The 24th Academic Exchange Seminar Between Shanghai Jiao Tong University And Osaka University

General Opening Session

Date:	November 29th, 2022
Time:	CST13:00～13:30 / JST14:00～14:30 (online)

13:00 (JST14:00) Opening words by moderator:
Dai Yanjun Professor, Shanghai Jiao Tong University
13:02 (JST14:02) Opening remarks:
HUANG Zhen, Dean of College of Smart Energy Former Vice President,
Shanghai Jiao Tong University, Academician of the Chinese Academy of Engineering
13:07 (JST14:07) Opening remarks:
KAWAHARA Genta, Vice President, Osaka University
13:12 (JST14:12) Brief explanation of collaboration in each field:
Materials Science (OU), Joining & Welding (SJTU)
Information Sciences (OU), Naval Architecture & Ocean Engineering (SJTU)
Industrial Biotechnology (OU), Chemistry (SJTU),
Combustion Engineering (OU),Smart City Studies (SJTU)
13:25 (JST14:25) Closing words by moderator / Group photo
Dai Yanjun, Professor, Shanghai Jiao Tong University

Date: November 29, Tuesday, 2022

Time: CST15:00～17:30 / JST16:00～18:30

Industrial Biotechnology "4th Industrial Biotechnology Symposium"

Co-organizer: Industrial Biotechnology Division, Institute for Open and Transdisciplinary Research Initiatives, OTRI, Osaka U.

Zoom address https://zoom.us/j/93415620551

Meeting ID: 934 1562 0551 PW: 452565

Opening remarks Prof. Takeshi Omasa (Grad Sch of Eng and OTRI, Osaka U)

CST 15:00-16:20 (JST 16:00-17:20)

20 min Presentation, including Discussion

15:00-15:20 (JST 16:00-16:20) Chairperson: Prof. Takeshi Omasa

Professor Susumu Uchiyama

(Department of Biotechnology, Graduate School of Engineering, Osaka University)

"Biophysical characterizations of AAV vectors for gene therapy"

15:20-15:40 (JST 16:20-16:40) Chairperson: Prof. Linquan Bai

Professor Fei Tao

(Shanghai Jiao Tong University)

"Carbon-negative synthetic biology: biomanufacture of biodegradable plastics"

15:40-16:00 (JST 16:40-17:00) Chairperson: Prof. Takeshi Omasa

Associate Professor Masaru Kojima

(Department of Materials Engineering Science, Graduate School of Engineering Science, Osaka University)

"Measuring the mechanical properties of cell using a two-fingered microhand system"

16:00-16:20 (JST 17:00-17:20) Chairperson: Prof. Linquan Bai

Tenure-track Associate Professor Rui Qing

(Department of Bioengineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University)

"QTY code: the membrane protein solubilization tool and its applications"

CST 16:20-18:20 (JST 17:20-18:20) Student session (Zoom: breakout room 1 and 2)

6min Presentation, 3min Discussion

Chairperson: Associate Prof. Chen-Guang Liu, Associate Prof. HiroyaTomita

Room 1 : Associate Prof. HiroyaTomita	Room 2: Associate Prof. Chen-Guang Liu
16:20-16:30 (JST 17:20-17:30) **Soichiro Ikuta** "*In situ* visualization of glutamate decarboxylase activity in germinating plant seed using mass microscope" 16:30-16:40 (JST 17:30-17:40) **Rui Wang** "Lipid turnover and SQUAMOSA promoter-binding proteins mediate variation in fatty acid desaturation under early nitrogen deprivation revealed by lipidomic and transcriptomic analyses in *Chlorella pyrenoidosa* " 16:40-16:50 (JST 17:40-17:50) **Shunsuke Ito** "Metabolic engineering of *Solanum* species for efficient production of useful steroidal compounds" 16:50-17:00 (JST 17:50-18:00) **Kaikai Tian** "Synergistic effect of fibrous structure and stiffness of designer protein hydrogels on bone marrow mesenchymal stem cell behaviors" 17:00-17:10 (JST 18:00-18:10) **Kai Torng Ang** "Effect of methylglyoxal on growth maintenance of human mesenchymal stem cells" 17:10-17:20 (JST 18:10-18:20) **Ru-Xiang Deng** "Characterization of the lomofungin biosynthetic gene cluster from *Streptomyces lomondensis* S015" 17:20-17:30 (JST 18:20-18:30) **Ryo Otsuka** "Activation of cryptic Streptomyces secondary metabolism by site-directed mutagenesis of ribosomal RNA gene"	16:20-16:30 (JST 17:20-17:30) **Yiqun Huang** "Community-integrated multi-omics faciliates screening and isolation of the organohalide dehalogenation microorganism" 16:30-16:40 (JST 17:30-17:40) **Chukwuebuka Maxwell Ononugbo** "Engineering the β-sandwich domain 1 of a thermostable protease for improved soluble expression for novel target binding" 16:40-16:50 (JST 17:40-17:50) **Kai Jiang** "Expanding the chemical diversity of fasamycin via genome mining and biocatalysis" 16:50-17:00 (JST 17:50-18:00) **Kulachatr Panyawechamontri** "The transient expression in *Nicotiana benthamiana* of human acid sphingomyelinase for enzyme replacement therapy" 17:00-17:10 (JST 18:00-18:10) **Ayturk Ali** "Identification of sucrose utilization genes for ansamitocin over-production from *Actinosynnema pretiosum*" 17:10-17:20 (JST 18:10-18:20) **Ayano Hara** "Development of CRISPR/dCas9-VPR transcriptional activation system in *D. magna*" 17:20-17:30 (JST 18:20-18:30) **Tian-Jie Ao** "Cascade utilization of carbon resource in corn stover to produce renewable energy products"

Closing remarks: Prof. Linquan Bai

The 24th Academic Exchange Seminar
Between Shanghai Jiao Tong University and Osaka University

Presentation Title: Biophysical characterizations of AAV vectors for gene therapy

Keywords：Gene Therapy, Virus Vector, Adeno Associated Virus, Mass Spectrometry

Name: Susumu Uchiyama

Affiliation and position:

Department of Biotechnology, Graduate School of Engineering, Osaka University

Short biography and publications (up to three)

Prof. Dr. Uchiyama is a biophysical chemist with over 25 years of experiences in studying solution biophysics of proteins and protein complexes, by using various kinds of biophysical methods. His research interest includes development of characterization methods and formulation for therapeutic proteins and virus vectors. Container closure system for biopharmaceuticals and vectors with better quality and more safety is also in his research scope. Recent years he is leading a Japan national project for the development of characterization and quality control of virus vectors for gene therapy. He has published more than 250 peer-reviewed papers including AAV characterizations and reviews. He serves on the Editorial Advisory Boards for Journal of Pharmaceutical Sciences.

1) Yoneda S, Torisu T, Uchiyama S. Development of syringes and vials for delivery of biologics: current challenges and innovative solutions. *Expert Opin Drug Deliv.* 18, 459-470 (2021).

2) Uchiyama S., Noda M., and Krayukhina E. Sedimentation velocity analytical ultracentrifugation for characterization of therapeutic antibodies. *Biophy. Rev.* 10, 259-269 (2018).

3) Uchiyama, S. Liquid formulation for antibody drugs. *Biochim Biophys Acta.* 1844, 2041-2052 (2014).

Abstract

Adeno-associated virus (AAV) vector is one of the most frequently used viral vector platforms used in gene therapies. As the scalable production of AAV vector is developed and the number of clinical trials is increased, more detailed and accurate characterizations of AAV vector are required. Unlike biopharmaceuticals, a certain number of species with similar properties to authentic AAV full particle are inevitably included in AAV product even after all purification processes. The difficulty of AAV characterizations originates not only from that AAV is composed of proteins and nucleic acid but also sample amount that can be subjected to biophysical analysis is limited. We have been developed several analytical methods for AAVs. For example, the confirmation of primary structure is performed by liquid chromatography mass spectrometry (LC-MS) and the ratio of VP proteins can be accurately determined by the combination approach using capillary electrophoresis CE-SDS and LC-MS. In this presentation, status and future perspectives of biophysical characterizations of AAV vectors will be introduced.

[Reference] Oyama H, Ishii K, Maruno T, Torisu T, Uchiyama S. Characterization of Adeno-Associated Virus Capsid Proteins with Two Types of VP3-Related Components by Capillary Gel Electrophoresis and Mass Spectrometry. Hum. Gene Ther. 32, 1403-1416 (2021).

As of Nov. 6, 2022

Presentation Title: Carbon-negative synthetic biology: biomanufacture of biodegradable plastics

Keywords: synthetic biology, carbon capture, biodegradable plastic, cyanobacteria

Name: Fei Tao

Affiliation and position:
Shanghai Jiao Tong University, Professor

Short biography and publications (up to three)

Fei Tao was born in Anhui Province of China in 1983. He went to Shandong University to study and obtained a Bachelor's degree in pharmacy (2004) and a Doctoral degree in microbiology (2009). After that, he joined Shanghai Jiao Tong University and has worked till now. From 2013 to 2014, he was a postdoctoral researcher at the Massachusetts Institute of Technology. His current main interests include synthetic biology, metabolic science, and biosensors.

1. Tan, C., Xu, P. & Tao, F.*, 2022 Carbon-negative synthetic biology: challenges and emerging trends of cyanobacterial technology. Trends Biotechnol. 40, 1488-1502.
2. Tan, C., Tao, F.* & Xu, P.*, 2022 Direct carbon capture for the production of high-performance biodegradable plastics by cyanobacterial cell factories. Green Chem., 24, 4470-4483.
3. Liu, H., Ni, J., Xu, P., Tao, F.*, 2018. Enhancing light-driven 1,3-propanediol production by using natural compartmentalization of differentiated cells. ACS Synth. Biol., 7, 2436-2446.

Abstract

Carbon emissions since the Industrial Revolution have significantly changed the Earth's climate, causing more serious environmental problems such as extreme weather. Synthetic biology is an emerging advanced technology. Taking advantage of it, people are expected to develop new and advanced green carbon-capture technologies to achieve carbon reduction. Plastic pollution is another serious environmental problem faced by humanity. Deleting and using degradable plastics is considered the ultimate solution for plastic pollution. In this report, we will present and discuss our research work in the production of degradable plastics using synthetic biotechnology. First, we will explore the basic concepts and scope of carbon-negative manufacturing and light-driven synthetic biology. We then will discuss the links between plastic pollution, degradable plastics, and sustainable production. Afterward, we use experiments as research work to explore the realization of carbon-negative production of degradable plastics. Finally, we will propose our prospects for the future of carbon-negative manufacturing of materials.

As of Nov. 29, 2022

The 24th Academic Exchange Seminar
Between Shanghai Jiao Tong University and Osaka University

Presentation Title: Measuring the mechanical properties of cell using a two-fingered microhand system

Keywords : Micromanipulation, Cell stiffness, Force measurement, Mechanobiology of single cells

Name: Masaru Kojima

Affiliation and position:

Department of Materials Engineering Science, Graduate School of Engineering Science, Osaka University, Associate professor

Short biography and publications (up to three)

Masaru Kojima received his Ph.D. from Nagoya Univ. in 2006. He had been Research Fellow with the Department of Biology, Nagoya Univ. from 2006, Assistant Professor on GCOE with the Department of Micro-Nano Systems Engineering, Nagoya Univ. from 2008, Assistant Professor in Department of Systems Innovation, Osaka Univ. from 2012, Assistant Professor in Department of Materials Engineering Science, Osaka Univ. from 2019. His research interests include development of microrobotic system for biological application, integration of robotics and biomaterials engineering for tissue regeneration and bio-robotics.

1) Kawakami M., Kojima M. et al., Automated Microhand System for Measuring Cell Stiffness By Using Two Plate End-Effectors, *IEEE Robotics and Automation Letters* 7(2), 2385-2390 (2022).
2) Mubarok W., Nakahata M., Kojima M., Sakai S., Nematode surface functionalization with hydrogel sheaths tailored in situ, *Materials today. Bio* 15, 100328-100328 (2022).
3) Hidaka M., Kojima M., Nakahata M., Sakai S., Visible light-curable chitosan ink for extrusion-based and vat polymerization-based 3d bioprintings, *Polymers* 13(9), 1382 (2021).

Abstract

In vivo, cells are constantly exposed to various stimuli, and these stimuli play an important role in determining the fate of cells. Therefore, there is a increasing demand to establish techniques for manipulating the environment around cells and tissues, in addition to measuring their characteristics. In particular, various techniques have been proposed to measure specific cellular responses to various external stimuli and to realize unprecedented analysis. In this background, we have developed a compact microhand system that can approach cells dexterously and rapidly. Furthermore, based on this microhand technology, we also developed a new end-effector that can measure the stiffness of cells and small tissues. We are still developing the system and realizing a variety of stimulation, such as chemical and thermal stimulation, in addition to force stimulation. In this seminar, we introduce a microhand system that enables the application of multimodal stimuli such as force stimulation as well as local chemical stimulation, and report an actual example of application of force stimulation response measurement to cell evaluation.

As of Nov. 11, 2022

Presentation Title: QTY code: the membrane protein solubilization tool and its applications

Keywords : membrane proteins, protein design, GPCR, therapeutics, biosensing

Name: Rui Qing

Affiliation and position:

Tenure-track associate professor, Department of Bioengineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University.

Short biography and publications (up to three)

Rui Qing earned his Ph.D. degree in Materials Science and worked on advanced battery technology at University of Florida, received postdoctoral training in Media Lab and was a research scientist at Koch Institute for Integrative Cancer Research at MIT. Dr. Qing has a multi-disciplinary scientific background in both material science and molecular biology. He co-developed with Dr. Shuguang Zhang the QTY code for membrane protein solubilization, and expanded its application in therapeutics and biosensing. His current research focus is to advance the design methodology of membrane proteins and the molecular level integration in bioelectronics, so as to address the societal challenges in human health and sustainability.

1. R. Qing, *et al.*, 2022, Protein design: From the aspect of water solubility, Chemical Reviews, (issue cover).
2. R. Qing, *et al.*, 2020, Non-full-length water-soluble $CXCR4^{QTY}$ and $CCR5^{QTY}$ chemokine receptors: implication for overlooked truncated but functional membrane receptors, iScience, 23-12: 101670.
7. R. Qing, *et al.*, 2019, QTY code designed thermostable and water-soluble chimeric chemokine receptors with tunable ligand affinity, Proceedings of the National Academy of Sciences, 116-51: 25668-25676.

Abstract

Membrane proteins comprise about 30% of the open reading frames in the genomes of higher eukaryotes and represent indispensable sets of biological functions that make up over 60% of therapeutic targets for drugs. Yet large-scale synthesis, characterization and utilization of these functional molecules lag far behind soluble proteins as they are difficult to overexpress and tend to aggregate in aqueous conditions due to molecules' hydrophobicity, especially for multi-pass TM proteins like GPCRs (G-protein couple receptor). Traditional means to stabilize the protein for their study requires arduous, non-transferrable efforts that are expensive and time-consuming, while limit their use beyond lab benches.

Here we devise a simple tool named QTY code to regulate the solubility of these hydrophobic receptors, by pairwise amino acid substitution in the transmembrane region. Over 10 membrane receptors were successfully solubilized, expressed and experimentally verified for ligand binding functions. The functional soluble variants of membrane receptors enable further biomedical use as 1), antibody-like decoy receptors for immunoregulation *in vivo*; 2) specific and sensitive probes in affinity-based biomimetic sensing platform with designated diagnostics purposes. Our approach not only enables the readily design of membrane proteins for functional and mechanistic study, but also produce a novel type of bio-nanomaterials that was not previously not attainable.

As of Nov. 29, 2022

Photo	**Presentation Title:** *In situ* visualization of glutamate decarboxylase activity in germinating plant seed using mass microscope **Keywords：** mass microscope, glutamate decarboxylase, enzymatic activity
	Name: Soichiro Ikuta
	Affiliation and position: Department of Biotechnology, Osaka University 3rd year PhD student

Abstract

Visualizing the distribution of enzymes is vital for understanding physiological phenomena. Recently, mass spectrometry imaging (MSI)-based enzyme histochemistry has been developed as a novel method to visualize the localization of enzymatic activity. It can be applied to enzyme histochemistry as it detects products from the supplied substrate using enzymes present on the tissue sections. However, enzyme histochemistry using MSI has not been applied to plant tissue samples.

Glutamate decarboxylase (GAD, EC:4.1.1.15) is an enzyme that catalyzes the decarboxylation reaction of L-glutamic acid to produce γ-aminobutyric acid(GABA). GABA biosynthesis is important both in the field of food chemistry and plant physiology. Therefore, the objective of this study was to develop a method to visualize the localization of GAD enzyme activity in germinating legume seeds using enzyme histochemistry with MSI. To achieve this objective, conversion by GAD enzyme was confirmed on the sections, and then the enzymatic reaction parameters were optimized to visualize the localization of GAD activity on germinated soybean seed sections using MSI. Subsequently, the results were evaluated using liquid chromatography-mass spectrometry (LC/MS) analysis. In addition, tissue-specific localization of GAD activity was further investigated in detail. Finally, the applicability of this method was verified using germinated alfalfa seeds. As a result, GAD activity in legume seeds was successfully visualized using MSI. This report is the first example of an MSI-based enzyme histochemistry technique applied to plant samples.

As of Nov.29, 2022

Presentation Title: Lipid turnover and SQUAMOSA promoter-binding proteins mediate variation in fatty acid desaturation under early nitrogen deprivation revealed by lipidomic and transcriptomic analyses in *Chlorella pyrenoidosa*

Keywords: microalgae, nitrogen deprivation, fatty acid desaturation, lipid turnover

Name: Rui Wang

Affiliation and position:
School of Life Sciences and Biotechnology, Shanghai Jiao Tong University
Ph.D. candidate

Short biography and publications (up to three)

Rui Wang is a Ph.D. candidate at Shanghai Jiao Tong University. His research focuses on fatty acid desaturation of microalgae affected by availability of nitrogen. He applied lipidomic, transcriptomic analyses, and functional characterization to identify key transcription factors in determining fatty acid desaturation.

Publications:

Wang R and Miao X (2022) Lipid turnover and SQUAMOSA promoter-binding proteins mediate variation in fatty acid desaturation under early nitrogen deprivation revealed by lipidomic and transcriptomic analyses in *Chlorella pyrenoidosa*. *Front. Plant Sci.* 13:987354. doi: 10.3389/fpls.2022.987354

Abstract

Nitrogen deprivation induces variations in fatty acid desaturation in microalgae, which determines the performance of biodiesel and the nutritional value of bioproducts. However, the detailed scenario and the underlying regulatory mechanism remain unclear. In this study, we attempt to outline these scenario and mechanisms by performing biochemical, lipidomic, and transcriptomic analyses in *Chlorella pyrenoidosa* and functional characterization of transcription factors in *Yarrowia lipolytica*. We found that early nitrogen deprivation dramatically reduced fatty acid desaturation without increasing lipid content. The contents of palmitic acid (16:0) and oleic acid (18:1) dramatically increased to 2.14 and 2.87 times that of nitrogen repletion on the second day, respectively. Lipidomic analysis showed the transfer of polyunsaturated fatty acids from phospholipids and glycolipids to triacylglycerols, and an increase in lipid species with 16:0 or 18:1 under nitrogen deprivation conditions. Upregulated stearoyl-ACP desaturase and oleyl-ACP thioesterase promoted the synthesis of 18:1, but restricted acetyl-CoA supply revealed that it was the intensive lipid turnover instead of an attenuated Kennedy pathway that played an important role in the variation in fatty acid composition under early nitrogen deprivation. Finally, two differentially expressed SQUAMOSA promoter-binding proteins (SBPs) were heterologously expressed in *Y. lipolytica*, demonstrating their role in promoting the accumulation of total fatty acid and the reduction in fatty acid desaturation. These results revealed the crucial role of lipid turnover and SBPs in determining fatty acid desaturation under early nitrogen deprivation, opening new avenues for the metabolic engineering of fatty acid desaturation in microalgae.

As of Nov. 29, 2022

Presentation Title: Metabolic engineering of *Solanum* species for efficient production of useful steroidal compounds

Keywords : metabolic engineering, potato, *Solanum*, yamogenin

Name: ITO Shunsuke

Affiliation and position:

Department of Biotechnology, Graduate School of Engineering, Osaka University, Osaka, Japan. Master's student.

Abstract

Yamogenin is one of useful compounds that show a variety of biological activities and synthesized in yam plants (*Dioscorea* spp.). However, Yam-based efficient production is difficult because it takes 4 to 5 years to grow and limited growing environment. Our laboratory has revealed that *CYP88B1* knockout potato accumulates steroidal saponins (SSs), Protoneodioscin and 25-*epi*-Indioside D instead of toxic steroid glycoalkaloids (SGAs), α-solanine and α-chaconine. These SSs can be converted to Yamogenin by hydrolysis (Fig. 1). In this study, I aim to enhance productivity of protoneodioscin and 25-*epi*-Indioside D in tissue culture of *CYP88B1* knockout potato.

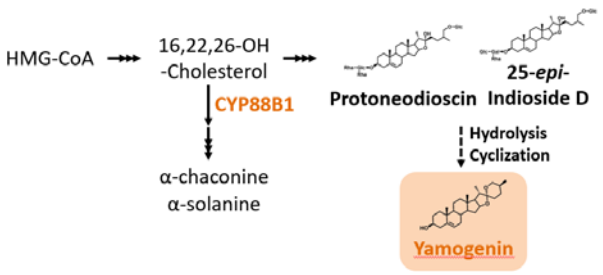

Fig. 1 Biosynthetic pathway of SSs and SGAs

I induced microtuber formation in plastic-bag-culture (Fig. 2). To test the effect of light on SS production in microtubers, harvested microtubers were incubated under different light conditions for 7 days, however, there was no significant changes in the contents of SS among examined condition. I also studied the effect of methyl jasmonate (MeJA) on the formation of microtubers and SS contents. As a result, 100 μM MeJA treatment during microtuber induction significantly increased the SS contents and the number of microtuber. These reults indicate that tissue culture of potato microtubers is effective for the production useful SS. In the future, further genetic modification and optimization of culture conditions could lead to higher productivity of those SS.

Fig. 2 Microtuber

As of November 16th, 2022

The 24th Academic Exchange Seminar
Between Shanghai Jiao Tong University and Osaka University

Presentation Title: Synergistic effect of fibrous structure and stiffness of designer protein hydrogels on bone marrow mesenchymal stem cell behaviors

Keywords: Protein hydrogels, fibrous structure, stiffness, bone marrow mesenchymal stem cell

Name: Kaikai Tian

Affiliation and position: State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic and Developmental Sciences, and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, PhD candidate.

Short biography and publications (up to three)

Kai-Kai Tian received his BS and MS degree from Yantai University and Dalian University of Technology, respectively. Now, he is studying for the PhD degree at Shanghai Jiao Tong University. His research is focused on design and fabrication of protein polymer-based hydrogels for tissue engineering.

Publications:

1. **Tian KK,** Huang SC, Xia XX, Qian ZG. Fibrous Structure and Stiffness of Designer Protein Hydrogels Synergize to Regulate Endothelial Differentiation of Bone Marrow Mesenchymal Stem Cells. *Biomacromolecules*, 2022, 23(4): 1777-1788.

2. Huang SC, Fan RX, **Tian KK,** Xia XX, Qian ZG. Controllable Fibrillization Reinforces Genetically Engineered Rubberlike Protein Hydrogels. *Biomacromolecules*, 2021, 22(2): 961-970.

Abstract

Stiffness and fibrous structure provided by native extracellular matrix have been proven as important cues in regulating cell fates. However, an understanding of how stiffness and fibrous structure synergize to regulate cell behaviors remains a challenge due to the inherent difficulties in making hydrogels with well-defined compositions, tunable stiffness and structures. Here, we fabricate two series of fibrous and porous hydrogels based on engineered resilin-silk-like and resilin-like protein polymers with similar stiffness.These two series of hydrogels were utilized as two-dimensional culture platforms for BMSCs to explore the effects of stiffness and fibrous structure on their proliferation and endothelial differentiation. For both hydrogel series, increasing compression modulus from about 8.5 to 23 kPa consistently promoted cell proliferation and endothelial differentiation. Nonetheless, the promoting effects were more pronounced on the fibrous gels than their porous counterparts at all three stiffness levels, which may be attributed to the differences in promoting the activation of transcription cofactor Yes-associated protein (YAP). Then, it was found that the stiffness signal activated YAP via spreading of focal adhesions and cytoskeleton, whereas fibrous structure reinforced YAP activation by promoting the maturation of focal adhesions and associated F-actin alignment. Therefore, our results clarify the synergistic effect of stiffness and fibrous structure on stem cell behaviors and may guide the fabrication of designer proteinaceous matrices toward tissue engineering.

Presentation Title: Effect of methylglyoxal on growth maintenance of human mesenchymal stem cells

Keywords : Human mesenchymal stem cells; Extracellular matrix; Growth behavior; Methylglyoxal

Name: Kai Torng ANG

Affiliation and position:

Department of Biotechnology, Graduate School of Engineering, Osaka University, Ph.D. student

Abstract

In order to apply human mesenchymal stem cells (hMSCs) for practical uses in regenerative medicine, culture strategies for producing a sufficient quantity of cells with stable quality are required. During the expansion culture of hMSCs, local cell density exponentially increases in the culture vessel along with changes in cell behaviors, causing cell contact inhibition and restricting cell proliferation. Previous studies on stem cell proliferation have reported that secretion and structural changes of the extracellular matrix (ECM) are important for maintaining cell proliferative potential. In this study, we investigated the proliferative effect of hMSCs using methylglyoxal (MG), a precursor of advanced glycation end products which is capable of modifying ECM structure.

hMSCs were seeded at a seeding density of 3.0×10^3 cells/cm^2 and cultured for 144 h. At t = 48 h, hMSCs were exposed to MG at 0.125 nM for 24 hours, and MG was removed from the culture at t = 72 h. In cells without MG exposure, the apparent specific growth rate μ^{app} (h^{-1}) increased to $(2.05 \pm 0.3) \times 10^{-2}$ h^{-1} in the early phase of culture (t = 48-72 h), while the apparent specific growth rate decreased to $(0.89 \pm 0.04) \times 10^{-2}$ h^{-1} in the late phase of culture (t = 72-144 h). On the other hand, the apparent specific growth rate of cells with MG exposure in the late phase of culture was $(1.50 \pm 0.1) \times 10^{-2}$ h^{-1}, confirming the maintenance of apparent specific growth rate of cells throughout the culture. The assembly and structural changes of the ECM main components, fibronectin and collagen type I after MG exposure and withdrawal (t = 72 h and 144 h) were examined by immunofluorescence staining. We confirmed that MG exposure disrupted fibronectin and collagen fibril formation and it prevented its deposition in the ECM, however it did not influence fibronectin and collagen type I secretion. These results suggest that MG could reform ECM and the reconstruction of the scaffold environment leads to the maintenance of cell proliferative capacity, indicating a new potential in establishing an expansion culture system.

As of Nov 29, 2021

Presentation Title: Characterization of the lomofungin biosynthetic gene cluster from *Streptomyces lomondensis* S015

Keywords: natural phenazines, biosynthetic pathway, cluster refactoring

Name: Ru-Xiang Deng

Affiliation and position:

School of Life Sciences and Biotechnology, Shanghai Jiao Tong University
PhD student

Short biography and publications (up to three)

Ru-Xiang Deng received the BS degree from Shandong University in 2017. He began as a master student (2017-2020) under the supervision of Professor Wei Wang at Shanghai Jiao Tong University. He continued studying for his PhD degree with Professor Xue-Hong Zhang at Shanghai Jiao Tong University. His research interests are metabolic engineering of *Streptomyces* for the production of secondary metabolites, microbial fermentation and identification of novel natural compounds.

Publications:

Deng RX, Zhang Z, Li HL, Wang W, Hu HB, Zhang XH. Identification of a Novel Bioactive Phenazine Derivative and Regulation of *phoP* on Its Production in *Streptomyces lomondensis* S015. J Agric Food Chem. 2021 Jan 27;69(3):974-981.

Li HL, Deng RX, Wang W, Liu KQ, Hu HB, Huang XQ, Zhang XH. Biosynthesis and Characterization of Medium-Chain-Length Polyhydroxyalkanoate with an Enriched 3-Hydroxydodecanoate Monomer from a *Pseudomonas chlororaphis* Cell Factory. J Agric Food Chem. 2021 Apr 7;69(13):3895-3903.

Abstract

Natural phenazines are a class of multifunctional secondary metabolites that play an important role in the biocontrol of various pathogens. Lomofungin is a highly substituted phenazine derivative that exhibit significant bioactivities against several tumor cell lines and can be employed for the construction of biosensor. However, the biosynthetic pathway of lomofungin has not been fully understood. In this study, we firstly analyzed the putative gene cluster and proposed a possible biosynthetic pathway. Then, the intact gene cluster was identified by heterogeneous expression combined with gene cluster refactoring. The function of modification enzymes was further verified by combinatorial expression percursor genes with modification enzymes. Specifically, SAM-dependent methyltransferase Lomo11 catalyzed the methylation of carboxyl group at C-1 position. Acyl-CoA dehydrogenase Lomo9 was responsible for the hydroxylation of the phenazine ring at positions 4 and 9. Finally, lomofungin biosynthetic pathway was revised and extended. This study provided valuable reference for the identification of phenazine biosynthetic pathways and discovery of phenazine modification enzymes.

As of Nov. 29, 2022

Presentation Title: Activation of cryptic *Streptomyces* secondary metabolism by site-directed mutagenesis of ribosomal RNA gene

Keywords : Base-editing; Anti-Shine-Dalgarno sequence; Actinomycetes

Name: OTSUKA Ryo

Affiliation and position:

Department of Biotechnology, Graduate School of Engineering, Osaka University, Osaka, Japan. Master's student.

Abstract

Streptomyces spp. produce a wide variety of bioactive compounds as secondary metabolites. However, most of the genes for the biosynthesis of secondary metabolites remain "cryptic". Activation of these cryptic secondary metabolism may lead to the discovery of new bioactive compounds. Ribosomal mutagenesis is a technique to activate cryptic secondary metabolism in actinomycetes. The introduction of drug-resistant mutations into the ribosome has enabled the discovery of new bioactive compounds. Here, we used Target-AID, a genome editing tool that replaces cytosines with thymines in target sequences, to introduce mutations in anti-Shine-Dalgarno (anti-SD) sequences and activate cryptic *Streptomyces* secondary metabolism. In the transformants of *Streptomyces coelicolor* A3(2), the cytosine residues of anti-SD sequence of the *rrnF* gene were replaced by thymine residues. Mutant strain #1 produced more actinorhodin and undecylprodigiosin than the wild-type strain, while no such differences were observed in mutant strain #2, #3 and the wild-type strain. These results suggest that secondary metabolism might be changed in the mutant strain #1.

As of November 14, 2022

Presentation Title: Community-integrated multi-omics faciliates screening and isolation of the organohalide dehalogenation microorganism

Keywords : Microbial community, Wastewater, Metagenome assembled genome, Organohalide dehalogenation

Name: Yiqun Huang

Affiliation and position:

School of Life Sciences and Biotechnology ,Shanghai Jiao Tong University
PhD student

Short biography and publications (up to three)

Short biography:

Yiqun Huang, using bioinformatics and molecular biology tools to investigate the environmental microbiome, Is focusing on the functional genes, metabolic pathways, and bacteria resources of the polluted environment microbial community (*e.g.*, chemical plant wastewater).

Publications:

1. Huang Y, Wen L, Zhang L. et al. Community-integrated multi-omics faciliates screening and isolation of the organohalide dehalogenation microorganism. **The innovation**. (pre-accpected).

2. Wen L+, **Huang Y+**, Wang W. et al. A novel *Diaphorobacter* sp. strain isolated from saponification wastewater shows highly efficient phenanthrene degradation. **Environmental Research**. 214, 114047. (2022).

3. Hu H, Wang M, **Huang Y**. et al. Guided by the principles of microbiome engineering: Accomplishments and perspectives for environmental use. **mLife**.12043 (2022)

Abstract

A variety of anthropogenic organohalide contaminants generated from industry are released into the environment, and thus cause serious pollution that endangers human health. In the present study, we investigated the microbial community composition of industrial saponification wastewater using 16S rDNA sequencing, providing genomic insights of potential organohalide dehalogenation bacteria (OHDBs) by whole-metagenome sequencing. We also explored yet-to-culture OHDBs involved in the microbial community. Microbial diversity analysis reveals that Proteobacteria and Patescibacteria phyla dominate microbiome abundance of the wastewater. In addition, a total of six bacterial groups (Rhizobiales, Rhodobacteraceae, Rhodospirillales, Flavobacteriales, Micrococcales, and Saccharimonadales) were enriched in the key organohalide removal module. Ninety-four metagenome-assembled genomes (MAGs) were reconstructed from Proteobacteria, Bacteroidota, Patescibacteria, and Actinobacteria, and 105 dehalogenase genes within 42 MAGs were identified, suggesting that the potential for hydrolytic organohalide dehalogenation is present in the microbial community. Subsequently, we characterized the organohalide dehalogenation of an isolated OHDB, *Microbacterium* sp. J1-1, which shows the dehalogenation activities of chloropropanol, dichloropropanol, and epichlorohydrin. This study provides a community-integrated multi-omics approach to collect functional OHDBs for industrial organohalide dehalogenation.

As of Nov. 29, 2022

Presentation Title: Engineering the β-sandwich domain 1 of a thermostable protease for improved soluble expression for novel target binding.

Keywords：SD1, target binding, *E. coli*, soluble expression, stability, rational design.

Name: Chukwuebuka Maxwell Ononugbo

Affiliation and position: Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan. PhD student (Omasa laboratory).

Short biography and publications (up to three)

Chukwuebuka Maxwell Ononugbo earned a bachelor's and master's degree in Microbiology from the Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka. He also received a master's degree in Biotechnology from the Department of Biotechnology, Graduate school of Engineering, Osaka University, Japan. He is currently a PhD student under the supervision of Prof. Takeshi Omasa and Prof. Yuichi Koga (Omasa laboratory), in the same program at Osaka University, with a bias in Molecular Biotechnology and Protein Engineering. His research interests include improving protein structure/function for novel target binding for applications in biomedicine and research.

Abstract

Over the years, the concept of target binding has been exploited significantly in diagnostics/therapeutics, medicine, and research. This field is dominated by monoclonal antibodies, as the most common biopharmaceuticals. However, this biologic faces several drawbacks, including large size, limited tissue penetration and high cost of production. These led to the extension of this concept to alternative protein frameworks leveraging their small size, stability, and expression in *E. coli*. The β-sandwich domain 1 of a thermostable protease Islandisin (SD1) provides a suitable framework, including exposed surface loops that could generate binding to several targets. A small-sized protein that folds like the variable domain of immunoglobulin with its hydrophobic core built by several hydrophobic residues, providing stability to the protein. It is expressible in *E. coli,* but most of the protein aggregates and accumulates in inclusion bodies. A limiting factor for binding applications. Therefore, in this study, SD1 surfaces were re-engineered to augment their net charge, reversibility of unfolding and generally reduce its aggregation. We identified the aggregation-prone regions (APRs), and the effect of aggregation gatekeeper residues on the protein stability was *in silico* analyzed. We also explored mutations within the core residues to improve the thermodynamic stability of the mutant(s). All mutants were confirmed soluble on SDS-PAGE and purified by affinity chromatography for further characterization. In conclusion, we could reduce the aggregation of SD1 and improve its soluble expression in *E. coli* by rational design. This study adds to the wealth of knowledge for understanding protein physicochemical properties and improvement strategies. The result of this study shows the possibility of the amenability of SD1 to novel target binding.

As of 9th November, 2022

The 24th Academic Exchange Seminar
Between Shanghai Jiao Tong University and Osaka University

Presentation Title: Expanding the Chemical Diversity of Fasamycin Via Genome Mining and Biocatalysis

Keywords: genome mining, biocatalysis, halogenase

Name: Kai Jiang

Affiliation and position: School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Ph.D. candidate

Short biography and publications (up to three)

Kai Jiang is currently a PhD student at Shanghai Jiao Tong University. He received his MS degree under the supervision of Professor Xudong Qu from Wuhan University in 2021. His research interest focuses on biosynthesis of the scaffold formation in the type II polyketide ABX and fasamycin.

Publications:

1. **Jiang K**, Yan X, Deng Z, Lei C, Qu X. Expanding the Chemical Diversity of Fasamycin Via Genome Mining and Biocatalysis. J Nat Prod. 2022 Apr 22;85(4):943-950.

Abstract

The rapid increase in multidrug-resistant pathogens has posed a great threat to global public health. Discovery of novel antibiotics with a mode of action that differs from those in current clinical use is therefore an urgent need. In this study, we activated the silenced microbial biosynthetic pathways through overexpression of the phosphopantetheinyl transferase (PPtase). PPtase is responsible for phosphopantetheinylation of the carrier proteins (CPs), which is a key step in the biosynthesis of polyketides (PKs), nonribosomal peptides (NRPs), fatty acids (FAs), lysine, and tetrahydrofolate. It has been demonstrated as able to activate both of the phosphopantetheinylation-dependent and -independent biosynthetic pathways. To discover bioactive secondary metabolites based on our developed PPtase strategy, we herein reported the identification of three new and four known fasamycin congeners from the Pptase-activated chemical constituents of *Streptomyces kanamyceticus*. By treating the fasamycin derivatives with two halogenases $Abx_{(-)}H$ and $FasV_{sk}$, we generated additional five new and one known fasamycins. A few of the analogues demonstrated significantly improved antimicrobial activity. The C29-methyl and C-2/C-22-halogen substitutions were revealed to be important for the bioactivity. Herein, we report the details of the genome mining, biocatalysis, structural elucidation, and antibacterial activities of these new antimicrobial products. This study increases the chemical diversity of bioactive fasamycin derivatives and provides useful halogenation tools for engineering their scaffolds.

Presentation Title: The transient expression in *Nicotiana benthamiana* of human acid sphingomyelinase for enzyme replacement therapy.

Keywords : Acid sphingomyelinase; Agroinfiltration; *Nicotiana benthamiana*

Name: Kulachatr Panyawechamontri

Affiliation and position:

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Abstract

Acid sphingomyelinase (ASM) converts sphingomyelin into phosphocholine and ceramide. Hereditary mutations of ASM result in the lysosomal storage disease called Niemann-Pick disease. Currently, enzyme replacement therapy (ERT) is considered as a new clinical trial for treating patients with Niemann-Pick disease type B. Plant-based expression system is an attractive and alternative system for recombinant protein production, because it enables post-translational modifications, and are safe from contamination by human pathogens. In this study, recombinant human ASM was produced in *Nicotiana benthamiana* as a functional protein using the Agrobacterium-mediated transient expression system. Moreover, ASM was successfully produced as a plasma membrane protein. Crude proteins were extracted from the infiltrated leaves using the revised microcentrifuge method. In addition, co-expression with p19 enhanced the production, resulted in showing the highest ASM activity in 6 days post-infiltration with the enzyme activity of 238.6 Units/mg total soluble protein. This study developed an alternative plant-expression system of recombinant ASM production in *N. benthamiana* whole plant that might be applied to other applications in the future. However, ASM purification method in plant is necessary to improve the purity of ASM.

As of Nov 29, 2021

The 24th Academic Exchange Seminar
Between Shanghai Jiao Tong University and Osaka University

Presentation Title: Identification of sucrose utilization genes for ansamitocin over-production from *Actinosynnema pretiosum*

Keywords: Carbon utilization, Sucrose hydrolase activity, Proteomic analysis, Ansamitocin

Name: Ayturk·Ali

Affiliation and position: Master candidate at Shanghai Jiao Tong University. (Supervisor: Prof. Linquan Bai)

Short biography and publications (up to three)

Ayturk·Ali is a master candidate at Shanghai Jiao Tong University. Her main research interest is the mechanism of sucrose utilization in *Actinosynnema pretiosum*. Guided by sucrose hydrolysis activity of fractionations of intracellular or extracellular proteins, she identified sucrose hydrolase proteins from *Actinosynnema pretiosum* and illustrated the underlying molecular mechanism for the ansamitocin over-production effect of sucrose supplementation.

Abstract

Sucrose, the most abundant disaccharide in nature, is a common carbon and energy source for the industrial biotechnology. However, sucrose is mostly used for pellet morphology control and rarely as a carbon source for antibiotic production in actinobacteria. Given the limited studies on the mechanism of sucrose utilization in actinobacteria, we investigated sucrose utilization by *Actinosynnema pretiosum* ATCC 31280 and illustrated the mechanism for effect of sucrose supplementation on the production of secondary metabolite ansamitocin P-3 (AP-3), a member of microbial maytansinoids with potential antitumor activity. The result showed that *Actinosynnema pretiosum* ATCC 31280 efficiently utilizes sucrose as the sole carbon source, and the optimal concentration of sucrose is 70 g/L with a 79.3% titer improvement of AP-3. Sucrose hydrolysis activity guided fractionation and subsequent proteomic analysis identified 1 intracellular and 2 extracellular sucrose utilization proteins in *Actinosynnma pretiosum* ATCC 31280. We also genetically engineered the identified sucrose utilization genes, and achieved a 36.9% titer improvement of AP-3 and a 44.3% decrease of residual sucrose in the medium. Our work elucidated the sucrose utilization mechanism and provided a new strategy for natural product over-production in actinobacteria.

As of Nov. 29, 2022

Presentation Title: Development of CRISPR/dCas9-VPR transcriptional activation system in *D. magna*

Keywords：genetic engineering, CRISPR, transgenic

Name: Ayano Hara

Affiliation and position:

Bioenvironmental Science Laboratory, Department of Biotechnology, Graduate School of Engineering, Osaka University.

Abstract

The crustacean *Daphnia magna* is a model organism for environmental and toxicological studies. In response to environmental stimuli, they are known to switch phenotypes variously. To understand these mechanisms at the genomic level, useful tools of loss-of-function (LOF) studies were established. On the other hand, gain-of-function (GOF) techniques still have some problems, so there are limitations to genetic analysis in *D. magna*. In this study, we aimed to develop CRISPR/dCas9 VPR system that activates the expression of the target gene from the endogenous genomic locus. We constructed a plasmid expressing dCas9-VPR protein ubiquitously and designed gRNA, which targets to activate green fluorescence protein (GFP) expression in transgenic line. Next, we injected the plasmid and gRNA into the embryo and found that GFP expression was activated by CRISPR/dCas9-VPR system. Now we are trying to generate transgenic *D. magna*, which expresses dCas9-VPR protein in their whole body. For the knock-in experiment, I optimized the promoter region and added a fluorescent protein gene as a reporter in the donor plasmid. This study might accelerate to reveal of the genetic function of *D. magna*.

As of 16st November, 2022

Presentation Title: Cascade utilization of carbon resource in corn stover to produce renewable energy products

Keywords: cascade utilization, corn stover, ethanol, methane , microalgae

Name: Tian-Jie Ao

Affiliation and position:

Affiliation: State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic and Developmental Sciences, and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University

Position: Ph.D. candidate

Short biography and publications (up to three)

Short biography:

Tian-Jie Ao is a Ph.D. candidate at Shanghai Jiao Tong University. His research interests are focused on biomass resources and biorefinery, organic waste re-utilization, and wastewater treatment.

Publications

Ao, T., Li, K., Mehmood, M.A., Zhao, X.-Q., Bai, F.-W., Boopathy, R., Liu, C.-G., 2022. Process optimization for acidic deep eutectic solvent pretreatment of corn stover to enhance enzymatic saccharification. Biomass Conversion and Biorefinery.

Ao, T., Chen, L., Chen, Y., Liu, X., Wan, L., Li, D., 2021. The screening of early warning indicators and microbial community of chicken manure thermophilic digestion at high organic loading rate. Energy 224, 120201.

Ao, T., Chen, L., Zhou, P., Liu, X.F., Li, D., 2021. The role of oxidation-reduction potential as an early warning indicator, and a microbial instability mechanism in a pilot-scale anaerobic mesophilic digestion of chicken manure. Renewable Energy 179, 223-232.

Abstract

Corn stover (CS) is the most widely distributed agricultural residue in China, which can be utilized as the feedstock for cellulosic ethanol production. Nonetheless, nearly half of the carbon resource in the CS will be released into the atmosphere in the form of CO_2 during the cellulosic ethanol fermentation process. Besides, the residual vinasse still contains a certain amount of carbon-based compounds derived from CS that cannot be utilized in the cellulosic ethanol fermentative process, which finally results in the loss of carbon resources in CS. Here we proposed an effective scheme via cascade utilization of carbon resources in corn stover to achieve the improvement of carbon utilization from 40% to 75%. CS was first effectively pretreated by deep eutectic solvent (DES) to deconstruct the refractory structure of CS. Second, the pretreated CS was selected as the substrate for ethanol fermentation by the genetically engineered *Saccharomyces cerevisiae* that can assimilate mixed sugar simultaneously. Third, the residual vinasse from the ethanol production process was utilized by combining microbial electrolysis cell (MEC) with anaerobic digestion (AD) to transfer the organic waste to methane to achieve the carbon resource re-utilization and clean energy production, simultaneously. Finally, the released CO_2 from the ethanol fermentative process and the digestate from the MEC-AD process were collected as the substrates for self-flocculating microalgae cultivation to achieve the cascade utilization of carbon resources in CS.