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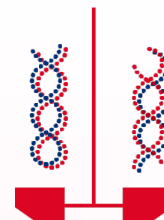

The Society for Biotechnology, Japan

2021 Sakura-Bio Meeting

May 29, 2021

Venue:

Zoom

Participation is free
(no conference fee).

Co-Chairs:

Hideo Nakano
(Nagoya Univ.)

Tohru Suzuki
(Gifu Univ.)

Keynote speakers:

Nobuya Itoh (Toyama Pref. Univ.)
Togo Uchida (ICLEI, Japan)

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~ 2021 Sakura-Bio Meeting ~

Venue: Online (Zoom)

May 29 (Sat), 2021

10:00-10:05 Opening remarks by the conference Co-chair
Dr. Hideo Nakano (Nagoya Univ.), Professor

10:05-10:55 Keynote lecture (Chair: Dr. Nakano)
Dr. Nobuya Itoh (Toyama Prefectural Univ.), Professor
Screening of gene-specific amplicons from metagenomes for biocatalysis application

10:55-12:00 Oral presentations
6 presenters x 20 min (2 breakout rooms)
Room A (Dr. Runguphan, Dr. Yoon, Dr. Chen)
Room B (Dr. Nakatani, Dr. Altaib, Dr. Damnjanovic)

12:00-13:00 Lunch break

13:00-13:50 Keynote lecture (Chair: Dr. Suzuki)
Dr. Togo Uchida (ICLEI, Japan), Executive director
The role of local governments in "sustainable development"

13:50-14:50 Oral presentations
6 presenters x 20 min (2 breakout rooms)
Room A (Mr. Oka, Mr. Nakamichi, Mr. Obata)
Room B (Mr. Honjo, Ms. Jia, Ms. Hori)

14:50-15:10 Break

15:10-16:30 Oral presentations
4 presenters x 20 min (1 breakout room)
Room A (Ms. Kihara, Mr. Mohd Din, Mr. Sakurai, Mr. Muangmaraong)

16:30-16:35 Closing remarks by the conference Co-chair
Dr. Tohru Suzuki (Gifu Univ.), Professor

*Oral presentations time schedule: 15 min for presentation, 4 min for Q&A, 1 min for speaker change.

Keynote lectures

Screening of Gene-specific Amplicons from Metagenomes for Biocatalysis Application

Nobuya Itoh (presenter) *

Biotechnology and Pharmaceutical Engineering Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan

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Metagenomics is an emerging tool for isolating novel and homologous genes. However, most previous approaches have involved metagenomic DNA extraction and *Escherichia coli* library construction, followed by sequence- or function-based library screening. Such approaches are time-consuming, especially for target gene detection. Although mining genomic databases for target genes has proved more effective for finding or identifying useful genes, this technique requires the construction of an in-house metagenomic database for enzyme screening. The screening of gene-specific amplicons from metagenomes (S-GAM) approach that we have developed is a powerful technique for efficiently isolating target genes from metagenomes (1, 2). This approach overcomes a major disadvantage of previous techniques, namely, their low efficiency in obtaining target genes from metagenomes. S-GAM also permits the omission of the time-consuming subcloning and expression-optimization procedures. In this paper, we describe the construction of a metagenomic library of enzyme genes using the S-GAM technique, with a focus on the *Leifsonia* sp. *adh* gene (*lsadh*) (1), the *Rhodococcous* sp. phenylacetaldehyde reductase gene (*par*) (2), and the bacterial multi-copper oxidase gene (*mco*). The genes obtained using this approach encode a wide range of amino acid sequences: 45%–99% for *Hladh* (homology to *Leifsonia* *adh*), 97%–99% for *Hpar*, and 47%–100% for bacterial *mco.* Thus, the S-GAM approach showed high effectiveness for obtaining gene resources from metagenomes. We also discuss the potential use of novel enzymes as biocatalysts.

References:

(1) Itoh, N., Kariya, S., and Kurokawa, J.: *Appl. Environ. Microbiol.*, **80**, 6280-6289 (2014). /Erratum **82**, 61 (2016).

(2) Itoh, N., Kazama, M., Takeuchi, N., Isotani, K., Kurokawa, J.: *FEBS Open Bio.*, **6**, 566-575 (2016).

The Role of Local Governments in "Sustainable Development"

Togo UCHIDA[1] (presenter)

[1]ICLEI-Local Governments for Sustainability, Japan Office

Started as International Council for Local Environmental Initiatives (ICLEI), ICLEI – Local Governments for Sustainability (hereafter "ICLEI") was established in 1990 at a "World Congress of Local Governments for a Sustainable Future", organized by UNEP and Center for Innovative Diplomacy (CID) and with the presence of The International Union of Local Authorities (IULA).

Since its establishment, ICLEI has been working on global sustainability agenda and has grown to a global network working with more than 2,500 local and regional governments committed to sustainable urban development across over 125 countries. Our activity has evolved alongside the global discussion on sustainability, and we influence sustainability policy and drive local action for low emission, nature-based, equitable, resilient, and circular development.

In this presentation, I will introduce a brief history of how global sustainability agenda evolved and how local governments intervened in this process with relation to the SDGs. In addition, I will touch upon the recent approaches taken by local government in it pursuance on sustainability.

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Oral presentations

Systematic Engineering of *Saccharomyces cerevisiae* for D-lactic Acid Production with Near Theoretical Yield

Weerawat RUNGUPHAN[1], **Akaraphol WATCHARAWIPAS**[1], **Kittapong SAE-TANG**[1], **Kitisak SANSATCHANON**[1], **Pipat SUDYING**[1], **Kriengsak BOONCHOO**[1], **Sutipa TANAPONGPIPAT**[1], **Kanokarn KOCHARIN**[1]

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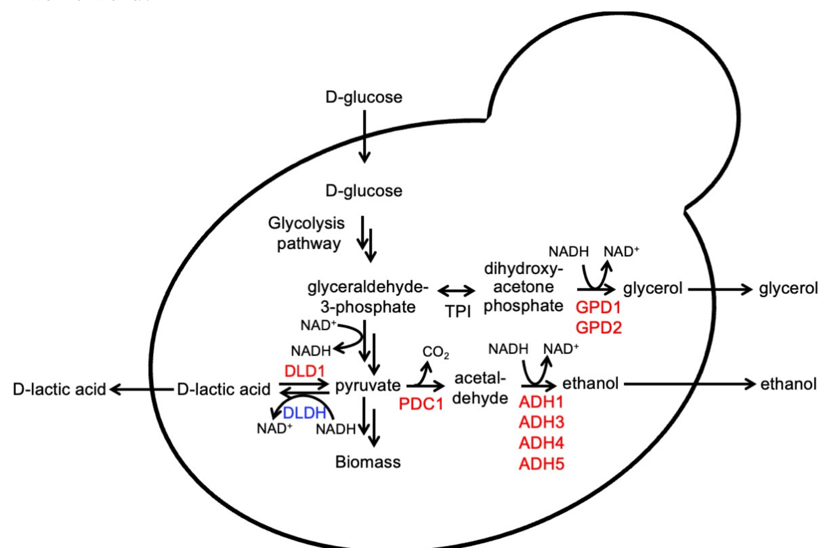
D-lactic acid is a chiral three-carbon organic acid that can improve the thermostability of polylactic acid. Here, we systematically engineered *Saccharomyces cerevisiae* to produce D-lactic acid from glucose, a renewable carbon source, at near theoretical yield. Specifically, we screened D-lactate dehydrogenase (DLDH) variants from lactic acid bacteria in three different genera and identified the *Leuconostoc pseudomesenteroides* variant (*Lp*DLDH) as having the highest activity in yeast. We then screened single-gene deletions to minimize the production of the side products ethanol and glycerol as well as prevent the conversion of D-lactic acid back to pyruvate. Based on the results of the DLDH screening and the single-gene deletions, we created a strain called ASc-d789M which overexpresses *Lp*DLDH and contains deletions in glycerol pathway genes *GPD1* and *GPD2* and lactate dehydrogenase gene *DLD1*, as well as downregulation of ethanol pathway gene *ADH1* using the L-methionine repressible promoter to minimize impact on growth. ASc-d789M produces D-lactic acid at a titer of 17.09 g/L in shake-flasks (yield of 0.89 g/g glucose consumed or 89% of the theoretical yield). Fed-batch fermentation resulted in D-lactic acid titer of 40.03 g/L (yield of 0.81 g/g glucose consumed). Altogether, our work represents progress towards efficient microbial production of D-lactic acid.



References:
(1) *FEMS Yeast Research*, **2021** (in press); doi.org/10.1093/femsyr/foab024

Formate production from CO_2 and H_2 with biohybrid catalyst

Ki-Seok Yoon[1,2]* (presenter), **Nguyen Khac Hung**[1,2], **Takuo Minato**[1,2], **Seiji Ogo**[1,2]

[1]Advanced Energy Materials Thrust, International Institute for Carbon-Neutral Energy Research (WPI-I2CNER), Kyushu University, [2]Biomimetics Laboratory, Dept. of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University

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Catalytic reduction of CO_2 to formic acid by using H_2 has received increasing attention because of the growing importance of carbon-neutral and hydrogen energy technology. To develop efficient systems for catalytic production of formate from CO_2 and H_2, various types of homogeneous and heterogeneous catalysts have been synthesized. However, these synthetic catalyst systems still require expensive noble metals as active sites and their catalytic reactions should be carried out at high-pressure and temperature conditions. On the other hand, biological catalysts for CO_2 hydrogenation into formate can occur at mild reaction conditions with very high activity. In this study, we have purified and characterized two membrane-bound proteins of [NiFe]-hydrogenase[1)] for H_2 activation and [Mo]-formate dehydrogenase[2)] for CO_2 reduction from our isolated bacterium *Citrobacter* sp. S-77. Inspired by their remarkable O_2-tolerant and catalytic behaviors[3,4)], we developed a well-ordered enzyme-immobilized system (**Fig. 1**) that efficiently generates formate from CO_2 and H_2 under ambient conditions.

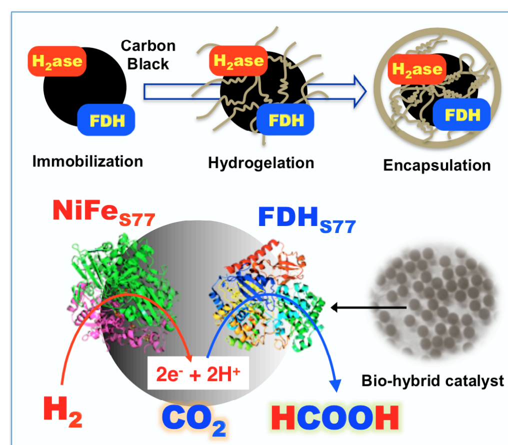

Fig. 1. A platform for formate production from CO_2 and H_2 with biohybrid catalyst.

References:

[1)]*J. Biosci. Bioeng.* O_2-stable membrane-bound [NiFe]-hydrogenase, 114, 479-484, **2012**; [2)]*J. Biosci. Bioeng.* Membrane-bound [Mo]-formate dehydrogenase, 118, 386–391, **2014**; [3)]*Angew. Chem. Int. Ed.*, [NiFe]-hydrogenase for PEMC catalyst, 53, 8895–8898, **2014**; [4)]*Chem. Commun.*, Structural basis of O_2-tolerant mechanism of Hyb-type [NiFe]-hydrogenase, 54, 12385–12388, **2018.**

Efficient Bioconversion of CH_4 Gas-to-Liquid CH_3OH using a Microbial Gas-Phase Bioreactor with Methanotrophs

Yan-Yu CHEN[1] (presenter), **Masahito Ishikawa**[1], **Katsutoshi Hori**[1]*

[1]Department of Biomolecular Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

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Bioconversion of methane gas (CH_4) to liquid methanol (CH_3OH) has received great attention since only a single step using methanotrophs, methane-oxidizing bacteria, is necessary under mild conditions. However, the conventional methanotrophic bioprocess, conducting in an aqueous phase bioreaction, required stirring or bubbling to enhance the water solubility of CH_4 and O_2, which is energy-consuming. A microbial gas-phase bioreaction has been proposed for a high efficiency of gas delivery. It has been demonstrated that the CH_4 degradation rate in a gas-phase reaction without active supply of gases was much higher than that in an aqueous-phase reaction with stirring[1]. Since there is no bulk liquid phase in the system and cells are immobilized on a solid support, it is difficult to harvest high boiling-point products from the system. Here we constructed the inverse membrane bioreactor (IMBR) with an efficient strategy to supply reagents and harvest liquid products through a liquid phase and simultaneously to conduct a gas-phase bioreaction (**Figure 1**). We successfully demonstrated that bioconversion from CH_4 gas to liquid CH_3OH by methanotrophs was achieved in the IMBR. A continuous CH_3OH bioproduction can be conducted over 72 hrs and the CH_3OH productivity was higher than that in other systems in literatures. As a result, the proposed system facilitates the development of gas-to-liquid bioconversion using microbial gas-phase bioreactions.

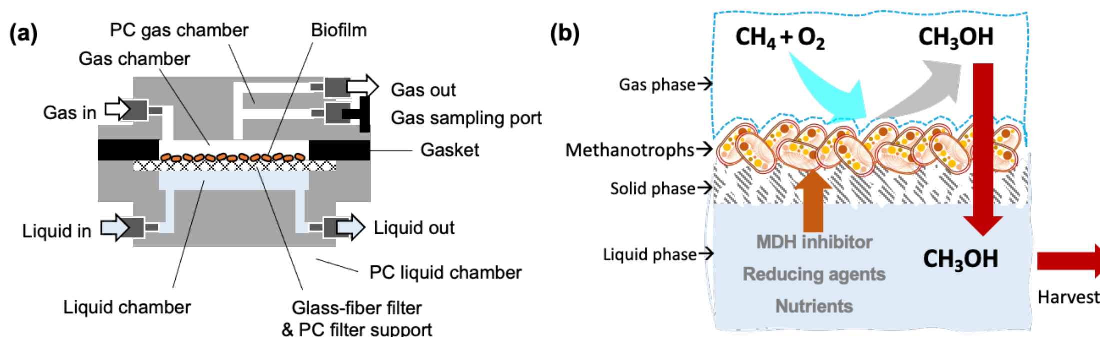

Figure 1. The illustration (a) and the working principle (b) of the inverse membrane bioreactor for CH_4-to-CH_3OH bioconversion.

References: (1) *Biochem. Eng. J.*, 154, 107441, **2020**.

Establishment of a percutaneous infection model using zebrafish and *Yersinia ruckeri*, a fish pathogen causing enteric redmouth

Hajime NAKATANI (presenter), **Katsutoshi HORI***

Graduated School of Engineering, Nagoya University Furo-cho, Chikusa, Nagoya 464-8603, JAPAN

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The epidermis and mucus layer of fish act as barriers that protect them against the pathogens in water, and provide niches for symbiotic microorganisms which benefit the host's health. However, our understanding of the relationship between fish skin bacterial flora and fish pathogen infection is limited. In order to elucidate this relationship, an experimental model for infection through fish skin is necessary.

To reveal the relationship between skin bacterial flora and pathogen infection, we have developed a percutaneous infection model using zebrafish and *Yersinia ruckeri*, a pathogen causing enteric redmouth disease in salmon and in trout (Figure 1). Pathogen challenge, either alone or together with pricking by a small needle, did not cause infection of the fish. However, cold stress given by temperature shift of the breeding water from the optimum 28°C for zebrafish to 20°C caused infection of injured fish following pathogen challenge. We investigated the effects of cold stress, injury, and pathogen challenge, alone and in combination, on fish skin bacterial flora using 16S rDNA metagenomics. We found that cold stress drastically altered the skin bacterial flora, which was dominated by *Y. ruckeri* on infected fish. In addition, fish whose indigenous skin bacterial flora was disrupted by anti-biotics had their skin occupied by *Y. ruckeri* following a challenge with this pathogen, although the fish survived without injury to create a route for invasion into the fish body. Our results suggest that the indigenous skin bacterial flora of fish protects them from pathogen colonization, and that its disruption by stress allows pathogens to colonize and dominate their skin.

This percutaneous infection model can be used to study the interaction between fish skin bacterial flora and fish pathogens in water, or the relationship between pathogens and the host's skin immune system.

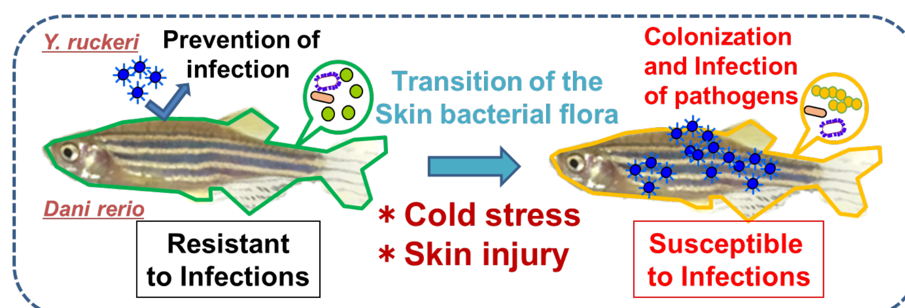

Figure 1 Percutaneous infection model using zebrafish and *Yersinia ruckeri*

The role of the Microbiota Member *Bifidobacterium* in Modulating Fecal Gamma-aminobutyric Acid (GABA) Concentration in Healthy Human Subjects; Observational Analysis and *in vitro* Study

Hend Altaib [1,3](presenter), **Kohei Nakamura** [1,2,3], **Mayuko Abe** [2], **Yassien Badr** [3], **Emiko Yanase** [1,2,3], **Izumi Nomura** [3] and **Tohru Suzuki** [1,2,3,*]

[1]The United Graduate School of Agricultural Science, Gifu University

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Background and objective

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Numerous gut bacteria have shown the ability to produce GABA. Microbial produced GABA is thought an important carrier for the signal between gut and brain. Here, we analyzed the relationship between fecal GABA concentration and microbial composition in more than 70 human participants. A further *in vitro* approach was applied to those with low GABA content to improve fecal GABA content.

Results

The GABA level was detected in a broad range (0–330 μg/g feces). The participants' samples were classified into high (>100 μg/g), medium (10–100 μ g/g), and low (<10 μg/g) groups, based on fecal GABA concentration. The results reveal that the microbiome of the high-GABA samples had lower alpha diversity than the other samples. Beta diversity analysis showed significant ($p < 0.05$) separation between the high-GABA samples and others. Furthermore, we surveyed the abundance of specific GABA producer biomarkers among the microbiomes of tested samples. The family Bifidobacteriaceae exhibited high abundance in the microbiome of the high-GABA group. To validate this finding, a fecal isolate-GABA producer *Bifidobacterium adolescentis* 4-2 was co-cultured with low GABA producing microbiomes, which enhanced GABA productivity, especially when combined with manno-oligosaccharides (MOS). Gene cluster responsible for MOS assimilation was identified in the genome of *B. adolescentis* 4-2, indicating the potentiality of both reactants to act as a GABA enhancer symbiotic formula. The summary of the study is shown in Fig. 1.

Conclusion

This study demonstrated that *bifidobacterium* abundance was associated with high fecal GABA content in healthy human subjects. These results may aid the development of potential probiotics and/or symbiotics to improve microbial GABA production, which can support the maintenance of the physical and psychiatric health of the host.

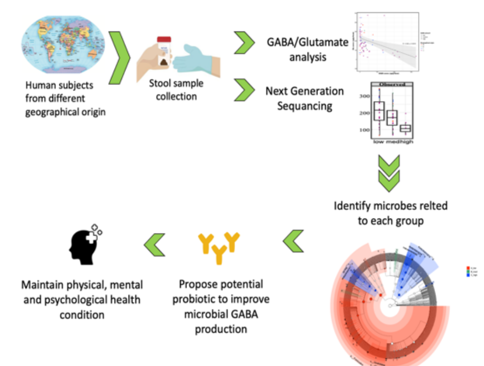

Fig.1 Summary of study

cDNA display coupled with next-generation sequencing for activity-based selection: Comprehensive analysis of transglutaminase substrate preference

Jasmina DAMNJANOVIC[1] (presenter), **Nana ODAKE**[1], **Takaaki KOJIMA**[1], **Naoto NEMOTO**[2], **Kiyotaka HITOMI**[3], **Hideo NAKANO**[1]

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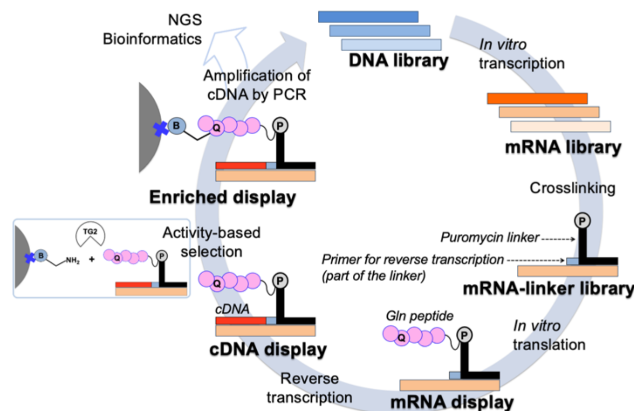
cDNA display is a well-established *in vitro* display technology based on a covalent linkage between a protein and its corresponding mRNA/cDNA, used for affinity-based selection of peptides and antibody fragments in protein engineering[1,2]. A great advantage of cDNA display is the ability to handle enormous library size (10^{12}) in a microtube scale, in a matter of days. Furthermore, increasing availability of next-generation sequencing (NGS) makes the library screening even more high-throughput and information-driven.

Here, we aimed at developing a platform coupling the advantages of cDNA display with high-throughput and accuracy of next-generation sequencing for the selection of preferred transglutaminase 2 substrate peptides (Figure 1). After the optimization of the platform by the repeated screening of binary model libraries, one round of screening and selection of fully randomized four-amino-acid library at positions -1, +1, +2, and +3 from the glutamine residue was carried out. Enriched cDNA complexes were analyzed by next-generation sequencing with the help of bioinformatics, revealing the comprehensive amino acid preference of the transglutaminase 2 in each position of the peptide backbone.



References:

1) Suzuki *et al.*, *Biochem. Biophys. Res. Commun.* (2018) 503: 2054.

2) Terai *et al.*, *ACS Omega* (2019) 4: 7378.

Prediction of binding motif function based on DNA shape features extracted from transcription factor AoXlnR-binding sites

Hiroya Oka[1] (presenter), **Takaaki Kojima**[1], **Kunio Ihara**[2], **Tetsuo Kobayashi**[1] and **Hideo Nakano**[1]

[1] Department of Applied Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University

[2] Center for Gene Research, Nagoya University

By integrating information obtained from genomic SELEX-Seq (gSELEX-Seq) and expression differences analysis, we genome-widely identified genes that are regulated directly by a transcription factor (TF) in our previous work[1]. The study also showed that thousands of genes with one or more binding motifs of XlnR, a TF from *Aspergillus oryzae,* in their promoter regions displayed no response to the overexpression of AoXlnR in *A.oryzae*. Given this, we designed this study to construct several predictive models for determining whether binding motifs in genes can exhibit a differential response to changes in AoXlnR expression (Figure 1). These models were constructed using 3D DNA shape information determined using the sequence around the AoXlnR binding motifs with classification as functional or nonfunctional.

In this study, our parameters related to DNA structure, HelT, ProT, MGW, and Roll were calculated for each sequence region using the R package "DNAshapeR.". We tried constructing a discriminant model by a support vector machine using the obtained DNA structure values as input parameters and an expression level in response to high expression of AoXlnR as an output parameter. These DNA shape-based models can correctly classify functional motifs in terms of area under the curve. Furthermore, we found that the number of motifs in the promoter region links differently to the parameters that contribute to the differential expression. The results show that the differential expression levels of genes located downstream of the AoXlnR motif were closely related to specific DNA shape information around the binding motifs.

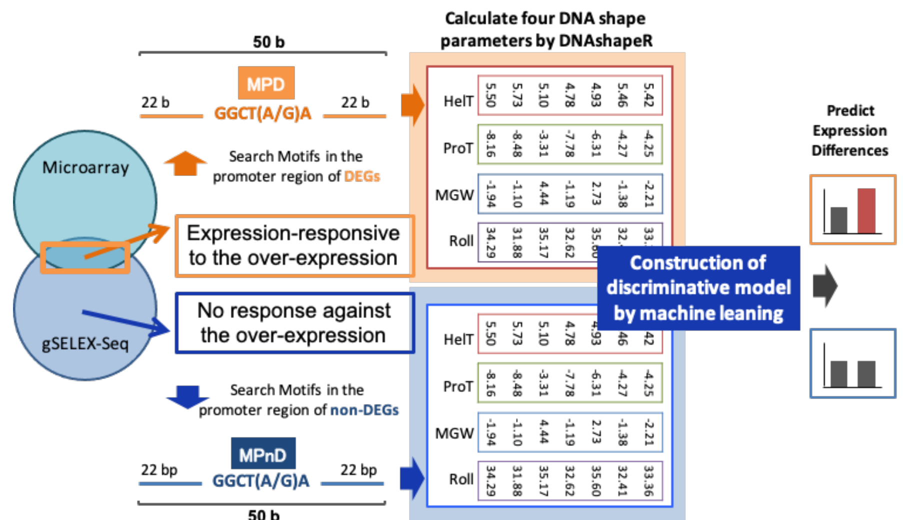


Figure 1. The scheme of prediction of Expression differences

1) Oka, H., Kojima, T., Ihara, K., Kobayashi, T., and Nakano, H. (2019) Comprehensive investigation of the gene expression system regulated by an Aspergillus oryzae transcription factor XlnR using integrated mining of gSELEX-Seq and microarray data. BMC Genomics 20, 16.

A novel factor for membrane vesicle formation in a hypervesiculating strain *Buttiauxella agrestis* DSM 4586

Nao NAKAMICHI[1] (presenter), **Ryota MORIUCHI**[2], **Hideo DOHRA**[2,3], **Hiroyuki FUTAMATA**[1,2,4], **Yosuke TASHIRO**[1,2,5]*

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Bacteria release membrane vesicles (MVs) from their cell surface to the environment. MVs have similar composition with the cell membrane and contain bacterial cell-derived component, therefore they are expected for various biotechnological applications including enzyme careers, vaccines and drug delivery systems. The mechanism of MV formation has been studied in various bacterial species and growth conditions, but the whole picture is not clear. *Buttiauxella agrestis* DSM 4586^T is a gram-negative bacterium, that produces high amount of MVs[1,2]. High MV production is a common characteristic in *Buttiauxella* spp, but not *B. agrestis* DSM 9389. In this study, we investigated the mechanism on hypervesiculation in *Buttiauxella* spp. With a transposon mutant analysis using DSM 4586^T, we found that the deletion of lipopolysaccharide (LPS) synthesis genes increased MV production and membrane permeability, suggesting that the presence of LPS contributes to outer membrane stability and the repression of MV formation. The LPS synthesis gene cluster *waaQ-waaL* is conserved in *B. agrestis*, but not in other *Buttiauxella* species. On the other hand, MV formation was decreased in the transposon mutant of an uncharacterized gene *omvA*, which is conserved in DSM 4586^T but not in other *Buttiauxella* spp. OmvA is predicted to be localized in the periplasm and associated with peptidoglycan. The complementary analysis showed that MV production was recovered by the expression of *omvA* in the mutant, suggesting that OmvA promotes MV formation. In conclusion, we identified that the modification of LPS structure changes MV production and OmvA is an important factor of MV production in *B. agrestis* DSM 4586^T.

References:
(1) *Front. Microbiol.*, 8:571, **2017**.
(2) *Appl. Environ. Microbiol.*, 86: e01131-20, **2020**.

Acquisition of new antibody genes using goldfish

Koshiro OBATA[1], Sarina YAMADA[1], Yutaka TAMARU[1,2]*

[1]Department of Life Sciences, Graduate School of Bioresources, Mie University,
[2] Mie University Smart Cell Innovation Research Center

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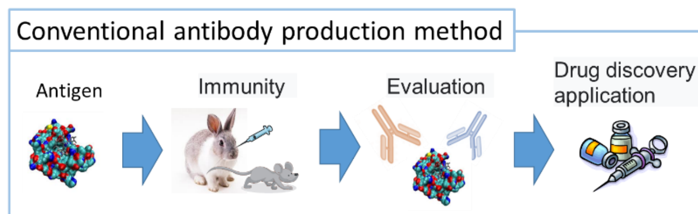
Antibodies used in a wide range of fields are mainly produced using mammals such as mouse and rabbits as host animals. However, in evolutionarily closely related organisms, antibodies with high specificity and titer against substances already present in the living body of the host animal, such as protein antigens, sugars, and lipids that are well conserved between species is often difficult to obtain. Examples of a method for avoiding this include using systematically distant animal species and devising an antigen administration method. Therefore, in our laboratory, as a method of avoiding the above problems, we focused on evolutionarily distant fish that have acquired immunity similar to that of mammals. The target is goldfish, which has a small breeding space and is easy to handle. Among them, a variety called bubble eye (blister eye), which has a bag (blister) with a huge cornea like a balloon under both eyes, is used. In previous studies, we have succeeded in obtaining scFv using the IgM gene as a template by injecting fluorescent protein (EGFP) into a water bubble as an antigen and performing PCR. Therefore, in this presentation, we changed the antigen from EGFP to TF-EGFP and attempted to obtain the scFv gene with an antigen having a large molecular weight. As a result, it was necessary to change the Ig heavy chain variable part Second PCR conditions. In addition, the PCR conditions prior to the second immune sample did not amplify well in the third immune sample, suggesting that the PCR conditions may need to be changed in the third immune sample.



Bubble eye

The fluctuation of microbial interactions is one of key factors for stable function of microbial community

Masahiro Honjo[1], **Kenshi Suzuki**[2], **Koki Amano**[3], **Yasuhisa Saito**[4], **Kazuhiro Takeda**[3], **Motohiko Kimura**[3], **Hidehiro Ishizawa**[5], **Yosuke Tashiro**[3], **and Hiroyuki Futamata**[1, 3, 5, *]

[1]Graduate School of Science and Technology, Shizuoka University,
[2]Biotechnology Research Center, The University of Tokyo,
[3]Depertment of Applied and Chemistry and Biochemical Engineering, Graduate School of Engineering, Shizuoka University,
[4]Depertment of Mathematics, Shimane University,
[5]Research Institution of Green Science and Technology, Shizuoka University

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It is valuable to understand bacterial homeostatic mechanisms for applying microbial complex system to biotechnology. Synthetic bacterial communities (SBCs) have been used to analyze the homeostatic mechanisms because microbial ecosystems are too complex to understand the mechanisms. Previous our researches demonstrate that an SBC constructed with three kinds of phenol-utilizing bacteria under chemostat conditions using phenol as a sole carbon source maintains phenol-degrading activity with coexistence using metabolic networks irrespective of all phenol-degraders [(1)]. We focused on interspecies interactions to understand how bacteria changes their roles in the SBC, which was based on Lotka-Volterra equations including parameters of interspecies interactions. Of 5,075 coexisting points, bacterial population densities at the 17 points were similar to those of the SBC but interspecies interactions were different from each other, suggesting mathematically that an interspecies interaction fluctuates even in a stable population dynamics. It was investigated effects of supernatants of the SBC collected in time series on the growth of distinct strain. *J* value, which is used to evaluate survival power of a bacterium in complex system under resource limiting conditions, was calculated with growth parameters. Population densities were monitored by real-time qPCR targeting genes encoding phenol hydroxylase. Positive correlation between dynamics of population densities and *J* values of three strains was observed: The population densities changed significantly corresponding to the *J* values change. These dry and wet approaches indicated that the fluctuation of interspecies interactions contribute to stability of the SBC, resulted in homeostatic phenol-degrading ability.

References:

(1) JBB, 131, p.77, 2021.

Rapid tuning of peptide binding by PURE ribosome display and NGS

JIA beixi[1], **Takaaki Kojima**[1], **Hideo Nakano**[1]

[1] (Laboratory of Molecular Biotechnology, Graduate School of Bioagricultural Science, Nagoya University)

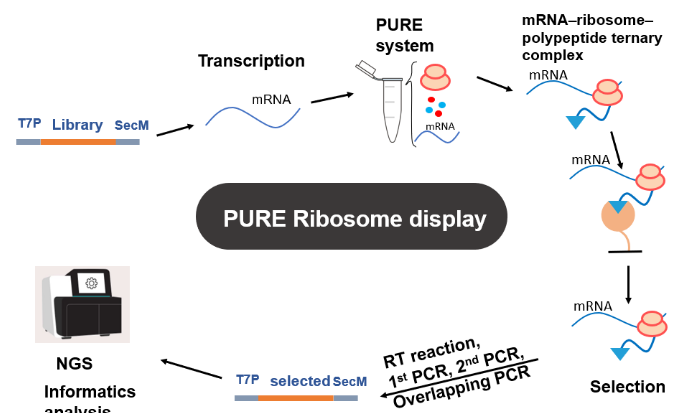
【The purpose of this research】

Ribosome display is an *in vitro* single-molecule display technology for selection and screening of huge variety of library, which physically links genotype (mRNA) and phenotype (protein) using ribosome. With the help of PURE (protein synthesis using recombinant elements) system, the efficiency of ribosome display has been increased greatly. In addition, with the utilize of next-generation sequencing (NGS), we can monitor the enriched mRNA sequences in each selection step of the ribosome display. Here we tried to establish a combination scheme of PURE ribosome display, NGS analysis and bioinformatics to optimize a binding peptide, strep-tag II, against a streptavidin mutant, strep-tactin, as a model binder.

【Methods】

The procedure of PURE ribosome display was shown in the figure.

A mutant peptide library of the strep-tag II sequence with partially doped NNK codons was constructed and displayed on ribosome with its mRNA. The mRNA-ribosome-peptide complex was loaded to strep-tactin column, and the bounded mRNA was recovered. The sequence of initial and the selected mRNAs were analyzed by NGS, followed by bioinformatics analysis to reveal preference of the binding peptide sequence. Several peptides containing preferred amino acid residues were constructed and the binding to strep-tactin was measured by ELISA and biosensor.



【Results】

NGS analysis of the selected sequences and the initial doped library indicated that five positions in the strep-tag II sequence (totally 8 residues) seems to be optimum because the original amino acids in above five positions were enriched. However, the original amino acids in the other three positions were slightly eliminated from the selected library and other amino acids were preferentially selected in the three positions. The constructed variants based on the analysis showed a slight increase in its binding to strep-tactin protein, which suggesting the applicability of the ribosome display selection scheme in peptide optimization to target molecules.

Identification and characterization of a β-1,3-xylanase from the human gut bacterium *Bacteroides cellulosilyticus*

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β-1,3-Xylan is a homologous polymer of β-1,3-linked D-xylose units that are found only in the cell walls of red algae and green algae[1)]. β-1,3-Xylanases (1,3-β-D-xylan xylanohydrolase; EC 3.2.1.32) catalyze hydrolysis of β-1,3-glycosidic linkages of β-1,3-xylan to produce several short-chain β-1,3-xylooligosaccharides[2)]. To date, the only functionally characterized β-1,3-xylanase are those from marine bacteria.

In this study, we characterized the enzymatic properties of putative β-1,3-xylanse (*Bc*Xyn26B) from the human gut bacterium, *Bacteroides cellulosilyticus* strain WH2[3)]. The *Bc*Xyn26B gene has been cloned and expressed as a soluble protein in *Escherichia coli*. The recombinant *Bc*Xyn26B was purified using immobilized metal affinity chromatography and anion exchange chromatography. This enzyme showed specific hydrolytic activity against β-1,3-xylan and released various β-1,3-xylooligosaccharides with β-1,3-xylobiose as the main product. (Fig. 1). It displayed maximum activity at 50 °C during a 10-min incubation at pH 6.0. Enzyme activity was stable in the pH range 6.0 ~ 10.0, with pH 6.0 being optimal.

This is the first report of the expression and characterization of β-1,3-xylanase from human gut microbes, and the substrate specificity of *Bc*Xyn26B imply that the human gut *Bacteroides* species have unknown metabolic pathways for β-1,3-xylan utilization.

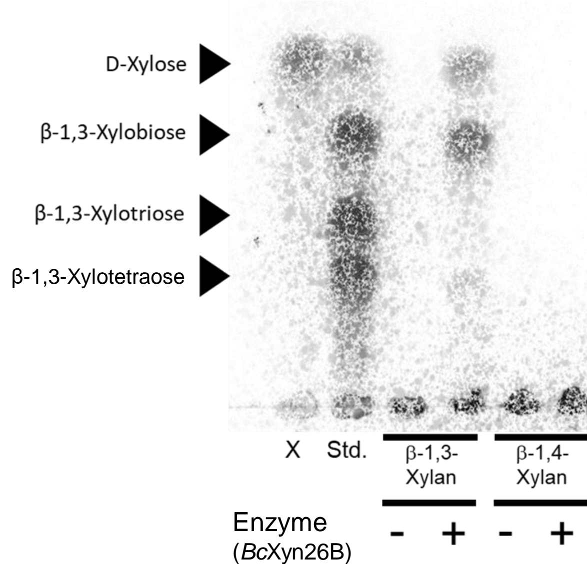

Fig. 1. Substrate specificity of *Bc*Xyn26B

References:

(1) *Nature*, **187**, 82-83, **1960**.
(2) *Appl. Microbiol. Biotechnol.*, **97**, 6749-6757, **2013**
(3) *PLoS Biol.*, **11**, e1001637, **2013**

Development of a method for obtaining rabbit monoclonal antibodies using DNA immunization

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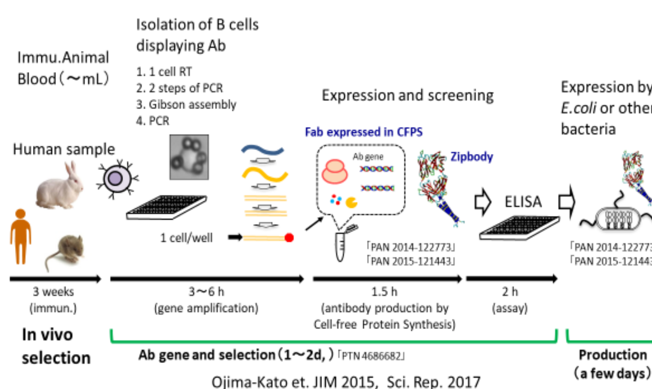
【Purpose】

Monoclonal antibody (mAb) is defined as an antibody molecule, which is cloned from a single B cell, recognizing an antigen molecule specifically. The hybridoma technology is generally used to produce mAb. However, the technology has several drawbacks such as low efficiency of cell fusion and long-time cultivation of hybridoma cells for the selection of desired antibody-producing cells. A new platform called Ecobody$_{(1)}$ technology has been established that enables screening and selection of mAbs in typically two days using single-cell RT, PCR, and cell-free protein synthesis system (CFPS), without any cell cultivation.

Here we have tried the combination of Ecobody technology and DNA immunization that gives immune response of proteins encoded by the injected DNA without preparation of antigen proteins. Green Fluorescent Protein (GFP) and Meflin$_{(2)}$, a potent biomarker for the suppression of pancreatic cancer were used as model antigen.



【Result】

Four-week-old rabbit was repeatedly immunized with a plasmid expressing GFP or Meflin by DNA electroporation. An increase in antibody titers was detected by ELISA using serum collected against at each week Meflin-expressing HEK293T cells and its culture supernatant. Then the rabbit was euthanized to collect B cells from blood and spleen. We are going to select B cells binding the Meflin-expressing cells and to obtain rabbit monoclonal antibodies from thus isolated single B cells by the Ecobody technology.

References:
(1) *Scientific reports, 7(1), 1-9.***2017**
(2) *Cancer science, 111(4), 1047.***2020**

Mutualistic relationship is a critical factor for microbial coexistence

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It is indispensable for efficient bio-conversion to maintain functional stability of bacterial complex systems. Phenol-utilizing *Comamonas testosteroni* strain R2 is able to grow under batch culture conditions with phenol as sole carbon source, whereas the chemostat culture of strain R2 collapses suddenly. Fortuitously, we found that strain R2 had maintained the phenol-degrading activity with non-phenol degrader *Stenotrophomonas* sp. strain Y even under phenol-fed chemostat condition. While the phenol-degrading activity was inhibited by the supernatant of strain R2-chemostat culture, the activity was not inhibited by the supernatant strain Y was inoculated in. These results suggested that the growth of strain R2 was negatively affected by metabolites produced from itself, and a mutualistic relationship between strains R2 and Y enables coexistence, resulted in the functional stability. Non-phenol degrader *Escherichia coli* strain BW25113 coexisted with strain R2 under chemostat condition and had maintained phenol-degrading activity of the chemostat culture. KEIO library mutants were used to investigate how strain BW25113 made the mutualistic relationship with strain R2. The growth of strain R2 with KEIO library mutants deleted genes encoding some amino acids synthesis metabolisms and carbohydrate metabolisms were lower than that of strain R2 with strain BW25113. These results suggested that we need for efficient bio-conversion to shed light overlooked microorganisms, e.g., target compounds non-degraders. It is unknown yet about the mutualistic metabolisms between strains R2 and Y/BW25113, and the negative effect of metabolite produced from strain R2. These subjects are currently under investigation.

Effect of temperature on metabolic activity and cell viability in *Streptomyces*

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Streptomyces are well known as a producer of secondary metabolites such as antibiotics. However, studies on primary metabolism as well as basic characteristics such as cell morphology in *Streptomyces* are limited. Here, we investigated the effect of temperature on primary metabolism and cell morphology in *Streptomyces lividans* 1326. Cell growth rate monitored by optical density at 600 nm (OD_{600}) and glucose consumption rate increased with increasing culture temperature from 28 °C (standard growth temperature) to 40 °C, but decreased significantly at 44 °C. The glucose consumption rate at 40 °C was 45% higher than that at 28 °C (Fig. 1A). Production of trehalose and α-ketoglutarate was maximized at 36 °C, with increased titer of 2.6 and 2.2-fold higher than that at 28 °C, respectively. In contrast, production of ribulose was maximized at 40 °C, with 4.3-fold higher titer than that at 28 °C. These results showed that the production of primary metabolites in *S. lividans* differed greatly depending on culture temperature. Next, we investigated the effect of temperature on cell morphology and viability. As a result, cells were shortened with increased number of dead cells at 40ºC (Fig. 1B). This result was inconsistent with the cell growth determined by cell turbidity at OD_{600}, suggesting that cell turbidity may not accurately reflect the *Streptomyces* biomass. Therefore, we measured cell dry weight and counted colonies from the cell cultures for verifying the accurate number of live cells. Consequently, the maximum cell dry weight at 40 ºC was 26% lower than at 28ºC. Also, the number of colonies from 40 ºC cultures was continuously lower than that from 28 ºC cultures. These results showed that cell turbidity did not accurately reflect the biomass of *S. lividans* when the culture temperature was elevated. Moreover, the results showed that the number of live cells at 40 °C is less compared to that at 28 °C. Together with the fact that the glucose consumption rate at 40 ºC was higher than that at 28ºC, the biomass specific glucose consumption rate was increased at the elevated temperature. In conclusion, *S. lividans* accelerated metabolic activity in primary metabolism for glucose consumption at high temperature, at which a number of cells cannot be survived.

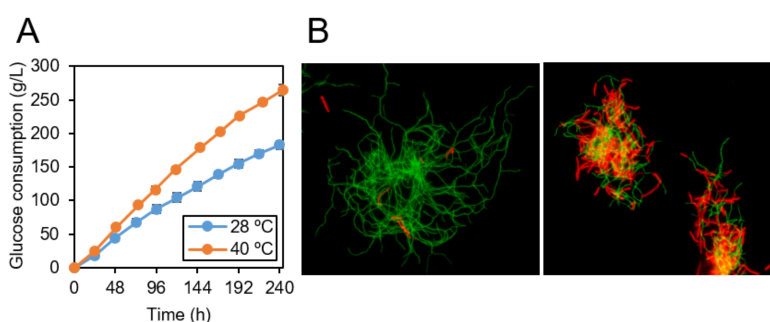

Fig. 1 Glucose consumption (A) and fluorescent microscopy (B) of *S. lividans* 1326 at 28ºC (orange, left), and 40°C (blue, right). Green and red represent live and dead cells, respectively.

Collecting of self-transmissible plasmids harboring antibiotic resistance genes from different environments

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Exceeding usage of antibiotics increased the occurrence of resistant bacteria, which usually showed multidrug resistances. The resistant bacteria can spread resistance genes by mobile genetic elements including plasmids. The objective of this study is to understand which plasmids transmit antibiotic resistance genes among bacteria in different environments. Plasmid capturing by biparental mating method (using tetracycline as a selective marker) was used to obtain self-transmissible plasmids from different environmental samples. By this technique, several plasmids were obtained from the Sanaru lake sediment samples.

There were seven incompatibility group IncA (or IncC) plasmids and one IncP-1delta plasmid. Complete nucleotide sequence of one of the seven were determined, named pBMDG108. This plasmid carried different resistance gene cassettes surrounded by a transposon and an integron. Phylogenetic analysis based on the nucleotide sequence of *repA* gene encoding replication initiation protein in pBMDG108 showed that this plasmid belongs to IncC group plasmid. Six resistance genes against sulfonamide, tetracycline, chloramphenicol, streptomycin, and aminoglycoside were found. Host range of the plasmid is now being investigated.

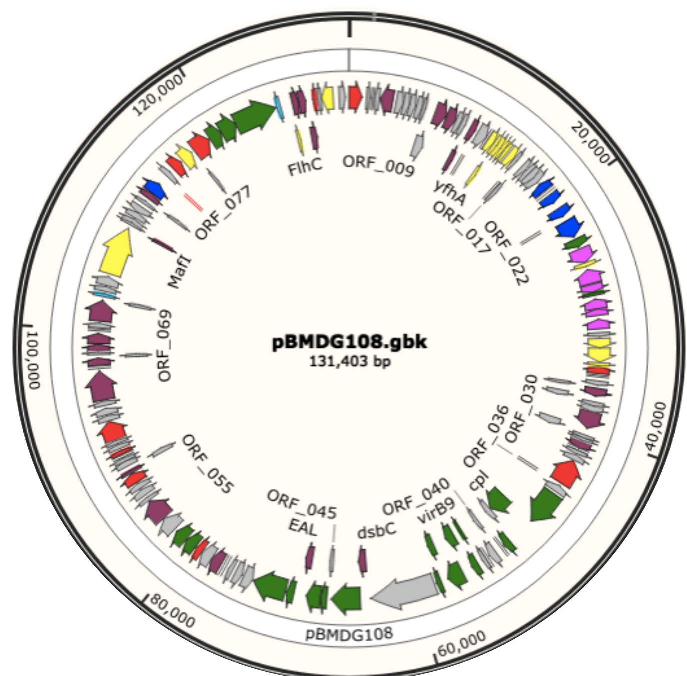

Figure 1 The circular map of pBMDG108. The color indicates different functions. The replication, maintenance, conjugation, antibiotic resistant gene, transposon, accessory genes and hypothetical protein depicted into red, yellow, green, pink, blue, magenta and gray, respectively.