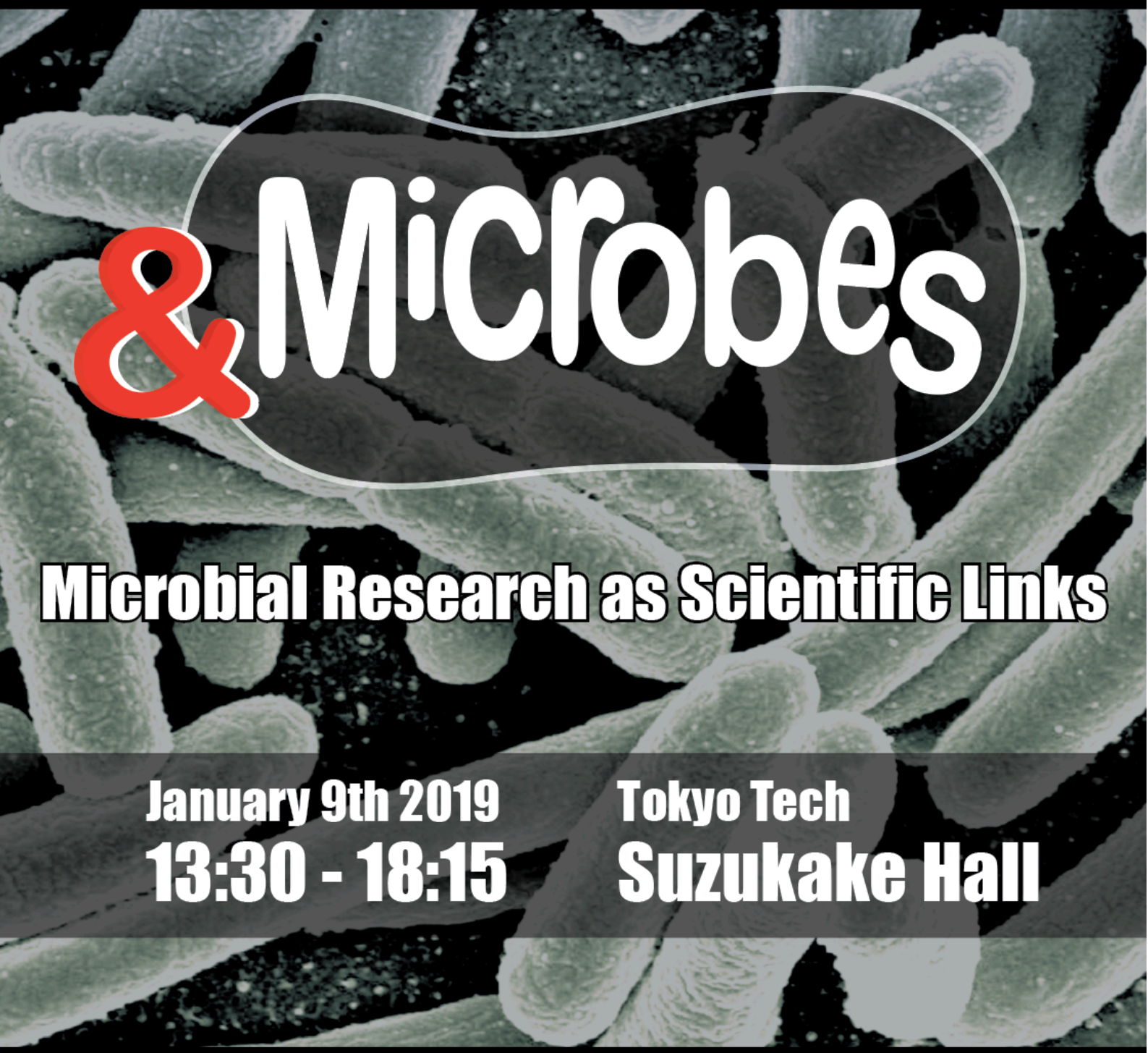
7th Tokyo Tech International Symposium on Life Science and Technology



Microbial Research as Scientific Links

January 9th 2019
13:30 - 18:15

Tokyo Tech
Suzukake Hall

School of Life Science and Technology, Tokyo Institute of Technology

Abstract

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Program

Symposium (3F Main hall)

13:30–13:35 Welcome address by **Hisakazu Mihara** (Tokyo Tech, LST, Dean)

13:35–13:40 Overview of symposium by **Takuji Yamada** (Tokyo Tech, LST)

13:40–14:20 **Shota Atsumi** (University of California, Davis, USA)
Biological chemical production from CO_2

14:20–15:00 **Masayuki Su'etsugu** (Rikkyo University, Japan)
In vitro repetition of chromosome-replication cycle and its applications

15:00–15:20 Coffee break

15:20–15:50 **Naoyuki Yamamoto** (Tokyo Tech, LST)
Proteolytic system of lactobacilli and functional peptides in the fermented milk

15:50–16:30 **Yun-Gi Kim** (Keio University, Japan)
Role of gut microbiota in health and disease

16:30–16:50 Coffee break

16:50–17:30 **Jihyun F. Kim** (Yonsei University, Republic of Korea)
Genomics and systems/synthetic biology of microbes and microbiomes

17:30–18:10 **Ken Takai** (Japan Agency for Marine-Earth Science & Technology, Japan)
Dark energy ecosystem predicted and not predicted by chemical disequilibrium

18:10–18:15 Closing remarks

18:15–18:25 Group photo

Banquet (2F Mototeca)

18:45–18:50	Opening greeting by **Takuji Yamada** (Tokyo Tech, LST)
18:50-18:55	Toast by **Eiry Kobatake** (Tokyo Tech, LST, Associate Dean)
20:15	Closing remarks by **Hisakazu Mihara** (Tokyo Tech, LST, Dean)

Abstract

Biological Chemical Production from CO_2

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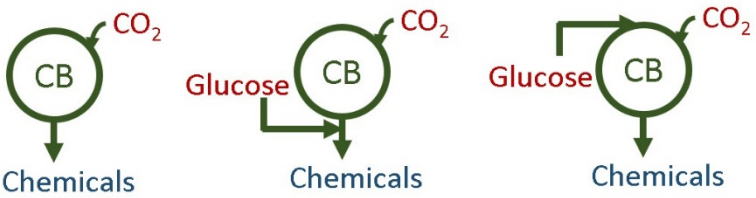
Chemical production in photosynthetic organisms is a nascent technology with great promise for renewable chemical production. Cyanobacteria are under investigation as a means to utilize light energy to directly recycle CO_2 into renewable chemical compounds currently derived from petroleum. However, while genetic engineering tools are readily available for model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*, this is not the case for cyanobacteria. We have previously engineered production of the chemical feedstock 2,3-butanediol (23BD) from an obligate photoautotrophic cyanobacterium, *Synechococcus elongatus* PCC 7942 [1]. We subsequently explored the optimization of 23BD production by varying ribosomal binding site and promoter strength, operon organization, and gene expression at the transcriptional and translational level [2]. The resulting engineered strains exhibited enhanced total carbon fixation and 23BD production under continuous light conditions. Any large-scale cyanobacterial production scheme may rely on natural sunlight for energy, thereby limiting production time to only lighted hours during the day. To overcome this limitation we engineered *S. elongatus* for production of 23BD in continuous light, diurnal light, and continuous dark conditions via supplementation with sugars [3, 4]. To improve glucose utilization, enhance CO_2 fixation and increase chemical production, modifications were introduced to glycolytic pathways and the Calvin Benson cycle [5]. These modifications are designed to increase carbon flux and redirect it towards carbon fixation. The engineered strain efficiently uses both CO_2 and glucose, and produces 12.6 g/L of 23BD with a rate of 1.1g/L/d under continuous light conditions [5]. This presentation will cover ongoing work with this system, which focuses on improving 23BD titers in variety of lighting and media conditions through further exploration of modifications to sugar metabolism and CO_2 fixation.



Light conditions	Continuous light	Continuous light Diurnal (12h:12h light-dark) Continuous dark	Continuous light Diurnal (12h:12h light-dark) Continuous dark
Titer	2.4 g/L	3.0 g/L	**12.6 g/L**
Productivity	120 mg/L/day	300 mg/L/day	**1.1 g/L/day**
Carbon source	CO_2	CO_2 & glucose	CO_2 & glucose
Reference	Oliver et al. *PNAS* (2013)	McEwen et al. *Metab Eng* (2016)	Kanno et al. *Nat Commun* (2017)

Fig. 1 Summary of cyanobacterial 23BD production developed in the Atsumi lab

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In vitro repetition of chromosome-replication cycle and its applications

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Self-replication is a fundamental property of living systems inherited from the origin of life. To achieve self-replication, cells must replicate their genetic information. In *Escherichia coli*, replication of the circular chromosome (4.6 Mb) starts from a single replication origin, *oriC*. A series of replication reactions have been reconstituted *in vitro* using purified proteins more than 30 years ago (Kaguni and Kornberg, 1984 *Cell*). However continuous repetition of the replication cycle has not been achieved yet. Recently we developed the replication cycle reaction (RCR) by *in vitro* reconstitution of the whole replication cycle of the *E. coli* chromosome using 25 proteins (Fig. 1) [1, 2]. The cycle consists of replication initiation at *oriC*, bidirectional progression of replication forks, completion of replication, and segregation of two daughter circular DNA molecules. The segregation process produces monomeric circular DNA that is topologically identical to the input template DNA, thus allowing autonomous repetition of the replication cycle under isothermal conditions. Indeed, RCR can propagate circular DNA exponentially even from a single DNA molecule. Because RCR uses the chromosome-replication system, large DNAs (longer than 200 kb) can be propagated as intact circular DNA molecules, and the replication fidelity is extremely high (approximately 1.2×10^{-8} errors per base per cycle) [1].

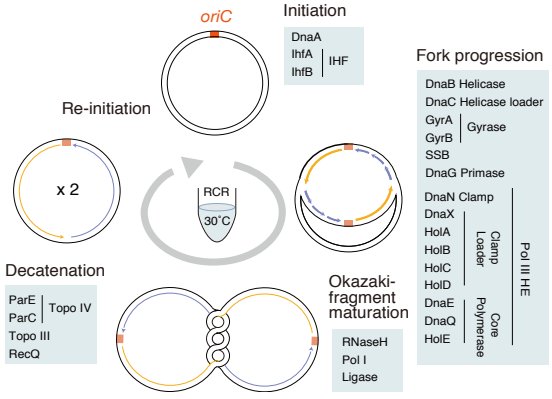

Fig. 1 Replication Cycle Reaction (RCR)

So far, conventional DNA cloning relies on living host cells like *E. coli*, which is time-consuming and labor-intensive. In addition, some sequences are toxic or unstable in the cells. RCR now provides a powerful tool to generate large circular DNA without

relying on living hosts. We here developed a novel DNA assembly reaction, termed RA (Recombination Assembly), in which multiple fragments with overlapping ends are efficiently ligated in a single-step isothermal reaction. When circular assembly products of RA was subjected directly to the RCR propagation, only the target circular DNA molecules but not linear intermediate molecules were selectively propagated because RCR requires a circular form of template DNA (Fig. 2). Using this two-step reaction, termed RA-RCR, we have successfully constructed a 27 kb plasmid from 50 fragments. The RA-RCR method could provide cell-free approach towards whole genome synthesis (or Genome Project-write) from scratch.

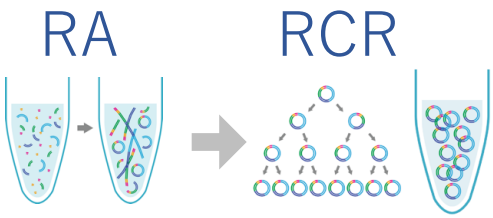

Fig.2 Two step cell-free cloning using RA-RCR

My ultimate research goal is to reconstruct the self-replication phenomenon of living cells using biological building blocks. The “transcription” and “translation” reaction has been reconstituted *in vitro* and commercially available as PURE system (Shimizu et al., 2001 *Nat. biotech.*). RCR provides the remaining Central-Dogma component, “replication.” I will show our approach towards self-replication reaction by integration of RCR and PURE system (Fig 3), and also discuss about *in vitro* evolution of genetic information during the self-replication reaction.

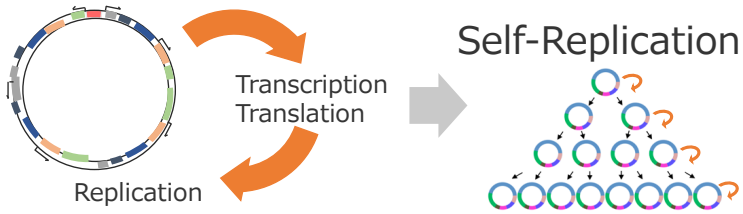

Fig. 3 Self-replication reaction by integration of “Central-Dogma” sub-systems

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Proteolytic system of lactobacilli and functional peptides in the fermented milk

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Lactic acid bacteria have proteolytic system to decompose milk proteins and utilize the peptides as nitrogen sources. Among lactic acid bacteria, *Lactobacillus helveticus* has the highest proteolytic activity and releases various functional peptides in the fermented milk. Many kinds of antihypertensive peptides originating from food protein hydrolysates have been reported ever. Hypertension is a major risk factor in cardiovascular disease, such as heart disease and stroke. Most of the reported antihypertensive peptides have inhibitory activities against angiotensin I-converting enzyme (ACE) that catalyzes release of the potent vasoconstrictor, angiotensin II from angiotensin I.

In our study, antihypertensive peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) were isolated from *L. helveticus* fermented milk. These peptides showed significant antihypertensive effects on subjects with high blood pressure and also improved arterial stiffness which is a crucial parameter for cardiovascular risk.

For about proteolytic system involved in the processing of VPP and IPP, a novel protein with affinity to upstream of proteolytic enzyme genes in the presence of BCAA was identified in *L. helveticus*. Topics about the regulatory system and the impact on VPP and IPP productions will be discussed in my talk.

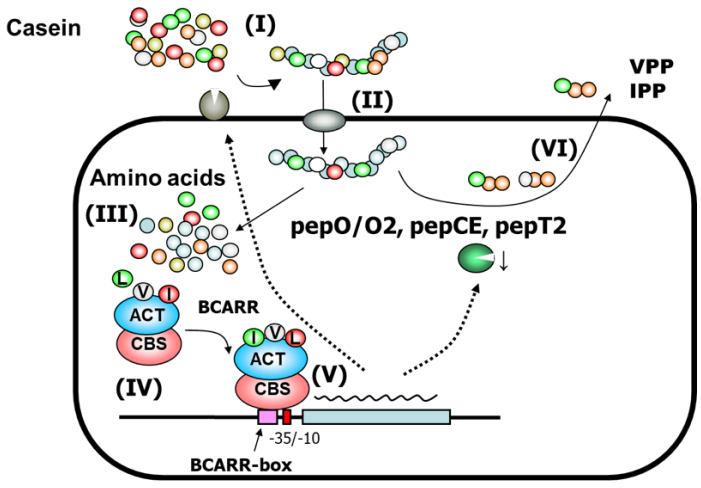

Figure. Regulatory system in *L. helveticus* proteolytic system

Role of gut microbiota in health and disease

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In human intestine, 100 trillion bacteria comprising more than 100 different species live together in dense, interdependent communities, which is known as gut microbiota. Gut microbiota performs numerous important functions such as nutrient acquisition, development and maintenance of gut immune system, and protection against exogenous pathogens. Innovation of analytical technologies including next generation sequencing and '-omics' (transcriptomic, proteomic and metabolomic) approaches allowed us to get much deeper insights into the functional role of gut microbiota. As a result, there has been growing evidence that imbalances in gut microbial communities, described as dysbiosis, are associated with pathogenesis of both intestinal and extra-intestinal disorders. Based on the accumulating knowledge, many health care companies especially in USA and Europe started to pay attention to gut microbiota as drug candidates.

In this seminar, I would like to introduce recent findings in the reciprocal crosstalk between host and gut microbiota in health and diseases.

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Genomics and Systems/Synthetic Biology of Microbes and Microbiomes

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Powered by high-speed high-throughput next-generation genomic technologies, life science and biotechnology are being transformed. In our laboratory, we apply genomic and metagenomic tools to study model microbes and microbial communities. Multi-omics systems-level understanding of the *Escherichia coli* cell factory may open the door to synthetic biology and next-generation biotechnology. Analysis of genomes sampled from a long-term evolution experiment revealed that the coupling between genomic and adaptive evolution is complex and can be counterintuitive even in a constant environment [1]. The microbiome, comprised of the microbiota and its collective genomes called the metagenome, is an integral part of our body and the ecosystem. Systems understanding of host physiology can be possible only if the microbial counterparts that reside in are fully appreciated and both are considered as a unit, i.e. holobiont. Recent analyses reveal that a myriad of microbial members, mutualistic, commensal, or pathogenic to the host, play pivotal roles in health and disease by producing diverse macromolecules and metabolites. Host-microbiota relationships in the plant rhizosphere [2] and the human gastrointestinal tract, as well as the dynamics of microbial communities, will be presented as examples. In the talk, efforts to develop probiotics or more preferably pharmabiotics for the prevention or treatment of gastrointestinal cancers will also be presented. Synthetic biology concepts and toolkits enable us to modulate the microbiome to maintain (eubiosis) or regain (rebiosis) homeostasis, and even to transform it to become preventive or curative.

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Dark energy ecosystem predicted and not predicted by chemical disequilibrium

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Over the past 35 years, researchers have explored seafloor deep-sea hydrothermal vent environments around the globe and studied a number of microbial ecosystems there, which is now called as Dark Energy Ecosystems. Bioinformatics and interdisciplinary geochemistry-microbiology approaches have provided new ideas on the diversity and community composition of microbial life living in deep-sea vents. In particular, recent investigations have revealed that the community structure and productivity of chemolithotrophic microbial communities in the deep-sea hydrothermal environments are controlled primarily by variations in the geochemical composition of hydrothermal fluids.

This was originally predicted by a thermodynamic calculation of energy yield potential of various chemolithotrophic metabolisms in a simulated hydrothermal mixing zone. The prediction, called as McCollom and Shock's prediction, has been finally justified by the relatively quantitative geomicrobiological characterizations in various deep-sea hydrothermal vent environments all over the world. Thus, there should be a possible principle that the thermodynamic estimation of chemolithotrophic energy yield potentials could predict the realistic chemolithotrophic living community in any of the deep-sea hydrothermal vent environments in this planet. Once such a principle is realized, the principle can be applied not only to exploration of extant dark energy ecosystem but also to understanding of the most ancient dark energy ecosystem in the Earth and even the likely extraterrestrial dark energy ecosystems in our solar system.

In addition, recent electrochemical studies of deep-sea hydrothermal mineral deposits and environments have pointed to the existence of microbial ecosystem beyond McCollom and Shock's prediction.