The 90th Anniversary Meeting Program & Abstracts

The 90th Anniversary Meeting

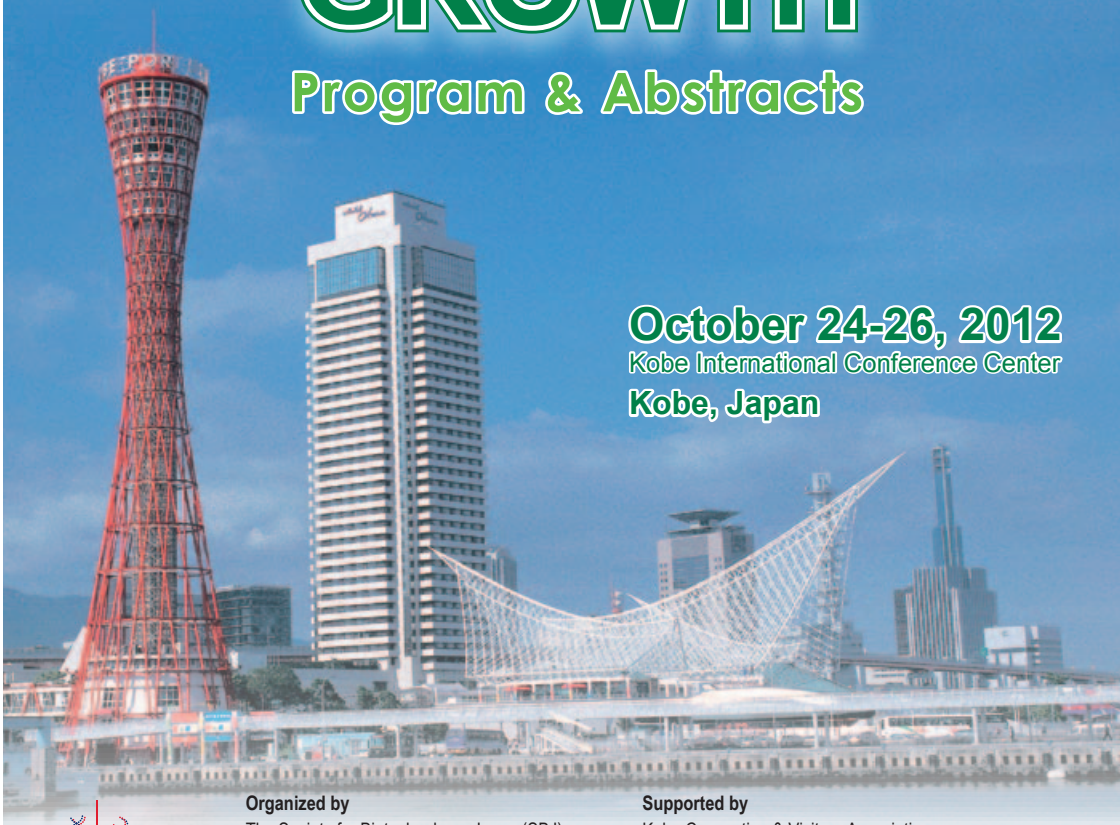
The Society for Biotechnology, Japan

International Symposium on

BIOTECHNOLOGY FOR

GREEN GROWTH

Program & Abstracts



October 24-26, 2012
Kobe International Conference Center
Kobe, Japan

Organized by
The Society for Biotechnology, Japan (SBJ)
Japan Science and Technology Agency (JST)

Supported by
Kobe Convention & Visitors Association
Nakauchi Tsutomu Convention Shinko Zaidan Foundation
Suntory Institute for Bioorganic Research

The Society for Biotechnology, Japan

ERRATA

◆The presentation below has been cancelled.

Abstract: Page 50

3Fa04 Production of the bio-butanol based on the continuous in situ　fermentation and adsorption (ISFA) technology　○Hanjie YING[1,2], Yong CHEN[2,3], Jinglan WU[2,3]([1]State Key Laboratory of Materials-Oriented Chemical Engineering,China, [2] College of Biotechnology and Pharmaceutical Engineering,　Nanjing University of Technology, China, [3] National Engineering　Technique Research Center for Biotechnology, China)

ご迷惑をおかけしましたことをお詫びし、ここに訂正いたします。

We apologize for any inconvenience this may have caused.

Table of Contents

Greetings

Dear Colleagues,

We would like to welcome all of you to the 90th Anniversary Meeting of Society for Biotechnology, Japan (SBJ), which will be held at the Kobe International Conference Center (KICC), Kobe Port Island, from October 23rd (Tue) to 26th (Fri), 2012. To commemorate the 90th anniversary with all the participants, the meeting is organized with the concepts “from academia to industry”, “from Japan to the world”, and “from senior to junior”.

At the opening day (October 23rd), the commemorative ceremony for the 90th anniversary will be held in the morning, followed by award presentation ceremony in the afternoon at the Main Hall of KICC. The celebration dinner will be held at Kobe Portopia Hotel in the evening of October 23rd. From October 24th to 26th, symposia, general sessions, and luncheon seminars will be held at the KICC conference rooms, and technical exhibitions, including SBJ commemoration displays, will be convened at the Reception Hall of KICC. There will also be the international symposium (all presentations and discussions are given in English) on Biotechnology for Green Growth.

KICC and Kobe Portopia Hotel are both located in the Kobe Convention Complex which is central to Port Island. Many restaurants, fashion towns, parks, and museums are scattered around Kobe, the city of convention, culture, and leisure. The four-day long meeting will be an excellent opportunity for you to meet with SBJ members and colleagues from abroad.

We are very pleased to welcome you to the exciting meeting which will be remembered for years to come. Finally, we would like to thank all the members of the Executive Committee, as well as our colleagues who contributed to the success of this memorable event.

Hisao Ohtake
Chair of the Executive Committee
The 90th Anniversary Meeting

Satoshi Harashima
President
The Society for Biotechnology, Japan

About SBJ

The Society for Biotechnology, Japan (SBJ), formerly the Society of Fermentation Technology, Japan, was founded as the Osaka Brewing Society in 1923. SBJ consists of approximately 3200 individual members, 250 institutional and supporting members, representing throughout Asia as well as other regions.

SBJ monthly publishes journals in both English and Japanese, also provides information and services online across the study of biotechnology. The Journal of Bioscience and Bioengineering (JBB), first published as the Journal of Fermentation Technology in 1973, is now highly appreciated by scientists throughout the world (Impact factor in 2011: 1.793).

Events and seminars are conducted throughout a year. The annual meeting is held in autumn covering for 3 days. Approximately 650 topics are discussed with more than 1000 participants.

Acknowledging the world information is becoming more globalized each day. SBJ aims to promote opportunities to network and exchange ideas among its members to disseminate SBJ's approaches; "the academia to the industry" and "to contribute to the society" to the public.

Please visit our web site at http://www.sbj.or.jp/e/ for more information.

The 90th Anniversary Meeting of the Society for Biotechnology, Japan

Board Members

President:	Satoshi Harashima
Vice President:	Kenji Sonomoto
	Kenzo Yanagi
Chairperson:	Hisao Ohtake
Vice-Chairperson:	Eiichiro Fukusaki
	Masahito Taya

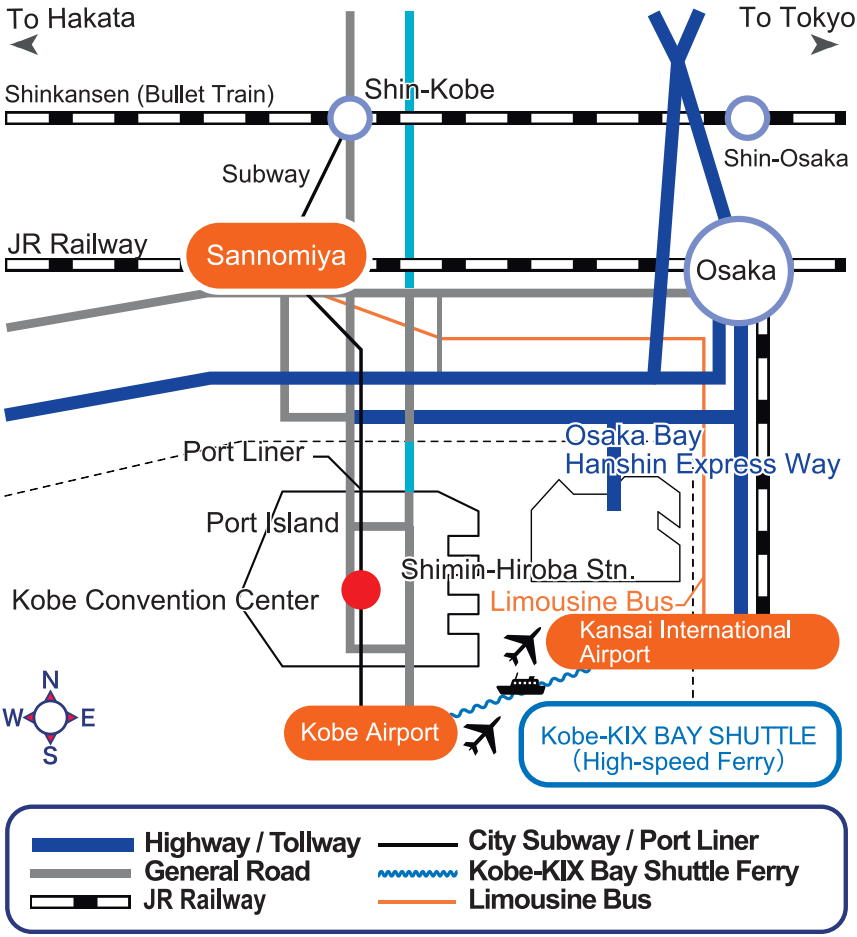
International Symposium Organizing Committee:

Kohsuke Honda
Shigeru Kitani
Ryo Misaki
Takuya Nihira
Hiroshi Umakoshi
Hitoshi Wake

International Symposium Secretariat:

The Society for Biotechnology, Japan
c/o Department of Biotechnology, Graduate School of Engineering,
Osaka University
2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
Tel: +81-6-6876-2731, Fax: +81-6-6879-2034
E-mail: sympo2012@sbj.or.jp

Access



International Flight (Arriving Kansai Int'l Airport)

Kansai International Airport (KIX) is the closest international gateway for Kobe and is located on the Osaka Bay.

Transfer from KIX to Kobe:

By Taxi: It takes about 70 min. and costs approx. 22,000yen (From KIX to Sannomiya, downtown Kobe).

By Limousine Bus: It takes about 70 min. and costs 1,900yen (KIX to Sannomiya). The bus runs every 10 to 20 min. from 6:20am till 10:40pm.

By Bay Shuttle Ferry (to Kobe Airport): It takes about 30 min. and costs 1,800yen. The ferry runs every 45 to 60 min. from 7:15am till 10:00pm. Free bus transfer is available from KIX to KIX Ferry dockland. From Kobe airport, take Port Liner monorail to Shimin Hiroba, the station closest to the Kobe International Conference Center.

JR Shinkansen (Bullet) Train (Arriving at Shin-Kobe Station)

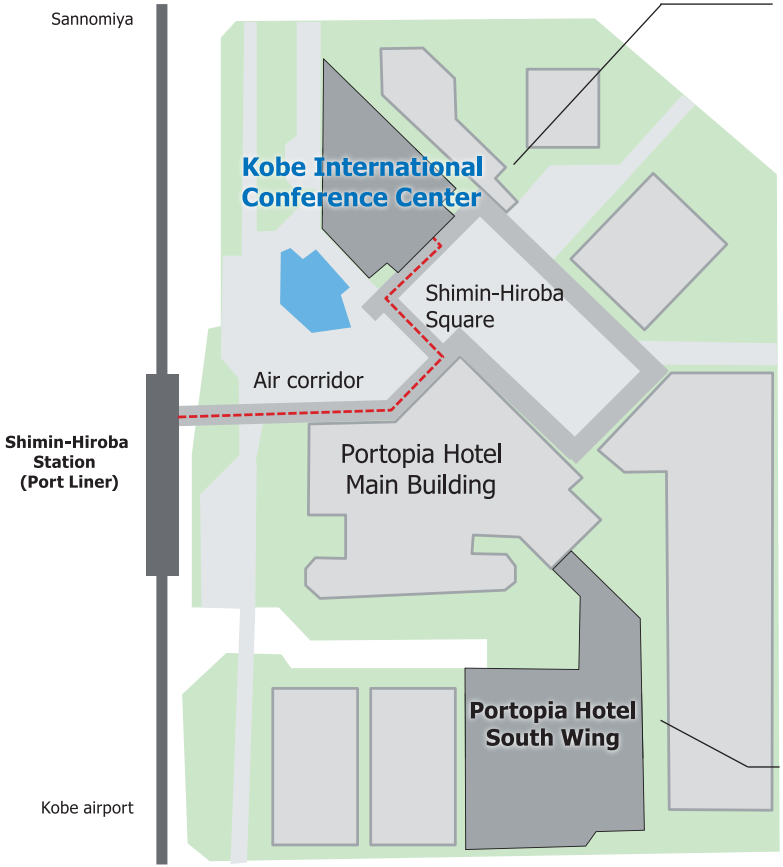
It takes about 2 hours and 50 min. and costs approx. 15,000yen from Tokyo to Shin-Kobe by bullet train. The bullet train bound for Shin-Kobe from Tokyo operates from 6am till 8:30pm. From Nagoya, it takes about 70 min. and costs approx. 8,000yen. From Kyoto, it takes about 30 min. and costs approx. 3,500yen.
Transfer from Shin-Kobe to downtown Kobe, Sannomiya:
By Taxi: It takes about 5 min. and costs approx. 660yen.
By City Subway: It takes about 2 min. and costs 200yen.

Local or Express Rail (Arriving at downtown Kobe, Sannomiya Station)

It takes about 20 min. and costs approx. 400yen by JR Lines from Osaka.
It takes about 50 min. and costs approx. 1,050yen from Kyoto.

ABOUT SANNOMIYA & GETTING TO PORT ISLAND

“Port Liner” monorail: The monorail bound for Kobe Airport from Sannomiya station runs every 5 min. and stops at “Shimin Hiroba”, the station closest to the Kobe International Coference Center. From Sannomiya station to Shimin Hiroba station, it takes about 10 min. and costs 240yen by Port Liner.
By Taxi: It takes about 10 min. and approx. 1500yen from Sannomiya.



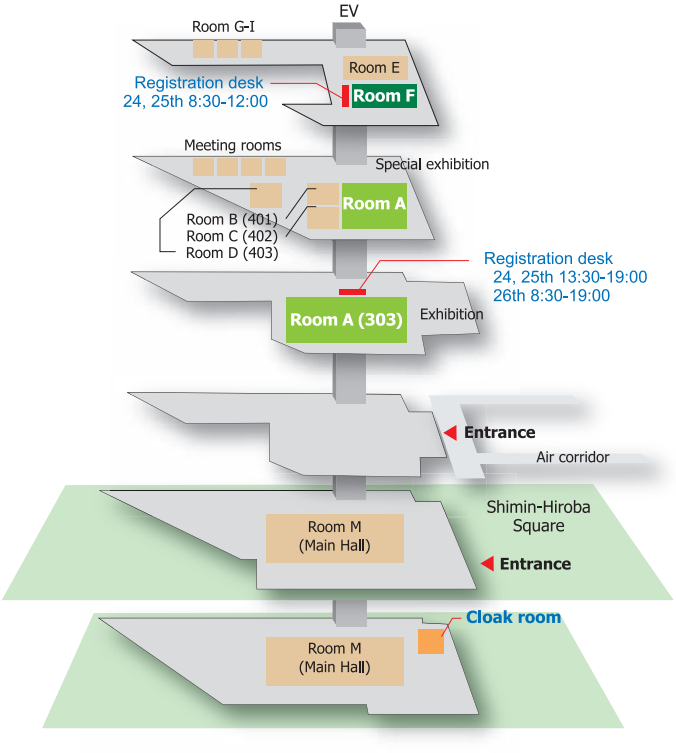
Kobe International Conference Center

Reception for 90th anniversary ceremony	1F Foyer (23rd Oct)
90th Anniversary ceremony, Award ceremony, Award lectures	1F Room M (Main hall)
Registration desk for international symposium	**3F Entrance hall** **24th Oct 13:30–19:00** **25th Oct 13:30–19:00** **26th Oct 8:30–19:00** **5F Room F** **24th Oct 8:30–12:00** **25th Oct 8:30–12:00**
Symposium/ **International symposium**	**3F Room A** **5F Room F** Room E
General presentations	4F Room B～D 5F Room G～I
Young researchers committee for biotechnology	4F Room B
Luncheon seminars	5F Room E, F 4F Room B～D
Exhibition	3F Reception hall
Special exhibition	4F Lounge
Cloak room	**BF Rehearsal room**

Portopia Hotel

90th Anniversary celebration dinner	South Wing, Ohwada 18:00–20:00 23rd Oct

Floor Plan



5F

Registration desk
(24, 25th 8:30-12:00)
Room F
Room E, G-I

4F

Room A (Balcony seats)
Room B-D
Special exhibition
Meeting rooms

3F

Registration desk
(24, 25th 13:30-19:00)
(26th 8:30-19:00)
Room A
Exhibition

2F

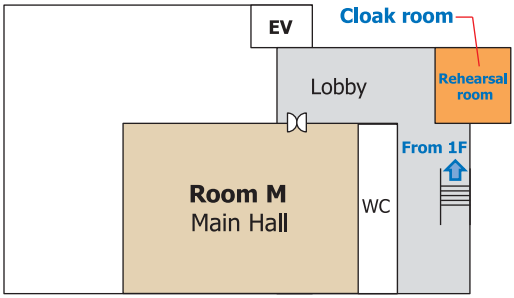
Entrance

1F

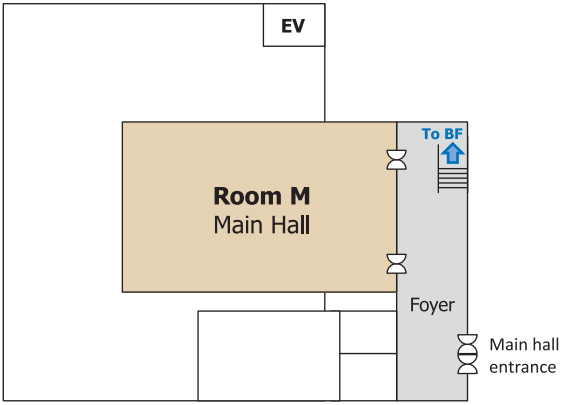
Entrance

BF

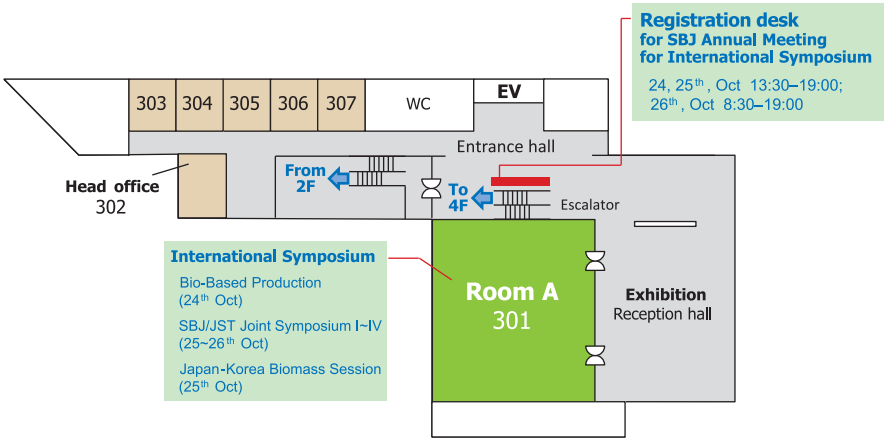
Cloak room
Room M



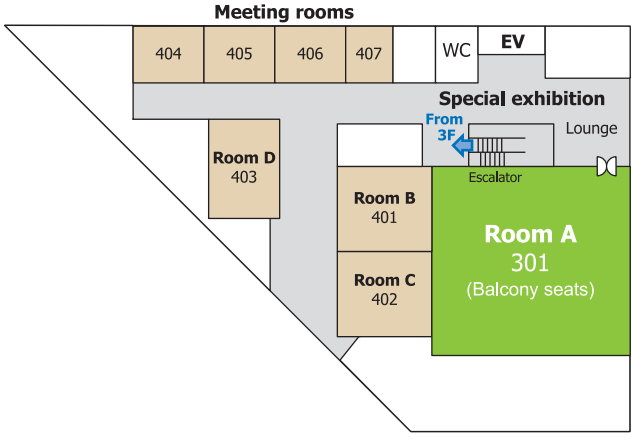
Kobe International Conference Center BF



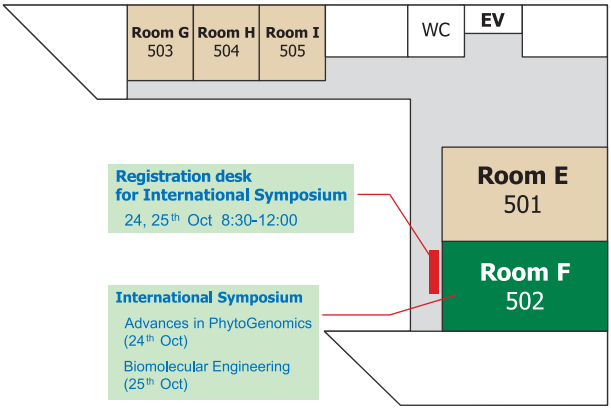
Kobe International Conference Center 1F



Kobe International Conference Center 3F



Kobe International Conference Center 4F



Kobe International Conference Center 5F

Program at a Glance

	10/24 (WED)	10/25 (THU)	10/26 (FRI)
	Advances in PhytoGenomics **Room F 502**	Biomolecular Engineering (co-organized by YABEC) **Room F 502**	SBJ/JST Joint Symposium II -Biorefinery- **Room A 301**
9:00	9:00-12:00	9:00-9:30 **3Fa01**	9:00-10:40 **4Aa01-4Aa04**
9:30		9:30-9:40 **BREAK**	
10:00		9:40-11:45	
10:30	**2Fa01-2Fa08**	**3Fa02-3Fa05**	10:40-10:45 **BREAK**
11:00			10:45-12:00
11:30		11:45-14:00	**4Aa05-4Aa07**
12:00	12:00-13:50 **BREAK**	**BREAK**	12:00-14:00 **BREAK**
	Bio-Based Production **Room A 301**	SBJ/JST Joint Symposium I -General Session- **Room A 301**	SBJ/JST Joint Symposium III -Biofuel Production by Microalgae- **Room A 301**
13:50	13:50-16:20		
14:00		14:00-15:25	14:00-15:15
14:30	**2Ap01-2Ap05**	**3Ap01-3Ap03**	**4Ap01-4Ap03**
15:00			15:15-15:25 **BREAK**
15:30		15:25-15:35 **BREAK**	15:25-16:20
16:00		15:35-16:20 **3Ap04**	**4Ap04-4Ap05**
	16:20-16:30 **BREAK**	16:20-16:40 **BREAK**	16:20-16:40 **BREAK**
16:30	16:30-19:00	Japan-Korea Biomass Session **Room A 301**	SBJ/JST Joint Symposium IV -System & Synthetic Biotechnology- **Room A 301**
17:00	**2Ap06-2Ap10**	16:40-17:45 **3Ap05-3Ap07**	16:40-17:55 **4Ap06-4Ap08**
17:30		17:45-17:55 **BREAK**	
18:00		17:55-19:00	17:55-18:05 **BREAK**
18:30		**3Ap08-3Ap10**	18:05-19:00 **4Ap09-4Ap10**
19:00			

Program

10/24 (Wednesday)

Advances in PhytoGenomics

Organizers: Toshiya MURANAKA (Osaka University)
Kiichi FUKUI (Osaka University)

Chairperson: Kiichi FUKUI (Osaka University)

9:00–9:25	**2Fa01**	Functional chromosome dynamics with a link to synthetic green biotechnology ○Ingo SCHUBERT (Department of Cytogenetics and Genome Analysis, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany)
9:25–9:45	**2Fa02**	Manipulation of plant traits using novel gene silencing system, CRES-T (Chimeric REpressor gene Silencing Technology) ○Nobutaka MITSUDA[1], Masaru OHME-TAKAGI[1,2] ([1]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan, [2]Institute for Environmental Science and Technology (IEST), Saitama University, Japan)
9:45–10:05	**2Fa03**	A top-down approach to function the *Synechocystis* PCC6803 genome in a novel cloning host *Bacillus subtilis* 168 ○Mitsuhiro ITAYA (Institute for Advanced Biosciences, Keio University, Japan)

Chairperson: Toshiya MURANAKA (Osaka University)

10:05–10:30	**2Fa04**	Decoding the chemical diversity and evolution written in the genomes of the Asteraceae ○Dae-Kyun RO[1], Don Trinh NGUYEN[1], Nobuhiro IKEZAWA[2], Vince QU[1] ([1]Department of Biological Sciences, University of Calgary, Alberta, Canada, [2]Department of Biotechnology, Osaka University, Japan)
10:30–10:55	**2Fa05**	Cassava molecular breeding and functional genomics –Challenges and opportunities– ○Jarunya NARANGAJAVANA (Center for Cassava Molecular Biotechnology, Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand)
10:55–11:15	**2Fa06**	KOMICS: metabolomics databases and tools for biotechnology ○Daisuke SHIBATA, Takeshi ARA, Hideyuki SUZUKI, Nozomu SAKURAI (Kazusa DNA Research Institute, Chiba, Japan)

Chairperson: Kazuhito FUJIYAMA (Osaka University)

11:15–11:40	**2Fa07**	Dammarene-type ginsenoside metabolic engineering –Characterization of genes involved in dammarenediol saponin biosynthesis– Jung-Yeon HAN, ○Yong-Eui CHOI (Department of Forest Resources, College of Forest and Environmental Science, Kangwon National University, South Korea)
11:40–12:00	**2Fa08**	Production of plant triterpenoids in engineered yeast ○Toshiya MURANAKA, Hikaru SEKI (Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan)

Bio-Based Production

Organizers: Hiroshi SHIMIZU (Osaka Univeristy)
Eiichiro FUKUSAKI (Osaka University)

Chairperson: Hiroshi SHIMIZU (Osaka Univeristy)

13:50–14:20	**2Ap01**	Living microbes as selective and efficient redox biocatalysts ○Andreas SCHMID, Katja BÜHLER, Mattijs JULSING, Bruno BÜHLER (Laboratory of Chemical Biotechnology, TU Dortmund University, Germany)
14:20–14:50	**2Ap02**	Toward biological replacement of petroleum ○James C. LIAO (Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, USA)
14:50–15:20	**2Ap03**	*Bacillus subtilis* cell factory for production of *scyllo*-inositol promising for Alzheimer's disease ○Ken-ichi YOSHIDA[1], Chumsakul ONUMA[2], Shu ISHIKAWA[2], Naotake OGASAWARA[2] ([1] Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, Japan, [2] Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), Japan)
15:20–15:50	**2Ap04**	Engineering of signal transduction for enhanced production of biopharmaceuticals and bioenergy ○Jian-Jiang ZHONG[1], Yang-Chun YONG[2] ([1] School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, China, [2] School of the Environment, Jiangsu University, China)

15:50–16:20	**2Ap05**	Synthetic biology for the comprehension of biomolecular networks ◯Masahiro OKAMOTO[1,2] ([1] Department of Bioinformatics, Graduate School of Systems Life Sciences, Kyushu University, Japan, [2] Synthetic Systems Biology Research Center (SSBRC), Kyushu University, Japan)
16:20–16:30		BREAK

Chairperson: Eiichiro FUKUSAKI (Osaka University)

16:30–17:00	**2Ap06**	Strategies and technologies towards tailor made fuels from biomass ◯Jochen BUECHS[1], Tobias KLEMENT[1], Martin KUNZE[1], Gernot JAEGER[1], Sandra WEWETZER[1], Frederike CARSTENSEN[2], Matthias WESSLING[2], Philipp GRANDE[3], Pablo Domínguez DE MARIA[3], Antje SPIESS[4] ([1] AVT - Biochemical Engineering, RWTH Aachen University, Germany, [2] AVT - Chemical Process Engineering, RWTH Aachen University, Germany, [3] Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Germany, [4] AVT - Enzyme Process Technology, RWTH Aachen University, Germany)
17:00–17:30	**2Ap07**	Actinobacteria tyrosinase and its applications to biomaterials ◯Byung-Gee KIM[1,2,3], Nahum LEE[1,3], SangHyuk LEE[2,3], ChangHyun SONG[1,3] ([1] School of Chemical and Biological Engineering, Seoul National University, Korea, [2] Institute of Molecular Biology and Genetics, Seoul National University, Korea, [3] Institute of Bioengineering, Seoul National University, Korea)
17:30–18:00	**2Ap08**	Cyberinfrastructure for metabolomics and synthetic biology ◯Masanori ARITA[1,2] ([1]Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Japan, [2] RIKEN Plant Science Center, Japan)
18:00–18:30	**2Ap09**	Bioprocess engineering on biofuels and bio-based chemicals production from microalgae ◯Jo-Shu CHANG[1,2,3], Chun-Yen CHEN[2] ([1]Department of Chemical Engineering, National Cheng Kung University, Taiwan, [2]Center for Bioscience and Biotechnology, National Cheng Kung University, Taiwan, [3]Research Center for Energy Technology and Strategy, National Cheng Kung University, Taiwan)

18:30–19:00	**2Ap10**	Systems metabolic engineering –Rational design of microbial cell factories ◯Hiroshi SHIMIZU[1], Chikara FURUSAWA[1,2], Takashi HIRASAWA[1], Naoaki ONO[3], Katsunori YOSHIKAWA[1], Yoshihiro TOYA[1] ([1] Department of Bioinformatic Engineering, Osaka University, Japan, [2] Quantitative Biology Center (QBiC), RIKEN, Japan, [3] Graduate School of Information Science, Nara Institute of Science and Technology (NAIST), Japan)

10/25 (Thursday)

Biomolecular Engineering [co-organized by Young Asian Biochemical Engineers' Community (YABEC)]

Organizers: Takeshi OMASA (University of Tokushima)
Noriho KAMIYA (Kyushu University)

Chairperson: Takeshi OMASA (University of Tokushima)

9:00–9:30	**3Fa01**	Reaction evaluation and new process design in composting of biological wastes~Young Asian Biotechnologist Prize~ ○Jingchun TANG[1], Arata KATAYAMA[2] ([1]College of Environmental Science and Engineering, Nankai University, China, [2] EcoTopia Science Institute, Nagoya University, Japan)
9:30–9:40		BREAK

Chairperson: Noriho KAMIYA (Kyushu University)

9:40–10:05	**3Fa02**	Organic solvent stable enzymes ○Hiroyasu OGINO (Department of Chemical Engineering, Osaka Prefecture University, Japan)
10:05–10:30	**3Fa03**	Engineering of aminoacyl-tRNA synthetases for residue-specific incorporation of amino acid analogues into proteins ○Tae Hyeon YOO (Department of Molecular Science and Technology, Department of Applied Chemistry and Biological Engineering, Ajou University, Korea)
10:30–10:55	**3Fa04**	Production of the bio-butanol based on the continuous in situ fermentation and adsorption (ISFA) technology ○Hanjie YING[1,2], Yong CHEN[2,3], Jinglan WU[2,3] ([1]State Key Laboratory of Materials-Oriented Chemical Engineering, China, [2] College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, China, [3] National Engineering Technique Research Center for Biotechnology, China)

Chairperson: Takeshi OMASA (University of Tokushima)

10:55–11:20	**3Fa05**	Functional properties of diet ginger (*Zingiber officinale* Roscoe, Zingiberaceae) for antioxidant, anti-pigmentation, anti-cancer, anti-bacterial and wound repair Pei-Fang WU, Yi-Ting CHOU, ○Hui-Min WANG (Department of Fragrance and Cosmetic Science, Kaohsiung Medical University, Kaohsiung, Taiwan, ROC)
11:20–11:45	**3Fa06**	Enzyme engineering with the aid of ionic liquids ○Noriho KAMIYA[1,2] ([1]Center for Future Chemistry, Kyushu Univeristy, Japan, [2]Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, Japan)

SBJ/JST Joint Symposium I –General Session–

Organizers: Tadashi MATSUNAGA (Tokyo University of Agriculture and Technology)
Hisao OHTAKE (Osaka University)
Shigeru ISHIMASA (JST)

Chairperson: Kohsuke HONDA (Osaka University)

14:00–14:05		Welcome adress ○Hisao OHTAKE (Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan)
14:05–14:15	**3Ap01**	Introduction of the JST CREST/PRESTO program research area “Creation of basic technology for improved bioenergy production through functional analysis and regulation of algae and other aquatic microorganisms” ○Tadashi MATSUNAGA (Tokyo University of Agriculture and Technology, Japan)

Chairperson: Masafumi YOHDA (Tokyo University of Agriculture and Technology)

14:15–14:50	**3Ap02**	Can biotechnology address the global energy crisis? ○Jonathan D. TRENT[1,2] ([1] Bioengieering Branch, NASA Ames Research Center, USA, [2] Biomolecular Engineering, University of California Santa Cruz, USA)

Chairperson: Akira SATO (Yamaha Motor, Co.)

14:50–15:25	**3Ap03**	Food and fuel from microalgae: Integration of photosynthesis and photovoltaics allows to achieve a positive energy balance of algae biomass production ◯Mario R. TREDICI (University of Florence, Italy)
15:25–15:35		BREAK

Chairperson: Tsuyoshi TANAKA (Tokyo University of Agriculture and Technology)

15:35–16:10	**3Ap04**	Biorefinery of microalgae ◯René H. WIJFFELS (Wageningen University, Bioprocess Engineering, the Netherlands)

Chairperson: Kohsuke HONDA (Osaka University)

16:10–16:20		Closing remarks ◯Shigeru ISHIMASA (Japan Science and Technology Angency (JST), Japan)

Japan-Korea Biomass Session

Organizers: Atsuhiko SHINMYO (Nara Institute of Science and Technology)
Shigeaki FUJIKAWA (NPO Kinki Bio-Industry Development Organization)

Chairperson: Shigeaki FUJIKAWA (NPO Kinki Bio-Industry Development Organization)

16:40–16:45		Opening remarks ◯Atsuhiko SHINMYO (Nara Institute of Science and Technology, Japan)
16:45–17:05	**3Ap05**	Improvement of ethanol yield from xylose by breeding of industrial yeast ◯Haruyo HATANAKA, Tomohide MAEDA, Yuri YASUE, Kazue NISHIYAMA (Suntory Business Expert Limited, Institute for Microbial Science, Japan)

17:05–17:25	**3Ap06**	Applications of microbial factory technology to production of bioethanol from cellulosic biomass Jung-Hyun JO[1], Sang-Min JUNG[1], Yong-Cheol PARK[2], Yong-Su JIN[3], ○Jin-Ho SEO[1] ([1]Department of Agricultural Biotechnology, Seoul National University, Korea, [2] Department of Advanced Fermentation Fusion Science and Technology, Kookmin University, Korea, [3] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, USA)
17:25–17:45	**3Ap07**	Potential in bioethanol production from waste paper sludge in pulp-based biorefinery ○Enoch Y. PARK[1,2], Joni PRASETYO[1], Kazuya NARUSE[2], Tatsuya KATO[2] ([1] Laboratory of Biotechnology, Graduate School of Science and Technology, Shizuoka University, Japan, [2] Laboratory of Biotechnology, Faculty of Agriculture, Shizuoka University, Japan)
17:45–17:55		BREAK

Chairperson: Moon-Hee SUNG (Kookmin University)

17:55–18:15	**3Ap08**	Application of Metabolomics to high resolution phenotype analysis ○Eiichiro FUKUSAKI (Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan)
18:15–18:35	**3Ap09**	Biological production of 3-hydroxypropionic acid from glycerol ○Sunghoon PARK (School of Chemical and Biomolecular Engineering, Pusan National University, Korea)
18:35–18:55	**3Ap10**	Biodiesel production by enzymatic process and the utilization of crude glycerol Hah Young YOO, Laxmi Prasad THAPA, Ja Hyun LEE, Sang Jun LEE, ○Seung Wook KIM (Department of Chemical and Biological Engineering, Korea University, Korea)
18:55–19:00		Closing remarks ○Sunghoon PARK (Pusan National University, Korea)

10/26 (Friday)

SBJ/JST Joint Symposium II –Biorefinery–

Organizers: Haruko TAKEYAMA (Waseda University)
Yoshihiro SHIRAIWA (Tsukuba University)

Chairperson: Haruko TAKEYAMA (Waseda University)

9:00–9:25	**4Aa01**	Understanding and altering the unique metabolism of hyperthermophilic archaea ◯Haruyuki ATOMI[1,2], Kunio MIKI[2,3], Tadayuki IMANAKA[2,4] ([1]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Japan, [2]CREST, Japan Science and Technology Agency (JST), [3]Department of Chemistry, Graduate School of Science, Kyoto University, Japan, [4]Department of Biotechnology, College of Life Sciences, Ritsumeikan University, Japan)
9:25–9:50	**4Aa02**	Blue ocean for blue biotechnology – Marine bioenergy production ◯Choul-Gyun LEE[1,2] ([1]Department of Biotechnology, Inha University, Korea, [2]Korea National Consortium on Marine Bioenergy Research)
9:50–10:15	**4Aa03**	Establishment of innovative technology to create new microalgal strains increasing biofuel production by polyploidization and heavy-ion beam irradiation ◯Shigeyuki KAWANO[1,2], Koichi WATANABE[1,2], Shuhei OTA[1,2] ([1]Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Japan, [2]CREST, Japan Science and Technology Agency (JST), Japan)

Chairperson: Yoshihiro SHIRAIWA (Tsukuba University)

10:15–10:40	**4Aa04**	New dimensions in microalgae biomass production for bioenergy ◯Otto PULZ (IGV, Nuthetal, Germany)
10:40–10:45		BREAK

Chairperson: Yoshihiro SHIRAIWA (Tsukuba University)

10:45–11:10	**4Aa05**	Development of sustainable biorefinery based on microalgae ○Akihiko KONDO[1,2] ([1]Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, Japan, [2]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Japan)
11:10–11:35	**4Aa06**	*Chlamydomonas reinhardtii* - A powerful host for the production of biofuels and high value products ○Olaf KRUSE (Bielefeld University, Department of Biology/ Center for Biotechnology, Algae Biotechnology & Bioenergy Group, Germany)

Chairperson: Haruko TAKEYAMA (Waseda University)

11:35–12:00	**4Aa07**	Alkenone biosynthesis by marine Haptophytes as biorefinery for renewable energy production ○Yoshihiro SHIRAIWA[1,2] ([1]Faculty of Life & Environmental Sciences, University of Tsukuba, Japan, [2] CREST, Japan Science and Technology Agency (JST), Japan)

SBJ/JST Joint Symposium III –Biofuel Production by Microalgae–

Organizers: Masayuki OHMORI (Chuo University)
Shigeru OKADA (The University of Tokyo)

Chairperson: Masayuki OHMORI (Chuo University)

14:00–14:20	**4Ap01**	Characterization of hydrocarbon biosynthesis and secretion mechanisms by the green microalga, *Botryococcus braunii* to control biofuel production ○Shigeru OKADA[1,5], Ikuro ABE[2,5], Tetsuko NOGUCHI[3,5], Takeshi OHAMA[4,5] ([1]Department of Aquatic Biosciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan, [2] Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan, [3] Department of Biological Sciences, Faculty of Science, Nara Women's University, Japan, [4] Department of Environmental Systems Engineering, Kochi University of Technology, Japan, [5] CREST, Japan Science and Technology Agency (JST), Japan)

14:20–14:55	**4Ap02**	Metabolic engineering of cyanobacteria for the light-powered production of hydrogen from water ◯Matthias RÖGNER (Plant Biochemistry, Faculty of Biology & Biotechnology, Ruhr-University Bochum, Germany)
14:55–15:15	**4Ap03**	Selection strategies for the promising microalgal strains for algae-fuel production ◯Hideaki MIYASHITA[1], Norihide KURANO[2] ([1]Department of Interdisciplinary Environment, Graduate School of Human and Environmental Studies, Kyoto University, Japan, [2]Research laboratories, DENSO CORPORATION, Japan)
15:15–15:25		BREAK

Chairperson: Shigeru OKADA (The University of Tokyo)

15:25–16:00	**4Ap04**	Elucidation of novel triterpene pathways in *Botryococcus* and engineering plants for high-value oil production Tom D. NIEHAUS[1], Scott KINISON[1], Shigeru OKADA[2], Shuiqin WU[1], Timothy P. DEVARENNE[3], David S. WATT[4], Vitaliy SVIRIPA[4], ◯Joe CHAPPELL[1] ([1]Plant Biology Program, University of Kentucky, USA, [2]Department of Aquatic Biosciences, University of Tokyo, Japan, [3]Department of Biochemistry and Biophysics, Texas A&M University, USA, [4]Department of Cellular and Molecular Biochemistry, University of Kentucky, USA)
16:00–16:20	**4Ap05**	The Cyanofactory™~ a novel cyanobacterial bioprocess based on the synthetic biology concept ~ ◯Koji SODE[1,2] ([1]Tokyo University of Agriculture & Technology, Japan, [2] Japan Science and Technology Agency, CREST, Japan)

SBJ/JST Joint Symposium IV –System & Synthetic Biotechnology–

Organizers: Daisuke UMENO (Chiba University)
Tomohisa HASUNUMA (Kobe University)
Kohsuke HONDA (Osaka University)

Chairperson: Daisuke UMENO (Chiba University)

16:40–17:00	**4Ap06**	Bio-refinery from microalgae through fermentation and metabolic engineering ◯Tomohisa HASUNUMA[1,2] ([1]Organization of Advanced Science and Technology, Kobe University, Japan, [2]PRESTO, Japan Science and Technology Agency (JST), Japan)
17:00–17:35	**4Ap07**	Synthetic biology approaches to produce C3-C5 alcohols from microorganisms ◯Shota ATSUMI (Department of Chemistry, University of California Davis, USA)
17:35–17:55	**4Ap08**	Synthetic metabolic engineering –A novel, simple technology for designing a chimeric metabolic pathway– ◯Kohsuke HONDA[1,2], Xiaoting YE[1], Kenji OKANO[1], Hisao OHTAKE[1] ([1] Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan, [2] PRESTO, Japan Science and Technology Agency (JST), Japan)
17:55–18:05		BREAK

Chairperson: Tomohisa HASUNUMA (Kobe University)

18:05–18:40	**4Ap09**	Production of Biolsoprene™ monomer ◯Derek H. WELLS (Du Pont Industrial Biosciences, USA)
18:40–19:00	**4Ap10**	Evolutionary pathway engineering: toward the fast-track construction of long-step, but high-fidelity pathway for non-natural compounds ◯Daisuke UMENO[1,2], Maiko FURUBAYASHI[1], Kyoichi SAITO[1] ([1]Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University, Japan, [2]PRESTO, Japan Science and Technology Agency (JST), Japan)

Abstracts

Functional chromosome dynamics with a link to synthetic green biotechnology

○Ingo SCHUBERT

Department of Cytogenetics and Genome Analysis, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), D-06466 Gatersleben, Germany

One of the research focuses of the IPK in Gatersleben is dynamics of plant genomes. In the frame of this topic, groups of the Department of Cytogenetics and Genome Analysis work on several aspects of chromosome biology applying interphase cytogenetics, chromosome painting and other cutting-edge technologies. Aims are to elucidate i) karyotype evolution, e.g. how large or how small can a chromosome be, which mechanisms shape the dipoid chromosome complement, how to trace the chromosomal 'diploidisation' of (allo)polyploids (mesopolyploidy), but also ii) structural arrangement of chromosomes and their distinct functional domains (centromeres, telomeres, nucleolus organizers, eu- and heterochromatin) during interphase and during nuclear divisions, iii) correlations of specific epigenetic modifications with competent *versus* incompetent functional states; iv) degree and functional importance of dynamics of interphase nuclear architecture. I will provide a glancing light on some selected examples of this research and eventually on aspects of potential applicability, such as the generation of artificial (mini)chromosomes for breeding and biotech purposes.

[Keywords] Chromosome biology, Interphase chromatin dynamics, Centromere maintenance, Artificial chromosomes, Synthetic centromeres

Manipulation of plant traits using novel gene silencing system, CRES-T (Chimeric REpressor gene Silencing Technology)

○Nobutaka MITSUDA[1], Masaru OHME-TAKAGI[1,2]

[1]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)
[2]Institute for Environmental Science and Technology (IEST), Saitama University

A number of transcription factors have been shown to play important roles for the regulation of growth and development and act as master regulator of various phenotypes in plants. Thus, identification of biological function of each transcription factor is an important subject for elucidation of transcriptional network, signaling cascade and manipulation of plant traits. However, plant genes are frequently duplicated and this structural and functional redundancy often interferes with efforts to identify the functions of these factors. To overcome these difficulties, we developed a novel gene silencing system, called *C*himeric *RE*pressor Gene-*S*ilencing *T*echnology (CRES-T), in which a transcription factor that is converted into strong repressor by fusion with EAR-motif repression domain (SRDX) suppresses the expression of target genes dominantly over the activation activity of endogenous and functionally redundant transcription factors, resulted in the induction of phenotype similar to loss-of-function of the alleles of the gene for transcription factor. Using this CRES-T system, we are comprehensively analyzing function of transcription factors encoded in *Arabidopsis* genome. In addition, we isolated a number of CRES-T lines that exhibit tolerance to various abiotic stresses, such as high salinity, high osmotic pressure, heat, freezing and drought. Not only in model plants, we found that the chimeric repressor is a useful to manipulate various plant traits and actually produced horticultural plants with different shape of flowers. We present that the CRES-T system is not only useful for functional analysis of redundant transcription factors in plants but also is a powerful tool for the manipulation of plant traits.

[Keywords] Transcription factor, Chimeric repressor, Gene regulation

A top-down approach to function the *Synechocystis* PCC6803 genome in a novel cloning host *Bacillus subtilis* 168

○Mitsuhiro ITAYA

Institute for Advanced Biosciences, Keio University

Gene networks of exiting genomes are well regulated to make cellular lives in various environments. Genome engineering recently achieved enables top down approach to examine all the genes included in one genome species albeit limited to small bacteria [1, 2]. Highlighted was a demonstration of chimera genome comprised with a 3.5-Mb genome of a nonpathogenic, unicellular photosynthetic bacterium *Synechocystis* PCC6803 and a 4.2-Mb genome of *Bacillus subtilis* 168 [1].

The chimera genome cell, stemmed from *B. subtilis* to which the entire cyanobacterium genome was assembled, has not grown yet in photosynthesis media. Most of genes from the cyanobacterium genome were dormant and their expression mode was found unregulated in both transcription and translation and molecular regulation mechanism is essential to produce cells carrying designed genomes [3]. Certain biological significance raised by the newly constructed chimera genome and our attempts to draw scenarios for conversion of the two network systems existing in one cell will be presented.

In contrast to *E. coli* host-vector systems, giant DNA equivalent to bacterial genome are being effectively handled in our system using the *Bacillus GenoMe* (BGM) vector derived from the genome of *B. subtilis* 168 [1, 3]. Methods to handle other giant DNAs form plants and animals and their plausible applications would be shown.

[1] Itaya, et al., (2005) PNAS, 102, 15971.
[2] Gibson, et al., (2010) Science, 329, 52
[3] Itaya, (2009) p155. In Systems Biology and Synthetic Biology, Pengcheng et al., Eds, John Wiley & Brothers, Inc.

[Keywords] Genome design, Whole genome cloning, Gene expression, Ribosomal RNA

Decoding the chemical diversity and evolution written in the genomes of the Asteraceae

○Dae-Kyun RO[1], Don Trinh NGUYEN[1], Nobuhiro IKEZAWA[2], Vince QU[1]

[1]Department of Biological Sciences, University of Calgary, Alberta, Canada
[2]Department of Biotechnology, Osaka University, Japan

A recent development in next generation sequencings (NGS) broke the boundary between “model” and “non-model” organisms and accordingly has provided unique opportunities to elucidate the biosynthetic mechanisms of many untapped phytochemicals in medicinal plants. Sunflower (or Asteraceae) family is one of the under-studied plant families, despite that it constitutes ~8% of all angiosperms (~24,000 species). Asteraceae has evolved from the South America at about 50 million years ago and has rapidly radiated to other continents to become a dominant plant family on earth. Among many specialized metabolites, two main chemical constituents in Asteraceae are sesquiterpene lactone (STL) and natural rubber (NR). However, their biosynthesis and regulation remain unknown. We conjectured that the studies of STL and NR metabolism in 50 million years of time-frame will help us better understand how specialized metabolisms evolve and diversify in plant. Using the NGS data, the genes coding for the central oxidizing enzyme in the STL biosynthesis were identified from several sub-families of Asteraceae, including a fossil plant, *Barnadesia spinosa* and a recently evolved species, *Artemisia annua*. Comparative studies of their enzymatic activities have shown that the primordial enzyme from *B. spinosa* displays a broader range of substrate specificity than recently emerged enzymes. Novel terpene compounds could be synthesized by pairing the primordial enzyme with various sesquiterpene substrates. Genomics analysis in lettuce further identified a cytochrome P450 which catalyzes the formation of the simplest STL, costunolide, but its homolog in sunflower catalyzes distinct regio- and stereoselective hydroxylation on the same sesquiterpene substrate. Homology structural modeling of these two P450 enzymes revealed a small number of residues differing in their substrate-binding sites. However, simple exchange of those residues resulted in the loss of activities but did not swap their activities. This result implies that an additional layer of residues contribute to the specificity. In addition to STL, lettuce and sunflower can synthesize terpene-polymer NR with different polymer lengths. Genomics and reverse genetics approach in lettuce to elucidate NR biosynthesis will be also discussed.

[Keywords] Next generation sequencing, Sesquiterpene, Natural rubber, Cytochrome P450, Asteraceae

Cassava molecular breeding and functional genomics –Challenges and opportunities–

○Jarunya NARANGAJAVANA

Center for Cassava Molecular Biotechnology, Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in the tropics, after rice and maize. The high starch content makes cassava a desirable energy source for human consumption as well as for various industrial bio-products including biofuel applications. Cassava breeding efforts throughout the world have made significant impact on cassava production, however it is anticipated that additional gains in breeding efficiency, which would translate into genetic gain, could be made through the application of advanced molecular breeding technologies. The cassava whole genome sequence, recently was developed by the US-DOE Joint Genome Institute Community Sequencing Program, facilitates the implementation of molecular tools. It allowed the identification of candidate simple sequence repeat and single nucleotide polymorphism markers which can be used for genetic mapping and marker assisted selection for further improvement. The availability of a well-annotated, full-length cassava reference genome has been instrumental in facilitating valuable transcriptome, proteome, and functional genomics applications in cassava. We focus on identifying genes and quantitative trait loci controlling economically important traits. Better understanding of how genes play such important roles involved in several traits has been investigated. It is critical for policy-makers, the research community, and donors to understand the challenges and opportunities of developing this crop for the future. The regional cooperation should help in clearly defining the target problems and challenges to rise up to the expectations of stake holders.

[Keywords] Cassava, Energy crop, Genome, Molecular breeding, Functional genomics

KOMICS: metabolomics databases and tools for biotechnology

○Daisuke SHIBATA, Takeshi ARA, Hideyuki SUZUKI, Nozomu SAKURAI

Kazusa DNA Research Institute, Chiba, Japan

Metabolomics approaches have been prevailing in biotechnology research during the past decade. However, archiving information of metabolites found in various organisms and integrating the metabolome data to the transcriptome data of each organism are still a challenging field in bioinformatics. With recent advance in technologies of mass spectrometry (MS), low molecular weight metabolites are analyzed both in a high resolution and sensitivity, which leads to reliable quantification of each metabolite, with help of advanced software for handling chromatogram data, which is prerequisite for archiving metabolite data as metabolomics research. Due to the large chemical diversity found in metabolites, various MS machines with state-of-the-art technologies have prevailed for metabolite analysis. Over the past decade our laboratory has been analyzing metabolites from various organisms and authentic chemicals for metabolite identification using eight MS machines and accumulated more than 100,000 chromatograms in our servers. With intension to provide the whole datasets to public in a unified manner to accelerate the metabolomics science, we have designed archives for data depository, as the databases MassBase for MS chromatogram datasets and KomicMarket for annotated metabolite datasets. Furthermore, we have been devoting to the development of genetic information databases and tools. One of the databases, the KaPPA-View systems are for overviewing the metabolome and transcriptome data on the pathway maps, as an option, with the gene-to-gene or metabolite-to-metabolite co-relationships. These databases and tools are available through our web portal KOMICS (Kazusa Metabolomics Database) (http://www.kazusa.or.jp/komics/en/).

[Keywords] Metabolome analysis, Transcriptome, Bioinformatics, Mass spectrometry

Dammarene-type ginsenoside metabolic engineering –Characterization of genes involved in dammarenediol saponin biosynthesis–

Jung-Yeon HAN, ○Yong-Eui CHOI

Department of Forest Resources, College of Forest and Environmental Science, Kangwon National University, Chunchon 200-701, South Korea

Ginseng (*Panax ginseng* C.A. Meyer) is one of the most popular medicinal herbs and contains pharmacologically active components, ginsenosides, in their roots. Ginsenosides, a class of tetracyclic triterpene saponins, are synthesized from dammarenediol-II after hydroxylation by the cytochrome P450 (CYP) enzymes and then glycosylation by glycosyltransferases. The first step in biosynthesis of dammarane-type ginsenosides is cycling of 2, 3-oxidosqualene to dammarenediol-II, a reaction that is catalyzed by an enzyme from dammarenediol synthase. We functionally characterized the dammarenediol synthases (*PgDDS*) from *P. ginseng*. Dammarenediol-II is thought to be converted to two ginsenoside aglycones (protopanaxadiol and protopanaxatriol) after hydroxylation by cytochrome P450 (CYP) enzymes. Two CYP genes are thought to be involved in dammarene-type ginsenoside biosynthesis. One of these genes is involved in protopanaxadiol production by hydroxylation of dammarenediol at the C-12 position. Another gene is protopanaxatriol synthase which involved in protopanaxadiol hydroxylation at the C-6 position conferring protopanaxatriol production. We reported that *CYP716A47* is involved in the hydroxylation of dammarenediol-II at the C-12 position to yield protopanaxadiol (Han et al. 2011). The next step of protopanaxatriol production (protopanaxdiol 6-hydroxylase) is also characterized by our group recently. We will discuss the possible metabolic engineering using those genes involved in dammarene-type ginsenoside saponin biosynthesis in *Panax ginseng*.

[Keywords] *Panax ginseng*, Tetracyclic triterpene, Saponin biosynthesis, Dammarane-type ginsenoside

Production of plant triterpenoids in engineered yeast

○Toshiya MURANAKA, Hikaru SEKI

Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Plant triterpenoids represent a large and structurally diverse class of natural products. Among these chemicals, glycyrrhizin, a triterpenoid saponin derived from underground parts of *Glycyrrhiza* plants (*G. uralensis* and *G. glabra*, Fabaceae; licorice), is one the most important crude drugs in the world. Glycyrrhizin and its aglycon glycyrrhetinic acid exhibit various pharmacological activities, including anti-inflammatory and hepatoprotective activities. Glycyrrhizin is also known to be 150 times sweeter than sucrose. Therefore, a large amount of licorice and its extracts are on the world drug market as medicinal materials and sweetening agents, although their production largely depends on the collection of wild licorice plants, and this has caused a decrease in licorice reserves and an increase in desertification where it is harvested. Here, we show the engineering of *Saccharomyces cerevisiae* to produce glycyrrhetinic acid. This was achieved by redirecting a portion of the native 2,3-oxidosqualene pool to heterologous pathway leading to glycyrrhetinic acid, by expressing three pathway enzymes, β-amyrin synthase (bAS), which constructs the triterpene skeleton β-amyrin, and two cytochrome P450 monooxygenases (CYP88D6 and CYP72A154) these perform subsequent oxidations of β-amyrin at positions C-11 and C-30, respectively. Furthermore we could isolate another type of P450 categorized into CYP716A subfamily, which functions as multifunctional oxidase in triterpenod 2biosynthesis. CYP716A12 was able to generate oleanolic acid, ursolic acid and betulinic acid coexpressed with bAS, α-amyrin synthase and lupeol synthase in yeast, respectively. Our results provide proof-of-concept for the engineering of the production of high-value triterpenoid products in yeasts.

[Keywords] P450, Metabolic engineering, Synthetic biology, Triterpenoid

Living microbes as selective and efficient redox biocatalysts

○Andreas SCHMID, Katja BÜHLER, Mattijs JULSING, Bruno BÜHLER

Laboratory of Chemical Biotechnology, TU Dortmund University, Germany

Redox biocatalysis enables a large and steadily growing number of industrially interesting highly specific reactions. Technically demanding reactions, e.g. oxyfunctionalizations of hydrocarbons, make use of complex enzyme systems like oxygenases. Here, the application of living microbial cells is most attractive, as they allow efficient cofactor regeneration, self-renewal (enzyme synthesis and growth), and handling of reactive oxygen species. Furthermore, multiple reactions can be coupled inside the cell functioning as microreactor. Such whole-cell systems pose challenges with respect to catalyst optimization and process control. Beside technical aspects such as mass transfer and reaction kinetics, a large number of concurrent and highly cross-linked biological processes such as recombinant gene expression, energy metabolism, and toxification have to be considered.

With the aim to construct the ideal microbial cell for recombinant oxygenase catalysis, we follow a systems biotechnology approach also considering technical implications. The coupling of specific hydrocarbon oxyfunctionalization and transaminase catalysis will be presented as an example for orthologous reaction engineering. Finally, three approaches for overcoming the inherent problem of substrate and O_2 mass transfer limitations will be introduced: substrate uptake systems, catalytic biofilms in microreactors and regiospecific, O_2-independent, aromatic hydroxylation.

[Keywords] Whole cell biocatalysis, Multistep reactions, Asymmetric epoxidation, Microreactors, Productivity

Toward biological replacement of petroleum

○James C. LIAO

Department of Chemical and Biomolecular Engineering, University of California, Los Angeles

Petroleum is the major source that provides energy and chemicals used today. Unfortunately, because of its unreliable supply, fluctuation in price, and negative environmental impacts, the need to replace petroleum with alternative sources has become increasingly important. Current biorefining schemes use plant biomass or algae to produce ethanol, biodiesel, and chemicals. While these are currently the leading processes, next-generation biorefinery should consider other options as the starting materials and manufacturing technology. For example, direct photosynthetic production of fuel or chemicals from CO_2 is attractive, as this scheme bypasses the need for lignocellulose deconstruction and algal lipid processing. Non-photosynthetic, electricity-powered CO_2 reduction to fuel or chemicals further by-pass the need for the need for light exposure surface areas in photo-bioreactor, and represents a feasible option for biochemical and biofuel production as well as electricity storage. Finally, decomposing waste proteins to ammonia and fuels or chemicals provides the possibility of closing the nitrogen cycle and increase the yield and productivity of biorefining.

[Keywords] Metabolic engineering, Synthetic biology, Biorefining, Photosynthesis

Bacillus subtilis cell factory for production of *scyllo*-inositol promising for Alzheimer's disease

○Ken-ichi YOSHIDA[1], Chumsakul ONUMA[2], Shu ISHIKAWA[2], Naotake OGASAWARA[2]

[1]Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University
[2]Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST)

Inositol stands for a class of compounds forming nine stereoisomers through epimerization of the six hydroxyl-groups. *myo*-Inositol (MI) is one of the isomers, most abundant in nature, and supplied cheap from phytin in rice-bran. Some of the other isomers are rare and thus very expensive but reported to possess interesting biological functions for the treatment of diseases difficult to treat. For instance, another isomer *scyllo*-inositol (SI) was shown to disperse the high-molecular-weight oligomeric aggregates of beta-amyloid peptide in brain, and thus appears promising for Alzheimer's disease.
We demonstrated that manipulating the inositol metabolism in *Bacillus subtilis* enabled the artificial interconversion among inositol stereoisomers secreted into the culture medium, materializing a *B. subtilis* cell factory for simple and inexpensive bioconversion of cheap MI to valuable SI. We investigated the effect of medium components on the SI bioconversion efficiency to find that increased concentrations of Bacto soytone, one of the major nutrients of the bioconversion medium, improved the efficiency without significant effect on the cell growth. We thus successfully defined an optimized medium composition for SI bioconversion, by which almost 50% of MI initially contained in the medium was converted to SI after 48-h culture of bioconversion. Effects of medium optimization on transcriptome were examined to understand the reasons for the efficient bioconversion, implying that a global change in metabolic pathways might occur to fulfill the demand of required coenzyme regeneration.

[Keywords] *Bacillus subtilis*, Inositol, Bioconversion, Alzheimer's disease

Engineering of signal transduction for enhanced production of biopharmaceuticals and bioenergy

○Jian-Jiang ZHONG[1], Yang-Chun YONG[2]

[1]State Key Laboratory of Microbial Metabolism, and Lab. of Molecular Biochemical Engineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dong-Chuan Road, Shanghai 200240, China
[2]Biofuels Institute, School of the Environment, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, Jiangsu Province, China

Signal transduction engineering has been successfully applied to industrial and environmental biotechnology as illustrated in our previous work. Here we report new progresses in production of biopharmaceuticals and bioenergy by using this interesting approach. In liquid fermentation of *Ganoderma lucidum*, a famous traditional medicinal mushroom, which produces interesting antitumor ganoderic acids, calcium signal transduction was found to be significant to the ganoderic acid biosynthesis. A possible model on the effect of external calcium ion on the ganoderic acid biosynthesis via calcineurin signal transduction pathway was proposed. Similarly, in bioactive ginsenoside biosynthesis, signal transduction under heavy metal elicitation led to higher accumulation of ginsenosides in *Panax ginseng* cell cultures. In bacterial world, quorum sensing is a wide-conserved cell-cell communication system and plays important roles in various biological processes. But it is yet poorly understood what its role is in a bioelectricity generation process. In a microbial fuel cell system, which is promising for simultaneous clean energy generation and pollutant treatment, by manipulation of *rhl* quorum sensing expression, we found that *Pseudomonas aeruginosa* could use different electron shuttles. Interestingly, the electron shuttle substitution (by phenazines) increased the maximum current output of the *rhl* overexpressed strain over the wild-type one. Further investigation is under way.

[Keywords] Bioprocess engineering, Signal transduction engineering, Quorum sensing, Biomedicine, Bioelectricity

Synthetic biology for the comprehension of biomolecular networks

○Masahiro OKAMOTO[1,2]

[1]Department of Bioinformatics, Graduate School of Systems Life Sciences, Kyushu University
[2]Synthetic Systems Biology Research Center (SSBRC), Kyushu University

In order to make the paradigm shift from the concept of “watched and analyzed biology” to that of “synthetic and analyzed or utilized biology”, the innovative research named Synthetic Biology was started from 2000 in US, such as designing synthetic genetic circuit by combining known interrelated biomaterials, realizing a certain bio-functional behaviors such as switch, oscillation *in vivo*, designing synthetic metabolic pathways by incorporating enzyme coded genes from other origins into the cells. However, these attempts have been done on a small scale and with a trial-and-error method. The objectives of this research project is to establish the coordination between the fundamental technologies for synthetic biology in order to comprehend biomolecular networks by integrating the following three missions: 1) design synthetic genetic circuit or metabolic pathway with using the methods of computational science, 2) construct the circuit *in vitro* with using the method of engineering, 3) construct the circuit *in vivo* or in the cell with using the methods of molecular biology. In order to construct and control a large scale of dynamic and complex synthetic genetic circuit or metabolic pathways, the following fundamental technologies for synthetic biology are essential: Biochemical Engineering, Embryological Engineering, Molecular Biology, Evolutional Molecular Engineering, Micro Fluid Engineering, Biomolecular Chemistry, Simulation Engineering, and Knowledge-based Engineering. In the first stage (2–3 years), our mission is to construct dynamic and multi-elements synthetic genetic circuit, followed by the construction of differentiation-induced system against stem cell and by the realization of cell factory, in which cells can produce the target metabolites by themselves according to the cell environment in the last 2-years. The research project is composed of the following four sub sections: (A01) fundamental technologies of molecular biology, (B01) fundamental technologies of engineering, (C01) fundamental technologies of computational science, and (X01) integrated section of A01, B01 and C01.

[Keywords] Synthetic metabolic engineering, Micro fluid engineering, Synthetic genetic circuit, Evolutional molecular engineering, Embryological engineering

Strategies and technologies towards tailor made fuels from biomass

○Jochen BUECHS[1], Tobias KLEMENT[1], Martin KUNZE[1], Gernot JAEGER[1], Sandra WEWETZER[1], Frederike CARSTENSEN[2], Matthias WESSLING[2], Philipp GRANDE[3], Pablo Domínguez DE MARIA[3], Antje SPIESS[4]

[1]AVT - Biochemical Engineering, [2]AVT - Chemical Process Engineering, [3]Institute of Technical and Macromolecular Chemistry, [4]AVT - Enzyme Process Technology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

Fuel and chemical industry is facing a large challenge, as its raw material has to be shifted from fossil to renewable resources in the coming years. Completely new logistic concepts and technologies to convert biomass to intermediates and finally to fuels and chemicals have to be developed. In the centre of excellence “Tailor Made Fuels from Biomass (TMFB)”, funded by the German Research Foundation, the conversion of different kinds of biomass to intermediates for subsequent fuels production is investigated. Solid biomass, pre-treated by mechanical means, is directly chemically fractionated or dissolved in ionic liquids and partially chemically hydrolysed to oligomers. Subsequently, the oligomers can enzymatically be converted to glucose. Enzymes are studied which retain their activity even in the presence of high concentrations of ionic liquids. This combined chemo/enzymatic route is completed in much shorter time than conventional methods. Another possibility to utilize the chemically fractionated material is a combined enzymatic hydrolysis coupled with a fermentation process. The robustness of the fermentation in the presence of the residual chemicals from the preceding pre-treatment step is crucial for an over-all economic process. In TMFB the itaconic acid production with the smut fungus *Ustilago mayadis* is investigated. This microorganism is relatively insensitive against different stress factors. With a wild type strain itaconic acid concentrations of 35 g/l were obtained. Currently, newly developed recombinant strains are investigated. This fermentation process is designed to be operated in continuous mode with cell retention, using microfiltration membranes. A new pulsed diafiltration technique is used to prevent membrane fouling.

[Keywords] Lignocellulose, Integrated process, Continuous culture, Cell retention, Membranes

Actinobacteria tyrosinase and its applications to biomaterials

○Byung-Gee KIM[1,2,3], Nahum LEE[1,3], SangHyuk LEE[2,3], ChangHyun SONG[1,3]

[1]School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea
[2]Institute of Molecular Biology and Genetics, Seoul National University, Seoul, Korea
[3]Institute of Bioengineering, Seoul National University, Seoul, Korea

Most *Streptomyces* species produce and secrete melanin, a dark pigment involved in the protection against UV rays and reactive oxygen species. The key enzyme to synthesize melanin is tyrosinase, which uses phenolic compounds and catechol derivatives. Here, we demonstrate the secreted tyrosinase from *Strepomyces avermitilis* involved in not only hydroxylation of catechol derivatives but also degradation of catechol derivatives. Generally, ortho-hydroxylated catechol derivatives such as 3'ODI and piceatannol (a kind of stilbene compounds) show higher antioxidant activity than their corresponding monophenolic compounds. To produce such ortho-hydroxylated catechol derivatives, their hydroxylation has been done by whole cell reaction of *Streptomyce avermitilis* by inhibiting extracellular tyrosinase activity using catechol as an inhibitior as well as a reducing reagent. Since the tyrosinase secreted by *S. avermitilis* has both functions of monooxygenase and dioxygenase, the kinetics of such hydroxylation reaction of tyrosinase were elucidated more in detail. We have expressed the tyrosinase (MelC2) from *S. avermitilis* in *E. coli* with the help of chaperon GroEL/GroES and its tyrosinase activity to produce ortho-hydroxylated catechol derivatives was confirmed. In addition, to enhance the ratio of monooxygenase function to dioxygenase function of tyrosinase, site directed mutagenesis of the helper protein melC1 and melC2 were attempted. We have compared the activities of monooxygenase and dioxygenase of the tyrosinase (MelC2) from *S. avermitilis* with those of a commercial tyrosinase from *Agaricus bisporus* (mushroom) using several substrates, and explained their structures based upon their substrate specificities. We will discuss on how the structures of tyrosinase affect their applications to biomaterials.

[Keywords] Tyrosinase, Cross-linking of biomaterials, ortho-Dihydroxylation, *Streptomyces*

Cyberinfrastructure for metabolomics and synthetic biology

○Masanori ARITA[1,2]

[1]Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo
[2]RIKEN Plant Science Center

A cyberinfrastructure, i.e., web-based research environment, for metabolomics and related areas is introduced. When a group of people collaboratively work on the same scope, web-based archives such as Wikipedia or the Registry of Standard Biological Parts (http://partsregistry.org/) are ideal information resources. The advantages of such wiki-based sites are the flexibility in its management and the less psychological barriers to join the community. The often-said drawbacks of such archives are the lack of security and of data-mining or visualization services due to unorganized formats or missing description. Since it is difficult to find a full-time administrator or curator for such collaborative projects, the latter drawback may be fatal for scientific data. To overcome this drawback, a MediaWiki-based data repository is designed and multiple extensions are prepared in programming languages such as Lua and JavaScript (the original language of MediaWiki is PHP). We standardize data inputs by using templates, and prepare functional pages so that compiled data are automatically subject to statistical or mining processes. Although our main focus is mass spectra from different biological sources, these data can be integrated with variety of other resources such as compounds, plant species, and gene structures. In this talk, we introduce several examples of automated data processing and explain benefits of such open data archives. The website is accessible at http://metabolomics.jp/.

[Keywords] Metabolomics, Database, Mass spectra

Bioprocess engineering on biofuels and bio-based chemicals production from microalgae

○Jo-Shu CHANG[1,2,3], Chun-Yen CHEN[2]

[1]Department of Chemical Engineering, National Cheng Kung University, Taiwan
[2]Center for Bioscience and Biotechnology, National Cheng Kung University, Taiwan
[3]Research Center for Energy Technology and Strategy, National Cheng Kung University, Taiwan

The future resources for food, fuels, and chemicals will potentially come from ocean. One of the major marine resources is microalgae, which have the ability to fix carbon dioxide at a much faster rate than that of terrestrial plants. The fixed carbon dioxide is converted to microalgal biomass, which has potential applications in producing biofuels, animal feed, health food, pharmaceuticals, and other high-value products. Therefore, using microalgae to mitigate CO_2 emissions is a promising strategy for CO_2 storage and re-utilization. We have identified various indigenous microalgae strains that utilize flue gas of a steel-making factory for growth with excellent CO_2 biofixation ability. We are able to adjust the composition of resulting microalgal biomass (lipid, carbohydrates, proteins, pigments, etc.) by using different cultivation strategies to meet the needs of downstream applications. To make the concept of microalgae industry a reality, new technologies and engineering approaches are developed; for instance, outdoor large-scale cultivation, biomass harvesting, product conversion technology, and so on. Some key technologies required for realizing commercialization of microalgae-based CO_2 emission mitigation and biofuels/chemicals production will be introduced.

[Keywords] Microalgae, Mitigation of CO_2 emissions, Lipid, Carbohydrate, Protein, Biofuels, Flue gas, Biorefinery

Systems metabolic engineering –Rational design of microbial cell factories

○Hiroshi SHIMIZU[1], Chikara FURUSAWA[1,2], Takashi HIRASAWA[1], Naoaki ONO[3], Katsunori YOSHIKAWA[1], Yoshihiro TOYA[1]

[1]Department of Bioinformatic Engineering, Osaka University
[2]Quantitative Biology Center (QBiC), RIKEN
[3]Graduate School of Information Science, Nara Institute of Science and Technology (NAIST)

In order to establish sustainable societies, production processes should shift from petrochemical-based processes to bioprocesses. Recently integration of *in silico* and experimental approaches for creation of microbial cell factrories is highly desired. Recently, on the basis of whole-genome information, the genome-scale metabolic reaction models (GSMs) of cells have been reconstructed for many organisms. Using the GSMs, a reliable prediction of metabolic fluxes is possible by using Flux Balance Analysis (FBA). The constructed GSMs are applied to prediction of metabolic fluxes under several environmental conditions and to design of genetic modification for valuable compounds production. Metabolic flux analysis (MFA) based on quantification of ^{13}C-labeling patterns of metabolites by mass spectroscopy (MS) is also a powerful tool to quantify fluxes experimentally in a metabolic network of microorganisms. In order to obtain stress tolerant strain, experimental evolution and multiomics analysis of evolved strains are also presented. These analyses provide deep understanding of cellular processes and upgrade designability of cell factories. Systems metabolic engineering gives rise to new knowledge about metabolic systems that can facilitate engineering of strains suitable for industrial applications.

[Keywords] Genome scale model, Metabolic flux analysis, Evolution engineering, Multiomics analysis, Metabolic engineering

Reaction evaluation and new process design in composting of biological wastes

○Jingchun TANG[1], Arata KATAYAMA[2]

[1]College of Environmental Science and Engineering, Nankai University
[2]EcoTopia Science Institute, Nagoya University

A combination of quinone profile method and denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal RNA genes was applied to characterize the microbial community of composting process. A higher quinone content and microbial diversity was found during thermophilic composting process as compared to mesophilic composting process. The mesophilic microbial community was characterized by the predominance of ubiquinones and menaquinone (MK)-8, which correspond to *Proteobacteria* and fungi. The thermophilic microbial community was characterized by the dominance of MK-7 in the initial period, and increases in the amounts of menaquinones with long and partially-saturated side chains in the later period, corresponding to *Firmicutes* and *Actinobacteria*, respectively. DGGE analysis also confirmed that diversity of microbial communities increased but differently in the two processes. Composting process was also applied to dispose marine seaweed waste. Alginate-degrading bacterium A7 was isolated from wakame (*Undaria pinnatifida*) compost and confirmed to belong to the genus *Gracilibacillus* by partial 16S rDNA analysis. Alginase was purified from *Gracilibacillus* A7 and evaluated for its ability to produce elicitor-active oligosaccharides. Composting of the *Undaria pinnatifida* (wakame) seaweed was conducted after inoculation with *Gracilibacillus* sp. A7 and halotolerant bacterium *Halomonas* sp. AW4. Inoculation with strains A7 and AW4 resulted in 27.8 and 24.7% degradation of wakame dry mass after 168 h of composting, whereas only 17.5% degradation occurred in the uninoculated control. Inoculation with A7 resulted in 2.8 times faster degradation of alginate and 1.2 and 1.6 times higher levels of reducing sugars and unsaturated sugars than inoculation with AW4. The compost produced from the inoculation with A7 had low plant toxicity as measured by germination experiment. Inoculation of wakame with alginate-degrading bacteria not only shortened the length of composting but also created seaweed compost with good fertilizer qualities.

[Keywords] Composting, Quinone profile method, Denaturing gradient gel electrophoresis, Alginate degradation

Organic solvent stable enzymes

○Hiroyasu OGINO

Department of Chemical Engineering, Osaka Prefecture University

Enzymes catalyze specific reactions without producing by-product under mild conditions. It is possible to construct environmentally-friendly sustainable bioprocesses, when enzymes are used as catalysts for chemical processes. Especially, chemical processes producing fine chemicals consume much energy and resource and produce much by-product. Although enzymes are good catalysts for these processes, enzymes are not stable in the presence of organic solvents used for production of fine chemicals. By the addition of organic solvents to the enzymatic reaction solution, the solubility of substrates and the reaction rate can often be markedly increased. In the presence of organic solvents hydrolytic enzymes can also perform synthetic reactions, which are the reverse reactions of hydrolysis. A further advantage of non-aqueous reaction solutions is that the risk of unwanted microbial contamination is normally very low. Therefore organic solvent-stable enzymes were developed. First, microorganisms which produce organic solvent stable enzymes were isolated from natural source. Using organic solvent stable enzymes, some reasons of organic solvent stability of enzymes were understood. Furthermore, high stable and high active enzymes were developed from organic solvent stable enzymes by protein engineering.

[Keywords] Organic solvent, Stability, Tolerance, Lipase, Protease

Engineering of aminoacyl-tRNA synthetases for residue-specific incorporation of amino acid analogues into proteins

○Tae Hyeon YOO

Department of Molecular Science and Technology, Department of Applied Chemistry and Biological Engineering, Ajou University, Suwon, Korea

In the past several decades, there have been significant advances in understanding of protein sequences, structures and functions which in turn have aided engineering of proteins with novel structures and/or functions. In addition, recent work from several laboratories has shown that an expanded repertoire of amino acid analogues can be incorporated into proteins. The recruitment of new amino acid constituents provides the protein engineering field with new tools and raises the prospects for creating novel proteins. In this talk, I will present a high-throughput screening method for engineering aminoacyl-tRNA synthetases to activate noncanonical amino acids and show examples how the engineered enzymes are utilized to study biology, especially to interrogate cellular protein synthesis.

[Keywords] Amino acid analogues, Protein engineering, Aminoacyl-tRNA synthetase

Production of the bio-butanol based on the continuous in situ fermentation and adsorption (ISFA) technology

○Hanjie YING[1,2], Yong CHEN[2,3], Jinglan WU[2,3]

[1]State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing, China
[2]College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing, China
[3]National Engineering Technique Research Center for Biotechnology, Nanjing, China

Since butanol is an important chemical and a potential energy-substitute, production of butanol based on the biological methodology has being obtained comprehensive attention from academic and industrial institutions all of the world. However, similar to the other bulk chemicals manufactured by biotechnology, the preparation of bio-butanol nowadays exists several problems, i.e., use of the foodstuffs as substrate, low economic efficiency, high energy-consumption as well as high environmental pollution.

This report focuses on the following two questions. The corresponding solutions were proposed and the main results were presented subsequently. Firstly, production of bio-butanol commonly suffered from poor strain tolerance, low productivity and butanol yield, which cause low butanol titers, large investment in equipment and consumption of raw materials. A novel method for cell immobilization by surface adsorption and strategies of cofactor manipulation were developed to address these problems. The fermentation time was significantly shortened from 40–50 h to 10–14 h while the butanol titer was increased from 12–13 g/L to 15–16 g/L, which resulted in a three-fold increase in productivity.

Secondly, due to the high energy-consumption and large amount waste-water disposal in the traditional distillation process, an innovative in situ product recovery (ISPR) technology was developed incorporation with the process simulation to recovery bio-butanol from the fermentation broth. As a result, the bio-butanol concentration was improved from 1.2% to 15–20%, the energy-consumption was reduced 25–30%, and the waste-water disposal was decreased around 50%, which provided the fundamental technology to realize the high-efficiency production of bio-butanol in an industrial scale.

[Keywords] Bio-butanol, Cofactor manipulation, Adsorption immobilization, ISPR

Functional properties of diet ginger (*Zingiber officinale* Roscoe, Zingiberaceae) for antioxidant, anti-pigmentation, anti-cancer, anti-bacterial and wound repair

Pei-Fang WU, Yi-Ting CHOU, ○Hui-Min WANG

Department of Fragrance and Cosmetic Science, Kaohsiung Medical University, Kaohsiung, Taiwan, ROC

Ginger, the rhizomes of the herb *Zingiber officinale* Roscoe (Zingiberaceae), is a perennial herb and is used widely as a spice throughout the world. There are many species of ginger, mainly cultivated in tropical countries of Asia. We published that biologically active compounds found in diet ginger has been shown to have a number of pharmacological activities including anti-oxidant, anti-pigmentation, anti-cancer, anti-bacterial and to reduce the metabolic syndrome. It has important economic value and a wide range of pharmacological effects.

- Chung-Yi Chen, Chien-Chih Chiu, Chi-Peng Wu, Yi-Ting Chou, Hui-Min Wang* Enhancements on skin cell proliferations and migrations via 6-dehydrogingerdione 2012, submitted to PLoS ONE
- Chung-Yi Chen, Kuo-Chen Cheng, Andy Y Chang, Ying-Ting Lin, You-Cheng Hseu, Hui-Min Wang*
  10-Shogaol, an Antioxidant from *Zingiber officinale* for Skin Cell Proliferation and Migration Enhancer
  International Journal of Molecular Sciences. 13, 1762-1777, 2012
- Hui-Min Wang, Chung-Yi Chen, Hsi-An Chen, Wan-Chun Huang, Wei-Ru Lin, Tun-Chieh Chen, Chun-Yu Lin, Hsin-Ju Chien, Po-Liang Lu, Chiu-Mei Lin, Yen-Hsu Chen*
  Zingiber officinale (ginger) Compounds with Tetracycline Have Synergistic Effects against Clinical Extensively-Drug Resistant *Acinetobacter baumannii*
  Phytotherapy Research. 24, 1825-1830, 2010
- Bing-Hung Chen, Pei-Yu Wu, Kuan-Ming Chen, Tzu-Fun Fu, Hui-Min Wang*, Chung-Yi Chen*
  Antiallergic Potential on RBL-2H3 Cells of Some Phenolic Constituents of *Zingiber officinale* (Ginger)
  Journal of Natural Products. 72, 950–953, 2009

[Keywords] Ginger, Anti-oxidant, Anti-pigmentation, Anti-cancer, Anti-bacterial

Enzyme engineering with the aid of ionic liquids

○Noriho KAMIYA[1,2]

[1]Center for Future Chemistry, Kyushu Univeristy
[2]Department of Applied Chemistry, Graduate School of Engineering, Kyushu University

For more than a decade, ionic liquids (ILs) have been extensively exploited as new reaction media for biocatalysts. The great potential of ILs is well recognized because the physicochemical characteristics can be tuned by altering the pair of cation/anions or by introducing functional substituents into the IL components. Indeed, a range of enzymes has proven to exhibit catalytic activity in ILs with little or only a few percent water content. Recently, the potential of ILs as unique solvents for the pretreatment of lignocellulosic biomass has been focused, which opens a new avenue to the utilization of renewable bioresources for enzyme engineers. Previous reports including ours clearly indicate that ILs can be employed not only as simple reaction media but also as potent chemicals for altering substrate characteristics in enzymatic catalysis. Here we report our ongoing efforts on how to utilize ILs in enzymatic saccharification of cellulosic biomass. The latest trial for the construction of new IL-based reaction media for biotransformation will be presented as well.

[Keywords] Ionic liquids, Lignocellulosic biomass, Solvent engineering

Introduction of the JST CREST/PRESTO program research area "Creation of basic technology for improved bioenergy production through functional analysis and regulation of algae and other aquatic microorganisms"

○Tadashi MATSUNAGA

Tokyo University of Agriculture and Technology

Biological energy production from solar energy has been a human dream for a long time. Ethanol produced from corn and sugar cane by yeast has already been commercialized as a biofuel. However, the use of these sources for bioethanol competes with food production. Therefore, the use of non-edible crop components and wood waste has become an important research target. Recently, biofuel production using algae and other aquatic microorganisms has attracted attention, because these organisms cannot only be used to produce ethanol but also to produce biodiesel fuel and other hydrocarbons. Use of the marine environment is an important advantage because the oceans cover 70% of the Earth. This Research Area aims to create innovative technologies for bioenergy production through the functional analysis of algae and other aquatic microorganisms based on recently developed "omics" technologies, using marine and freshwater organisms and specifically focuses on the production of biofuels, including biodiesel fuel, light oil (alkane and alkene), ethanol, methane, hydrogen, etc., as well as lipids and sugars that can be converted to biofuels. For that purpose, as well as the collaboration among researchers from various fields, including marine biotechnology, phycology, microbiology, bioinformatics, marine biology, biochemistry, genetic engineering, plant physiology, chemistry, chemical engineering, etc., challenging projects with creative ideas from new and active researchers are necessary. This Research Area is jointly operated with multiple CREST and PRESTO researchers. This Research Area is expected to generate basic technology for the low cost production of biofuels. The use of fossil fuels including crude oil will be decreased with the use of new biofuel production technologies. Furthermore, the establishment of new metabolic pathways in algae and other aquatic microorganisms that lead to the production of chemicals, including precursors of plastics, may change the current dependency on oil among chemical industries. Active mutual information exchange of each research progress enhances synergy between these projects and promotes further achievements, like new technologies within five to ten years through large-scale pilot plant experiments upon completion of this Research Area.

[Keywords] Biofuel, Algae, Aquatic microorganisms, Omics, Metabolic pathways

Can biotechnology address the global energy crisis?

○Jonathan D. TRENT [1,2]

[1]Bioengieering Branch, NASA Ames Research Center
[2]Biomolecular Engineering, University of California Santa Cruz

There are claims that biofuels produced by microalgae can provide a sustainable substitute for fossil fuels. Indeed, some known species of microalgae are fast growing and provide the highest yields of biodiesel. There are however, formidable challenges to meet the demands of large-scale production within the constraints of economics and without competing with agriculture for freshwater, fertilizer, or land. To meet these challenges, we have proposed a system called OMEGA (Offshore Membrane Enclosures for Growing Algae). In the OMEGA system, oil-producing freshwater algae are grown in flexible, clear plastic photobioreactors (PBRs) attached to a floating infrastructure anchored offshore in a protected bay. Domestic wastewater (sewage) and CO_2 from coastal facilities provide water and nutrients. The surrounding seawater controls the temperature inside the PBRs. The salt gradient between seawater and wastewater kills algae that escape from the system and drives forward osmosis to concentrate nutrients and facilitate algae harvesting. The OMEGA infrastructure supports traditional aquaculture and alternative energy (solar, wave and wind). Integrating algae cultivation, wastewater treatment, CO_2 sequestration, aquaculture, and alternative energy supports the economics of the system as a whole. By using domestic wastewater for water and fertilizer and by operating offshore, OMEGA does not compete with agriculture for water, fertilizer, or land. By treating wastewater, sequestering CO_2, and creating a “floating reef,” OMEGA has a positive impact on the local environment. Progress into the feasibility of OMEGA will be discussed.

[Keywords] Biofuels, Algae, Photobioreactor, Wastewater treatment, Aquaculture

Food and fuel from microalgae: Integration of photosynthesis and photovoltaics allows to achieve a positive energy balance of algae biomass production

○Mario R TREDICI

Dipartimento di Biotecnologie Agrarie, Università degli Studi di Firenze

In spite of the potential of microalgae, no company seems to possess a mature technology able to produce algae biomass at low cost and compete with traditional sources of food, feed and fuel. The high capital and operating costs of microalgae farming, the non-sufficiently positive energy balance and the not yet established sustainability still prevent the development of this technology to commercial scale. But new designs are emerging, which may contribute to closing the gap between algae feedstock and current alternatives.

Among the barriers that need to be cleared before fuel, feed and food from algae may become a reality, there is the sustainable cultivation of selected strains at large scale and the low EROI (Energy Return on Investment) of the process. The main reasons for the preference of photobioreactors (PBR) over ponds for algae cultivation is that they provide a close and more controllable environment, thus limiting the risk of contamination and ensuring a higher and more sustainable production. However, no PBR design has been developed and tested at the (large) scale necessary for a complete economic and energetic evaluation of the process, and at pilot scale PBR show a lower EROI compared to ponds.

The Green Wall Panel (GWP), a low-cost, flat PBR developed at the University of Florence and commercialized by Fotosintetica & Microbiologia S.r.l. (Italy), needs about 350 KJ m^{-2} d^{-1} to operate (mostly for mixing and cooling). In the summer, with an average productivity of 20–25 g m^{-2} d^{-1} (equivalent to an energy output of about 400 KJ m^{-2} d^{-1}), the biomass production process in this system achieves an EROI near unity, while an EROI of more than three would be necessary. An integrated GWP has been recently developed to reduce energy costs and enhance the EROI. Called SOLO, this stand-alone system uses part of the impinging photons for photovoltaic generation of about 1 MJ m^{-2} d^{-1} of electric energy, which are sufficient to cover all the needs of the process (including cultivation, harvesting and drying). In a particular application of the SOLO tested outdoors, it has been shown that a significant fraction (30–50%) of the impinging photons can be diverted to the photovoltaic elements without reducing algal productivity.

- Rodolfi L., Chini Zittelli G., Bassi N., Padovani G., Biondi N., Bonini G., Tredici M.R. (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.* 102: 100-112.
- Tredici M. R. (2010) Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. *Biofuels* 1(1): 143-162.

[Keywords] Microalgae, Energy return on investment, Green wall panel (GWP), Photobioreactor, Photosynthesis and photovoltaics

Biorefinery of microalgae

○René H. WIJFFELS

Wageningen University, Bioprocess Engineering, P.O. Box 8129, 6700, EV Wageningen, the Netherlands

Our mission is to develop sustainable biobased production strategies in which phototrophic microalgae are used for a single step conversion of light energy into functional products without depletion of natural resources (1,2).
Microalgae are considered one of the most promising feedstocks for sustainable production of commodities such as food, feed, chemicals, materials and biofuels. The technology for production is still immature, but if developed it is expected that biomass can be produced at commercial scale for a cost price less than 0,68 €/kg dry biomass (3). If the different biomass components are collected the total value for commodities in algal biomass is higher than 1.65 €/kg dry biomass (4).
Recently, we started the project AlgaePARC (www.AlgaePARC.com). AlgaePARC is a pilot facility with which we intend to bridge the gap between basic research and demonstration projects. In AlgaePARC we will compare state of the art technologies and develop new reactor concepts and process control strategies.
As a follow up projects are started to develop technology for disruption of algal biomass and fractionation of the biomass into different compounds with maintenance of their functionality. The technologies we develop should be mild, effective with respect to extraction efficiency and product purity and require a minimal amount of energy and costs. Algal biomass produced at AlgaePARC will be used for biorefining.
“Biorefinery” is a facility that integrates cell disruption, extraction, conversion and separation technologies of biomass. The biorefinery strategy is analogous to today’s petroleum refinery, in which multiple fuel products and chemicals are produced from crude petroleum. Biorefinery includes the selective isolation of products (proteins, carbohydrates, lipids) from crude biomass.
The co-production of multiple products from microalgae is a new challenge. Research and development needs to be done to explore scalable mild cell disruption and extraction technologies that keep the functionality of the different cell components.

1. Wijffels R.H., Barbosa M.J. (2010) Science, 379: 796-799
2. Wijffels R.H., Janssen M., Barbosa M.J. (2011) Microbial Biotechnol, 4: 132-134
3. Norsker N.H., Barbosa M.J., Vermuë M.H., Wijffels R.H. (2010) Biotechnology Advances, 29: 24-27
4. Wijffels R.H., Barbosa M.J., Eppink M.H. (2010) Biofuels, Bioproducts, & Biorefining, 4: 287-295

[Keywords] Microalgae, Biorefinery

Improvement of ethanol yield from xylose by breeding of industrial yeast

○Haruyo HATANAKA, Tomohide MAEDA, Yuri YASUE, Kazue NISHIYAMA

Suntory Business Expert Limited, Institute for Microbial Science

Xylose is one of main sugars abundant in biomass like switch grass, corn cobs, rice straws and wood chips. A xylose fermenting yeast has been constructed by recombining genes of xylose reductase (XR) and xylitol dehydrogenase (XDH) from *Pichia stipitis*, and a gene of xylulose kinase (XKS) from *Saccharomyces cerevisiae* under the control of strong promoter. When this recombinant yeast ferments xylose to ethanol, substantial quantities of xylitol and glycerol are also produced as by-products, resulting in less efficient ethanol production, compared with that from glucose. It is considered that cofactor imbalances might be the reason for the inefficient ethanol production, because XR requires NADPH as a cofactor, while XDH uses NAD as a cofactor. *S. cerevisiae* uses NAD for the oxidative reactions and NADPH for the reductive reactions in order to synthesis biomass. *S. cerevisiae* reoxidizes NADH to NAD by the reaction to make glycerol under anaerobic condition. *S. cerevisiae* has three NAD(H) kinases, Utr1p and Yef1p in the cytosol, and Pos5p in the mitochondria. These NAD kinases have different preferences for cofactors as substrates. In this study, we tested whether the deletions or overexpressions of these NAD kinase genes change the cofactor balance and improve the ethanol yields from xylose. The strain disrupted *UTR1* and *YEF1* showed the highest ethanol yield and produced the lowest xylitol and glycerol among the bred strains. It indicates that the shortage of NAD is one of the reasons for a high accumulation of xylitol. Moreover, we found that xylitol was excreted by glycerol channel, Fps1p, at some extent, and the disruption of Fps1p decreased xylitol production and resulted in higher ethanol yield. Industrial yeast often has the ability of high ethanol production, or high stress tolerance. However most of them are multiploid and have no spolulation ability. We tried to delete each set of *UTR1, YEF1* and *FPS1* from one industrial diploid strain by using a marker recycling system, and confirmed these breeding for the fermentation from xylose were also available for industrial yeast of diploid.

[Keywords] Bioethanol, Xylose, NAD kinase, Glycerol channel

Applications of microbial factory technology to production of bioethanol from cellulosic biomass

Jung-Hyun JO[1], Sang-Min JUNG[1], Yong-Cheol PARK[2], Yong-Su JIN[3], ○Jin-Ho SEO[1]

[1]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea
[2]Department of Advanced Fermentation Fusion Science and Technology, Kookmin University, Seoul 136-702, Republic of Korea
[3]Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Microbial factory technology (MFT) is composed of engineering of the key metabolic pathways, enforcement of cofactor biosynthetic capacities and global optimization from genetic modification at a gene level to fermentative strategies at a process scale. MFT has been used to produce bioethanol from cellulose biomass by metabolically engineered *Saccharomyces cerevisiae*.

As cellulosic biomass hydrolyzates are composed pentoses (C-5 sugar) and hexoses (C-6 sugar), economic production of cellulosic bioethanol requires utilization of both sugars, primarily glucose and xylose. Wild type *Saccharomyces cerevisiae* cannot convert xylose to ethanol because the yeast does not have a xylose metabolizing activity. One of the key problems with using the recombinant *S. cerevisiae* transformed with the *Scheffersomyces stipitis* derived genes coding for NADPH-dependent xylose reductase (XR) and NAD^+-dependent xylitol dehydrogenase (XDH) as a cellulosic bioethanol producer includes the cofactor mismatch between XR and XDH.

Protein engineering was employed to make NADH-preferring XR and $NADP^+$-dependent XDH. A synthetic isozyme system was generated by expressing both wild and mutant XR or both wild and mutant XDH and characterized for the ability of xylose metabolism in an effort to construct a metabolic highway for xylose utilization.

S. cerevisiae has transporters (proteins responsible for sugar transport) with high affinity for glucose relative to xylose. Xylose transport is strongly inhibited by the presence of glucose in the fermentation broth. A mixture of cellobiose and xylose was used instead of a mixture of glucose and xylose to avoid competitive transport between glucose and xylose. A recombinant *S. cerevisiae* was constructed for simultaneous utilization of both cellobiose and xylose by introducing the two genes encoding a cellobiose-specific transporter and β–glucosidase able to hydrolyze cellobiose to glucose. Engineering optimization was done to determine the optimum profiles of cellobiose and xylose with fermentation time for the maximum productivity of ethanol.

[Keywords] Cellulosic biomass, Bioethanol, Recombinant *Saccharomyces cerevisiae*, Synthetic isozyme system, Sugar transport

Potential in bioethanol production from waste paper sludge in pulp-based biorefinery

○Enoch Y. PARK[1,2], Joni PRASETYO[1], Kazuya NARUSE[2], Tatsuya KATO[2]

[1]Laboratory of Biotechnology, Graduate School of Science and Technology, Shizuoka University

[2]Laboratory of Biotechnology, Faculty of Agriculture, Shizuoka University

Ethanol production from waste papers or paper sludge (PS) of pulp industry is considered to be the most appropriate way to the biorefinery process, because it does not require any delignification process. In this study, cellulase production from cellulase producer, saccharification of paper wastes using produced cellulase and ethanol production from hydrolysate of waste papers were investigated. Especially, a simultaneous saccharification and fermentation (SSF) was conducted using a cellulase produced from PS by the hyper cellulase producer, *Acremonium cellulolyticus* C-1 for PS saccharification, and a thermotolerant ethanol producer *Saccharomyces cerevisiae* TJ14 for ethanol production. Using cellulase of PS origin minimizes biofuel production costs, because the culture broth containing cellulase can be used directly. When 50 g PS cellulose/l was used in SSF, the ethanol yield was 0.23 g ethanol/g PS cellulose, and was two times higher than that in separated hydrolysis and fermentation (SHF). Cellulase activity throughout in SSF remained around 50% to the initial activity. The ethanol yield in the range from 50 to 150 g PS cellulose/l in SSF was 0.22–0.24 g ethanol/g PS cellulose. Ethanol production was 40 g/l under the condition of 161 g/l of PS cellulose using 15 (filter paper unit/g PS cellulose) at 80 h in SSF. This indicates that the utilization of PS as raw material for bioethanol production offers a good prospect. On the other hand, one-pot bioethanol production including both productions of cellulase and ethanol was conducted using the hyper cellulase producer, *Acremonium cellulolyticus* C-1 and an ethanol producer *S. cerevisiae* with Solka flock (SF), crystal cellulose as a sole carbon source. Cellulase activity remained 8–12 FPU/ml during one-pot process. Ethanol concentration increased with the SF concentration. When 50–300 g SF/l was used in 500 ml Erlenmeyer flask scale, the ethanol concentration and yield based on initial SF were as 8.7–46.3 g/l and 15.4–17.5% (g ethanol/g SF), respectively. This indicates that one-pot ethanol process, producing cellulase and ethanol with two different microorganisms, is an option for bioethanol production.

[Keywords] Paper sludge, Biorefinery, Bioethanol, Cellulase, Saccharification

Application of Metabolomics to high resolution phenotype analysis

○Eiichiro FUKUSAKI

Department of Biotechnology, Graduate School of Engineering, Osaka University.

Metabolomics is applicable without genome sequence information. Therefore, Metabolomics would be powerful analytical tool for commercially available plant and microorganisms. According to the above context, Metabolomics means exhaustive relationship analysis between 'data matrix of metabolic profile' and 'data matrix of performance of samples'. Among those operation of Metabolomics, metabolic fingerprinting, in which metabolic profile is used as fingerprint for samples' performance prediction and classification, is one of the most important application of Metabolomics. On this occasion, practical operation of Metabolomics for high resolution phenotype analysis would be presented. To reveal a power of Metabolomics, a possibility of Metabolomics based prediction of "Life span", which is highly integrated and complicated quantitative phenotype, was studied to find strong relationship between metabolome and lifespan by means of PCA. Orthogonal projection on latent structure (OPLS) based model can predict yeast life span using metabolome as fingerprint. Using information of principal component vector in PCA life span related metabolites were nominated and life span related genes were speculated. The result implied Metabolomics would be applicable for high resolution analysis of a complicated quantitative phenotype, and metabolome information might be useful for strategic enhancement of useful quantitative phenotype for biomass production.

[Keywords] Metabolomics, Metabolic profiling, Metabolome, GC/MS, Multi variate analysis

Biological production of 3-hydroxypropionic acid from glycerol

○Sunghoon PARK

School of Chemical and Biomolecular Engineering, Pusan National University

For every 9 kg of biodiesel produced, about 1 kg of crude glycerol is left behind which poses a challenge and opportunity to find the best use of it. Here we present the production of a valuable platform chemical, 3-hydroxypropionic acid (3-HP), from glycerol using genetically engineered *Escherichia coli* and *Klebsiella pneumoniae* strains. In one approach, we screened and expressed various aldehyde dehydrogenases (ALDHs) along with glycerol dehydratase (GDHt) in *E. coli* and demonstrated that a recombinant bearing GDHt and an ALDH produced 3-HP above 50 g/L in 72 h with an average yield of 33% on glycerol. To improve the yield, the recombinant *E. coli* strain was further developed by disrupting the gene *glpK* (glycerol kinase), *ldhA* (lactate dehydrogenase), *frd* (fumarate reductase AB). The resting cells of the recombinant mutant produced 3-HP with an average yield of 80.5% on glycerol. In the other approach, NAD^+-dependent gamma-glutamyl-gamma-aminobutyraldehyde dehydrogenase (PuuC) of *K. pneumoniae* DSM 2026 which oxidizes 3-hydroxypropionaldehyde to 3-HP was homologously overexpressed in various *K. pneumoniae* DSM 2026 and a newly isolated *K. pneumoniae* J2B. The *K. pneumoniae* strains have been engineered to different genetic background such as Δ*dhaT*, Δ*yqhD,* Δ*ldhA* Δ*adhE,* Δ*glpK*. etc individually and in combination. Recombinant *K. pneumoniae* could successfully produce 3-HP and 1,3-propanediol (1,3-PDO) separately or simultaneously. This talk will summarize our recent work on the production of 3-HP and 1,3-PDO from glycerol.

[Keywords] 3-Hydroxypropionic acid, 1,3-Propanediol, Glycerol, Metabolic engineering

Biodiesel production by enzymatic process and the utilization of crude glycerol

Hah Young YOO, Laxmi Prasad THAPA, Ja Hyun LEE, Sang Jun LEE,
○Seung Wook KIM

Department of Chemical and Biological Engineering, Korea University, Seoul, Korea

Currently environmental, economical and security issues demanded alternative energy sources of fossil fuel and petroleum products. Therefore, nowadays biofuels like biodiesel and bioethanol became more attractive sources of energy due to its renewable and environmental benefits. In this work, advanced enzymatic immobilization method and optimal reaction conditions for biodiesel production were investigated. Non-edible oil sources, including waste oil were utilized as biodiesel feedstock for environmental friendly production process. Circulation and continuous processes by using co-immobilized lipase were investigated for the improvement of biodiesel production in a packed-bed reactor.
Secondly, crude glycerol which is by-product of biodiesel production was used for cephalosporin C (CPC) and bioethanol production. *Acremonium chrysogenum* M35 showed high levels of differentiation of hyphal fragments into arthrospores when cultured in the presence of glycerol. The glycerol utilization led to significantly higher CPC production as compared to rice oil.
Similarly, bioethanol was produced continuously from *Enterobacter aerogenes* using immobilized cells in bioreactor. To enhance the production of bioethanol from crude glycerol we optimized various process parameters by using statistical method. We also developed a glycerol utilizing *Escherichia coli* BL21 (DE3) strain for the production of bioethanol in the presence of ethanol producing gene set of *Enterobacter aerogenes*.
The results presented here suggest that low-priced glycerol takes the place of natural oil and methionine for CPC and bioethanol production in the industrial scale.

[Keywords] Biodiesel, Bioethanol, Cephalosporin C, Enzyme immobilization

Understanding and altering the unique metabolism of hyperthermophilic archaea

○Haruyuki ATOMI[1,2], Kunio MIKI[2,3], Tadayuki IMANAKA[2,4]

[1]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University
[2]CREST, Japan Science and Technology Agency (JST)
[3]Department of Chemistry, Graduate School of Science, Kyoto University
[4]Department of Biotechnology, College of Life Sciences, Ritsumeikan University

Archaea represent the third domain of life and display unique biological properties not found in Bacteria and Eucarya. Their membrane lipids consist of isoprene molecules covalently bound to glycerol molecules through ether bonds. The methanogens, chemoautotrophs that rely on the conversion of hydrogen and carbon dioxide to methane as an energy source, are all members of the archaea. Our studies are mainly focused on the hyperthermophilic archaeon, *Thermococcus kodakarensis*. This organism is an obligate anaerobe and heterotroph, and grows at extremely high temperatures with an optimum at 85˚C. We have determined its genome sequence and have also established a genetic system through which we can disrupt, insert and modify genes of interest. The genetic system allows us to directly examine and confirm gene function *in vivo*. We are presently interested in the unique metabolism of this archaeon and its regulation. The organism can degrade a variety of sugar polymers with enzymes and pathways distinct to eukaryotes/bacteria. *T. kodakarensis* can utilize elemental sulfur as a terminal electron acceptor, but can also dispense electrons via hydrogenases, resulting in the generation of molecular hydrogen. Here we will present our recent findings on the unique metabolism of this organism, giving attention to enzymes/pathways related to glycolysis/gluconeogenesis, hydrogen production, pentose metabolism and coenzyme A biosynthesis. We will also present some initial attempts to alter the metabolism of *T. kodakarensis* utilizing the gene manipulation system developed in this archaeon.

[Keywords] Archaea, Hyperthermophile, Metabolism, Regulation

Blue Ocean for Blue Biotechnology – Marine Bioenergy Production

○Choul-Gyun LEE[1,2]

[1]Department of Biotechnology, Inha University, Incheon 402-751, Korea
[2]Korea National Consortium on Marine Bioenergy Research

Algal biotechnology is drawing increasing interest due to its potential as a source of sustainable clean liquid energy. Microalgal biotechnology has also shown enormous potentials in valuable pharmaceuticals, pigments, carbohydrates, and other fine chemicals. Its application has been extended to the areas of wastewater treatment and agriculture. Recent development in various algal biotechnology found microalgal mass culture can be a useful solution in treatment of wastewater, fixation of carbon dioxide and production of biofuel. However, despite all these advantages of microalgal biofuels, there are quite a number of challenges to overcome before economic production of microalgal biofuel can be achieved: (i) finding/constructing algae strain(s) suitable for mass culture and for wide range of climate; (ii) maximizing solar conversion efficiency in mass culture; (iii) achieving both high oil content and high productivity in mass culture; (iv) designing and engineering of cost effective sustainable mass culture systems; (v) harvesting microalgae and extracting microalgal oils with minimal use of energy; (vi) finding cheap (and renewable) sources for methanol and nutrients (such as phosphate and nitrate). Most of the culture systems and bioprocesses available today would be suitable for the products that cost over $20 USD/kg. Bioenergy must be produced much cheaper than most of the biologically-driven products. One of the possible solutions for some of these challenges is mass culturing microalgal in large ocean area. All the opportunities and challenges of microalgal biofuels will be discussed. Possible ranges for different biofuels will be estimated using the maximum energy conversion efficiency from Sun to biomass and/or biofuels. Then, the feasibility of various bioenergy production technologies will be compared by economic analysis.

[Keywords] Marine microalgae, Ocean culture, Photobioreactor, Microalgal biodiesel

Establishment of innovative technology to create new microalgal strains increasing biofuel production by polyploidization and heavy-ion beam irradiation

○Shigeyuki KAWANO[1,2], Koichi WATANABE[1,2], Shuhei OTA[1,2]

[1]Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo
[2]CREST, Japan Science and Technology Agency (JST)

To make the biofuel production using microalgae practicable, it is necessary to breed their strains which can be mass-produced just like grains and horticultural crops. However, there was no conception of breeding in the microalgae so far, or their genomes have not been decoded in most of species. In the present study, the microalgae irradiated with the heavy-ion beams, which have the successful results by the selective breeding of horticultural crops, will be selected and bred based on the quantitative data concerning their forms. We aim for the establishment of the breeding scheme, which is innovative, advanced and specialized in microscopic algae, based on complete genome information.
The green microalgae, *Chlorella* and *Haematococcus*, have been focusing on their own natural height-productivity of starch and oil. *Chlorella* species under sulfur-deficient conditions promoted transient accumulation of starch followed by a steady increase in lipid storage. Transmission electron microscopy (TEM) indicates an increase and decrease in starch granules and subsequent enlargement of lipid droplets under sulfur-deficient conditions. They accumulate 1.5–2.7-fold higher amounts of starch and 1.5–2.4-fold higher amounts of lipid under sulfur-deficient conditions than under sulfur-sufficient conditions. *Haematococcus pluvialisi* is known by accumulating “astaxanthin” in the stressful environment like light and oligotrophic conditions, and it is used widely in the biomass industry in recent years. Ultrastructural 3D reconstruction based on 300–350 serial sections for TEM has applied to analyze the dynamics of astaxanthin-accumulation and chloroplasts-degradation during encystment under the continuous light. There are no developing chloroplasts but many large starch grains surrounded by small fragmented residues of the thylakoid membrane. They decrease gradually whereas many large oil drops, which seemed to accumulate astaxanthin, appeared in the cytoplasm outside the chloroplast. They occupied finally not less volume than 52% of cell. We are now isolating many mutant strains of *Chlorella* and *Haematococcus* by polyploidization and heavy-ion beam irradiation.

[Keywords] *Chlorella*, *Haematococcus*, 3D reconstruction, Sulfur-deficient, Astaxanthin

New dimensions in microalgae biomass production for bioenergy

○Otto PULZ

IGV, Nuthetal, Germany

Recent developments in mass production of microalgae as phototrophic microorganisms are demonstrated.
In recent years there arose an urgent need for new solutions for phototrophic biotechnology out of the development of technologies for carbon capture (CO_2) and bioenergy.
The most widespread technology for the mass production of microalgae in raceway ponds is with a productivity of 15–20 g/m^2×d not productive enough to fit in the demands of CO_2-emitting companies like power plants.
Two goals are in focus for the emerging phototrophic technologies:
- high productivities in mass cultivation (up to 80 g/m^2×d)
- low cost area demand
The key issue is to find effective ways to introduce light into photobioreactors.
The most important designs of photobioreactors with their R&D demands are described.

[Keywords] Microalgae, Mass cultivation, Photobioreactors

Development of sustainable biorefinery based on microalgae

○Akihiko KONDO[1,2]

[1]Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University
[2]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST)

Bio-refinery, the utilization of biomass as the starting material for the production of fuels and chemicals, has received considerable attention in recent years. Polysaccharides present in lignocellulosic materials are of great interest as alternative feedstocks for second-generation ethanol production, because they are abundant, inexpensive, and renewable sources of sugars for microbial fermentation. However, the use of terrestrial plants will soon be limited by the unavailability of vegetated regions on earth and fresh water required for growing plants. Microalgae have attracted attention as an alternative feedstock because they convert solar energy to algal biomass more efficiently than higher plants. Furthermore, microalgae grown in an aquatic environment provide additional benefits of year-round cultivation without requiring arable land. In particular, the use of salt-tolerant microalgae grown in seawater as a feedstock has the advantage of eliminating the load on freshwater resources for their cultivation. The halophilic cyanobacterium *Arthrospira* (*Spirulina*) *platensis* is a potential microalgal feedstock because it accumulates a large amount of glycogen and grows faster than other microalgae. Moreover, *A. platensis* cultures have the advantage of being resistant to bacterial contamination because they can grow under conditions of high pH and high salinity. Accordingly, *A. platensis* is expected to be a promising supplier of carbohydrate in the form of glycogen. Also, we developed direct ethanol production from microalgal carbohydrate by using one of our core technologies, cell surface engineering of *Saccharomyces cerevisiae*. In this symposium, we will introduce our project, development of bioethanol production from marine microalgae, which is constructed from 1) development of mass cultivation system of *A. platensis* under marine water condition, 2) development of a novel genome modification technology for microalgae, 3) system biology analysis of microalga, 4) improvement of photosynthetic capacity of microalgae through genetic engineering, 5) development of ethanol production system from microalgal glycogen by using cell surface engineering.

[Keywords] Bio-refinery, Bio-ethanol, Microalgae

Chlamydomonas reinhardtii - A powerful host for the production of biofuels and high value products

○Olaf KRUSE

Bielefeld University; Department of Biology/ Center for Biotechnology
Algae Biotechnology & Bioenergy Group

The increasing interest on the application of phototrophic microorganisms as a production host for sun energy based bio-products is reflected by a variety of worldwide established collaborative research initiatives working in the field of algae biotechnology.
This presentation will summarize recent advances achieved in our group with the microalga *C. reinhardtii* and includes new molecular engineering approaches for more efficient sun-to-biomass conversion efficiency and the establishment of a mechanism for the production/secretion of a high value product. In addition it will be demonstrated that *C. reinhardtii* with its cellulose-free cell wall has the ability of cellulose degradation and assimilation for growth, a phenomenon which has been previously only shown for heterotrophic bacteria and fungi. Phototrophic microbes like *C. reinhardtii* may thus serve as bio-catalysts in cellulosic biofuel production approaches.
Finally, the identification of a new microalgal candidate for an efficient bio-refinery concept including rapid cell growth, high lipid content and good biogas production yields will be presented.

[Keywords] Microalgae, *C. reinhardtii*, Biofuels, Cellulose, Light harvesting

Alkenone biosynthesis by marine Haptophytes as biorefinery for renewable energy production

○Yoshihiro SHIRAIWA[1,2]

[1]Faculty of Life & Environmental Sciences, University of Tsukuba, Japan
[2]CREST, Japan Science and Technology Agency (JST), Japan

Marine microalgae have contributed to change in global environment by reducing carbon dioxide concentration and increasing oxygen concentration. Such changes have been the motive force for the evolution of microalgae during three billion years. Coccolithophorids (Haptophytes), marine unicellular calcifying algae producing calcium carbonate crystals as cell covering and lipid droplet as storage compound, are known to be sources for lime stones and petroleum produced at the Cretaceous era. According to literatures, the white cliff in Dover and petroleum in the Middle East countries were produced from huge mass of inorganic and organic cell components of such coccolithophorids, respectively. Coccolithophorids are still producing a huge bloom even in the present ocean especially at high latitude ocean such as the North Atlantic Ocean and the Bering Sea. In this talk, I will focus on “the potential of haptophytes” for the reduction of atmospheric CO_2 and especially the production of renewable energy source. Some of haptophyte algae do not produce triglycerides but produce oil droplets such as long-chain ketones, namely alkenones and alkens, which are associated with bio-oil production, in the cells. These metabolites occupy ca. 30% of cell dry mass and are candidates for liquid and gaseous hydrocarbons as biofuels. Our researches on alkenone producing haptophytes, their growth regulation, mechanism for metabolic analysis of alkenone biosynthesis and efficient production of useful metabolites and the development of lipid metabolomics will be introduced.

[Keywords] Alkenone biosynthesis, Biofuel, Biorefinery, Coccolithophorid, Haptophyte algae, Lipid, Long-chain ketone, Metabolome

Characterization of hydrocarbon biosynthesis and secretion mechanisms by the green microalga, *Botryococcus braunii* to control biofuel production

○Shigeru OKADA[1,5], Ikuro ABE[2,5], Tetsuko NOGUCHI[3,5], Takeshi OHAMA[4,5]

[1]Department of Aquatic Biosciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo
[2]Graduate School of Pharmaceutical Sciences, The University of Tokyo
[3]Department of Biological Sciences, Faculty of Science, Nara Women's University
[4]Department of Environmental Systems Engineering, Kochi University of Technology
[5]CREST, Japan Science and Technology Agency (JST)

The green microalga *Botryococcus braunii* produces unusually large amounts of liquid hydrocarbons and secretes them outside of cells. One of the chemical races of this alga produces specific triterpenes, botryococcenes and methylsqualenes, that are promising as an alternative fuel source since they are chemically very similar to petroleum. The genes coding for enzymes responsible for the synthesis of these triterpenes, squalene synthase-like proteins (SSL)-1, -2, and -3, were recently identified (Niehaus *et al*., 2011). The objective of our research is to uncover "why and how" these triterpenes are produced and secreted by this alga not only at the cellular but also the molecular level by four strategies; 1) Characterization of the mechanism for gene expression related to triterpene biosynthesis in order to regulate hydrocarbon production, 2) Analysis of the reaction mechanisms for SSL-1, -2, and -3 based on X-ray crystallography to modify their functions for more effective hydrocarbon production, 3) Microscopic observations of intercellular organelles in terms of hydrocarbon production and secretion to understand the unique system of hydrocarbon secretion by the alga, and 4) Establishment of techniques for transformation of the *B. braunii* in order to identify functions of unknown genes or to generate new algal strains more suitable for biofuel production. Information on expression patterns of the genes concerned with hydrocarbon production during culture periods, the important amino acid residues in SSL-1 that would recognize carbon chain length of the substrate, and the relation between hydrocarbon secretion and cell division cycles were accumulated. Techniques that may enhance introduction of exogenous genes were also developed.

T. D. Niehaus, S. Okada, T. P. Devarenne, D. S. Watt, V. Sviripa, and J. Chappell (2011) *PNAS*, **108**: 12260-12265

[Keywords] Microalgae, *Botryococcus*, Hydrocarbon, Biosynthesis, Transformation

Metabolic engineering of cyanobacteria for the light-powered production of hydrogen from water

○Matthias RÖGNER

Plant Biochemistry, Faculty of Biology & Biotechnology, Ruhr-University Bochum, Bochum, Germany

In order to produce hydrogen as potential future renewable energy source from water, we propose to engineer cyanobacterial photosynthesis towards increased bioenergy instead of biomass production. Besides the implementation of a highly active, oxygen tolerant hydrogenase from other organisms such as green algae (this requires a special strategy...), especially the photosynthetic electron metabolism has to be engineered in many individual steps towards this goal. The result of each single engineering step (such as antenna size reduction, partial uncoupling of the thylakoid membrane, re-routing of electrons at the photosystem 1 acceptor site) has to be monitored by both functional (for instance spectroscopy) as well as metabolic characterization on the whole cell level (for instance by an in depth quantitative proteome, lipidome- and metabolome analysis). The direction of engineering is supported by studying protein-protein-interactions in isolated model systems – for instance the affinity of Ferredoxin (Fd) for Ferredoxin-NADP-Oxidoreductase (FNR) *vs*. hydrogenase, which is a decisive step for re-routing electrons from water for hydrogen production instead of CO_2-fixation.
Performance of such engineered cells has to be optimized by improving fermentation conditions and by an optimal photobioreactor design. Optimal culture conditions can be found and kept constant for several months by using continuous flow fermentation techniques which allow the systematic optimization of each individual parameter. Provided such systems are optimized both on the individual cell level and on the systems level, a more than 100-fold increase of hydrogen production in comparison with the most productive natural systems existing to date can be estimated, which would be a promising basis for an economically competitive H_2-production.

Esper, B., Badura, A. & Rögner, M. (2006) *Trends in Plant Sci.* 11, 543-549
Bernat, G., Waschewski, N. & Rögner, M. (2009) *Photosynth. Res.* 99, 205-216
Waschewski, N., Bernat, G. & Rögner, M. (2010) *in: "Biomass to Biofuels – Strategies for Global Industries" (Vertes, A., Qureshi, N., Yukawa, H., Blaschek, H.P. eds.) John Wiley & Sons, Chichester, UK,* p. 387-401
Imashimizu, M., Bernát, G., Isato, K., Broekmans, M., Konno, H., Sunamura, E.-I., Rögner, M., Hisabori, T. (2011) *J. Biol. Chem.* 286, 26595-26602
Badura, A., Kothe, T., Schuhmann, W. & Rögner, M. (2011) *Energy Environ. Sci.* 4, 3263-3274

[Keywords] Biohydrogen production, Design cell, Cyanobacteria, Photosynthesis, Biobattery, Photoelectrochemistry

Selection strategies for the promising microalgal strains for algae-fuel production

○Hideaki MIYASHITA[1], Norihide KURANO[2]

[1]Department of Interdisciplinary Environment, Graduate School of Human and Environmental Studies, Kyoto University
[2]Research laboratories, DENSO CORPORATION

Bio-fuel production using microalgae is a sustainable system based on oxygenic photosynthesis using solar irradiation, CO_2 and water. Therefore, the algae-fuel has been actively investigated as the alternative source of energy against the oil crisis in the future, as well as for a CO_2 recovering process to prevent the green house effects on the Earth. However, only a few large-scale systems for commercial production have been established, since the algae-fuel is uneconomic since the production cost is very high at present. Interdisciplinary researches including phycology, physiology, molecular biology, chemistry, chemical engineering and so on are required to cut down the production cost. Strain selection of microalgae is one of the effective strategies for the cost reduction. Even though more than 35,000 species of algae have already been described, genera that have been evaluated for large-scale production are not so many even now. Moreover, the number of species described until recently was reported to be just several % of estimated algal species on the Earth. Therefore, continuous efforts to isolate new algae from natural environments as well as the systematic selection of promising oil-producers are extremely important. We have continued to isolate and evaluate microalgae which are suitable for large-scale fuel production using open pond system, and successfully obtained several strains which are efficient for fuel production. On the way of the selection, we set microalgal abilities required for open pond system. Those are high biomass productivity at low pH under high (>35˚C) or low (<15˚C) temperatures, and oil productivity. In this presentation, we would like to introduce our strategies for algae isolation and selection for fuel production using open pond system, in addition to the growth characteristics of selected microalgae.

[Keywords] Microalgae-fuel, Strain selection strategy, Open pond

Elucidation of novel triterpene pathways in *Botryococcus* and engineering plants for high-value oil production

Tom D. NIEHAUS[1], Scott KINISON[1], Shigeru OKADA[2], Shuiqin WU[1], Timothy P. DEVARENNE[3], David S. WATT[4], Vitaliy SVIRIPA[4], ○Joe CHAPPELL[1]

[1]Plant Biology Program, University of Kentucky, Lexington, KY, 40546-0312, USA
[2]Department of Aquatic Biosciences, University of Tokyo, Tokyo 113-8657, Japan
[3]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, 77843-2128, USA
[4]Department of Cellular and Molecular Biochemistry, University of Kentucky, Lexington, KY, 40536, USA

Assuming biofuels generated via the fermentation of sugars derived from cellulosic and non-cellulosic constituents of biofuels crops will provide a substantial contribution to our future energy needs, augmenting and amending the productivity of these biofuel crops is now a major research thrust worldwide. One way of enhancing these biofuels crops will be to engineer them for value-added components such as oils that can be used for efficient fuel production and the manufacturing of other high-value products currently derived from petroleum oils. Towards this end, we have developed an engineering strategy for optimized production of long, branched-chain hydrocarbon biosynthesis in plants. Branched chain hydrocarbons, like methylated triterpenes, are readily cracked into paraffins and naphthenes that can either be distilled to combustible fuels (gasoline, jet and diesel), or can be used directly for the synthesis of plastics, nylons, paints and other oil-derived products manufactured by diverse chemical industries. Our working hypothesis has been that success in generating high level production platforms for triterpene oils in plants can be accomplished by targeting this metabolism to unique compartments within the cell, thus eliminating the regulatory mechanisms that normally operate to control this metabolism occurring in the cytoplasm, and providing a means for the direct channeling of photosynthetically fixed CO_2 to the biosynthesis of novel, value-added products. Preliminary experiments suggests that we have been able to create plants yielding up to 1% of their dry weight as triterpenes. These results have important ramifications for furthering our understanding of basic metabolism in plants, as well as the development of novel chemical production platforms in plants.

[Keywords] Triterpenes, Botryococcene, Metabolic engineering

The Cyanofactory™ ~ a novel cyanobacterial bioprocess based on the synthetic biology concept ~

○Koji SODE[1,2]

[1]Tokyo University of Agriculture & Technology, 2-24-16 Naka-cho, Koganei-shi, Tokyo, 184-8588, Japan
[2]Japan Science and Technology Agency, CREST, 2-24-16 Naka-cho, Koganei-shi, Tokyo, 184-8588, Japan

Our research group is engaged in the development of a novel bioprocess based on the synthetic biology concept, for bioenergy production, designated as the "*Cyanofactory*™". The *Cyanofactory*™ is composed of **1)** synthetic marine cyanobacterial host strains, **2)** synthetic operons for the production of biofuel-related compounds, and **3)** the employment of ionic liquids for downstream processing.
"Synthetic marine cyanobacterial host strains" are the engineered cyanobacteria whose functions are highly controlled by the artificial signal transduction system, which is composed of the light sensors / histidine kinases, the response regulator and the riboregulator / riboswitch systems. This system counts light stimulation frequency and controls cell growth, biofuel-related compound production, self-aggregation and auto-lysis by regulation of the gene expression necessary for each process. Finally, the produced biofuel-related compounds are extracted by ionic liquids (ILs), which is recognized as a "Green solvents". The combination of downstream processes employing ILs realizes the sustainable production of biofuel-related compounds based on synthetic cyanobacterial processes with minimal energy and waste.

[Keywords] Cyanofactory, Cyanobacteria, Synthetic biology, Ionic liquid, Signal transduction

Bio-refinery from microalgae through fermentation and metabolic engineering

○Tomohisa HASUNUMA [1,2]

[1]Organization of Advanced Science and Technology, Kobe University
[2]PRESTO, Japan Science and Technology Agency (JST)

Bio-refinery, the utilization of biomass as the starting material for the production of fuels and chemicals, has received considerable attention in recent years. Environmental concerns and the depletion of oil reserves have resulted in governmental actions and incentives to promote research on environmentally benign and sustainable bio-fuels such as bio-ethanol. Cyanobacteria has emerged as a feedstock for producing bio-fuels since many microalgal species have the ability to assimilate CO_2 and accumulate large amounts of carbohydrate in the form of starch or glycogen in cells.
The halophilic cyanobacterium *Arthrospira* (*Spirulina*) *platensis* was investigated for its feasibility to serve as feedstock for fermentative production of bio-ethanol. The use of salt-tolerant microalgae grown in seawater as a feedstock has the advantage of eliminating the load on freshwater resources for their cultivation. Moreover, *A. platensis* cultures have the advantage of being resistant to bacterial contamination because they can grow under conditions of high pH and high salinity. With optimal control of both light intensity and nitrate supply, glycogen production of *A. platensis* reached nearly 1.03 g/L (a glycogen productivity of 0.29 g/L/d), which is, to the best of our knowledge, the highest α-polyglucan production performance ever reported in microalgae. In the present study, glycogen produced by *A. platensis* was directly converted to ethanol by a recombinant *Saccharomyces cerevisiae* strain expressing both *Streptococcus bovis* α-amylase and *Rhizopus oryzae* glucoamylase with more than 80% of theoretical yield.
For the enhancement of glycogen productivity in cyanobacteria, glycogen metabolism would be preferred to be modified through metabolic engineering. Glycogen is significantly accumulated in the cyanobacterial cell by the depletion of nitrate. We have developed an analytical system of dynamic metabolic profiling in cyanobacteria with metabolomics and *in vivo* ^{13}C-labeling technique. In the present study, metabolic intermediates involved in the Calvin cycle, glycolysis, TCA cycle, amino acid biosynthesis have determined during the perturbation by nitrate depletion. Based on the metabolic profiling system, putative mechanism of glycogen biosynthesis was successfully assumed under the nitrate depleted condition.

[Keywords] Microalgae, Metabolic engineering, Bio-refinery, Bio-ethanol

Synthetic biology approaches to produce C3-C5 alcohols from microorganisms

○Shota ATSUMI

Department of Chemistry, University of California Davis

Biofuels synthesized from renewable resources are of increasing interest because of global energy and environmental problems. Compared to the traditional biofuel, ethanol, higher alcohols such as isobutanol and 1-butanol offer advantages such as higher energy density, lower hygroscopicity, lower vapor pressure, and compatibility with existing transportation infrastructure. Some Clostridia species are known to naturally produce isopropanol and 1-butanol. However, these fuels are not synthesized economically using native organisms. Additionally, other C3-C5 alcohols are not produced in large quantities by natural microorganisms. Synthetic biology offers an alternative approach in which synthetic pathways are engineered into user-friendly hosts for the production of these fuel molecules. We built up unnatural synthetic pathways to produce higher-order alcohols. Moreover, we improved the productivity by combining gene deletion and overexpression techniques. Our demonstration shows that the strategy enables the exploration of biofuels beyond those naturally accumulated to high quantities in microbial fermentation.

[Keywords] Isobutanol, Biofuel, Synthetic biology

Synthetic metabolic engineering –A novel, simple technology for designing a chimeric metabolic pathway–

○Kohsuke HONDA[1,2], Xiaoting YE[1], Kenji OKANO[1], Hisao OHTAKE[1]

[1] Department of Biotechnology, Graduate School of Engineering, Osaka University
[2] PRESTO, Japan Science and Technology Agency (JST)

A novel, simple technology, designated as “synthetic metabolic engineering”, for freely designing a chimeric metabolic pathway for bio-based production was proposed. In this technology, genes encoding thermophilic enzymes were overexpressed in mesophilic host. The excellent thermal stability of thermophilic enzymes allows us to obtain whole-cell biocatalysts as highly selective as purified enzymes by simply heating the resulting recombinant mesophiles. The *in vitro* synthetic metabolic pathways can be constructed by rational combination of these biocatalytic modules since this approach is applicable to all thermophilic enzymes as long as they can be functionally expressed in heterologous mesophiles. Owing to their independence from biological activity of living cells, synthetic pathways no longer play the physiological roles of natural metabolism involving an energy generation (catabolism) and energy consuming synthesis of biomolecules (anabolism), and thus a bespoke pathway specialized to chemical conversion can be constructed. In this work, we constructed a chimeric glycolytic pathway, in which the consumption and regeneration rates of ATP and ADP are balanced, by combinatorially using 7 glycolytic enzymes of *Thermus thermophilus*, the cofactor-independent phosphoglycerate mutase of *Pyrococcus horikoshii*, and the non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase of *Thermococcus kodakarensis*. By coupling this pathway with a thermophilic malate/lactate dehydrogenase, a stoichiometric amount of lactate could be produced from glucose with an overall ATP turnover number of 32.

[Keywords] Synthetic metabolic engineering, Thermophile, Embdem-Meyerhof pathway

Production of BioIsoprene™ Monomer

○Derek H. WELLS

DuPont Industrial Biosciences

DuPont Industrial Biosciences and The Goodyear Tire & Rubber Company are developing a bio-based alternative to petroleum-derived isoprene, a key chemical required to produce a diverse range of products including specialty elastomers, motor mounts and fittings, rubber bands, golf balls, shoes and tires. The annual world market potential for polymer grade isoprene is approximately 2 billion dollars. Biological production of isoprene (BioIsoprene™ monomer) is a non-petrochemical process that directly addresses the lack of supply and price instability of this valuable commodity.

The biosynthetic production of isoprene presents significant metabolic engineering challenges, such as pathway enzyme regulation, carbon to product channeling, redox and energy balancing, and selection of appropriate enzymes. High titers of BioIsoprene™ monomer have been achieved by the introduction of an isoprenoid pathway and an engineered isoprene synthase into *Escherichia coli*. Analysis has demonstrated that the BioIsoprene™ product is >99% pure prior to recovery and purification. Pre-pilot work has led to the manufacture of prototype passenger car tires demonstrating the functional process from start to finish.

[Keywords] DuPont, Isoprene, Isoprenoid, Metabolic engineering, *Escherichia coli*

Evolutionary pathway engineering: toward the fast-track construction of long-step, but high-fidelity pathway for non-natural compounds

○Daisuke UMENO[1,2], Maiko FURUBAYASHI[1], Kyoichi SAITO[1]

[1]Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University
[2]PRESTO, Japan Science and Technology Agency (JST)

Rapid expansion of available biosynthetic gene is enabling synthetic biologists to start constructing various pathways for non-natural compounds. However, targeted synthesis of non-natural compounds remains a challenge for two reasons. First, non-natural pathways fatally contain non-existing biochemical steps, and we have to create all and each of them. Second, due to the promiscuity or imperfectness of biosynthetic parts, simple assemblage of biosynthetic genes generates hyper-branched pathway that divert precursors into numerous unwanted compounds. Here, we demonstrate the construction of long-step pathway to a series of carotenoids and steroids with non-natural backbones through the iterative rounds of directed evolution to create novel biochemical steps, generates diversity in substrate/ product specificity, followed by the strategic assemblage of the variant enzymes. We show that this way we can construct highly-specific pathways toward a targeted molecule out of the promiscuous enzymes.

[Keywords] Directed evolution, Secondary metabolism, Specificity, Gene regulatory networks, Carotenoids, Isoprenoids

Index

Authors Index

Keywords Index

Journal of Bioscience and Bioengineering

Published by the Society for Biotechnology, Japan
/ Distributed outside Japan by Elsevier

http://www.elsevier.com/locate/jbiosc

Print ISSN 1389-1723 Online ISSN 1347-4421 CODEN: JBBIF6


mosaic