploid cells of *Ogataea polymorpha*, a-type cells and α-type cells. Under nitrogen starvation conditions, a-cells and α-cells mate and form diploid cells and subsequently undergo meiosis to form four haploid spores. There are two known types of mating in yeast: outcrossing and inbreeding. Outcrossing refers to the mating between cells that are derived from distinct ancestral cells and inbreeding the mating of cells that are isogenic except for the mating type gene. Homothallic *O. polymorpha* cells can undergo outcrossing mating as well as inbreed mating. However, the frequency of outcrossing has not been investigated.

【Methods and Results】This study aims to establish a method to determine the ratio of outcross mating and inbreed mating. Because it is necessary to distinguish diploid colonies and haploid colonies, we modified the standard synthetic medium so that cells first undergo vegetative growth to form colonies and then commit to the meiosis/sporulation. We found that diploid and haploid colonies are distinguishable in the pink or white colony color on the nitrogen starved NaKG medium containing 0.02 mM $(NH_4)_2SO_4$. We could detect 1.21% inbreeding in homothallic a and 3.75% in homothallic α.

B07	**Elucidation of secretory mechanism of the multiple bacteriocin transporter, EnkT** ○TAKEUCHI A , WADA N , SUSHIDA H , NAKAYAMA J , ZENDO T （Kyushu Univ.・Bioscience and Biotechnology）

【Introduction】*Enterococcus faecium* NKR-5-3 produces multiple bacteriocins. Among them, enterocins NKR-5-3A, C, D, and Z (Ent53A, C, D, Z) were found to be matured and secreted by an ATP-binding cassette (ABC) transporter, EnkT, although an ABC transporter generally secretes only a cognate bacteriocin. In previous study, three α-helix structures (Helix 1, 2 and 3) and four conserved amino acids in the N-terminal peptidase domain of EnkT were identified as important regions for recognition of the bacteriocin precursor and cleavage of the leader peptide. In this study, we intend to characterize the function of the important regions to elucidate the secretory mechanism of EnkT.

【Methods and Results】To characterize the functions of the conserved amino acids (Q9 and D110) and to assess the effects of negative charged amino acids in Helix4 of the EnkT peptidase domain on bacteriocin secretion, substituted mutants of these residues were constructed. Substitution of D110 to Q or K affected bacteriocin secretion, indicating that the negative charge of D110 is important for the peptidase activity. In addition, in order to conduct *in vitro* analysis of interaction between the EnkT peptidase domain and substrates, his-tagged Ent53C precursor was heterologously expressed in *Escherichia coli*. As a result of denaturation by urea and guanidine-HCl, the Ent53C precursor was successfully purified using Ni Sepharose.

B08	**Analysis of glycolipids of *Pseudomonas aeruginosa* under phosphate starvation conditions** ○ZHU H , ISHIBASHI Y , OKINO N （Kyushu Univ.・Bioscience and Biotechnology）

【Introduction】

In natural environment, microbials often suffer from phosphorus scarcity, and some of microorganisms evolved a system to against the phosphate starvation. *Pseudomonas aeruginosa* is a famous human opportunistic pathogen associates with some illnesses. We analyzed the glycolipids of *P. aeruginosa* under phosphate starvation conditions and revealed that this strain produces glucuronosyl diacylglycerol and monoglucosyl diacylglycerol (MGDG), which were not expressed under phosphate sufficient conditions. In this study, we report the MGDG synthase found in the genome of *P. aeruginosa*.

【Methods and Results】

The putative MGDG synthase of *P. aeruginosa* (PA3218) was cloned and expressed it in *E. coli*. The recombinant protein was purified and used for biochemical analysis. Substrate specificity of PA3218 was analyzed using various UDP-sugars as a sugar donor and 7-nitrobenz-2-oxal-1,3-diazole-labeled ceramide (NBD-Cer) and NBD-diacylglycerol (DG) as a sugar acceptor. The purified PA3218 showed glycosyltransferase activity when UDP-glucose and NBD-DG were used as sugar donor and acceptor substrates, respectively. This result indicates that PA3218 encoded diacylglycerol glucosyltransferase.

B09	**Elucidation of mechanism of antifungal bacteria Anti-G isolated from palm co-compost.** ○KONDO Y, NAKANO T, ASAHARA R, TASHIRO Y, SAKAI K （Grad. Sch. Biosci. Biotechnol. Sci,. Kyushu Univ.）

【Introduction】*Ganoderma boninense* is a phytopathogenic fungus that infects palm and causes Basal stem rot (BSR). On the other hand, we isolated and identified 100 multifunctional bacterial strains from palm co-compost of methane fermented with palm waste and reported that 12 strains showed antifungal activity against *G. boninense* (Anti-G) (Chin et al., J. Biosci. Bioeng, 2017). Therefore, this study aimed to elucidate the mechanism of 12 strains with Anti-G.

【Methods and Results】First, the antifungal activity of Anti-G was quantitatively evaluated by the paper disc method. It was suggested that Anti-G differed depending on the dosage and culture period of antifungal substances. Furthermore, we investigated the presence of chitinase activity but chitinase activities were not detected, so it indicated that chitinase was not involved in the antifungal activity. In addition, *Enterobacter cloacae* subsp. *dissolvens* B3, *Bacillus tequilensis* CE4, and *B. subtilis* subsp. *spizizenii* TSASi1 were subjected to the Illumina MiSeq. AntiSMASH analysis showed that 4, 11, and 15 known antifungal biosynthetic gene clusters were identified, respectively.

B10	**Development of a new gene expression vector for *Thermus thermophilus* using a silica-inducible promoter** ○IIDA T , FUJINO Y , SUEMATSU Y , DOI K （Kyushu Univ.・Microbial generic resource）

【Introduction】

To produce thermostable enzymes with correct folding, a thermophilic host for heterologous expression is needed. Previously, we reported a strong and inducible promoter which works under the condition with supersaturated silica in *Thermus thermophilus*. Using this promoter (*Psip*), we have developed a new **s**ilica-**i**nducible e**x**pression vector.

【Methods and Results】

Putative *Psip* region and thermostable 🚲-galactosidase gene were inserted into *Thermus* vector pYK596 (Hyg^R) at *Nhe*I and *Eco*RI sites. Promoter deletion assay revealed that 100 bp upstream region of *Psip* was necessary for strong expression. As these plasmids seemed to be unstable due to the truncation of pYK596 backbone, we finally constructed an expression vector named pSix4 which contains a 100 bp upstream region of *Psip* and complete pYK596 backbone. Plasmid stability and promoter activity was confirmed by qPCR and β-galactosidase reporter assay. pSix4 showed the higher plasmid stability and the stable expression of target gene. This system achieved highest expression level among the expression system in *T. thermophilus.*

B11

Structural identification and characterization of novel bacteriocins

○YOSHIDA H[1], NOMIYAMA T[1], KUWAHARA M[1], FUKAMI K[2], NAKAYAMA J[1], ZENDO T[1,2]
([1]Faculty of Agriculture, [2]Material Manager Center, Kyushu Univ.)

【Introduction】

Bacteriocins are bioactive antimicrobial peptides synthesized on the ribosome and released extracellularly by numerous bacteria. Bacteriocins produced by lactic acid bacteria (LAB) are safe for human use, resistant to heat, low pH and proteolytic enzymes, and active against Gram-positive bacteria including drug-resistant bacteria. The purpose of this study is to discover a wide variety of novel LAB bacteriocins and to elucidate their structures and characteristics. Here, the bacteriocins produced by two LAB isolates, *Pediococcus pentosaceus* DW41 and *Lactobacillus plantarum* No. 9, are being analyzed for their structures and characteristics.

【Methods and Results】

P. pentosaceus DW41 isolated from silage was found to show antibacterial activity specifically against strains belonging to *Pediococcus*. Among 16 isolates from the pickled dried radish, *L. plantarum* No. 8 and No. 9 were found to show antibacterial activity. Some genetic analysis revealed the both strains possessed putative genes encoding bacteriocins. Their bacteriocins are being purified from the culture supernatants by hydrophobic interaction chromatography, cation exchange chromatography and RP-HPLC, and the MWs and the amino acid sequences will be analyzed.

B12

Analysis of unconventional protein secretion for AoSod1 in *Aspergillus oryzae*

○KUBOTA K , TAKEGAWA K , HIGUCHI Y
(Kyushu Univ.・Applied Microbiology)

【Introduction】*Aspergillus oryzae* is used in the Japanese fermentation and brewing industry because it can safely secrete useful enzymes. In general, secreted proteins are transported to the plasma membrane via the endoplasmic reticulum and the Golgi apparatus. However, it has been recently reported that there are certain proteins that do not contain a signal peptide but may be secreted. This pathway is called unconventional protein secretion (UPS). In this study, UPS analysis was performed by targeting AoSod1, the *A. oryzae* ortholog of *Saccharomyces cerevisiae* superoxide dismutase Sod1.

【Methods and Results】Strains expressing AoSod1 tagged with HA epitope were first cultured in rich nutrient medium and thereafter exchanged to carbon-source or nitrogen-source starvation media. After cultivation, cells and culture supernatants were collected. After SDS-PAGE, proteins were detected by CBB staining and HA-tagged proteins were detected by Western blotting using an anti-HA antibody. As a result, AoSod1 was not secreted into the culture supernatant of rich and nitrogen-source starvation media under the condition in which cell lysis did not occur; in contrast, AoSod1 was secreted into the supernatant of carbon starvation medium. Furthermore, we are analyzing an effect of AoSod1 secretion by suppressing the expression of *Aosso1* that is involved in vesicle transport.

JSBBA WEST 3rd Student Forum Executive Committee

Chair person	**Hayato NYUNOYA**
Vice-chair person	**Kota WATANABE**
members	**Reiko HIASA**
	Riko MISHIMA
	Pui Yan YEUNG
	Yuki MORITA
	Azusa YAMADA

JSBBA WEST Student Committee
E-Mail: jsbbawsf2018@gmail.com
http://nishinihon.jsbba.or.jp/