Q_Synthetic Biology and Metabolic Engineering

Q-1

Improvement of Lycopene Production from C1 Substrate in *Methylorubrum extorquens* by Engineering Non-Mevalonate Pathway

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Formate, which can be synthesized from CO_2 or CO, is soluble C1 substrate so that it is more easier to use as microbial feedstock than C1 gas. In this study, we produced lycopene from formate using *M. extorquens* by engineering non-mevalonoate pathway, also called MEP pathway, for effective lycopene production. First, LMZ01 strain was constructed by deleting *crtCD*, genes for consuming lycopene, and used as a parental strain, showing improved lycopene production. Then, *dxs*, a rate-limiting step of MEP pathway, was overexpressed under *lac*, *tac*, and *mxaF* promoter, respectively. Expression level was shown to be strong in the order of *mxaF*, *tac*, and *lac* promoter by expressing EGFP. Interestingly, while the *mxaF* promoter with strong expression level was ineffective, the *tac* promoter with moderate level showed 45% increase in lycopene production. This showed lycopene could be produced reasonably from formate and suitable expression level of MEP pathway is crucial.

Keywords : Methylorubrum extorquens, formate, lycopene

Q-2

Syntrophic Microbial Systems for Mevalonate Synthesis from Mmethane

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Methane has been recognized not only as a potent greenhouse gas that should be reduced but also as an important renewable resource for the production of biochemicals and fuels. Methanotrophs have emerged as promising biocatalysts because of their ability to utilize methane as a sole carbon and energy source at ambient temperature and pressure. Methanotrophic bacteria modulate non-methanotroph metabolism by secreting organic acids in nature. By mimicking the natural methane ecosystem, we developed co-culture system of methanotrophs and syntrophic partners to increase methane utilization capacity with high flexibility. In this study, we optimized the conditions such as media, gas transfer and inoculation ratio, and observed the successful growth of both *Methylococcus capsulatus* Bath and *Escherichia coli* SBA01. *M. capsulatus* Bath is a highly efficient methane biocatalyst, and *E. coli* SBA01 is an evolved strain that has high organic acid tolerance and utilization ability. By using the co-culture system of *M. capsulatus* Bath and recombinant *E. coli* SBA01 having mevalonate pathway, mevalonate was produced from methane. We envision that this methane-based syntrophic system has various potential applications and it will increase our understanding of methanotroph-heterotroph interactions.

Keywords : Methane, methanotroph, fermenter

Q-3

High-Throughput Part Characterization Using Combinatorial Library Assembly Approach

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For the successful design of a genetic circuit, simulation techniques are required for selecting an appropriate circuit that shows the desired output among various combinations of parts. The prediction technique reduces the time and effort required for design, build, and test unnecessary part combinations. To do this, quantitatively characterized parts are essential. In conventional methods to quantify the parts, the strength of every part is measured by the expression ratio of green fluorescence protein involving the target part, and the reference red fluorescent protein. However, this approach has a problem in that the measured quantity can be varied depending on the combination of other parts in the same circuit. Also, the quantification process takes time as the number of parts increases. In this study, we built the combinatorial library of parts using the Golden gate assembly technique and quantified the parts in the combinatorial library by measuring their fluorescence in the colony state. The genotype of the colonies was obtained by a long-read sequencing technique using tagging primer. The proposed technique can calculate quantitative values of the DNA parts considering the interaction among the parts, and quantify multiple parts in a high-throughput manner with a single experiment with combinatorial libraries.

Keywords : Part characterization, long read sequencing, high-throughput

Q-4

A Mathematical Modeling for Quantitative Prediction of Genetic Circuit Behavior

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One of the main challenges in designing a genetic circuit is predicting its functional behavior that we expect. This behavior prediction significantly reduces the time and labor of circuit design processes by screening the most appropriate designs instead of exploring the whole combinatorial search space. Here, we built a mathematical model explaining the quantitative changes of a genetic circuit in a cell-free condition. The model consists of 51 biochemical reaction equations with seven unknown parameters, estimated by a simulation-based optimization approach. All other parameters were assumed with fixed constants from published information. The inferred model successfully simulated the genetic circuit behavior, including sfGFP expression with different circuit DNA concentrations. This research will help to predict a genetic circuit consisting of various DNA parts in specific host cells and fill the gap between in vivo and in vitro systems of the artificially designed biochemical networks. We anticipate that the proposed model eventually promote to establish design rationale of genetic circuits in the field of synthetic biology.

Keywords : Genetic circuit, modeling, prediction

Q-5

Production and Characterization of Monoclonal Antibody for the Rapid Detection of DDT

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DDT has been banned in most countries because it is persistence in environment and hazardous effect on animal, human. In this study, a simple and rapid immunoassay was described for detection of organochlorine pesticides (DDT). Also, anti-DDT antibodies (IgG) were developed and characterized for the specific binding to DDT. Gold nanoparticles were first conjugated with anti-DDT antibodies, and then FITC-labeled antigen (FITC-DDA) was reacted with the immobilized antibodies. The fluorescence quenching of FITC was strongly occurred due to the specific interaction between the antibodies and DDA. However, in the presence of DDT, fluorescence intensity was recovered because the FITC-DDA was release from the surface of AuNPs owing to competitive immunoreaction. Under optimized condition, the fluorescence intensity was measured with a linear dynamic range and a low detection limit. Furthermore, we used small, inexpensive, and portable equipment which can be used for point-of-care testing of fluorescence intensity. This simple strategy could be applied to various quenching-based methods for pesticide detection in environmental samples.

Keywords : Monoclonal antibody, DDT, gold nanoparticle

Q-6

Biological Conversion of Methane to Threonine Using Methanotrophs

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Nowadays, natural gas has gained increasing attention as an eco-friendly and low-cost resource for value-added productions. Methane, a primary content of natural gas, is a carbon and energy source for methanotrophs. Methanotrophic bacteria can be utilized as an efficient biocatalyst to produce metabolites or non-native compounds from methane under mild temperature and pressure conditions. To increase productivity, methanotrophs are often genetically engineered using synthetic biology approaches. Here, we aimed to synthesize threonine from methane using *Methylococcus capsulatus* Bath and *Methylosinus trichosporium* OB3b. We engineered these bacteria via recombinant expression of threonine aldolase. We observed the successful expressions of three different types of enzymes in both strains. We compared the enzyme activities and optimized the reaction conditions (pH, temperature, time). We expect that methanotrophs with novel threonine aldolase have high potential for ecological and biotechnological applications.

Keywords : Methanotrophs, methane conversion, threonine aldolase

Q-7

Metabolic Engineering of *Enterobacter aerogenes* for Reduced Byproducts Production to Improve 2,3-Butanediol Yield and Productivity from Glucose-Xylose Mixture

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The pathway engineering of *Enterobacter aerogenes* was attempted to improve its production capability of 2,3-butanediol using glucose and xylose mixture as carbon source. The gene deletion of *pflB* and *ldhA* improved 2,3-butanediol production yield, while the deletion of *ptsG* increased xylose consumption rate significantly in the presence of glucose. The constructed strain, EMY-22, achieved high production yield with low aeration. However with higher aeration, production yield became lower and productivity became higher, also shorter fermentative time with 2-ketogluconate accumulation. To solve the problem, strain was overexpressed glucose transporter gene (*galP*) and 2,3-butanediol production operon (*budABC*). With gene overexpression, strain achieved higher yield with higher aeration condition. Also pyruvate synthesis pathway was overexpressed by *pykF* and malic enzyme gene overexpression. With *pykF* and malic enzyme gene overexpression, strain produced 2,3-butanediol with lower 2-ketoglconate accumulation with stable pH condition, adding $CaCO_3$.

Keywords : *Enterobacter aerogenes*, 2,3-butanediol, metabolic engineeering

Q-8

Novel Organophosphorus Pesticide Detection Whole-Cell Biosensor using Bio-part Library

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The organophosphorus pesticide like fenitrothion is widely used in the farmland, since it is cheap and effective as an insecticide that affects the insect's nervous system as an acetylcholine esterase inhibitor. But, accumulation of the pesticide in human causes harmful effects like cancer induction. Various methods for detecting residual pesticides have been developed for last decades. However, conventional methods require expensive equipment with expertise for detecting, and laborious pre-treatment step. Here, we present a whole-cell biosensor that easy-to-use and highly sensitive can detect 0.5 ppm fenitrothion. This whole-cell biosensor was sorted from the combinatorial library constructed with various promoters, ribosomal binding sites, terminators and coding genes (oph, mutant dmpR, sfGFP). The selected biosensor was applied to natural samples, like soil and agricultural products and we confirmed that the biosensor can detect with minial 1 ~ 5 ppm fenitrothion. We anticipate that the combinatorial library based screening technique could be powerful and effective for constructing highly sensitive and low detection limit whole-cell biosensor that can be used for practical applications.

Keywords : Organophosphorus pesticide, bio-part library, whole-cell biosensor

Q-9

Metabolic Engineering of *Corynebacterium glutamicum* for the Enhanced Production of Glutaric Acid

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Glutaric acid is a dicarboxylic acid that is used as a platform chemical for the production of plastics and plasticizers. Currently, glutaric acid is produced through a chemical process that involves toxic compounds like potassium cyanide. In this aspect, the use of microbial fermentation is a promising sustainable and eco-friendly alternative process for glutaric acid production. In this presentation, we report the development of a recombinant *Corynebacterium glutamicum* strain for the production of glutaric acid from glucose via cadaverine and 5-aminovalerate (5-AVA) pathway. To establish glutaric acid production via lysine and 5-AVA pathway, 5-aminovalerate transaminase, glutarate semialdehyde dehydrogenase, lysine 2-monooxygenase and δ-aminovaleramidase was expressed in recombinant *C. glutamicum*. But this system require molecular oxygen in the pathway. For glutaric acid production via cadaverine and 5-AVA pathway, 5-aminovalerate transaminase, glutarate semialdehyde dehydrogenase, putrescine transaminase and gamma-aminobutyraldehyde dehydrogenase were expressed in recombinant *C. glutamicum*. To prevent remaining cadaverine putrescine importer were introduced. The detailed strategies and results will be presented in this presentation.

Keywords : Glutaric acid, *Corynebacterium glutamicum*, metabolic engineering

Q-10

One-Step Multiplex Genome Editing for Cell Factory Engineering of *Bacillus subtilis*

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Bacillus subtilis is an one of the most important industrial strain to produce antibiotics, pharmaceutical proteins, and industrial enzymes due to its excellent protein secretory capability and GRAS (generally regarded as safe). For cell factory engineering to improve the quantity and quality of commercial enzymes or metabolites, it must be engineered through mutations of multiple genes, especially gene knock-out. Although the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats/associated protein 9)-assisted genome editing tool has been developed for efficient genome engineering in *B. subtilis*, simultaneously modulating multiple genes on the chromosome remains challenging due to time-consuming process and low-efficiency. Among the recently developed various genome editing tools, the cytidine base editor (CBE), which rapidly induces C-to-T conversion in target DNA locus, is expected to have a strong potential for multiplexing because it does not rely on homologous recombination and double strand break (DSB). In this study, we evaluated the multiplexing capacity of CBE in *B. subtilis* for one-step multiplex genome editing. We first developed CBE system showing the robustly mutational efficiency (98.25%) for *B. subtilis*. Next, when multiplexing CBE system, simultaneous editing efficiency of target position for double, triple, quadruple, and quintuple targets was confirmed to be 100%, 100%, 83.3%, and 75.5%, respectively. Whole genome sequencing of the all multi-edited strains showed that they had an average of 8.5 off-target single-nucleotide variants (SNVs) at gRNA-independent positions and the number of SNVs was not proportional to the number of gRNAs. The results suggest that the CBE is a powerful tool for multiplex genome editing in *B. subtilis* and can facilitate cell factory engineering.

Keywords : Multiplex genome editing, cytidine base editor, *Bacillus subtilis*

Q-11

S-Methylmethionine Production in *E. coli* Using MMT Gene from *Arabidopsis thaliana*

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The sulphur containing amino acid S-methylmethionine plays an important roles in human health. S-methylmethionine is also called as Vitamin U. S-methylmethionine helps the working of ulcer. There have been no other research about S-methylmethionine producing in *E. coli*. In our laboratory, Methionine S-methyltransferase(MMT) from *Arabidopsis thaliana* was used for producing S-methylmethionine. And other gene works to produce precursors, methioinine and S-adenosylmethionine, were done. metJ, the methioninie biosynthesis repressor, mmuM, the recycling pathway of S-methylmethionine, mmuP, the importer of S-methylmethionine were deleted from wild type strain. And for the overflow of precursor, we overexpressed genes which are related to producing methionine in *E. coli*. (metF, metA*, metH, metK). Finally the strain, dJMP_aKFcMsA*H, was constructed and 0.078g/L of S-methylmethionine was produced in 25mL flask culture with M9 media.

Keywords : S-methylmethionine, methionine, Methionine S-methyltransferase

Q-12

A Chemo-Microbial Hybrid Process for the Production of 2-Pyrone-4,6-Dicarboxylic Acid as a Promising Bioplastic Monomer from PET Waste

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2-pyrone-4,6-dicarboxylic acid (PDC) is one of the valuable compounds that can only be synthesized biologically, and because of its unique structure (pseudo-armatic ring and two carboxylic acid groups), it is used in combination with diols, acids and alcohols and can be synthesized into a PDC based polyester which has better biodegradability or better material properties than conventional biodegradable polyester. In this study, PDC was synthesized from the PET waste derived TPA that was synthesized through thiol functionalized and SiO_2 catalysis with economical microwave-assisted neutral hydrolysis without highly acidic or basic waste water which is usuallly used in PET hydrolysis. TPA from PET waste and two recombinant *Eschericia coli* were used for PDC biosynthesis. The strain (*E. coli* PCA strain) which is harbouring the genes derived from TPA degradation microorganism, *Comamonas sp. strain E6* and the other strain (*E. coli* PDC_{PCA} strain) which was introduced genes from *Sphingomonas parcimobilis* that has aromatic compound metabolism including PDC. As a result , TPA was synthesized from PET waste over 97 % efficiency, and PDC was synthesized with 96.08 % of the overall efficiency from chemo-microbial hybrid process.

Keywords : 2-pyrone-4,6-dicarboxylic acid (PDC), bio-degradable plastics, PET waste

Q-13

Mass Production of Scaffold DNA

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M13 phage is a filamentous phage that infects *Eschericia coli* through F pilus. It is mainly used in phage display to find new antibodies. Also, its single strand circular DNA is used in DNA nanotechnology as a building block. When M13 phage enters in *E.coli*, it replicates itself using *E.coli*'s enzymes such as DNA polymerase, RNA polymerase, and ligase. This is called rolling circle replication. There are three stages in rolling circle replication. In stage 1, single strand phage DNA enters in *E.coli* through F pili. Enzymes in *E.coli*, such as DNA polymerase and ligase make single strand phage DNA into double strand DNA. In stage 2, the production of single-strand DNA follows the Fibonacci increase. And in stage 3 it follows an arithmetic increase on the assumption that the replication rate is constant. When production of single strand DNA is plotted simply, longer stage 2 is favorable. In this rolling circle replication, p5 concentration is a branch point between stage 2 and stage 3. If the accumulation of p5 is fast, shift to stage 3 occurs fast. However, accumulation of p5 is slow, shift to stage 3 occurs lately. It means that we can get longer stage2. Accumulation of p5 can be controlled at translational level. A bottleneck in translation is ribosome binding. The ribosome binds to RBS(ribosome binding site) in mRNA. Ribosome binding affinity is affected by RBS sequence, spacer sequence, and coding sequence. The spacer is about 5-6bp sequence between RBS and start codon. RBS can be expected by the program. If the spacer sequence is changed, ribosome binding affinity is changed, and the protein expression level is changed. We engineered 5' UTR of protein V in M13 phage and screened 4 most efficient mutants. Production of M13 phage increased to 2.5 to 4.5-fold, and ssDNA production increased in 3.5 to 4.5-fold. Furthermore, we measured the engineered spacer expression levels by fusion of protein V with GFP. The fluorescence of the good producers is lower than the bad producers and the wild type. Also, the artificial expression of P5 using an inducible promoter shows that the concentration of P5 is inversely proportional to M13 phage and ssDNA production

Keywords : M13 phage, scaffold DNA, RCA

Q-14

Development of Closed-Recirculating Aquaculture System for Productivity Improvement and Safety of Marine Products

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Annual domestic fisheries production amounted to 1.4 million tons and cultivated aquaculture production of 1.181 million tons as of 2016, but production is decreasing every year due to marine environmental pollution, and safety issues are also emerging. Therefore, In order to solve the adverse balance of import and export of fisheries products worth 3 trillion won and to secure national food security in the 21st century, attention is being paid not only to increase quantity but also to eco-friendly sustainable forms. The closed recirculating aquaculture (RAS) system is capable of breeding even with a small amount of seawater, so it is possible to prevent damages from various natural disasters, save energy due to the inflow of large amounts of seawater, and block disease sources from outside. In addition to securing safety, it is possible to maintain a constant water temperature at all times, which is very advantageous for the environment in Korea where the temperature change is large, and high production is expected. The purpose of the study is to develop of the closed recirculating aquaculture system (RAS) for increase quantity of food puoducts using the sequencing batch reactor (SBR). The pre-developed SBR can reduce nitrogen and phosphorus in marine wastewater by using high efficiency marine bacteria and marine sediment as eco-friendly and effective biological materials. The pilot plant-scale SBR set up in Gyeonggi Province Maritime and Fisheries Research Institute(71, Gaegeonneo-gil, Danwon-gu, Ansan-si, Gyeonggi-do, Korea; 37°13'08.9"N 126°37'38.8"E,) and operated 2.5 h cycle-1 of TC with following four stages: the influence stage (0.5 h) within the aeration/mixing stage (1.0 h), settlement stage (0.5 h), and idle/effluence stage (1.0 h). The system operated with 10.5 h hydraulic retention time (HRT), 24.5 d solids retention time (SRT) and exchange ratio (VF/VT) of 0.47, since the filling volume (VF) was 14.0 m3 cycle-1. As reducing reaction time, Q was 15 m3 d-1. Based on previous studies, MLSS was adjusted and routinely maintained at 1,500 mg L-1, and the mixed liquor volatile suspended solids (MLVSS) were adjusted and routinely maintained at approximately 1,200 mg L-1 . The treatment performance was 32 m3 d-1 of treatment amount in 30 m3 of the reactor with the average removal efficiency of 84% for total nitrogen and 52% for total phosphorus at the optimal operation condition.

Keywords : SBR

Q-15

Metabolic Profile of *Clostridium carboxidivorans* (DSM 15243) Using Heterotrophic and Mixotrophic Fermentation under pH Control

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Clostridium carboxidivorans (DSM 15243) is capable of mixotrophic fermenting sugar (glucose, fructose, etc.) and synthesis gas (H_2, CO_2, and CO) to produce various acids and alcohols (acetate, butyrate, ethanol, butanol, etc.) under anaerobic condition. This study describes metabolic profile in three different condition (H_2/CO [60/40], H_2/CO_2 [80/20] and Ar) under glucose fermentation. The cultures were operated at 37 ℃ in 1 L bottle with 300 mL working volume containing 10 g/L glucose, and the pH was automatically controlled at 6.0 by 3 M NaOH. A low reducing power in the Glycolytic pathway can be covered by adding syngas. In addition, the enough electron donor/reducing equivalents such as H_2, CO contributed to be re-assimilated via the Wood-Ljungdahl pathway. During the test, $C_{2\text{-}6}$ acids, alcohols, and lactate was produced by *C. carboxidivorans*,. Acetate yield was 0.106 ± 0.012 g/g with Ar and the acid production can increase with mixotrophic fermentation.

Keywords : *Clostridium carboxidivorans*, metabolic pathway, mixotrophic fermentation

Q-16

Investigation of Metabolic Rewiring with CK2 Overexpression in Colon Cancer Cells by Carbon Isotope Using GC/MS

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CK2 is a serine/threonine kinase that is constitutively active in multiple cancer cells.Previously, we reported that stable increase of CK2 in cancer cells could induce EMT. Using colon cancer cell lines such as HT29 and SW620, CK2 also induced EMT and the transitioned cells became more proliferative than control cells. We assumed that CK2 could affect cancer cell growth by modulating energy metabolism. Therefore, we examined reprogrammed metabolic fluxes of CK2 on glucose metabolism. To measure intracellular metabolite, we fed U-13C6 glucose, U-13C5 glutamine to glucose, glutamine free DMEM respectively, and cultured until cells reached to metabolic steady state. The results of isotope analysis showed that continuous activation of CK2 in colon cancer cell lines facilitates the Warburg effect and anaplerotic pathways. This study suggests that because oncogenes regulate important metabolic enzymes and metabolism signaling pathways, CK2 is excellent metabolic target for cancer therapy.

Keywords : Serine/threonine kinase, GC/MS, CK2

Q-17

^{13}C-MFA with Co-Culture for Resveratrol Production in *E. coli*

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^{13}C-MFA (Metabolic Flux Analysis) is a technique for decoding fluxes of central carbon metabolism using a C source marked ^{13}C. C source, marked ^{13}C, is provided in strain and the label information of the metabolite is measured. It is then possible to track label information based on a model that decodes flux using specific enzymes in the strain. Resveratrol is a beneficial compound found in plants. It has variuus effects such as cancer, antioxidants and life extension. Resveratrol can be created using acyl-CoA in the EMP pathway and tyrosine in the PP pathway. However, the road has a different temper and is difficult to turn into a single strain. Thus, an attempt was made to produce resveratrol using co-culture. In this study, two *E. coli* were designed to supply more resveratrol precursor based on previous studies. Then resveratrol will be produced with co-culture. Finally, using ^{13}C-MFA, the metabolic rate of co-culture will be analyzed, and ways to increase resveratrol will be discussed.

Keywords : Resveratrol, 13C-MFA

Q-18

Construction of Minicell-Producing Mutants for Production of Toxic or Membrane-Based Chemicals

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Minicells are small sphere-shaped cells formed by aberrant cell division, which are capable of other cellular processes as well as protein synthesis. However, because bacterial minicells have no chromosomal DNA, there are no bacterial growth and cell division. In this study, we genetically engineered *ftsZ*, *ftsA*, *plsB*, *plsC* and *minCD* genes related bacterial cytokinesis to increase the formation of minicells in *Escherichia coli*. Through increased minicell formation, the improved tolerance was exhibited in the mutant strains, which brings about the enhanced production yield about toxic compounds. Furthermore, the production of membrane-based chemical such as lycopene is improved in minicell-forming mutants whose size is longer than wildtype's because of abnormal cell division. Based on these characteristics, minicell mutants has great potential as chassis for production of toxic compounds such as isobutanol, isobutyraldehyde, isobutyl acetate and membrane-based product such as lycopene with high production yield.

Keywords : Minicell, cytokinesis, toxic compound

Q-19

In vivo DNA Assembly of FK506 BGC through TAR Cloning in *Saccharomyces cerevisiae*

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Due to the developments of next-generation sequencing technologies, recognition of microbial genomes as a rich resource for novel natural product discovery make it possible. Heterologous expression of natural product biosynthetic gene clusters (BGCs) gives an approach not only to decode biosynthetic logic behind natural product (NP) biosynthesis, but also to discover new chemicals from unknown BGCs. Transformation-associated recombination (TAR) cloning make use of the natural *in vivo* homologous recombination of *Saccharomyces cerevisiae* to directly capture large genomic loci. FK506 (tacrolimus) is known as immunosuppressant and antifungal activity, which can be produced by *Streptomyces tsukubaensis*. FK506 biosynthetic gene cluster spans 83.5 kb in size and consists of 26 genes. Though we have already known sequence or length of FK506 gene cluster, the synthesis of FK506 still represents several challenges. Here we report a TAR-based genetic platform that allows us to directly clone in *S.cerevisiae* as a cloning strain, refactor transcriptional region in biosynthetic gene clusters, and heterologous express a silent biosynthetic pathway to yield a FK506 in *Streptomyces coelicolor.*

Keywords : Streptomyces, biosynthetic gene clusters, transformation associated product

Q-20

Construction of FK506-Sensitive Biosensor Based on the Transcription Factor MphR

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FK506 is a 23-membered macrolide molecule which has various biological activities such as immunosuppressive, neural regenerative, hair growth and antifungal activities. Because of low FK506 productivity by WT *Streptomyces tsukubaensis*, some previous studies mutated the strain with UV or NTG and screened FK506 hyper-producer by comparing the inhibition zone of *Aspergillus niger* based on antifungal activity of FK506. However, this screening method could not compare FK506 production between hyper-producers because of limited dynamic range. Therefore, we are trying to develop high-throughput FK506-sensitive biosensor using transcription factor MphR. MphR is a repressor protein that is bound to promoter region and can be derepressed by binding with 12~16-membered macrolide molecules such as erythromycin. We constructed an *E. coli* plasmid system containing *mphR* gene and MphR-sensitive reporter gene. By introducing random mutations on *mphR* gene region and screening the appropriate mutant, it would be possible to develop FK506-sensitive biosensor system that can screen FK506 hyper-producer with small amounts of *Streptomyces* extract.

Keywords : FK506, transcription factor MphR, biosensor

Q-21

Production of Poly(3-Hydroxybutyrate-*co*-3-Hydroxyvalerate) Using Engineered *Methylorubrum extorquens* AM1 from Formate as a Sole Carbon Source

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Formate is environmentally sustainable C1 feedstock, synthesized from electrochemical reduction of CO_2 or hydration of CO. For production of biopolymer from formate, *Methylorubrum extorquens* AM1 was used. It utilizes one-carbon compounds as a sole carbon source and produces poly-3-hydroxybutyrate (PHB). However, it is stiff and fragile due to high crystallinity, limiting its application. To improve the properties of biopolymer, polymerization of other monomers is necessary. For production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)), *bktB*, *phaJ1* and *phaC2* originated from *C. necator*, *P. aeruginosa* and *R. aetherivorans*, respectively, were expressed and native *phaC* was deleted. For higher portion of 3HV, UTR sequence of *bktB* was engineered to increase expression level and native *phaA*, which is similar function with *bktB* and has smaller binding pocket than *bktB*, was deleted. As a result, P(3HB-co-3HV) was synthesized with high portion of 3HV by adding propionate as a precursor, based on the analysis of biopolymer composition using GC/MS. And small amount of butyrate (below 0.1 g/l) was added for increase of 3HV portion. It showed synergistic effect not only on 3HV portion but also on cell mass. Also, when using formate as a sole carbon source P(3HB-co-3HV) was synthesized with 10%(mol%) 3HV. This study showed PHA copolymer with high portion of 3HV could be synthesized using low-carbon sources, especially using only C1 carbon source.

Keywords : PHA copolymer, formate, *Methylorubrum extorquens*

Q-22

Enabling an Efficient Metabolism in *Methylobacterium extorquens* AM1 by the Reductive Glycine Pathway

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While known for its one-carbon (C1) substrate (e.g. formate and methanol) utilization capabilities, the methylotrophic microbial strain *Methylobacterium extorquens* AM1 exhibits a comparatively slow growth rate, reducing the competitiveness of the strain as a conventional industrial platform. With the metabolic inefficiency of the Serine Cycle and the Ethylmalonyl-CoA Pathway (EMCP) specified as the known contributors to this problem, this study attempts to both restructure and add various metabolic pathways to achieve enhanced metabolic efficiency of the strain. By diverting the EMCP carbon flux into gluconeogenesis, solving the consequent lack of glycine via the recently recognized Reductive Glycine Pathway (RGP) and providing required reductive power through modifying the Serine Cycle, a metabolically efficient , EMCP-independent *M. ethylobacterium* extorquens AM1 strain is proposed.

Keywords : C1, *Methylobacterium*, reductive glycine pathway

Q-23

Engineering Glucose Uptake Rate and Its Effect on Metabolites Production by Glucose Transporters Engineering in *Escherichia coli*

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Most bacteria have evolved to maximize growth rate, with rapid consumption of carbon sources from the surroundings. However, fast growing phenotypes feature secretion of organic acid or ethanol and have reduced biomass yield in aerobic conditions. Several glucose uptake pathways exist in *Escherichia coli*, including the glucose phosphotransferase system, ABC transporters, symporters, and non-specific pathways. As these transporters or their subunits were knocked out in *E. coli*, the growth and glucose uptake rates decreased but acetate overflow was relieved and biomass yield was improved. Alteration of intracellular metabolism caused by the mutations was investigated with transcriptome analysis and 13C metabolic flux analysis (13C MFA). Various transcriptional and metabolic perturbations were identified in the sugar transporter mutants. Transcription of genes related to glycolysis, chemotaxis, and flagella synthesis was downregulated, and that of gluconeogenesis, Krebs cycle, alternative transporters, quorum sensing, and stress induced proteins was activated in the sugar transporter mutants. Based on these findings, robust production hosts were constructed using the sugar transporter mutants. When pathway genes for producing value-added compounds (γ-aminobutyrate, lycopene) were introduced, the specific production yields of the compounds were improved significantly in the sugar transporter mutants, suggesting that controlling the sugar uptake rate is a good strategy for ameliorating metabolite production.

Keywords : Glucose transporter, acetate, 13C

Q-24

Development of Bio-Refinery Platform Technology Based on CO_2-Uptake Microorganisms

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Afterward the Paris Climate Agreement, demand for CO_2 capture and carbonization continues to rise in the post-2020 climate change policy. Most CCS technologies do not offer the eventual solution because of the high expense of energy or the heavy burden of trapped CO_2 in the ground. Biological conversion technologies that do not require high energy costs and can capture and transform CO_2 at the same time may provide a solution. Generally CO_2-utilizing microorganisms use solar energy to conduct photosynthesis, which is environmentally limited in their cultivation and expects a large incubation area for large quantity production. Furthermore, the photosynthesis mechanism using Rubisco has a disadvantage that delay due to mechanism intricacy. To surmount these technical limitations, we suggest a technology to convert CO_2 into high-value products through a new pathway that provides external reducing power (electricity or H_2) instead of solar energy and can assimilate CO_2 rapidly. Eventually, we hope to make a new industrial areas that can produce various high-value metabolites from CO_2 using the CO_2-utilizing microorganisms obtained through this work, and will be able to solve both climate change response and energy problem at the same time.

Keywords : Bio-refinery, CO_2 conversion, CO_2-consuming microorganisms

Q-25

System Development for Production of Antimicrobial Peptides in *Escherichia coli* Using a Bacterial Ferritin

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A bacterial production system for toxic protein such as microbial peptides, which could be effectively used for various research or applications, has been constantly developed. However, high-yield production of antimicrobial peptides was limited due to their toxicity to host cells and proteolytic degradation. In this study, we describe a production system based on fastening antimicrobial peptides to bacterial ferritin applied as a structural protein to avoid the harmful effects on the expression host. Bacterial ferritin, ion-storage protein, generate inclusion body particles retain the original functions in cells. We attempted to generated ferritin inclusion bodies displaying functional leucine zipper proteins as bait for localizing cytosolic recombinant antimicrobial peptides in *Escherichia coli*. As a result of applying mastoparan V1(MP-V1) from the *Vespular vulgaris* and beta-defensin DF8 from the *Gallus gallus* into our system, production yields of 50 mg/ml or more were confirmed. Thus, we consider that this system was lead to improvements in the AMP bio-manufacturing platforms.

Keywords : Antimicrobial peptide, bacterial ferritin, genetic circuit

Q-26

Delivery of Protein Cargos for Biotechnological Engineering in Prokaryotes

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Here, we developed a method to deliver protein cargos into living bacteria using cell-penetrating peptides (CPP). We evaluated the cell penetration efficiency of 98 CPPs with our optimized delivery method. We found that cell penetration efficiency was enhanced for the CPP-tagged proteins up to 12-fold in *E. coli* compared with untagged protein. To demonstrate the applicability of the CPP-conjugated strategy for protein delivery, we used meganuclease I-SceI for plasmid removal in *E. coli* harboring different plasmid copy numbers. CPP-conjugated I-SceI successfully eliminated low-to-high copy number plasmids. In addition, we developed a marker gene excision method based on a CPP-conjugated Cre recombinase/loxP system. Our method showed high efficiencies in markerless gene editing for metabolic engineering and ease of conducting experiments in *Methylomonas* sp. DH-1. Taken together, the CPPs can be used to deliver other proteins as well for microbial engineering.

Keywords : Cell-penetrating peptides, plasmid removal, microbial engineering

Q-27

Single Base Genome Editing in *E. coli* by Target-Mismatched CRISPR/Cas9

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Genome editing technology has been revolutionized by the CRISPR/Cas9 system. CRISPR/Cas9 is composed of single molecular guide RNA (sgRNA) and a proteinaceous Cas9 nuclease, which recognizes a specific target sequence and a PAM (protospacer adjacent motif) sequence and, subsequently, cleaves the targeted DNA sequence. This CRISPR/Cas9 system has been used as an efficient negative-selection tool to cleave non-edited or unchanged target DNAs during site-specific mutagenesis and, consequently, obtain cells with desired mutations. Here, we revisited the genome editing efficiency of the CRISPR/Cas9 system for *in vivo* oligonucleotide-directed mutagenesis. Various multiple mutations can be introduced efficiently in cells using this system. However, it was not able to obtain any single point mutation in a target gene, which might have resulted from mismatch tolerance. To solve this problem, we designed one or two base mismatches in the sgRNA sequence that recognizes the target sequence. A single point nucleotide mutation was successfully introduced when single base-mismatched sgRNAs were used. Our study shows that the target-mismatched sgRNA method is very effective for single nucleotide editing in microbial genomes.

Keywords : CRISPR/Cas9, mismatch tolerance, sgRNA

Q-28

Engineering Cell Wall Integrity Enables Enhanced Squalene Production in Yeast

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Microbial production of many lipophilic compounds is often limited by product toxicity to host cells. Engineering cellwalls can help mitigate the damage caused by lipophilic compounds by increasing tolerance to those compounds. To determine if thecell wall engineering would be effective in enhancing lipophilic compound production, we used a previously constructed squaleneoverproducingyeast strain (SQ) that produces over 600 mg/L of squalene, a model membrane-damaging lipophilic compound. ThisSQ strain had significantly decreased membrane rigidity, leading to increased cell lysis during fermentation. The SQ strain wasengineered to restore membrane rigidity by activating the cell wall integrity (CWI) pathway, thereby further enhancing its squaleneproduction efficiency. Maintenance of CWI was associated with improved squalene production, as shown by cell wall remodelingthrough regulation of Ecm33, a key regulator of the CWI pathway. Deletion of *ECM33* in the SQ strain helped restore membranerigidity and improve stress tolerance. Moreover, *ECM33* deletion suppressed cell lysis and increased squalene production byapproximately 12% compared to that by the parent SQ strain. Thus, this study shows that engineering of the yeast cell wall is apromising strategy for enhancing the physiological functions of industrial strains for production of lipophilic compounds.

Keywords : Cell wall integrity, *Saccharomyces cerevisiae*, squalene

Q-29

Tailoring the *Saccharomyces cerevisiae* Endoplasmic Reticulum for Functional Assembly of Terpene Synthesis Pathway

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The endoplasmic reticulum (ER) is a highly dynamicorganelle that plays crucial roles in protein synthesis and *precise* folding. When inherent capacity of ERis exceeded, it causes ER stress which triggers the unfolded protein response.The ER size is an important factor that can decide its protein synthesis andfolding capacity, thereby restoring normal ER functions via size adjustment. Inspiredby such inherent genetic programming, we engineered *Saccharomyces cerevisiae* to expand the ER by overexpressing a keyER size regulatory factor, INO2. ER expansion led to increasing capacity tosynthesize endogenous and heterologous ER-associated proteins, and theavailable space to accommodate them. Harnessing the yeast ER for metabolicengineering, we ultimately increased the production of squalene and cytochrome P450-mediated protopanaxadiol by 71-fold and 8-fold, compared to theirrespective control strains without overexpression of INO2. Furthermore,genome-wide transcriptome analysis of the ER-expanded strain revealed that thesignificant improvement in terpene production was associated with globalrewiring of the metabolic network. Our results showed that the yeast ER can beengineered as a specialized compartment for metabolic engineering of terpenebiosynthesis, representing new possibilities for high-level production of othervalue-added chemicals.

Keywords : Endoplasmic reticulum, *Saccharomyces cerevisiae*, terpene synthesis

Q-30

Production of Fructo-Oligosaccharides by Engineered Yeast

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Fructo-oligosaccharides (FOS) recently have been noted as promising sugar because of its various functional properties such as low calories, and prebiotics. Especially, it is useful as alternative sweeteners for obese and diabetic patients by supporting growth of healthy bacteria in digestive system. The FOS is consisted of linkage of fructose branches at sucrose and it can be obtained by glycosyltransferase reaction. In previous study, we developed *Saccharomyces cerevisiae* yeast strain to produce enzyme A. In this study, In this study, we tested FOS conversion titer by fermenting engineered strain and applied it to fermented beverages. As a results, we confirmed production of FOS by engineered strain and successfully fermented natural sweet beverages containing FOS. This study will be used as basic data for application to the genetically modified organism-food industry.

Keywords : Metabolic Engineering, enzyme secretion, artificial sweetner

Q-31

Rational Metabolic Engineering Strategies for Galacturonic Acid Fermentation in Engineered Yeast

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Pectin-rich biomass, such as a citrus peel waste, is expected to be a next-generation biomass for the conversion of biofuel through microbial fermentation because it has low lignin content and abundant in fermentable sugars (glucose, fructose, xylose, and arabinose) and galacturonic acid. In a previous study, we developed an engineered yeast strain fermenting three carbon sources (galacturonic acid, arabinose, and xylose), but it showed a low consumption rate of galacturonic acid. In this study, we investigated the limiting factors in galaturonic acid fermentation through the global intracellular metabolite analysis using GC/MS, and additional rational metabolic engineering strategy was performed in engineered *Saccharomyces cerevisiae* for effective galaturonic acid metabolism. As a result, the rational engineered strain improved the phenotype of galaturonic acid fermentation and reduced accumulation of limiting factors than control strain. Through this study, we can suggest that the possibility as a platform host to utilize pectin-rich biomass.

Keywords : Lignocelluolsic biomass, sugar acid, Cas9

Q-32

Kenaf Plant as Cellulosic Biomass for Ethanol Production by Engineered Yeast

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Kenaf (*Hibiscus cannabinus*) is a kind of fiber crop native to India and Africa. Because of its tougher and inflexible characteristics than other fibers, it has been utilized only for rope and fishing net. However, the kenaf has many other advantages, such as fast growth rate, excellent CO_2 absorptivity and large annual productivity per unit area. Thus, it is being newly noted as energy crop for renewable energy production, but little study has been done on it. In this study, we tested 11 different cultivars of kenaf samples to study their sugar content as well as fermentation profiles focusing on how efficiently the xylose fraction of the hydrolysates can be fermented into ethanol. First, we treated the samples using diluted acid (1% H_2SO_4, w/v), the most simple and cost-effective pretreatment method. Next, using engineered *Saccharomyces cerevisiae* strain developed to utilize xylose as carbon source in previous, the hydrolysates were processed by simultaneous saccharification and fermentation. Through this study, we evaluated the properties of kenaf as a novel biomass material and present potential of cellulosic bioenergy source which could replace fossil fuels.

Keywords : Lignocellulosic biomass, xylose fermentation, yeast

Q-33

Xylose Facilitates Lactic Acid Production of Engineered *Saccharomyces cerevisiae*

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Lactic acid is an organic acid that widely used in the food, pharmaceutical, textile and chemical industries. Also, it is a monomer of poly-lactic acid (PLA) which has increasing demand as an environmentally friendly and biodegradable plastic. In our prior study, a lactic acid-producing, xylose-fermenting *Saccharomyces cerevisiae* strain (SR8 LDH) has been developed. In the present study, we tested the SR8 LDH strain under various fermentation conditions to discover the most important condition determining lactic acid and ethanol production profiles. Using xylose as a substrate significantly improved lactic acid yields compared to using glucose under microaerobic conditions. High initial cell density improved lactic acid productivity, but it did not affect ethanol production. Lastly, when fermenting xylose, the *PDC1* gene encoding pyruvate decarboxylase was transcriptionally repressed, which might be associated with the production of low ethanol and high lactic acid. These results indicate that the type of a carbon source, i.e. using xylose instead of glucose could be a promising solution for lactic acid production by engineered *S. cerevisiae.*

Keywords : Transcriptome analysis, metabolic engineering, yeast

Q-34

Physicochemical Properties of Anti-Bacterial Crude Extract from *Zymomonas mobilis*

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Zymomonas mobilis has been known as an effective ethanol-producing bacterium in bioethanol production processes. However, other substances such as biosurfactant produced by this bacterium has been poorly investigated. Biosurfactant, the amphipathic substances produced by various microorganisms are used in various industries such as microbial enhanced oil recovery, antibiotics, mineral mining, cosmetic and food industries. Therefore, to screen the biosurfactant activity from this bacterium, the emulsification activity test on the culture broth from various strains of *Z. mobilis* including ZM4 (ATCC31821), ZM401 (ATCC31822) and their phosphodiesterase knockout mutants ZAM1 and ZAM2 respectively were carried out. The results indicated the higher biosurfactant activities present in ZM401 and ZAM2 culture broth as compared to that from their parental strain ZM4. Therefore, in order to further investigate the potential anti-bacterial activities of crude extract of biosurfactant, various crude extracts from the culture broth of both strains were prepared using hexane, ethyl acetate, and methanol etc. As the crude extracts were applied to Ultrasensitive Radial Diffusion Assay (URDA) using various pathogenic bacteria including *Escherichia coli* D31, *Bacillus subtilis* KCTC1021, *Aeromonas hydrophila* KCTC 2358, *Edwardsiella tarda* NUF251, *Streptococcus iniae* FP5229 and *Vibrio anguillarum*. The results indicated that ethyl acetate crude extracts from both strain showed highest anti-bacterial activities against *Bacillus subtilis* KCTC1021 and *Vibrio anguillarum* KCTC2711 . As far as we know, this study for the first time Consequently, this study showed further potential application of this bacterium such as antibiotic and biosurfactant.showed the presence of biosurfactant and anti-bacterial activities from this bacterium. Consequently, this study showed further potential application of this bacterium such as antibiotic and biosurfactant.

Keywords : Anti-bacterial activity, biosurfactant, *Zymomonas mobilis*

Q-35

Secretory Production Of Human Serum Albumin(rHSA) in *Saccharomyces cerevisiae*

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Since human serum albumin (HSA) is the major protein component of human plasma, it has been widely used as a medicinal protein. With the growing demand for HSA, development of an alternative recombinant HSA production system has been required because HSA produced from human plasma contains a potential risk of infection by blood-derived pathogens and human plasma supply can not cope with the demand for HSA. In this study, we developed a recombinant *S. cerevisiae* hyper-secreting HSA by employing target-protein specific translational fusion partner (TFP). TFP is a kinds of secretion enhancer consisted with signal peptide and pro-peptide promoting folding and trafficking of cargo proteins in the secretion pathway. When N-terminal 125 amino acids of Srl1p was used as TFP, the amount of secreted HSA was increased about two fold compared to secretion with native secretion signal. As a result of fed-batch fermentation of recombinant S. cerevisiae under sub-optimal conditions, approximately 0.62g/L of HSA was produced and directly purified by His tag affinity chromatography with 24% recovery.

Keywords : Human serum albumin, *Saccharomyces cerevisiae*, recombinant protein expression

Q-36

Effect of PelB Signal Sequences on Pfe1 Expression and ω-Hydroxyundec-9-enoic Acid Biotransformation in Recombinant *Escherichia coli*

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ω-Hydroxyundec-9-enoic acid (ω-HUA) is a valuable medium-chain fatty acid with industrial potentials. For bioconversion of ricinoleic acid to ω-HUA, in this study, an alcohol dehydrogenase (Adh) from *Micrococcus luteus*, a Baeyer-Villiger monooxygenase (BVMO) from *Pseudomonas putida* KT2440 and an esterase (Pfe1) from *Pseudomonas fluorescens* SIK WI were overexpressed in *Escherichia coli* BL21(DE3). In order to enhance accessibility of Pfe1 to the (*E*)-11-(heptanoyloxy) undec-9-enoic acid (11-HOUA) substrate, a truncated PelB signal sequence without the recognition site of signal peptidase (tPelB) was attached to the N-terminus of Pfe1, resulting in the construction of *E. coli* AB-tPE strain expressing Adh, BVMO and the tPelB-Pfe1 fusion protein. A batch-type biotransformation of ricinoleic acid by *E. coli* AB-tPE resulted in 1.8- and 2.2-fold increases in ω-HUA conversion yield and productivity, respectively, relative to those for the control strain without the PelB sequence (AB-E). By fed-batch-type biotransformation with glycerol feeding, the AB-tPE strain produced 23.7 mM (equivalent to 4.7 g/L) of ω-HUA with 60.8%(mol/mol) of conversion yield and 1.2 mM/h of productivity, which were 13.2, 2.9, and 2.6 times higher than those in a batch-type biotransformation using the AB-E strain. In conclusion, combination of the truncated PelB-Pfe1 fusion and fed-batch process with glycerol feeding provided the highest efficiency of ω-HUA biotransformation, of which strategies might be applicable for biotransformation of hydrophobic substances.

Keywords : ω-Hydroxyundec-9-enoic acid, ricinoleic acid, PelB signal sequence

Q-37

Bioconversion of Nonanoic Acid and Its Esters by Whole Cell Biocatalyst of *Candida tropicalis*

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Azelaic acid is an α,ω-dicarboxylic acid with nine carbons and has multiple applications in plastic and cosmetic industries. Chemical oxidation of oleic acid with ozone (called ozonolysis) allows the production of azelaic acid and a major byproduct of nonanoic acid. To increase the total yield of azelaic acid in the ozonolysis, in this study, sustainable biotransformation process using a whole cell biocatalyst was developed to directly convert nonanoic acid and its esters to azelaic acid. *Candida tropicalis* ATCC20962 immediately cleaved ethyl nonanoate to nonanoic acid after ethyl nonanoate addition, and then converted nonanoic acid into azelaic acid with the aid of nonane addition and continuous glucose supply. Finally, a fed-batch biotransformation by continuous feeding of pure nonanoic acid resulted in the production of 30.1 g/L azelaic acid with 0.30 g/L-h productivity and 90% molar yield. By combination of the ozonolysis and our process, a maximum of 95% molar carbon yield of azelaic acid from oleic acid was estimated. This is the first report that nonanoic acid and its esters were directly and biologically transformed to azelaic acid with over 90% yield, and would be a groundwork for the biotransformation of fatty acids with under nine carbons to the corresponding α,ω-dicarboxylic acids.

Keywords : Azelaic acid, nonanoic acid, *Candida tropicalis*

Q-38

Increased Xylose Consumption Rate of *Escherichia coli* BL21(DE3) by Adaptive Mutations in Anaerobic Condition

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Microbes uptake natural sugars and use them as energy. Most natural sugars consist of mixed sugars. When bacterial cells utilize mixed sugars in culture growth media, multi-growth phases can be observed due to the preference of sugar uptake and consumption, which is called diauxic growth. Although *E. coli* has been isolated from mammalian anaerobic guts, the physiology and metabolism of the model microorganism have been studied extensively under aerobic conditions because of convenience and manageability in the experiments. Anaerobic sugar metabolic regulation is still unclear and rarely studied. In anaerobic conditions, when D-glucose and D-xylose are given to BL21(DE3), that consumes glucose first, and xylose consumes very slowly. We observed bacterial adaptation in cell growth, and the acceleration of xylose utilization after glucose consumption. Genome sequencing revealed *xylR* missense mutations in the adapted cells, which are responsible for faster xylose consumption and increased succinate production. The adapted cells increased the transcription of *xylA* and *xylF* genes that encode xylose isomerase and xylose ABC transporter, respectively. This study will be helpful for understanding of the regulation mechanism of sugar utilization, and improving microbial fermentation biotechnology.

Keywords : Anaerobic fermentation, xylose, xylR

Q-39

Design and Application of Gluconate Inducible System for the Production of 3-Hydroxypropionate in *Pseudomonas putida* KT2440

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The problem of current production is that it requires inducers that are expensive. Substrate-inducible has advantage of using inducers that are in low price. Gluconate is preferred substrate for *Pseudomonas putida*, and it is cost-effective compared to conventional inducers such as IPTG and arabinose. We designed gluconate inducible system in order to control the metabolic flux of *P. putida* in the presence and absence of gluconate. Functionality of gluconate inducible system was first confirmed by expression of reporter gene under the control of promoter. Gluconate inducible system was determined to be active in the early exponential phase of the *P. putida*, suggesting the possibility of gluconate inducible system to be combined with the enzymes that are necessary for the growth phase, but not for the production phase. Tricarboxylic acid cycle is necessary for the cellular growth, but it is also competing pathway since acetyl-CoA serves as precursor for production of 3-hydroxypropionic acid. By integrating gluconate inducible system with the enzyme that is involved in the tricarboxylic acid cycle, acetyl-CoA would be used for growth of *P. putida* in the growth phase and used in the production of 3-hydroxypropionic acid in the production phase, resulting in improved production.

Keywords : Substrate-inducible, 3-Hydroxypropionate, *Pseudomonas putida KT2440*

Q-40

Improving Fatty Acid Production in *Escherichia coli* by Overcoming Toxicity of Short and Medium Chain Fatty Acids

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Oleochemicals, the substitute of petrochemicals, can be synthesized from plant oils and animal fats. Oleochemicals are on the rise as sustainable and bio-based chemicals as consumers become better well educated about environmental cost and importance. A variety of products such as lubricant, wax esters, interesterified fats, and cosmetics are derived from fatty acids. The production of transesterified fatty acids from biomass feedstocks are subject to subsequent physiochemical purification processes. The microbial production of free fatty acid can avoid the disadvantages of transesterification processes for fatty acid production from triglycerides. Several conditions have to be regulated for maximized and efficient free fatty acid production. Among them, the free fatty acid overproducing strain have shown that decreased viability and damaged inner membrane integrity. In this study, we introduce several mutations including WaaG(glucosyltransferase, adds the first glucose of the outer core of LPS) and RpoC(the gene express DNA-directed RNA polymerase subunit beta), which have been reported to improve fatty acid tolerance and also increase fatty acid production, into a fatty acid overproducing strain.

Keywords : Fatty acid, WaaG, RpoC

Q-41

Multidimensional Metabolic Engineering and Fermentation Optimization for High Production of Free Fatty Acids in *Escherichia coli*

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Free fatty acids can be readily converted to biofuel, such as alkane or fatty ethyl esters in the diesel range, by simple catalytic treatment. Therefore, microbial production of free fatty acid is expected to be an important share of alternatives of fossil fuel. In general, many studies have been conducted to produce free fatty acid from cheap biomass such as glucose in *Escherichia coli*, and this has made it possible to convert sustainable biomass into fuel. Fatty acid-based biofuels have been recently studied for further commercialization. Thus, various metabolic engineering efforts are being made to increase the titers and yields of free fatty acid production in *E. coli*. There have been numerous single strategies aimed at increasing fatty acid production such as heterologous expression or bypass of acetyl-CoA carboxylase, introduction of mutant Thioesterase I, disruption of membrane protein associated genes and overexpression of regulatory transcription factor FadR. However, studies in which those strategies have been used in combination has not been yet reported. Here we report the combinational genetic manipulation and fermenter optimization for high FFAs production in *E. coli* result in 10-fold increase than control strain.

Keywords : Free fatty acids, multidimensional metabolic engineering, fermentation optimization

Q-42

Redirection of Glycolytic Pathway in *Escherichia coli* for Production of NADPH-Dependent 3-Hydroxypropionic Acid

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In metabolic engineering, the growth of strains and the production of valuable chemicals require energy and reducing power, such as ATP, NADH and NADPH. Our target product, 3-hydroxypropionic acid (3-HP), is an important platform chemical that can be converted to valuable chemicals including acrylic acid and 1,3-propanediol. Previously reported that 3-HP can be synthesized in engineered *Eshcherichia coli* via malonyl-CoA with glucose as a carbon source. This synthetic pathway requires 2 moles of NADPH for 1 mole of 3-HP production. We anticipated to supply the reducing power required for 3-HP production by redirecting the glycolytic flux of *E. coli* from Embden-Meyerhof-Parnas pathway (EMPP) to pentose phosphate pathway (PPP) which is the main way of providing NADPH. However, redirection of glycolytic pathway in *E. coli* strain caused redox imbalance and as a consequence showed a serious reduction in a growth rate. In this study, we applied adaptive laboratory evolution (ALE) for redirected *E. coli* in order to recover its growth rate on glucose as a sole carbon source. By genotypic- and phenotypic-analysis, we characterized the mutations that contributed cell fitness recovery. The evolved strain harboring 3-HP synthetic machinery successfully produced 2.7 g/L 3-HP in flask scale and 11.5 g/L 3-HP in 1.5 L fermenter.

Keywords : Adaptive laboratory evolution, 3-hydroxypropionic acid, redirection of glycolytic pathway

Q-43

Improved Production of Azelaic Acid from Nonanoic Acid in *Escherichia coli* by Adaptive Laboratory Evolution and Rational Engineering

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Azelaic acid, a nine-carbon chain length α, ω-dicarboxylic acid, is used in chemical industry as chemical products and intermediates, such as polyesters and polyamides as well as medications. Production of azelaic acid by bioconversion is a highly promising process because it is environmentally friendly. In this study, a whole cell biocatalysis was proceeded to produce azelaic acid from nonanoic acid by introducing alkane hydroxylating system AlkBGT from *Pseudomonas putida* GPo1 in *Escherichia coli*. However, a trace amount of azelaic acid was produced due to inherently low performance of *E. coli* for utilizing nonanoic acid. Adaptive laboratory evolution strategy was established to obtain higher nonanoic acid-utilizing strain. We isolated *E. coli* mutants showing growth in minimal media supplemented with nonanoic acid as a sole carbon source. The isolated mutant, A11, was further engineered to block nonanoic acid degradation by deleting *fadE* gene, which encodes acyl-CoA dehydrogenase. After introducing AlkBGT into the engineered mutant A11_ER, the production of azelaic acid increased 9-fold compared to the wild-type strain. Eight mutations were identified in the mutant strain A11 and need to be further characterized.

Keywords : Bioconversion, adaptive laboratory evolution, azelaic acid

Q-44

Optimization of *Pseudomonas putida* Strains and Fermentation Processes to Produce Various Short-Chain-Length Polyhydroxyalkanoates from Levulinic Acid

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Polymers derived from microorganisms such as polyhydroxyalkanoates (PHAs) have gained attention due to the properties such as sustainability, biodegradability, and biocompatibility. Among them, polyhydroxybutyrate (PHB) which consists of 3-hydroxybutyrate (3HB) have been used for wide range of applications in industrial and medical fields. Due to its high brittleness and high melting point, incorporation of 3-hydroxyvlaerate (3HV) or 4-hydroxyvalerate (4HV) into PHA have been adopted to overcome this limitation. Levulinic acid (LA) is a five-carbon keto acid that can be readily obtained from cheap biomass and common feedstocks at a low cost. Recently, LA catabolic pathway of *Pseudomonas putida* was discovered and pathway intermediates such as 4-hydroxyvaleryl-CoA (4HV-CoA) and 3-hydroxyvaleryl-CoA (3HV-CoA) were revealed, making it possible to add and modulate the 3HV and 4HV monomer by engineering the pathway. In the previous study, a metabolically engineered *P. putida* strain was produced to synthesize various 3HV and/or 4HV-containing PHAs from LA. Herein, we focus on impairing the side pathways related to LA catabolism in *P. putida* and enhancing the production of various short-chain-length PHAs by improving the fermentation processes.

Keywords : *Pseudomonas putida*, levulinic acid, polyhydroxyalkanoates

Q-45

Screening of Soluble Expression of Psychrophilic Enzymes Using Chaperone System

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Psychrophiles are extremophiles, which can grow and reproduce at low temperatures ranging from −20℃ to +10 ℃. Psychrophilic enzymes are difficult-to-express (DTE) and heat-labile proteins that easily aggregates and forms inclusion bodies. DTE proteins do not emerge in fully soluble, well-folded, and active form in a heterologous expression system. Furthermore, folding assistance is required at various folding steps according to the protein size. To improve the yield of soluble psychrophilic enzymes, we used BL21(DE3) *E. coli* system that co-express the GroELS chaperone. The GroEL is a large double-ring-shaped oligomeric 14-mer chaperonin, which binds and encapsulates a substrate polypeptide along with its co-chaperonin GroES, which caps the central cavity of GroEL and facilitates the correct folding of proteins in the presence of ATP. In this study, we investigated the role of chaperone GroELS from *E. coli* and Antarctic psychrophilic bacterium *Psychrobacter* sp. PAMC21119 for the soluble expression of DTE 51 enzymes (7 mesophilic and 44 psychrophilic enzymes). Here, we present the preliminary screening results of soluble expression of DTE 51 enzymes.

Keywords : Difficult-to-express proteins, GroELS, soluble expression

Q-46

Engineering of Robust *Saccharomyces cerevisiae* Strain Regarding the Utilization of Undetoxified Lignocellulosic Hydrolysates for Efficient Bioethanol Production

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A current challenge of economically viable biofuels production is the presence of inhibitors in lignocellulosic hydrolysates which hampers the ethanol fermentation by *Saccharomyces cerevisiae*. As a major inhibitor derived from lignocellulosic biomass, acetic acid has toxic effect on yeast fermentation resulting in decreased cell growth, glucose/xylose utilization rate and ethanol yield. In order to develop the robust yeast strain, an evolutionary engineering strategy was first applied to improve xylose fermentation of yeast under acetic acid stress. Through evolutionary engineering, we obtained the acetate-tolerant strain harboring xylose isomerase (XI) pathway, XUSAE57, with high yields of 0.43-0.50 g ethanol/g xylose under 2-5 g/L of acetic acid stress, previously. This strategy not only achieved ~1.7 fold higher level of ethanol yields, but also improved xylose utilization rate by >2 folds. To further improve ethanol yield, we attempted to expand the substrate spectrum of XUSAE57 strain to include the non-sugar carbon source, acetate, by constructing a synthetic acetate catabolic pathway based on endogenous acetyl-CoA synthetase (ACS) and heterologous acetylating acetaldehyde dehydrogenase (AADH). The resulting recombinant strain produced ethanol from undetoxified lignocellulosic hydrolysates with the highest ethanol yields (0.49-0.51 g ethanol/g glucose and xylose) reported to date.

Keywords : *Saccharomyces cerevisiae*, lignocellulosic hydrolysate, bioethanol

Q-47

SKy BioFoundry: Constructing a Robotic Automated Platform for Developing Industrial Microorganisms with High-Throughput Genome Engineering System

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Biofoundries provide an integrated robotic platform to enable the rapid design, construction, and testing of genetically reprogrammed organisms for biotechnology applications and research. I present here the SKy Biofundry, Sungkyunkwan University as a domestic biofoundry infrastructure that focuses on massive genome engineering of industrial microorganisms. The SKy Biofoundry, as a member of Global BioFoundry Alliance(GBA), is capable of performing automatic actions for various experiments such as gene cloning, colony selections, and high-throughput cultivation using an integrated robotic and CRISPR-genetic platform in order to generate massive engineered strains and to collect the corresponding data. Our strength at the SKy Biofoundry is a seamless automated workflow that was optimized for CRISPR-assisted genome engineering including the CRISPR-recombineering and base editing. On the building the biofoundry, we established a sequence database for single guide RNA and additional DNAs which is essential for CRISPR-based genome engineering. Currently, the SKy Biofoundry is fully automated functioning on gene cloning and microbial transformations for *Escherichia coli* and *Corynebacterium glutamicum* and is active for constructing massive genetic variants for the industrial strains to accelerate a genetic understanding of industrial bacteria and to demonstrate the DBTL (Design-Build-Test-Learn) cycle. For more information, please visit the website of the SKy Biofoundry.

Keywords : Biofoundry, robotic platform, high-throughput

Q-48

Synthetic Metabolic Valves Designed from Transcript 3′ End Information Reveal an Optimal Solution for Bio-Production in *Escherichia coli*

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The design of biological devices requires a large number of well-characterized biological parts. As genetic circuitry becomes more sophisticated, the importance of the standardized regulatory parts is increasing. Collective efforts of many synthetic biologists resulted in thousands of biological parts available through literature and public repository. However, 3′-UTR bioparts are limited despite their critical role in gene expression. Here, we report 3′-UTR bioparts in *E. coli* designed by the genome-wide transcript 3′ ends information explored by Term-Seq. Comprehensive examination reveals multiple types of transcript 3′ end originated from transcription termination and RNA processing. Experimental characterization of rho-independent terminators showed a reduction of downstream gene expression ranging from 1.04 to 255-fold, providing a large repertoire of terminators with various strength. 3′-UTR bioparts designed by the rho-independent terminators provides a scalable means of controlling gene expression. Synthetic metabolic valves designed by 3′-UTR bioparts were able to alter metabolic flux directed toward branched metabolic pathways. The synthetic metabolic pathway with an optimal flux valve resulted in the titer of myo-inositol increased 8.92-fold. This study provides foundation for 3′-UTR engineering in synthetic biology applications.

Keywords : Term-Seq, 3' UTR biopart, synthetic biology

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Q-49

Functional Cooperation of the Glycine Synthase-Reductase and Wood-Ljungdahl Pathways for Autotrophic Growth of *Clostridium drakei*

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Among CO_2 fixing metabolic pathways in nature, the linear Wood-Ljungdahl pathway (WLP) in phylogenetically diverse acetate forming acetogens comprises the most energetically efficient pathway, requires the least number of reactions, and converts CO_2 to formate and then into acetyl-CoA. Despite two genes encoding glycine synthase are well conserved in the WLP gene clusters, the functional role of glycine synthase under autotrophic growth conditions has remained uncertain. Here, using the reconstructed genome-scale metabolic model, *i*SL771, based on the completed genome sequence, transcriptomics, ^{13}C isotope-based metabolite-tracing experiments, biochemical assays, and heterologous expression of the pathway in another acetogen, we discovered for the first time that the WLP and the glycine synthase pathway are functionally interconnected to fix CO_2, subsequently converting CO_2 into acetyl-CoA, acetyl-phosphate, and serine. Moreover, the functional cooperation of the pathways enhances CO_2 consumption and cellular growth rates via bypassing reducing power required reactions for cellular metabolism during autotrophic growth of acetogens.

Keywords : Acetogens, Glycine synthase-reductive pathway, Wood-Ljungdahl pathway

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Q-50

Characterization of a Novel Type III Polyketide Synthase from Piper Methysticum

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Type III polyketide synthases (PKSs) are the catalytic enzymes that produce the formation of an aromatic compounds in plant, bacteria, and fungi. A large number of functionally divergent Type III PKSs have been found in plants, including chalcone synthase(CHS), stilbene synthase (STS), 4-coumaroyl triacetic acid synthase (CTAS), benzalacetone synthase (BAS), and styrylpyrone synthase (SPS). These polyketides are a large class of natural products with biological activity and structural diversity. We identified one Type III PKS gene (t3PKS) in a Piper methysticum transcriptome. The deduced amino acid sequence of t3PKS showed 55-78% identity to those of other chalcone synthase superfamily enzymes of plant origin. Phylogenetic analysis shows that the t3PKS to be CTAS. However, bioconversion experiment with 4-coumaric acid CoA ligase(4CL) indicated that t3PKS exhibiting BAS activity that accepted both p-coumaroyl-CoA and feruloyl-CoA, but not caffeoly-CoA, as starter unit and one malonyl-CoA as extender unit and leaded to production of 4-hydroxy benzalacetone and vanillylidene acetone. Thus, the construction of artificial biosynthetic pathways using this novel BAS gene could be allowed for the synthesis of naturally-occurring flavoring phenylbutanoids.

Keywords : Type III polyketide, Benzalacetone synthase, 4-hydroxybenzalacetone

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Q-51

The CRISPR-dCas12a Iterference for Rpression of Esential Gnes in Cyanobacteria and Its Application

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Metabolic engineering of cyanobacteria has enabled photosynthetic conversion of CO_2 to value added chemicals as bio-solar cell factories. The using cyanobacterial for direct production of squalene is advantage, since they can just grow carbon dioxide from air and sunlight without energy sources based on glucose. However, metabolic engineering usingsynthetic biology tools is limited to build a bio-solar cell factory that converts CO_2 to value-added chemicals, as repression of essential genes has not been achieved. We developed a dCas12a-mediated CRISPR interference system (CRISPRi-dCas12a) in cyanobacteria that effectively blocked the transcriptional initiation by means of a CRISPR-RNA (crRNA) and 19-nt direct repeat, resulting in 53-94% gene repression. The repression of multiple genes in a single crRNA array was also successfully achieved without a loss in repression strength. In addition, as a demonstration of the dCas12a-mediated CRISPRi for metabolic engineering, photosynthetic squalene production was improved by repressing the essential genes of either *acnB* encoding for aconitase or *cpcB2* encoding for phycocyanin b-subunit in *Synechococcus elongatus* PCC 7942. The ability to regulate gene repression will promote for construction of bio-solar cell factories to produce value-added chemicals.

Keywords : CRISPR, cyanobacteria, metabolic engineering

Q-52

1,3-PDO by *Klebsiella pnemoniae* L17 Using ZVI as Reducing Agent from Glycerol

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1,3- PDO (1,3-Propanediol) is a high value-added product that is widely used as adhesives, laminates, and food additives. For efficient production of 1,3-PDO, sustainable and non-toxic regeneration of NADH is of great importance. ZVI (Zero-Valent Iron) can provide reducing equivalent for 1,3-PDO synthesis from glycerol as an electron donor. *Klebsiella pneumoniae* has a 1,3-PDO production pathway from glycerol and has been extensively investigated as exoelectrogens. In this study, we attempt to produce 1,3-PDO from glycerol by using an electrochemically active strain, *K. pneumoniae* L17, and ZVI as an electron donor. As a result, the production of 1,3-PDO using ZVI has increased significantly to 24.23 ±1.33 mmol/l. These results implicate that ZVI can regulate the bioconversion of electroactive strain such as *K. pneumoniae* L17, therefore improve glycerol conversion into value-added platform chemicals.

Keywords : ZVI, *Klebsiella pneumoniae* L17, 1,3-Propanediol(1,3-PDO)

Q-53

Microbial Production of Squalene in *Corynebacterium glutamicum* Engineered by the CRISPR-dCas9 and Cas12a

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Microbial production of squalene has driven the development of microbial cell factories, triggered by the limitation of low-yielding bioprocesses from plants and illegal harvesting shark liver. In addition, RNA-guided CRISPR technologies have been applied to microbial cell for rapid development of metabolic engineering in microbial cell factories for making it produce desired product like squalene. In this study, we have metabolically modified *Corynebacterium glutamicum* to produce squalene from glucose. For this, we have used three strategies, one of which is CRISPR-dCas9 based gene interference for precursor rebalancing, redox balancing and blocking the competing pathway by targeting combinatorial genes (gapA, gdh, and idsA). Second one is CRISPR-Cas12a based gene knockout by introducing STOP mutation to 16^{TH} codon (E) in idsA gene. The last one is a plasmid based over-expression of key enzymes (dxs, idi, and ispA) belong to MEP pathway and truncated squalene synthase. As a result, we found the best squalene production strain with blocking competingpathway by repressing the idsA gene using high-throughput cultivation. Using this strain, 5.4 ± 0.3 mg/g dry cell weight (DCW) and 105.3 ± 3.0 mg/L squalene were analyzed, which was a 5.2-fold increase over the parental squalene production strain. Thus, this study shows cost-effective and rapid strain development of metabolic engineering to build efficient microbial cell factories for industry.

Keywords : Squalene, dCas9, Cas12a

Q-54

Ultrasensitive Analysis of Biomarkers by Translational Amplification of Detection Signals

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Sensitive detection of biomarkers is one of the crucial factors required for early diagnostics and the efficient management of diseases. In this study, we attempted to increase the sensitivity of affinity-based bioassays by employing a signal amplification process based on a cell-free protein synthesis system. As a key reagent for this novel assay scheme, we developed bi-functional aptamers that not only selectively bind target molecules but also serve as the templates for cell-free synthesis of reporter enzymes. These aptamers, dubbed readable aptamers, were synthesized by simple PCR procedures, and readily implemented into existing platforms of the affinity-based assay. Through the pre-amplification of signal-generating enzymes, this method allowed an extremely low limit of detection in the ranges of aM.

Keywords : Readable aptamer, cell-free protein synthesis, biomarker detection

Q-55

Elaborate Flux Distribution in Glyoxylate Shunt Pathway to Improve 5-Aminolevulininc Acid Production in *Eshcerichia coli*

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Microbial fermentation of 5-aminolevulinic acid (ALA) has received much attention because of its potential in clinical applications. Overexpression of the related enzymes and an analogue transporter yielded remarkable achievements in ALA production, accompanying with deciphering its regulation. In spite of this, there is still significant room for carbon flux optimization to improve ALA production. This study aimed for precise carbon flux optimization for high ALA production in *Escherichia coli* expressing the ALA biosynthetic pathway. Initially. genes hemA and hemL were overexpressed with strong promoters and synthetic 5'-untranslated regions (5'-UTR). Then, the tricarboxylic acid (TCA) cycle was knocked-out for re-distribution of carbon flux toward the ALA production, additional precise tuning of carbon flux to the glyoxylate shunt by varying the transcriptional strength of aceA led to substantially improved cell growth and ALA production. Thus, this precise tuning of the glyoxylate cycle in a quantitative manner should also enable the efficient production of other valuable products derived from the TCA cycle.

Keywords : 5-Aminolevulinic acid, Glyoxylate shunt, Re-distribution of carbon flux

Q-56

The Effect on Enhancing Absorption Rate of Liposomal Vitamin C

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Vitamin C is oxidized and decomposed during digestion and is destroyed before reaching blood vessels and cells and cannot be properly absorbed by the body. In this study, liposomes with vitamin c capture were prepared, and the capture rate was found to be 40% through HPLC analysis. To confirm the absorption rate of liposomal vitamin c, SD-rat was divided into 4 groups (normal, control, vitamin c powder, liposomal vitamin c), and the sample was orally administered once. Blood was collected from the inferior vena cava every 0, 0.5, 1, 1.5, 2, 2.5, and 4 hours, and serum was separated, and the content of vitamin c was analyzed by HPLC. As a result of analysis, the concentration of vitamin c powder administered group decreased after 0.5 hours. However, the liposomal vitamin c group increased in concentration up to 2 hours and then decreased. These results suggest that liposomal vitamin c has a high absorption rate in the body and thus has high antioxidant capacity.

Keywords : Liposomal Vitamin C, absorption rate , antioxidant capacity

Q-57

High-Level Production of 4-Hydroxyvalerate from Levulinic Acid via Whole-Cell Biotransformation Decoupled from Cell Metabolism

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γ-Hydroxyvalerate (4HV) is an important monomer that has been used to produce various valuable polymers and products. In this study, an engineered 3-hydroxybutyrate dehydrogenase that can convert levulinic acid (LA) into 4HV was co-expressed with a cofactor (NADH) regeneration system mediated by an NAD^+-dependent formate dehydrogenase (CbFDH) in the *Escherichia coli* strain, MG1655. The resulting strain produced 23-fold more 4HV in a shake flask. The 4HV was highly produced at low aeration condition suggesting that ATP is not a limiting factor. All of these factors are considered beneficial characteristics for the production of target compounds, especially at an industrial scale. Under optimized conditions in a 5 L fermenter, the titer, productivity, and molar conversion efficiency for 4HV reached 100 g/L, 4.2 g/L/h, and 92%, respectively. Our system could prove to be a promising method for the large-scale production of 4HV from LA at low-cost and using a renewable biomass source.γ-Hydroxyvalerate (4HV) is an important monomer that has been used to produce various valuable polymers and products. In this study, an engineered 3-hydroxybutyrate dehydrogenase that can convert levulinic acid (LA) into 4HV was co-expressed with a cofactor (NADH) regeneration system mediated by an NAD^+-dependent formate dehydrogenase (CbFDH) in the *Escherichia coli* strain, MG1655. The resulting strain produced 23-fold more 4HV in a shake flask. The 4HV was highly produced at low aeration condition suggesting that ATP is not a limiting factor. All of these factors are considered beneficial characteristics for the production of target compounds, especially at an industrial scale. Under optimized conditions in a 5 L fermenter, the titer, productivity, and molar conversion efficiency for 4HV reached 100 g/L, 4.2 g/L/h, and 92%, respectively. Our system could prove to be a promising method for the large-scale production of 4HV from LA at low-cost and using a renewable biomass source.

Keywords : Levulinate, 4-hydroxyvalerate, *Escherichia coli*

Q-58

CRISPR Interference-Based Plasmid Selection System

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Selection of recombinant plasmids is fundamental to metabolic engineering and synthetic biology. Although antibiotics have been commonly used for selection of host cells carrying the plasmid, their use has multiple problems such as high cost on large-scale microbial fermentation and environmental transfer of antibiotic resistance genes. To solve these problems, we designed, built, and tested a double inverter logic gate-based plasmid selection system using CRISPR interference (CRISPRi). We first designed a CRISPRi plasmid named as single guide RNA landing pad (SLiP) which severely impairs host cell growth by repressing antibiotic resistance gene from its own plasmid or hindering essential gene expression on chromosome. We then implanted another sgRNA expression cassette on a different plasmid, which represses growth-inhibitory sgRNA from SLiP, resulting in recovery of host cell growth. Using this CRISPRi-based double inverter system, we successfully selected cells harboring the GFP-expressing recombinant plasmid, or enriched cells simultaneously containing two different fluorescent plasmids. Overall, the developed CRISPRi-based plasmid selection system will serve as a versatile tool for the antibiotic-free production of industrially valuable proteins, biochemicals, and therapeutic plasmids.

Keywords : CRISPRi, plasmid selection, antibiotic resistance

Q-59

Development of Nitrate Biosensor and Programmable Probiotic *Escherichia coli* for the Detection of Gut Inflammation

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Genetic circuit is valuable and promising tool for programmable control of gene expression within living cells and increasingly applied to probiotics to non-invasively monitor and treat diseases. Here, we engineered and optimized probiotic *Escherichia coli* Nissle 1917 for detection of nitrate emanating from inflammatory host response. Firstly, we optimized the expression level of two-component regulatory system to maximize the sensitivity against nitrate, and demonstrated that the recombinant *E. coli* Nissle 1917 strain harbouring nitrate biosensor can detect and respond to exogenous nitrate in the mucin containing media. Furthermore, we validated that orally delivered recombinant *E. coli* Nissle 1917 strain harbouring nitrate biosensor can detect and respond to nitrate originated from chemically-induced colitis mice, and the intensity of the reporter fluorescence were correlated with the amount of nitrate induced by inflammation *in vivo*. Finally, we constructed the engineered AND logic circuit as a proof-of-concept using orthogonal thiosulfate and nitrate biosensors to specifically respond to inflammation, and demonstrated that the engineered AND logic circuit in probiotic *E. coli* Nissle 1917 strain was activated in case of presence of both exogenous inducers are present. Overall, we developed the nitrate biosensor in probiotic *E. coli* Nissle 1917 strain which can be widely applicable both in the bacterial cell-based diagnostics or therapeutics for gut inflammation.

Keywords : Nitrate, biosensor, *Escherichia coli* Nissle 1917

Q-60

Improvement of Bioethanol Production Using CRISPR Cas9 from *Gracilaria verrucosa*

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Glucose and galactose are monosaccharides obtained from *Gracilaria verrucosa* (red algae). A total monosaccharide of 61 g/L was obtained from 120 g/L *G. verrucosa*. Those were used for the production of ethanol by *Saccharomyces cerevisiae*. *S. cerevisiae* consumes glucose first and then galactose. For effective fermentation, strains have been improved to enhance the consumption of galactose using CRISPR-Cas9 technique. The transcription factor in genetic switch *Gal4* initiates the galactose metabolic pathway. In this study, the production of *Gal3* was overexpressed and *Gal80* was deleted. *Gal3* and *Gal80* are genes that involved *Gal3*-triggered activation of the *Gal* gene family. *Gal3* is a gene that attaches to *Gal80* and Gal3-Gal80 complex detaches from the Gal4 gene. When *Gal80* binds to the *Gal4*, the complex deactivates transcription. Therefore, the *Gal80* was deleted that *Gal4* could be transcribed without interruption. The wild type of *S. cerevisiae* CEN-PK2 showed the ethanol yield coefficient (Y_{EtOH}) of 0.32. However, that of *GAL80* deleted type of *S. cerevisiae* CEN-PK2 increased to Y_{EtOH} = 0.44.

Keywords : Red algae, *Saccharomyces cerevisiae*, bioethanol

Q-61

Expression of Key MEP Pathway Enzymes from *Vibrio* sp. dhg to Improve Lycopene Production in *Escherichia coli*

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Microbial production is a promising method that can overcome major limitations in conventional methods of lycopene production, such as low yields and variations in product quality. Significant efforts have been made to improve lycopene production by engineering either the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway or mevalonate (MVA) pathway in microorganisms. To further improve lycopene production, it is critical to utilize metabolic enzymes with high specific activities. Two enzymes, 1-deoxy-d-xylulose-5-phosphate synthase (Dxs) and farnesyl diphosphate synthase (IspA), are required in lycopene production using MEP pathway. Here, we evaluated the activities of Dxs and IspA of *Vibrio* sp. dhg, a newly isolated and fast-growing microorganism. Considering that the MEP pathway is closely related to the cell membrane and electron transport chain, the activities of the two enzymes of *Vibrio* sp. dhg were expected to be higher than the enzymes of *Escherichia coli*. We found that Dxs and IspA in *Vibrio* sp. dhg exhibited 1.08-fold and 1.38-fold higher catalytic efficiencies, respectively. Consequently, the heterologous overexpression improved the specific lycopene production by 1.88-fold. Our findings could be widely utilized to enhance production of lycopene and other carotenoids.

Keywords : Lycopene, MEP pathway, *Vibrio* sp. dhg

Q-62

Novel Strategy to Exploit Glyoxylate Shunt for Chemical Production

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The glyoxylate cycle is a bypass of the TCA cycle without carbon loss and enables microorganisms to utilize simple organic compounds as their sole carbon source. Despite these characteristics, engineering strategies based on the cycle have been limited a few; the inactivation of a transcriptional regulator (*iclR*) which represses the expression of *aceBAK* in the cycle. Here, we have devised a novel strategy to precisely regulate the glyoxylate cycle rather than the simple amplification. The activity of isocitrate lyase (*aceA*) was taken under control for the overall regulation of the glyoxylate cycle considering its thermodynamic properties. The strategy has been successfully applied to the production of valuable chemicals in *Escherichia coli*. The strategy was initially applied to the production of 5-aminolevulinic acid (ALA). To enhance the availability of precursor, TCA cycle was blocked. Thereafter, the carbon flux was precisely optimized by exploiting the glyoxylate cycle and the resultant strain showed a 4.59-fold increase in ALA production. The strategy was further applied to chemical production from acetate. The strains with only production pathway amplification showed low production from acetate, whereas the tuning of glyoxylate cycle could lead to a significant increase. The production of itaconic acid and tyrosine could be enhanced by 6.46-fold and 1.62-fold, respectively. This is the first attempt to precisely control the glyoxylate cycle, and the results suggest that elaborate tuning of the cycle can be a powerful strategy to facilitate production of many other chemicals.

Keywords : Metabolic engineeirng, glyoxylate shunt, synthetic biology

Q-63

Stable and Tunable Maintenance of Multi-Copy Plasmid by Auxotrophic Control in Antibiotics-Free Condition

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Plasmids have been widely utilized for recent microbial biorefinery process as DNA shuttles that deliver genes to host cells. In plasmid-related systems, varying the plasmid copy number (PCN) enables controllable expression of target genes or pathways that achieves efficient production of biochemical. Although benefits of multi-copy expression of the genes, plasmids require the addition of antibiotics for their stable maintenance despite of the growing concerns on economic and environmental problems. However, heterogeneity of PCN in individual cells which greatly lowers the productivity of bioprocesses is inevitable even with the addition of antibiotics. Here, we developed the antibiotic-free auxotrophic plasmid maintenance system for stable and tunable gene expression by precise control of plasmid copy number (PCN) in *Escherichia coli*. To achieve this, we devised a system that expresses one of essential genes, *infA* encoding translation initiation factor (IF-1), on plasmid instead of chromosome. We named the developed strategy the Stable and TunAble PLasmid (STAPL) system. With the STAPL system, the gene expression was stably maintained for 40th generation with minimized cell-to-cell variation under antibiotic-free conditions. Moreover, varying the expression level of *infA* enabled us to rationally tune the PCN by more than 5.6-fold. This antibiotic-free PCN control system was applied for engineering *E. coli* to produce itaconic acid and lycopene. Notably, the STAPL system significantly improved the production of itaconic acid and lycopene (2-fold) compared to the conventional system based on antibiotics. Collectively, our results clearly indicated that the addition of antibiotics is not required to maintain the plasmids expressing many genes via the STAPL system. The STAPL system with its PCN control can effectively drive carbon flux toward biochemical production as well as provide population quality control.

Keywords : Synthetic auxotrophy, plasmid copy number, expression control