Posters

1. Yuri Kanbayashi, Kobe University

"Overexpression and characterization of Arabidopsis 5-aminolevulinic acid dehydratases in *E. coli*"

Short Summary:

5-aninolevulinic acid dehydratase（ALAD）, convert two 5-aminolevulinic acid to porphobilinogen, is an essential enzyme in the tetrapyrrole synthesis. Arabidopsis has two ALADs called ALAD1 and ALAD2. ALAD1 is an active enzyme, however, the roles of ALAD2 is unknown. We purified ALAD2 over-expressed in E. coli and analyze its biochemical features.

2. Kaho Tsuruyama, Kobe University

"5-aminolevulinic acid responsive genes related to increasing stress tolerance in *Arabidopsis*"

Short Summary:

5-aminolevulinic acid (ALA) is a common precursor for tetrapyrroles in plants. Feeding ALA promotes plant's growth and improves resistance to various abiotic stresses. We focus on its mechanism of action. We determined ALA upregulated and downregulated genes involved in stress response by qRT-PCR analysis.

3. Silai Zhang, Kobe University

"Development of 2,3-butanediol-high producing *Aspergillus oryzae* by pyruvate metabolism engineering"

Short Summary:

2, 3-butanediol (2,3-BDO) is one of value-added bio-based chemicals, and it can be produced biologically by pyruvate in cytosol. We constructed 2,3-BDO-producing A. oryzae strain, and improved the productivity by regulating pyruvate metabolic flux. As a result, the titer from glucose and starch reached to 70% and 60% of theoretical yields, respectively.

4. Satoshi Wakai, Kobe University

"Comparative genome analysis of genetically engineered *Aspergillus oryzae* strains by long-read sequencing using nanopore sequencer"

Short Summary:

Genetically engineered Aspergillus oryzae strains constructed by co-transformation have multiple copies of integrated genes on the genomes. In this study, genome structure analysis of these strains using nanopore sequencer showed long-tandem repeat structure of integrated gene.

5. Prihardi Kahar, Kobe University

"Development of high-efficiency lipid production system for *Lipomyces starkeyi*"

Short Summary:

Biolipids, including triacylglycerol produced by oleaginous yeast, have been confirmed to be one of the most important raw materials as feedstocks for biofuel and biochemicals. Therefore, the selection of oleaginous yeasts which can rapidly utilize all carbon sources released from such materials and then highly accumulate lipid in typical culture media was undertaken. As a result of screening, we found that Lipomyces starkeyi D35 is an oleaginous yeast meets with all needs. We have performed comprehensive gene expression analysis with this strain during the lipid production, confirmed that this strain can produce lipid as well as fatty acids independently with the mitochodrial activity. According to this

result, high growth and high lipid production is not connected directly, indicating that the high cell density production of lipid with this strain is possible to be established.

6. Shunsuke Takahashi, Kobe University

“A new method for procuring synthetic DNA fragments, suitable for constructing long DNA sequences”

Short Summary:

A de novo-designed long DNA sequences are allowed to be constructed by assembling a series of synthetic DNA fragments, which are constructed from chemically synthesized oligonucleotides. However, this means that it is not allowed to lack the assembly materials at least any one. Here, we developed a new multiple annealing step PCR method for synthesizing DNA fragments, which is suitable for procuring assembly materials used for constructing long DNA sequences. Using this method, we demonstrated that the synthetic DNA fragments used for constructing long DNA sequences can be obtained reliably and quickly. Combined with the OGAB method, a DNA assembly method, this method thus was demonstrated to provide the rapid synthesis of de novo-designed long DNA sequences.

7. Kento Maeda, Osaka University

“Efficient mevalonic acid production in high cell density fed-batch culture of *Escherichia coli*”

Short Summary:

[Background]

Microbial production of chemical products is expected to play an important role in creating a sustainable society as an alternative to petrochemical synthesis. In this research, we aim to achieve high mevalonic acid productivity by improving culture conditions. Mevalonic acid is a valuable chemical essential for Isoprenoid synthesis and is also used for producing drugs, cosmetics, and food-additives. We select E. coli as a mevalonic acid producing host considering high synthetic ability of acetyl-CoA, known as a precursor of mevalonic acid. Previous reports have indicated that culturing E. coli under sulfur limited conditions during stationary phase leads to high mevalonic acid production. Therefore, we anticipate high production by optimizing culture conditions during stationary phase in a fed-batch culture process.

[Materials and Methods]

In this experiment, the strain Escherichia coli MG1655 (DE3) which expresses mvaE and mvaS derived from Enterococcus faecalis was used. 1-L jar fermenter was used for fed-batch cultivation. In fed-batch mode, culture volume was set to 0.5 L, initially. Culture conditions were set at 37℃ and pH 7.0. Glucose was supplied appropriately so as not to exhaust. The concentrations of metabolites were measured by HPLC.

[Results and Discussion]

To realize high production, optimization of medium composition was considered as the first step. By investigating the effect of sulfate and glucose concentration on cell growth, we determined the optimal concentration of the two nutrients. Finally, we aimed to reach OD600=50 which was the highest among fed-batch culture using minimal medium as far as we were concerned. Culture profile of the fed-batch fermentation is shown in the following Figure. As expected, OD600 was maintained approximately to 50 during the stationary phase (from 24 to 48 hours). Glucose consumption and mevalonic acid production continued throughout the stationary phase. The strain produced 0.83 g/h of mevalonic acid (72.5% of theoretical yield).

8. Sheryl Joyce Grijaldo, University of the Philippines Manila

“Antidiabetic activities of *Antidesma bunius (bignay)*: *in vitro* and *in vivo* studies, and metabolite profile”

Short Summary:

Diabetes mellitus is a complex chronic condition characterized by hyperglycemia. In 2015, an estimated 1.6 million deaths worldwide were directly caused by diabetes. Antidesma bunius, locally known as bignay, is a fruit-bearing shrub tree ubiquitously found in the Philippines. In Philippine folk medicine, it is traditionally used as antidysentery, antioxidative and anticancer agents. Previous reports have shown its efficacy in lowering blood glucose levels in in vivo models. In vivo studies in alloxan-induced hyperglycemic mouse models performed by our group has shown the anti-hyperglycemic activities of both fruit and leaf extracts of bignay, comparable to the biguanide drug metformin. Follow-up blood chemistry and histopathology results has also shown that kidney damage, a side-effect of alloxan treatment as well as a complication of diabetes mellitus, was minimized by treatment with bignay extracts. In vitro glucosidase inhibition experiments showed mild inhibition by bignay extracts, suggesting a possible mechanism for its anti-hyperglycemic activities. In vitro antioxidant experiments also showed high antioxidant activities, suggesting a possible route for reducing kidney damage. Currently, our group is analyzing bignay extracts using ultra-performance

liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) to glean the types of compounds present in bignay. Metabolite profiling shown majority of terpenoid, steroid and polyphenol compounds present in the extracts.
Keywords: diabetes mellitus, Antidesma bunius, anti-hyperglycemic activity, glucosidase inhibition, antioxidant

9. Michael Russelle Alvarez, University of the Philippines Manila

"Mount Makiling Natural Products Library (MMNLP): Generation of Natural Products Library, Anticancer Screening, and Characterization by Tandem Mass Spectrometry and *in silico* Analyses"

Short Summary:

For decades, high-throughput screening coupled with bioassay-guided isolation of natural products has led to the discovery and development of drugs currently in the market. However, such methods are often time-consuming and costly. Thus, high-throughput bioactivity screening of plant extracts needs a robust method for characterization of the extracts, as well as a bioinformatics method to predict the bioactivity and correspondingly "filter" the required extracts needed to be tested. Here, we present the results of our project which involved creating a natural products library from the fruit trees found in Mt. Makiling, Laguna – a biodiversity hotspot and one of the ASEAN Heritage Parks. These extracts were screened against an in vitro lung cancer model; in our case, the non-small cell lung carcinoma cell line A549. Top extract "hits" were subsequently profiled using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS), producing a list of putatively identified and "unknown" compounds. Identified compounds were screened for possible anticancer activity by drug-likeness to known anticancer compounds using several software: CLC-Pred (http://www.way2drug.com/Cell-line/) and EGFR-Pred (http://crdd.osdd.net/oscadd/egfrpred/). Further analyses such as in silico docking models were done to validate the drug-target interactions and get a more comprehensive analysis.
Keywords: Natural products, anticancer, UPLC-MS/MS, CLC-Pred, EGFR-Pred, in silico docking

10. Kyoung Heon Kim, Korea University

"Production of 2'-fucosyllactose, a human milk oligosaccharide, by metabolically engineered *Saccharomyces cerevisiae*"

Short Summary:

2'-Fucosyllactose (2'-FL) is one of the most abundant human milk oligosaccharides. 2'-FL has potential applications in foods due to its health benefits of promotion of bifidobacterial growth but inhibition of pathogenic microbial binding to the human gut. Due to the limited amounts of 2'-FL in human milk, microbial production of 2-FL is considered promising. So far, microbial production of 2'-FL has been attempted mostly using Escherichia coli. In this study, 2-FL was produced by using a yeast Saccharomyces cerevisiae. By introducing a salvage pathway consisting of three genes of L-fucokinase, α-1,2-fucosyltransferase, and lactose permease into the yeast, fucose and lactose were used as the substrates, and a 2'-FL titer of 503 mg/L were obtained from 120-h fed-batch fermentation fed with ethanol as a carbon source. This is the first report on 2'-FL production using S. cerevisiae as a host for producing 2-FL industrially, via the salvage pathway.

11. Ryuichi Hirota, Hiroshima University

"Synthetic Phosphorus Metabolic Pathway for Biosafety and Contamination Management of Cyanobacterial Cultivation"

Short Summary:

Recent progress in genetic engineering has enabled us to develop various types of useful microalgae. However, engineered microorganisms, including microalgae strains for producing biofuels, must be cultivated within enclosed bioreactors due to biodiversity concerns. We developed a novel, simple and cost-effective biocontainment strategy that makes host microorganisms strictly dependent on an exogenous supplement of the environmentally rare-chemical, phosphite. Considering the high applicability, cost, and extremely high containment efficacy, this strategy can contribute to developing a reliable and practical biocontainment system that ensures the biosafety of engineered microalgae. Owing to the reality of phosphite-utilizing microorganisms in the environment, this strategy also offers the selective cultivation of the desired host strain.

12. Ryo Nishimura, Kobe University

"A transcriptional control system that can be turned on and off at any time"

Short Summary:

We modified GntR regulation in Bacillus subtilis to devise transient induction systems. GntR is the repressor antagonized by gluconate to induce transcription of the gntRKPZ operon for gluconate catabolism. On the other hand, the gnt operon is repressed by glucose via carbon catabolite repression involving CcpA/P-ser-HPr, which binds to two cre sites: one located in the gnt promoter region and the other within the gntR coding region. We initiated gntKPZ encoding of enzymes for gluconate catabolism expressed independently from the operon; this allowed constitutive degradation of gluconate. Both cre sites were mutated to abolish catabolite repression. The mutated gnt promoter was set up to drive the expression of the lacZ reporter under the control of GntR. Even in the presence of glucose, lacZ was induced upon the addition of gluconate and shut down again as gluconate was consumed. Thus, modified GntR regulation enables artificial transient induction. This will allow us to design a flexible metabolic engineering system with genes expressed only temporarily as desired.

13. Kotaro Mori, Kobe University

"Conjugal transfer that easily conveys giant DNA from a cell to another"

Short Summary:

The conjugative plasmid, pLS20, isolated from Bacillus subtilis natto, has an outstanding capacity for rapid self-transfer. In addition, it can function as a helper plasmid, mediating the mobilization of an independently replicating co-resident plasmid. In this study, the oriT sequence of pLS20cat (oriTLS20) was eliminated to obtain the plasmid, pLS20catΔoriT. This resulted in the complete loss of the conjugative transfer of the plasmid but still allowed it to mobilize a co-resident mobilizable plasmid. Moreover, pLS20catΔoriT was able to mobilize longer DNA segments, up to 113 kb of chromosomal DNA containing oriTLS20, after mixing the liquid cultures of the donor and recipient for only 15 min. The chromosomal DNA mobilization mediated by pLS20catΔoriT will allow us to develop a novel genetic tool for the rapid, easy, and repetitive mobilization of longer DNA segments into a recipient chromosome.

14. Kyosuke Kita, Kobe University

"Promotion of enzyme secretion by signal peptide optimization"

Short Summary:

Phytases comprise a group of phosphatases that trim inorganic phosphates from phytic acid (IP6). In this study, we aimed to achieve the efficient secretion of phytase by Bacillus subtilis. B. subtilis laboratory standard strain 168 and its derivatives exhibit no phytase activity, whereas a natto starter secretes phytase actively. The natto phytase gene was cloned into strain RIK1285, a protease-defective derivative of 168, to construct a random library of its N-terminal fusions with 173 different signal peptides (SPs) identified in the 168 genome. The library was screened to assess the efficiency of phytase secretion based on clear zones around colonies on plates, which appeared when IP6 was hydrolyzed. The pbp SP enhanced the secretion of the natto phytase most efficiently, i.e. twice that of the original SP. Thus, the secreted natto phytase was purified and found to remove up to 3 phosphates from IP6.

15. Ryosuke Mitsui, Osaka Prefecture University

"D-Lactic acid production by engineered lactic acid tolerant *Saccharomyces cerevisiae*"

Short Summary:

To reduce the amount of neutralizer in D-lactic acid fermentation, we constructed the lactic acid tolerant yeast by using CRISPR-Cas. Then, we modified the expression of glycolytic enzyme genes and D-ldh in the lactic acid tolerant yeast, and the resultant yeast was cultured to produce D-lactic acid in non- or semi-neutralizing condition.

16. Ruri Kitayama, Osaka Prefecture University

"Peptide synthesis of glycyl tyrosine precursor using organic solvent-tolerant PST-01 protease in the presence of organic solvents"

Short Summary:

Peptide synthesis of glycyl tyrosine precursor was carried out using the organic solvent-tolerant PST-01 protease in the presence of organic solvents under various reaction conditions.

17. Yuka Sasaki, Osaka Prefecture University

"Simultaneous use of CRISPR-δ-integration and multiple promoter shuffling enhances secretory overexpression of endoglucanase by *Saccharomyces cerevisiae*"

Short Summary:

Various recombinant proteins can be produced by the yeast Saccharomyces cerevisiae cell factories; therefore, efficient recombinant protein production techniques are desirable. In this study, to establish an efficient recombinant protein production technique in S. cerevisiae, the secretory production of recombinant protein endoglucanase II (TrEG) was tested. We developed 2 novel methods for TrEG production via clustered regularly interspaced short palindromic repeat (CRISPR) -δ-integration as well as multiple promoter shuffling, which involved the pre-breakdown of the δ-sequence by the CRISPR system and subsequent δ-integration as well as the conjugation of TrEG with various promoters and subsequent δ-integration, respectively. Moreover, simultaneous use of the CRISPR-δ-integration and multiple promoter shuffling methods was also examined. The CRISPR-δ-integration method was effective for improvement of the integrated TrEG copy number and its activity, and the multiple promoter shuffling method was also beneficial for enhancing the transcriptional level of TrEG and its activity. Furthermore, simultaneous use of CRISPR-δ-integration and multiple promoter shuffling methods was the most useful. Overall, the simultaneous use of CRISPR-δ-integration and multiple promoter shuffling can be useful and easily applied for recombinant protein production.

18. Hiroki Takagi, Kobe University

" Cis, cis-muconic acid production using metabolic engineering *S. pombe*"

Short Summary:

For efficiently cis, cis-muconic acid production, we engineered S. Pombe by CRISPR Cas9 system.

19. Miki Ito, Kobe University

"Beta-carotene production using genome edited *Schizosaccharomyces pombe*"

Short Summary:

Recently, as material production using microorganisms is expected, we consider utilizing genome editing Schizosaccharomyces pombe to produce beta-carotene efficiently. Because of insertion of carotenogenic genes, it is shown that the beta-carotene is successfully produced. It is assumed that the amount of production of beta-carotene increases by adjusting the Redox balance of metabolism.

20. Rena Matsuura, Kobe University

"Cadaverine production from cellobiose using *Corynebacterium glutamicum*"

Short Summary:

Cadaverine (1,5-diaminopentane) is a monomer for polyamides. Cadaverine was produced from cellobiose using metabolically engineered Corynebacterium glutamicum strains.

21. Naoki Sato, Kobe University

"Shikimic acid production from cellobiose using *Corynebacterium glutamicum*"

Short Summary:

Shikimic acid is a valuable hydroaromatic compound and a key metabolic intermediate. In this study, we successfully produced shikimic acid from cellobiose using metabolically engineered Corynebacterium glutamicum with BGL (β-

glucosidase). Deletiob of oyk and overexpression of aroB or aroG significantly improved shikimic acid productivity.

22. Kana Oku, Kobe University

"Fatty acid production from cellulosic biomass using *Corynebacterium glutamicum*"

Short Summary:

Fatty acid is made from natural fats and oils, but there are problems of raw matelials limitation. In this study, we succeeded in producing fatty acid from glucose and cellobiose using C. gutamicum.

23. Yuki Mori, Kobe University

"Production of n-butylamine in *Escherichia coli* by combining enzyme cascade reaction and metabolic pathway through acetyl-CoA"

Short Summary:

Amine is produced using a chemcal process. Therefore, we try to produce amine in bioprocess by using engineering E. coli. In this study, we demonstrate n-butylamine production. To produce n-butylamine, we combined enzyme cascade reaction to produce amine from alcohol and metabolic pathway through acetyl-CoA.

24. Daichi Satowa, Kobe University

"Metabolic Engineering of *Escherichia coli* for mevalonate production from cellobiose"

Short Summary:

Bioprocess is suitable for mevalonate production, but there still remain problems that yields of mevalonate is not sufficient and that cinversion biomass to glucose requires high cost. In this study, we tried to efficiently produce mevalonate from cellobiose by engineered E. coli.

25. Shogo Uchio, Kobe University

"Metabolic engineering of *Escherichia coli* for isopentenol production from glucose"

Short Summary:

Isopentenol is promising biofuel with favorable combustion properties and precursor for flavor compounds and isoprene. In this study, we tried to produce isopentenol from glucose by engineered E. coli.

26. Tatsuya Takahashi, Kobe University

"Metabolic engineering of *Escherichia coli* for production of p-aminobenzoic acid from glucose"

Short Summary:

p-aminobenzoic acid (PABA) is a building block for pharmaceuticals, and it has a great potential to serve as a raw material for aromatic polymers. In this study, we aimed PABA production from glucose with engineered E. coli.

27. Ryosuke Fujiwara, Kobe University

"Metabolic Design of *Escherichia coli* for Production of Shikimate Pathway Derivatives"

Short Summary:

We further modified the metabolism in E. coli to be confined the use of the glucose to the production of a target

compound, cis,cis-muconic acid. After that, to regain the capacity of cell growth, we introduced an exogenous pathway that supplements the metabolites necessary for cell growth with xylose into the strain.

28. Hikaru Mizuno, Kanazawa University

"Anaerobic glucose consumption is accelerated at temperature above the upper limit of cell growth in *Corynebacterium glutamicum*"

Short Summary:

Corynebacterium glutamicum grows at 30ºC as the optimal growth temperature, and ceases cell growth above 40ºC under aerobic conditions. In contrast, under anaerobic conditions, C. glutamicum produces lactate and succinate from glucose without cell growth. We found that glucose consumption rate under anaerobic conditions was the highest at 42.5ºC with 24% increase compared to that at 30ºC. Transcriptional analysis showed that genes involved in glycolysis and glucose uptake were upregulated at 42.5ºC. Moreover, the activity of some enzymes in glycolysis and TCA cycle was also upregulated at 42.5ºC. This study showed that the optimal temperature for cell growth and glucose consumption was largely different in *C. glutamicum*.

29. Shunsuke Kobayashi, Kanazawa University

"Bidirectional switching of carbon flow between glycolysis and pentose phosphate pathway by oxygen level in *Corynebacterium glutamicum*"

Short Summary:

Corynebacterium glutamicum is a facultative anaerobic bacterium used for the production of amino acids and organic acids under aerobic and anaerobic conditions, respectively. The carbon flow in *C. glutamicum* is passed through both glycolysis and pentose phosphate pathway (PPP) under aerobic conditions, whereas it is exclusively through glycolysis under anaerobic conditions. Because NADPH is mainly produced in PPP, splitting of carbon flow under aerobic conditions resulted in decreased titer and yield of a target product which requires NADPH for its synthesis. To improve titer and yield, increasing the carbon flow into PPP is required, for example by shutting off the carbon flow into glycolysis under aerobic conditions. In contrast, glycolysis needs to be reactivated for organic acids production under anaerobic conditions. To achieve this, the native promoter of glucose 6-phosphate isomerase was replaced with the anaerobic-specific *ldhA* promoter. Using this system, we demonstrated that the metabolic switching of carbon flow between glycolysis and PPP by oxygen level for efficient production of 1,5-diaminopentane and succinate under aerobic and anaerobic conditions, respectively, in a single *C. glutamicum* strain. This approach could be useful to switch the carbon flow by oxygen level in the other metabolic pathways.

30. Kengo Sasaki, Kobe University

"A model culture system for the in vitro human colonic microbiota of ulcerative colitis"

Short Summary:

Compositional alteration of the gut microbiota is associated with ulcerative colitis (UC). We established a model culture system for the *in vitro* human colonic microbiota of UC, which will be helpful for determining medical interventions for the disease. 16S rRNA sequencing confirmed that UC models successfully developed from the fecal inoculum and retained the bacterial species biodiversity of UC feces. UC models closely reproduced the microbial components,

although not completely, and successfully preserved distinct clusters from healthy subjects (HS), as observed in the feces. The relative abundance of bacteria belonging to family *Lachnospiraceae* was significantly decreased in the UC models compared to in the HS, as observed in the feces. Our system detected significantly lower butyrogenesis in the UC models than in HS, correlating with the decreased abundance of *Lachnospiraceae*. Interestingly, the relative abundance of *Lachnospiraceae* did not correlate with disease activity (defined as partial Mayo score), suggesting that *Lachnospiraceae* persisted in UC patients at a decreased level, irrespective of altered disease activity. Our model detects deregulation in the intestinal environment in UC patients and may be useful for simulating the effect of probiotics.

31. Gregory Guirimand, Kobe University

"Combined actions of lytic polysaccharide monooxygenases (LPMOs) and a cell surface-engineered strain of S. cerevisiae for xylitol production and nanofibrillation of Kraft pulp."

Short Summary:

Industrial production of xylitol from purified D-xylose involves a costly and polluting catalytic process. Biotechnological production of xylitol from lignocellulosic waste may therefore constitute an advantageous option. In this study, xylitol was produced from Kraft pulp by using a recombinant *S. cerevisiae* YPH499 strain expressing cytosolic xylose reductase (XR), along with β-D-glucosidase (BGL), xylosidase (XYL) and xylanase (XYN) enzymes co-displayed on the cell surface, in combination with two different lytic polysaccharide monooxygenases (LPMOs) respectively from the AA9, and AA14 families. These two LPMOs led to a significant improvement of the xylitol production, as well as increased biomass nanofibrillation.