The 13th Asian Congress on Biotechnology 2017 (ACB 2017)
“Bioinnovation and Bioeconomy”

http://www.acb2017thailand.org/
Organized by

Thai Society for Biotechnology (TSB)

Asian Federation of Biotechnology (AFOB)

Department of Biotechnology, Faculty of Technology, Khon Kaen University

Fermentation Research Center for Value Added Agricultural Products (FerVAAP), Khon Kaen University

Department of Chemical Engineering, Faculty of Engineering, Kasetsart University
GREETINGS

Preface and Welcome Address

It is my privilege to welcome our honorable guests and all participants to the 13th Asian Congress on Biotechnology (ACB 2017) organized by Asian Federation of Biotechnology (AFOB, www.afob.org), Thai Society for Biotechnology (TSB, www.biotec.or.th/tsb), Fermentation Research Center for Value Added Agricultural Products, Khon Kaen University (FerVAAP, fervaap.kku.ac.th) and Chemical Engineering Department, Kasetsart University (www.eng.ku.ac.th/en/).

ACB 2017 aims to provide a unique platform for scientists, academia and industry to present their current research findings in various biotechnological fields, particularly in Bioinnovation and Bioeconomy, as well as, to extend their existing networks to Asia, Europe and around the world. At ACB 2017, the participants will have chance to hear the updated bioinnovation and bioeconomy from 4 renown plenary lectures, 10 keynote speakers and 61 invited speakers, including 2 AFOB-EBF joint sessions on enzyme/catalysis and plant biotechnology, and 11 parallel sessions.

Not only the world leading speakers you will meet, but also leading scientists of over 300 delegates (two third is international participants) from 25 countries, namely Austria, Bangladesh, Cambodia, Canada, Czech Republic, France, India, Indonesia, Japan, Mainland China, Malaysia, Myanmar, Nepal, Norway, Pakistan, Philippines, Republic of Korea, Romania, Singapore, Sri Lankan, Switzerland, Taiwan, United State of America, Vietnam and Thailand. After academic incubating for 2 full days at the congress, the excursion will bring you out to the real experiences of different exploration of the rich culture of Vientiane in Laos and Cambodian influences of Phanom Rung Historical Park. You will see how the knowledge, people and culture become one without any boundary.

We are certain that you will have a premier time at ACB 2017, full of knowledge, great network strengthening and expansion, overwhelm of rich culture and enjoyable moment of selected Thai cuisine. Welcoming you again to ACB 2017 where bioinnovation and bioeconomic are in touchable distance.

Sincerely yours,

Associate Professor Dr. Penjit Srinophakun
Chairperson of the ACB 2017
Welcome message
from the President of Asian Federation of Biotechnology

Dear friends and colleagues,

On behalf of the President of Asian Federation of Biotechnology (AFOB), I would like to welcome you to the 13th Asian Congress on Biotechnology (ACB 2017) at Pullman Khon Kaen Raja Orchid, Khon Kaen, Thailand during July 23-27, 2017. I would like to thank the local organizers, Thai Society for Biotechnology (TSB), Fermentation Research Center for Value Added Agricultural Products (FerVAAP) and Chemical Engineering Department, Kasetsart University for their excellent job to make the remarkable success of ACB 2017. I would like to thank also for the great supports from AFOB members, EFB guests and Thai universities. Without your dedicated support and network this congress would have never been this success.

As the theme of the conference is bioinnovation and bioeconomy and we have over 300 people here, I would like to encourage the participants to find time to meet other delegates from 20 countries who come for the ACB 2017 comprising of professors, researchers, students, company executives, government staff and entrepreneurs to discuss about the startup projects. Hope we will have more startup companies after this congress. Finally, I wish you all have fruitful meeting. Welcome again to the ACB 2017!

Faithfully yours,

Professor Dr. George Fu Gao
President of Asian Federation of Biotechnology
Welcome message
from the President of Thai Society for Biotechnology

Dear friends and colleagues,

First of all, I would like to thank the Asian Federation of Biotechnology (AFOB) for giving the Thai Society for Biotechnology (TSB) the privilege to be the host of the 13th Asian Congress on Biotechnology (ACB 2017). Thanks also to the Fermentation Research Center for Value Added Agricultural Products (FerVAAP), Faculty of Technology, Khon Kaen University for being the co-host of this conference.

On behalf of the Thai Society for Biotechnology (TSB), I am delighted to welcome you to the 13th Asian Congress on Biotechnology (ACB 2017) on the theme of Bioinnovation and Bioeconomy which will be held in Khon Kaen, July 23-27, 2017.

As with the previous ACB Conferences, the ACB 2017 in each sub-division of AFOB will provide a wonderful forum for you to refresh your knowledge basis and explore the innovations in biotechnology. The conference will strive to offer plenty of networking opportunities, providing you with the opportunity to meet and interact with the leading professionals, academicians, researchers, friends and colleagues in the field of biotechnology, as well as sponsors and exhibitors. The first time of Joint Session of AFOB and EFB (European Federation of Biotechnology) in the ACB Conference (the 2nd Joint Session of AFOB-EFB) is also included in this ACB 2017.

Khon Kaen is situated in the center of the northeast of Thailand. It is a regional center for education, financial institutions, government offices, and transportation in the northeastern region. Khon Kaen University or KKU is a public research university in Thailand. It was the first university established in northeastern Thailand and remains the oldest and largest university in the region. The university is a hub of education in northeastern Thailand, which is also a widely recognized university in Asia. The President of KKU will be the host of the welcome dinner on 23rd July 2017 for the oversea participants.

Finally, a great conference is not remembered without an attractive social program. Vientiane, the capital of Loa RPD, is situated about 200 km from Khon Kaen. It is a slow life and pleasant city. Svelte and golden Pha That Luang in Vientiane is the most important national monument in Lao RPD; a symbol of Buddhist religion and Lao sovereignty. Phanom Rung or full name, Prasat Hin Phanom Rung, is a Khmer temple complex set on the rim of an extinct volcano at 402 metres (1,319 ft) elevation, in Buriram Province in the Northeastern region of Thailand. It was built of sandstone and laterite in the 10th to 13th centuries. I am sure that you will enjoy these 2 trips after full consumption of the scientific information.

We hope you will join us in this ACB 2017 Conference not only for a symphony of outstanding science, but also taking a little extra time to enjoy the spectacular and exciting of the “City of Center of Silk”, one of the symbolic of Khon Kaen.

With best wishes,

Assistant Professor Dr. Vichai Leelavatcharamas
President of Thai Society for Biotechnology
CONGRESS ORGANIZER

Chairperson:
Associate Professor Dr. Penjit Srinophakun

Secretary:
Professor Dr. Alissara Reungsang, Head of Biotechnology Department, Khon Kaen University

Advisory Board:
Professor Dr. Toshiomi Yoshida: former AFOB president
Professor Dr. Ho Nam Chang: former AFOB president
Professor Dr. Jung Keug Park: former AFOB secretary general
Professor Dr. Jian Jiang Zhang: former AFOB secretary general

Organizing Committee:
Assistant Professor Dr. Vichai Leelavacharamas: President of TSB
Professor Dr. George Fu Gao: President of AFOB
Professor Dr. Yoon Mo Koo: Secretary General of AFOB
Associate Professor Dr. Metta Charoen Panich, Head of Chemical Engineering Department, Kasetsart University
Associate Professor Dr. Chuenchit Boonchird, Advisory board member of TSB

Scientific Committee:
Chair: Associate Professor Dr. Sarote Sirisansaneeyakul, Thailand
Co-Chair: Professor Dr. Wen-Chien Lee, Taiwan

Scientific Committee members:
Professor Dr. Evo Frebort (EFB coordinator of ACB 2017)
Professor Dr. Masahiro Goto (DSG Publication), Japan
Professor Dr. Yinhua Wan (DSG Finance), Japan
Professor Dr. Duong Hoa Xo (Agricultural and Food Biotechnology), Vietnam
Professor Dr. Guo-Qiang Chen (Applied Microbiology), Mainland China
Professor Dr. Yuan Kun Lee (Applied Microbiology), Singapore
Professor Dr. Yew-Min Tzeng (Biopharmaceutical and Medical Biotechnology), Taiwan
Professor Dr. Teruyuki Nagamune (Biocatalyst and Protein Engineering), Japan
Professor Dr. Fengwu Bai (Bioprocess and Bioseparation Engineering), Mainland China
Professor Dr. Yoon Mo Koo (Bioprocess and Bioseparation Engineering), Korea
Professor Dr. Wen-Teng Wu (Bioenergy and Biorefinery), Taiwan
Professor Dr. Mohd Ali Hassan (Environmental Biotechnology), Malaysia
Professor Dr. Masao Fukuda (Environmental Biotechnology), Japan
Professor Dr. Choul-Gyun Lee (Marine Biotechnology), Korea
Professor Dr. Tai Hyun Park (Nanobiotechnology, Biosensors and Biochips), Korea
Professor Dr. Xian-En Zhang (Nanobiotechnology, Biosensors and Biochips), Mainland China
Professor Dr. Sang Yup Lee (Systems and Synthetic Biotechnology), Korea
Professor Dr. Zixin Deng (Systems and Synthetic Biotechnology), Mainland China
Professor Dr. I-Ming Chu (Tissue Engineering and Biomaterials), Taiwan
Professor Dr. Jung-Keug Park (Tissue Engineering and Biomaterials), Korea
Professor Dr. Satyahari Dey (Bioindustry Promotion and Bioeducation), India
Professor Dr. Chester Ho (Bioindustry Promotion and Bioeducation), Taiwan

**Scientific Local Committee members**
Professor Dr. Alissara Reungsang
Professor Dr. Vilai Rungsardthong
Associate Professor Dr. Mariena Ketudat-Cairns
Associate Professor Dr. Pawinee Chaiprasert
Associate Professor Dr. Ratchaneewan Aunpad
Associate Professor Dr. Aphichart Karnchanatat
Assistant Professor Dr. Prakit Sukyai
Assistant Professor Dr. Theppanya Charoenrat
Assistant Professor Dr. Rujikan Nasanit
Assistant Professor Dr. Tatsaporn Todhanakasem
Dr. Kuakoon Piyachomkwan
Dr. Watson Ariyaphuttarat
Dr. Adisak Romsang

**Sponsorship:**
Professor Dr. Sirirat Rengpipat
Assistant Professor Dr. Theppanya Charoenrat

**Proceeding**
Assistant Professor Dr. Prakit Sukyai

**Website:**
Assistant Professor Dr. Rujikan Nasanit
ACKNOWLEDGEMENTS OF SPONSORSHIP

The Organizing Committee gratefully thanks to the generous contributions from the following:

**Gold Level**

- Betagro Public Company Limited
- Kasetsart University Research and Development Institute (KURDI)
- Mitr Phol Innovation & Research Center

**Silver Level**

- The Thailand Research Fund
- National Research Council of Thailand
- Thailand Convention & Exhibition Bureau
- Ajinomoto Co., (Thailand) Ltd.
- PTT Global Chemical Public Company Limited (PTTGC)
- The National Center for Genetic Engineering and Biotechnology (BIOTEC)
- The Thai Association for Biotech Industries
- National Science Technology and Innovation Policy Office
## PROGRAMME AT A GLANCE

### Day 1: Sunday 23rd July 2017

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<th>Activity</th>
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<tr>
<td>14:00-17:00</td>
<td>Conference Registration and Poster Setup</td>
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<tr>
<td>17:00</td>
<td>Meet at the hotel lobby</td>
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<tr>
<td>17:00-18:30</td>
<td>Touring on the Bus to Khon Kaen University (20 min by bus from the hotel to the university)</td>
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<tr>
<td>18:30-20:00</td>
<td>Dinner hosted by Khon Kaen University at Chaturamook Building (only international participants)</td>
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### Day 2: Monday 24th July 2017

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<tr>
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<tr>
<td>07:30-08:30</td>
<td>Conference Registration and Poster Setup</td>
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<tr>
<td>08:30-09:10</td>
<td>Opening Session (Ballroom I &amp; II)</td>
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<tr>
<td></td>
<td>- Introduction of ACB 2017 by Chairperson: Associate Professor Dr. Penjit Srinophakun</td>
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<td>- Welcome address by the Founder of TSB: Professor Amaret Bhumirattana</td>
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<td>- Welcome address by the President of Khon Kaen University: Professor Kittichai Triratanasiriichai</td>
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<td></td>
<td>- Opening address by the President of AFOB: Professor George Fu Gao</td>
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<tr>
<td>09:10-09:20</td>
<td>Photo session</td>
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<tr>
<td>09:20-10:00</td>
<td>Plenary Lecture I: Professor Dr. Sang Yup Lee, KAIST, Korea</td>
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<tr>
<td>10:00-10:40</td>
<td>Plenary Lecture II: Dr. Chaya Chandavasu, PTT Global Chemical Public Company Limited, Thailand</td>
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<td>10:00</td>
<td>Coffee Break will be served.</td>
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<td>10:40-11:20</td>
<td>Plenary Lecture III: Professor Dr. Guoping Zhao, Chinese Academy of Sciences, China</td>
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<td>11:20-12:00</td>
<td>Plenary Lecture IV: Professor Dr. Roland Wohlgemuth, European Federation of Biotechnology</td>
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<tr>
<td>12:00-13:00</td>
<td>Lunch</td>
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<td>13:00-14:00</td>
<td>Poster Session (Ballroom III)</td>
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<tr>
<th>Time</th>
<th>Ballroom I (90 min)</th>
<th>Ballroom II</th>
<th>Erawan 1-2</th>
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<tr>
<td>14:00-15:30</td>
<td><strong>Bioenergy and Biorefinery (BEB)</strong> “Sustainable Biorefinery for Secondary Products”</td>
<td><strong>Bioprocess and Bioseparation Engineering (BBE)</strong></td>
<td><strong>Biopharmaceutical and Medical Biotechnology (BPMB)</strong></td>
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<td>Chairman: Prof. Wen-Teng Wu</td>
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<td>Invited speakers: Prof. Shu-Yii Wu</td>
<td>Prof. Fengwu Bai</td>
<td>Prof. Watanalai</td>
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<td>Prof. Suraini Abd Aziz</td>
<td>Invited speakers: Prof. Yoon Mo Koo</td>
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<td>5 Oral presentation</td>
<td>Prof. Virendra Swarup Bisaria</td>
<td>Invited speakers: Prof. Prasit</td>
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<td>Prof. Joseph Auresenia</td>
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<td>Prof. Cheng-Kang Lee</td>
<td>Prof. Guanghui Ma</td>
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<td>Prof. Masahiro Goto</td>
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<td>3 Oral presentation</td>
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<th>Ballroom I (90 min)</th>
<th>Ballroom II</th>
<th>Erawan 1-2</th>
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<tbody>
<tr>
<td><strong>AFOB-EBF Joint Session II on</strong></td>
<td><strong>Plant Biotechnology</strong></td>
<td><strong>Biotechnology, Biosensors and Biochips (NBB)</strong></td>
<td><strong>Young Scientists (YS)</strong></td>
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<td>Chairman: Dr. Duong Hoa Xo</td>
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<td>Invited speakers: Prof. Karel Dolezal</td>
<td>Prof. Xian-En Zhang</td>
<td>Prof. Quanfeng Liang</td>
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<td></td>
<td>Prof. Huynh Huu Duc</td>
<td>Keynote speaker</td>
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<td>Prof. Ondrej Novak</td>
<td>Prof. Chunhai Fan</td>
<td>Prof. Min Kyu Oh</td>
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<td>Prof. Bui Chi Buu</td>
<td>Invited speakers: Prof. Zongqiang Cui</td>
<td>Invited speakers: Prof. Benjamas Chiersilp</td>
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<td>Prof. Muhammad</td>
<td>Prof. Feng Li</td>
<td>Prof. Quanfeng Liang</td>
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<td>Manjural Karim</td>
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<td>Prof. Noriho Kamiya</td>
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<td>15:30-16:00</td>
<td><strong>Coffee Break</strong></td>
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<td>16:00-18:30</td>
<td><strong>Ballroom I, II</strong>&lt;br&gt;Room Preparation for Congress Dinner</td>
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<td><strong>Erawan 1-2</strong>&lt;br&gt;Biopharmaceutical and Medical Biotechnology (BPMB)&lt;br&gt;Chairman: Prof. Masahiro Goto&lt;br&gt;Co-Chairman: Prof. Jung Keug Park&lt;br&gt;Invited speakers: Prof. Watanalai Panbangred&lt;br&gt;8 Oral presentation</td>
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<td>Chat Tan 1-2&lt;br&gt;AFOB-EBF Joint Session I on “Enzyme/Catalysis”&lt;br&gt;Chairman: Prof. Teruyuki Nagamune&lt;br&gt;Co-Chairman: Prof. Md. Mozammel Hoq&lt;br&gt;Keynote speaker: Prof. Jung Bae Kim&lt;br&gt;Invited speakers: Prof. Francisc Peter&lt;br&gt;Prof. Pimchaisai Chaiyen&lt;br&gt;3 Oral presentation</td>
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<td>Fah Mui 3-5&lt;br&gt;Nanobiotechnology, Biosensors and Biochips (NBB)&lt;br&gt;Chairman: Prof. Feng Li&lt;br&gt;Prof. Dong Men&lt;br&gt;Prof. Jiaoyu Deng&lt;br&gt;Prof. Xiangwei Zhao&lt;br&gt;4 Oral presentation</td>
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<td>Iyara&lt;br&gt;Bioindustry Promotion and Bioeducation (BPB)&lt;br&gt;Chairman: Prof. Satyahari Dey&lt;br&gt;Co-Chairman: Dr. Goutam Ghosh&lt;br&gt;Invited speakers: Prof. Satyahari Dey&lt;br&gt;Assoc.Prof. Klanarong Sriseth&lt;br&gt;Dr. Goutam Ghosh&lt;br&gt;Mr. James Wang&lt;br&gt;Dr. Phathanon Prasitchoke&lt;br&gt;Dr. Watson Ariyaphuttarat&lt;br&gt;Dr. Rajkumar Rajagopal&lt;br&gt;Dr. J.N. Verma</td>
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<tr>
<td>19:00-21:00</td>
<td><strong>Welcome Reception and Dinner at Ballroom I and II (all participants)</strong></td>
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**Plenary lecture 40 min, Keynote speaker 30 min, Invited speaker 20 min, Oral presentation 10 min**

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**Day 3 : Tuesday 25th July 2017**

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<th>Time</th>
<th>Ballroom I</th>
<th>Ballroom II</th>
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<tr>
<td>09:00-10:30</td>
<td>Keynote speakers (3 persons)&lt;br&gt;Prof. Ivo Frebort, EFB&lt;br&gt;Prof. Fengwu Bai, China&lt;br&gt;Prof. Kalidas Shetty, USA</td>
<td>Keynote speakers (3 persons)&lt;br&gt;Prof. Jeong-Woo Choi, Korea&lt;br&gt;Prof. Wen-Yih Chen, Taiwan&lt;br&gt;Prof. Teruyuki Nagamune, Japan</td>
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<tr>
<td>10:30-11:00</td>
<td><strong>Coffee Break &amp; Networking</strong></td>
<td>Room Fah Mui 1-2&lt;br&gt;AFOB executive board meeting&lt;br&gt;(Coffee break &amp; lunch will be served.)</td>
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<tr>
<td>11:00-12:00</td>
<td><strong>Poster Presentation (Ballroom III)</strong></td>
<td><strong>Lunch &amp; Poster display</strong></td>
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<td>12:00-13:00</td>
<td><strong>Lunch &amp; Poster display</strong></td>
<td><strong>Lunch &amp; Poster display</strong></td>
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<td>13:00-15:00</td>
<td><strong>Bioenergy and Biorefinery (BEB)</strong></td>
<td><strong>Bioprocess and Bioseparation Engineering (BBE)</strong></td>
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<td>“Sustainable Biorefinery for Secondary Products”</td>
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<td>Chairman:</td>
<td>Assoc.Prof. Sarote</td>
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<td>Prof. Suraini Abd Aziz</td>
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<td>Co-Chairman:</td>
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<td></td>
<td>Prof. Jo-Chu Chang</td>
<td>Prof. Kyuya Nakagawa</td>
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<td>Invited speakers:</td>
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<td></td>
<td>Prof. Akihiko Kondo</td>
<td>Prof. Ho Nam Chang</td>
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<td>Prof. Jo-Shu Chang</td>
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<td>7 Oral presentation</td>
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<td><strong>Chat Tan 1-2</strong></td>
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<td><strong>AFOB-EFB</strong></td>
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<td><strong>Joint Session I on</strong></td>
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<td>“Enzyme/Catalysis”</td>
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<td>Chairman:</td>
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<td>Prof. Francis Peter</td>
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<td>Keynote speaker:</td>
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<td>Prof. Magali</td>
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<td>Prof. Patrick Shahgaldian</td>
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<td>Prof. Dietmar Haltrich</td>
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<td>3 Oral presentation</td>
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<td>15:00-15:30</td>
<td><strong>Coffee Break</strong></td>
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<td>15:30-17:30</td>
<td><strong>Bioenergy and Biorefinery (BEB)</strong></td>
<td><strong>Tissue Engineering and Biomaterials (TEB)</strong></td>
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<td></td>
<td>“Sustainable Biorefinery for Secondary Products”</td>
<td>Chairman:</td>
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<td>Chairman:</td>
<td>Assoc.Prof. Virendra Swarup Bisaria</td>
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<td>Prof. Shu-Yii Wu</td>
<td>Co-Chairman:</td>
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<td>Co-Chairman:</td>
<td>Prof. Joseph Auresenia</td>
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<td>Prof. Choul-Gyun Lee</td>
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<td>Invited speakers:</td>
<td>Prof. Duk Jae Oh</td>
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<td>Prof. Choul-Gyun Lee</td>
<td>Prof. Madihah Md. Salleh</td>
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<td>Prof. Yen-Han Lin</td>
<td>Mr. Suphashis Das</td>
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<td><strong>Systems and Synthetic Biotechnology (SSB)</strong></td>
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<td>Assoc.Prof. Cheunchit Booncherd</td>
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<td>Invited speakers:</td>
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<tr>
<td></td>
<td>Prof. Ki Jun Jeong</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr. Guankai Bian</td>
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<tr>
<td></td>
<td>1 Oral presentation</td>
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</tbody>
</table>
**Plenary lecture 40 min, Keynote speaker 30 min, Invited speaker 20 min, Oral presentation 10 min**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>17:30-18:00</td>
<td>Poster Award presented by Assist. Prof. Vichai Leelavatcharamas, the TSB president</td>
</tr>
<tr>
<td></td>
<td>Plaques of appreciation presented by Prof. Yoon Mo Koo, the AFOB secretary general</td>
</tr>
<tr>
<td></td>
<td>Closing Remarks by Prof. Ho Nam Chang, the former AFOB president</td>
</tr>
<tr>
<td>19:00-21:00</td>
<td><strong>Farewell Dinner at KOSA Hotel (Only international participants)</strong></td>
</tr>
</tbody>
</table>

**Day 4: Wednesday 26th July 2017**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>06:00-21:00</td>
<td>Route 1: Visit Vientiane, Laos (Please check your visa before applying)</td>
</tr>
<tr>
<td>05:00-16:00</td>
<td>Route 2: Visit Phanom Rung Historical Park (Prasat Hin Phanom Rung) at Buriram province</td>
</tr>
</tbody>
</table>

**Day 5: Thursday 27th July 2017**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>06:00-08:00</td>
<td>Giving food to the monks at Buddhist temples</td>
</tr>
<tr>
<td>10:00-11:30</td>
<td>Visit Wat Nong Wang (The biggest temple in Khon Kaen)</td>
</tr>
<tr>
<td>12:00-13:30</td>
<td><strong>Lunch</strong></td>
</tr>
<tr>
<td>13:30</td>
<td>Travel to Khon Kaen Airport (20 minutes)</td>
</tr>
</tbody>
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**Table 1: Iyara**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>16:30-17:30</td>
<td><strong>BEB division meeting</strong></td>
</tr>
<tr>
<td></td>
<td>Prof. Wen-Teng Wu Chairman</td>
</tr>
<tr>
<td>17:30-18:00</td>
<td>Poster Award presented by Assist. Prof. Vichai Leelavatcharamas, the TSB president</td>
</tr>
<tr>
<td></td>
<td>Plaques of appreciation presented by Prof. Yoon Mo Koo, the AFOB secretary general</td>
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<tr>
<td></td>
<td>Closing Remarks by Prof. Ho Nam Chang, the former AFOB president</td>
</tr>
<tr>
<td>19:00-21:00</td>
<td><strong>Farewell Dinner at KOSA Hotel (Only international participants)</strong></td>
</tr>
</tbody>
</table>

**Notes**

- Plenary lecture 40 min, Keynote speaker 30 min, Invited speaker 20 min, Oral presentation 10 min
- Route 1: Visit Vientiane, Laos (Please check your visa before applying)
- Route 2: Visit Phanom Rung Historical Park (Prasat Hin Phanom Rung) at Buriram province
- Giving food to the monks at Buddhist temples
- Visit Wat Nong Wang (The biggest temple in Khon Kaen)
- Travel to Khon Kaen Airport (20 minutes)
<table>
<thead>
<tr>
<th>SESSION</th>
<th>AGRICULTURAL AND FOOD BIOTECHNOLOGY (AFB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room</td>
<td>Erawan 1-2</td>
</tr>
<tr>
<td>25 July 2017</td>
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</tr>
<tr>
<td>Chairman</td>
<td>Prof. Sirirat Rengpipat</td>
</tr>
<tr>
<td>Co-Chairman</td>
<td>Prof. Rintu Banerjee</td>
</tr>
<tr>
<td>13:00-13:20</td>
<td>IV-AFB-01 Food and Probiotics</td>
</tr>
<tr>
<td></td>
<td>Prof. Sirirat Rengpipat (Chulalongkorn University, Thailand)</td>
</tr>
<tr>
<td>13:20-13:40</td>
<td>IV-AFB-02 Impact of Music and Sound on Lactobacillus amylophilus GV6 for Lactic Acid Production</td>
</tr>
<tr>
<td></td>
<td>Prof. Rintu Banerjee (Indian Institute of Technology Kharagpur, India)</td>
</tr>
<tr>
<td>13:40-13:50</td>
<td>O-AFB-01 Actinomycetes for Biocontrol of Crop Pathogens – Sharing Our Findings</td>
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<tr>
<td></td>
<td>Vikineswary Sabaratnam*, Tan Yee Shin, Md Yusoff Musa and Geok Yuan Annie Tan</td>
</tr>
<tr>
<td>13:50-14:00</td>
<td>O-AFB-02 Selection of Furfural Tolerant Lactic Acid Bacteria for Bioconversion of Lignocelluloses to Lactic Acid</td>
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<tr>
<td></td>
<td>Aungchararat Klongkaew and Chartchai Khanongnuch*</td>
</tr>
<tr>
<td>14:00-14:10</td>
<td>O-AFB-03 Protease Producing Lactic Acid Bacteria Isolated from Dry-Fermented Catfish for Antioxidant Peptides Preparation Nomchit Kaewthai Andrei*, Sirinat Srionnual, Tippayarat Songkroa and Jaruwan Sitdhipol</td>
</tr>
<tr>
<td>14:10-14:20</td>
<td>O-AFB-04 Probiotic Fortified Seaweed Silage as Improved Supplement in Marine Fish Hatchery Charles Sanchananaraj Vairappan*, Sangeetha Priya Anandan and Tan Hsin Lin</td>
</tr>
<tr>
<td>14:20-14:30</td>
<td>O-AFB-06 Influence of Starch Retrogradation on Synthesis of Resistance Starch, a Compound Vital for Diabetes Mellitus Management via Gut Microbiota Alteration Rike Tri Kumala Dewi*, Ihsan Iswaldi, Harum Fadhilaturnur, Nurhayati and Yalun Arifin</td>
</tr>
<tr>
<td>14:30-14:40</td>
<td>O-AFB-08 Utilization of Polysaccharides Extracted from Ficus awkeotsang Makino in Encapsulation Applications Yu-Shen Cheng*, Yu-Tzu Hu and Jhao-Syuan Gu</td>
</tr>
<tr>
<td>14:40-14:50</td>
<td>O-AFB-09 Role of Hemicelulose-B from Santalum album L. Suspension Cells in the Adherence of Lactobacilli in Vitro Moumita Patra and Satyahari Dey*</td>
</tr>
<tr>
<td>Chairman</td>
<td>Assist.Prof. Vichai Leelavatcharamas</td>
</tr>
<tr>
<td>Co-Chairman</td>
<td>Assist.Prof. Chartchai Khanongnuch</td>
</tr>
<tr>
<td>15:30-15:50</td>
<td>IV-AFB-03 Rice Husk as a Potential Substrate for Biofuel and Biorefinery</td>
</tr>
<tr>
<td></td>
<td>Assist.Prof. Chartchai Khanongnuch (Chiang Mai University, Thailand)</td>
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<tr>
<td>15:50-16:10</td>
<td>IV-AFB-04 Production of Recombinant Enzymes from Lactobacillus plantarum Food Grade Expression System</td>
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<td></td>
<td>Prof. Montarop Yamabhai (Suranaree University Technology Thailand)</td>
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<tr>
<td>16:10-16:20</td>
<td>O-AFB-10 Inhibitory Effect of Anti-browning Agents on Lethal Browning in Petal Tissue Culture of Dendrobium Sonia 'Earsakul’ Ananda Nuryadi Pratama, Jorge Sahagun, Anupan Kongbangkerd and Kumrop Ratanasut*</td>
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</tbody>
</table>
### SESSION AGRICULTURAL AND FOOD BIOTECHNOLOGY (AFB)

<table>
<thead>
<tr>
<th>Time</th>
<th>Room</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:20-16:30</td>
<td>O-AFB-11</td>
<td>Analysis of the Symptom of Grain Discoloration in Rice (Oryza sativa) (var. RD-61)</td>
<td>Inrbadhwi Said Omar Madi, Pimjai Meetum, Rachsawan Mongkol and Mana Kanjanamaneesathian*</td>
</tr>
<tr>
<td>16:30-16:40</td>
<td>O-AFB-12</td>
<td>Mycelial Cultivation of 5 Edible Mushrooms from Khao Kra-Dong Volcano Forest Park, Thailand</td>
<td>Tepupsorn Saensuka* and Suteera Suntararak</td>
</tr>
<tr>
<td>16:40-16:50</td>
<td>O-AFB-13</td>
<td>Angiotensin Converting Enzyme Inhibitory Activity of Enzymatic Bromelain Boletus Mushroom Protein Hydrolysate and The Membrane Ultrafiltration Fractions</td>
<td>Jindaporn Khongdetch, Natta Laohakunjit*, Orapin Kerdchoechuen, Khanok Ratanakhanokchai and Surapong Pinitglan</td>
</tr>
<tr>
<td>16:50-17:00</td>
<td>O-AFB-14</td>
<td>The Xa21 in the Backcross Introggression Lines, BC4F2, Derived from the Thai Rice Cultivar ‘RD47’/‘IRBB21’ Cross Enhances the Bacterial Blight Resistance Against Xanthomonas oryzae pv. oryzae Newly Isolated from Phitsanulok Province</td>
<td>Natta Suachaowna, Francois Grandmottet, Sirirat Sanyong, Suradet Palawisut and Kumrop Ratanasut*</td>
</tr>
</tbody>
</table>

### SESSION APPLIED MICROBIOLOGY (AM)

<table>
<thead>
<tr>
<th>Time</th>
<th>Room</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00-13:20</td>
<td>Iyara</td>
<td>Yeast Lipids for Sustainable Biodiesel Production</td>
<td>Prof. Zongbao K. Zhao (Dalian Institute of Chemical Physics P.R. China)</td>
</tr>
<tr>
<td>13:20-13:40</td>
<td>Iyara</td>
<td>Development of a CRISPR/CRI SRi Hybrid System for Metabolic Engineering of E. coli and Succinate Production</td>
<td>Prof. Li-Yu Sung (National Tsing Hua University, Taiwan)</td>
</tr>
<tr>
<td>13:40-13:50</td>
<td>Iyara</td>
<td>Optimization of Amino Acid Decarboxylation and Sugar Fermentation to Enhance Hydrogen Sulfide Production for Rapid Screening of Salmonella During Selective Enrichment</td>
<td>Juthamas Khueankhancharoen, Jureepan Saranak and Aluck Thipayarat*</td>
</tr>
<tr>
<td>13:50-14:00</td>
<td>Iyara</td>
<td>MicroRNAs in the Chloroplast of Unicellular Alga Chlamydomonas reinhardtii</td>
<td>Nazalan Najimudin*, Ghows Azzam, Japareng Lalung and Mohd Suhaimi Che Ani</td>
</tr>
<tr>
<td>14:00-14:10</td>
<td>Iyara</td>
<td>Screening, Isolation, and Characterization of Protease and Lipase Producing Bacteria Isolated from Fermented Shrimp Paste</td>
<td>Maureen Kumaunang, Wasana Savotha and Suppasit Maneerat*</td>
</tr>
<tr>
<td>14:10-14:20</td>
<td>Iyara</td>
<td>Constitutive and Methanol-Inducible Promoters from a Thermotolerant Yeast, Ogataea thermomethanolica, Suitable for Heterologous Gene Expression, Especially at Elevated Temperature</td>
<td>Piyanun Harnpicharnchai*, Peerada Promdonkoy, Kittapong Sae-Tang, Niran Roongsawang and Sutipa Tanapongpipat</td>
</tr>
</tbody>
</table>
### SESSION APPLIED MICROBIOLOGY (AM)

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<tbody>
<tr>
<td>14:20-14:30</td>
<td>Iyara</td>
<td>Comparative Genomics and Transcriptomics Analyses Revealed the Role of Significant Genes in Thermal and Ethanol Stress Tolerance in <em>Saccharomyces cerevisiae</em> SPSC01 Jian-Ren Xu, Chen-Guang Liu, Xin-Qing Zhao and Feng-Wu Bai*</td>
</tr>
<tr>
<td>14:30-14:40</td>
<td>Iyara</td>
<td>Screening and Characterization of High Ethanol-Producing Yeast from Selected Naturally Fermenting Fruits Kimberly L. Rodriguez*, Hosea L. Matel, Ma. Fatima I. Cruzada and Yolanda A. Ilagan</td>
</tr>
<tr>
<td>14:40-14:50</td>
<td>Iyara</td>
<td>A Comparative Study on Lipase Enzyme Immobilized on Acid and Glutaraldehyde Functionalized Multiwalled Carbon Nanotubes Ahmad Tariq Jameel* and Muhammad Arif</td>
</tr>
</tbody>
</table>

**Chairman** Prof. Zongbao (Kent) Zhao

15:30-15:50 IV-AM-03 ARTP Mutagenesis Tool for Life Science and Industry Prof. Xin-Hui Xing (Tsinghua University, P.R. China)

15:50-16:00 IV-AM-09 Nutraceutical Implication of Marine Carbohydrate from *Aphanothece* sp. Kumari Shanti Kiran and Satyahrari Dev* |

16:00-16:10 IV-AM-10 Antibacterial Efficacy of Tilapia By-products Against *Listeria monocytogenes* and *Salmonella Typhimurium* and Its Application in Fish Patties Huynh Tran Huyen Trang and Patimakorn Pasuwan* |

16:10-16:20 IV-AM-11 Evaluation of Lipase for its Formulation Additive in Bio-based Toothpaste and Contact Lens Solution Vijay Kumar Garlapati*, Nitish Vikram Shahi and Radhika Sharma |

### SESSION BIOENERGY AND BIOREFINERY (BEB)

**“Sustainable Biorefinery for Secondary Products”**

<table>
<thead>
<tr>
<th>Time</th>
<th>Room</th>
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</thead>
<tbody>
<tr>
<td>14:00-14:20</td>
<td>Orchid Ballroom I</td>
<td>Biological Biopreducts from Biomass Waste Prof. Shu-Yii Wu (Dean, College of Engineering, Feng Chia University (FCU), and CEO, APEC Research Center for Advanced Biohydrogen Technology (ACABT), Taiwan)</td>
</tr>
<tr>
<td>14:20-14:40</td>
<td>Orchid Ballroom I</td>
<td>Improved System for the Production of Activated Carbon from Oil Palm Kernel Shell Prof. Suraini Abd Aziz (University Putra Malaysia, Malaysia)</td>
</tr>
<tr>
<td>14:40-14:50</td>
<td>Orchid Ballroom I</td>
<td>Reactor Design for Levulinic Acid Production from Palm Oil Empty Fruit Bunches Jabosar Ronggar Hamonangan Panjaitan, Dewi Tristantini, Rizal Alamysah and Misri Gozan*</td>
</tr>
<tr>
<td>14:50-15:00</td>
<td>Orchid Ballroom I</td>
<td>Optimization of Sodium Hydroxide Pretreatment Enhanced Cellulose Saccharification in Napier Grass using Response Surface Methodology Paripok Phitsuwan*, Kazuo Sakka and Khanok Ratanaokonkhaichai</td>
</tr>
<tr>
<td>15:00-15:10</td>
<td>Orchid Ballroom I</td>
<td>Bioethanol Production by Batch and Repeated Batch using Immobilized Yeast Cells on Sugarcane Bagasse Apinya Sowatad and Tatsaporn Todhanakasem*</td>
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</tbody>
</table>

**Chairman** Prof. Wen-Teng Wu

24 July 2017
<table>
<thead>
<tr>
<th>SESSION</th>
<th>BIOENERGY AND BIOREFINERY (BEB) “Sustainable Biorefinery for Secondary Products”</th>
</tr>
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<tbody>
<tr>
<td>Room</td>
<td>Orchid Ballroom I</td>
</tr>
<tr>
<td>15:10-15:20</td>
<td>O-BEB-04 Hydrodeoxygenation of Bio-oil over NiMo/Al2O3 and CeO2, ZrO2 and TiO2 Additives Worada Moonsrikaew and Apinya Duangchan*</td>
</tr>
<tr>
<td>15:20-15:30</td>
<td>O-BEB-05 Recovering Activities of Inactivated Cellulases by the Use of Mannanase in Spruce Hydrolysis Donglin Xin, Ming Yang, Xiang Chen, Li Ma, Pai Peng, Jia Wang, Fangxia Yang, Jie Chu, Lili Jia and Junhua Zhang*</td>
</tr>
</tbody>
</table>

**25 July 2017**

| Chairman | Prof. Suraini Abd Aziz |
| Co-Chairman | Prof. Jo-Shu Chang |
| 13:00-13:20 | IV-BEB-03 Development of Microbial Cell Factories for Production of Aromatic Chemicals and Derivatives Prof. Akihiko Kondo (Kobe University, Japan) |
| 13:20-13:40 | IV-BEB-04 Microalgae as the Platform for Carbon Cycling and Circular Economy Prof. Jo-Shu Chang (National Cheng Kung University, Taiwan) |
| 13:50-14:00 | O-BEB-07 Effect of Pretreatment Agents on Improved Methane Recovery from Deoiled Grease Trap Waste Periyasamy Sivagurunathan*, Takuro Kobayashi and Kaiqin Xu* |
| 14:00-14:10 | O-BEB-08 Influence of Hydraulic Retention Time on Thermophilic Biogas Production from Palm Oil Mill Effluent in an UASB Bioreactor Safa Senan Mahmud, Jamaliah Md Jahim*, Peer Mohamed Abdul, Ahmad Jaril Asis and Shu-Yii Wu |
| 14:10-14:20 | O-BEB-09 Enhancement of Bioethanol Production via Hyper Thermal Acid Hydrolysis and Co-culture Fermentation with Optimal Yeasts Ratio using Waste Seaweed from Gwangalli, Busan, Korea In Yung Sunwoo, Trung Hau Nguyen, Pailin Sukwong, Gwi-Taek Jeong and Sung-Koo Kim* |
| 14:20-14:30 | O-BEB-10 Improved Fermentation Performance to Produce Bioethanol from Gelidi um amansii using Pichia stipitis Adapted to Galactose Pailin Sukwong, Chae Hun Ra, In Yung Sunwoo, Sumate Tantratian, Gwi-Taek Jeong and Sung-Koo Kim* |
| 14:30-14:40 | O-BEB-11 This work was not delivered on the conference schedule. |
| 14:40-14:50 | O-BEB-12 Effect of Microaeration on Kluyveromyces marxianus Fermentation with Lignocellulose Hydrolysate Wenjie Yuan* and Hualiang Feng |

| Chairman | Prof. Shu-Yii Wu |
| Co-Chairman | Prof. Choul-Gyun Lee |
| 15:30-15:50 | IV-BEB-05 An Update in World’s Largest Ocean Test-beds in Korea for Sustainable Marine Microalgal Biomass Production Prof. Choul-Gyun Lee (Inha University, Republic of Korea) |
| 15:50-16:10 | IV-BEB-06 Fermentation Process Design for Bioethanol Production Prof. Yen-Han Lin (University of Saskatchewan, Canada) |
### SESSION: BIOENERGY AND BIOREFINERY (BEB)

#### “Sustainable Biorefinery for Secondary Products”

<table>
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<tr>
<th>Room</th>
<th>Session</th>
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<tbody>
<tr>
<td><strong>O-EBE-15</strong></td>
<td>In-situ Synthesis of Canola Biodiesel Derived Estolides via</td>
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<td>Epoxidation Route</td>
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<td></td>
<td><em>Venu Babu Borugadda and Ajay K Dalai</em></td>
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<tr>
<td><strong>O-EBE-16</strong></td>
<td>Catalytic Pyrolytic for Bio-oil Production from Palm Kernel Shell</td>
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<td>using Respond Surface Methodology</td>
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<td></td>
<td><em>Poramate Prompun, Ronnagorn Anaporn and Wanida Koo-amornpattana</em></td>
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<tr>
<td><strong>O-EBE-17</strong></td>
<td>Oxidoreductases from <em>Kluyveromyces marxianus</em> Enhances</td>
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<td>Tolerance of Yeasts to Lignocellulose-derived Inhibitors</td>
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<td><em>Jiao-Qi Gao, Wen-Jie Yuan, Yi-Min Li and Feng-Wu Bai</em></td>
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<tr>
<td><strong>O-EBE-18</strong></td>
<td>RNAi Mediated Downregulation of Lignin Biosynthetic Pathway Gene</td>
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<td>Increases Saccharification Efficiency of Sweet Pearl Millet</td>
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<td><em>Anuttama Dutta</em> and Asitava Basu*</td>
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<tr>
<td><strong>O-EBE-19</strong></td>
<td>Consolidated Bioprocessing of Lignocellulosic Biomass for</td>
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<td>Biofuels Production using Engineered <em>Clostridium thermocellum</em></td>
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<td><em>Ranjita Biswas</em></td>
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<tr>
<td><strong>O-EBE-20</strong></td>
<td>Biogas Production from Water Lettuce in the Chao Phraya River</td>
</tr>
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<td><em>Netechanok Sombat, Suchat Leungprasert, Suriya Sawanon and Nusara Sinbuathong</em></td>
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</tbody>
</table>

### SESSION: BIOINDUSTRY PROMOTION AND BIOEDUCATION (BPB)

#### 24 July 2017

<table>
<thead>
<tr>
<th>Room</th>
<th>Session</th>
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<tbody>
<tr>
<td><strong>Iyara</strong></td>
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**Chairman** Prof. Satyahari Dey  
**Co-Chairman** Dr. Goutam Ghosh

<table>
<thead>
<tr>
<th>Session</th>
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</tr>
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<tbody>
<tr>
<td><strong>IV-BPB-01</strong></td>
<td>Mannan EPS from Arctic <em>Sphingobacterium</em> sp. for the Treatment of</td>
</tr>
<tr>
<td></td>
<td>Colitis Related Inflammation in BALB/c Mice</td>
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<tr>
<td></td>
<td><em>Prof. Satyahari Dey</em> (Indian Institute of Technology Kharagpur, India)</td>
</tr>
<tr>
<td><strong>IV-BPB-03</strong></td>
<td>The Role of Mitr Phol R&amp;D Innovation in Thailand’s Bioeconomy</td>
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<td></td>
<td><em>Assoc.Prof. Klanarong Sriroth</em> (Mitr Phol Sugar Corp., Ltd., Thailand)</td>
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<tr>
<td><strong>IV-BPB-02</strong></td>
<td>Opportunities in Research and Manufacturing of Bio-pharmaceuticals in</td>
</tr>
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<td>India</td>
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<td><em>Dr. Goutam Ghosh</em> (Panacea Biotec Ltd., India)</td>
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<tr>
<td><strong>IV-BPB-04</strong></td>
<td>Probiotics Business and Technology Promotion from Inside to Outside</td>
</tr>
<tr>
<td></td>
<td><em>Mr. James Wang</em> (SYNBIOTECH Inc., Taiwan)</td>
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<tr>
<td><strong>IV-BPB-06</strong></td>
<td>Production of Stereocomplex Poly (Lactic acid) from Microorganisms</td>
</tr>
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<td>Isolated in Thailand</td>
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<td><em>Dr. Phatthanon Prasitchoke</em> (PTT Global Chemical Public Company Limited, Thailand)</td>
</tr>
<tr>
<td><strong>IV-BPB-05</strong></td>
<td>The Thai Association for Biotech Industries (ThaiBIO) Promotes Thai-</td>
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<tr>
<td></td>
<td>Biotechnology Business to be Sustainable and Global Competitiveness</td>
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<tr>
<td></td>
<td><em>Dr. Watson Ariyaphuttarat</em> (The Thai Association for Biotech Industries, Thailand)</td>
</tr>
<tr>
<td>SESSION</td>
<td>BIOPHARMACEUTICAL AND MEDICAL BIOTECHNOLOGY (BPMB)</td>
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<tr>
<td>Room</td>
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<tr>
<td>Chairman</td>
<td>Prof. Watanalai Panbangred</td>
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<td>14:00-14:20</td>
<td>IV-BPMB-01 Large Scale Whole Genome Sequencing of <em>Mycobacterium tuberculosis</em> in Thailand: Implications for National End TB Strategy <strong>Prof. Prasit Palittaponkarnpim</strong> (Mahidol University, Thailand)</td>
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<td>14:20-14:40</td>
<td>IV-BPMB-02 Intelligent Particle Adjuvants for Advanced Vaccine Formulation <strong>Prof. Guanghui Ma</strong> (Chinese Academy of Sciences, P.R. China)</td>
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<td>14:40-15:00</td>
<td>IV-BPMB-03 Transdermal Drug Delivery Systems for Cancer Immunotherapy using Oil-based Nanocarrier <strong>Prof. Masahiro Goto</strong> (Kyushu University, Japan)</td>
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<td>15:00-15:10</td>
<td>O-BPMB-01 Cross Resistance Mechanisms between Antibiotic, Antiseptic, and Disinfectant in Human Pathogen <em>Pseudomonas aeruginosa</em> Adisak Romsang*, Thanaphat Auwattanamongkol, Jintana Duang-nkern, Jarupa Nakhadamrongwut and Skorn Mongkolsuk</td>
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<td>15:10-15:20</td>
<td>O-BPMB-02 Protein Hydrolysates and Partial Purified Peptides on Viability and Apoptosis of Liver Cancer Cell Ariya Khamwut and Nattanan Panjaworayan T-Thienprasert*</td>
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<td>Chairman</td>
<td>Prof. Masahiro Goto</td>
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<tr>
<td>Co-Chairman</td>
<td>Prof. Jung Keug Park</td>
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<td>16:00-16:20</td>
<td>IV-BPMB-04 This work was not delivered on the conference schedule.</td>
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<td>16:20-16:40</td>
<td>IV-BPMB-05 High Level Accumulation of Soluble Diphtheria Toxin Mutants (Crm197 and Triple-mutated Toxoid) with Co-expression of Molecular Chaperones in Recombinant <em>Escherichia coli</em> <strong>Prof. Watanalai Panbangred</strong> (Mahidol University, Thailand)</td>
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<td>16:50-17:00</td>
<td>O-BPMB-06 <em>Stephania</em> spp. Exerting Estrogenic and Anti-estrogenic Activities Nattitha Sophon, Jarunya Narangajavana, Patoomratana Tuchinda, Arthit Chaivongdua and Chuenchit Boonchird*</td>
</tr>
<tr>
<td>17:00-17:10</td>
<td>O-BPMB-07 Medically Important Compounds, Traditional Uses and Their Formulations for Healthcare Products Lamichhane Janardan*, Sharma Deepak, Pokharel Binita and Lamichhane Trishna</td>
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<td>17:10-17:20</td>
<td>O-BPMB-08 Bioactivity Measurement and Bioinformatics Analysis to Develop DNA Barcoding System in Himalayan Herbs of Nepal Sharma Deepak, Shrestha Tara, Lamichhane Trishna and Lamichhane Janardan*</td>
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<td>BIOINDUSTRY PROMOTION AND BIOEDUCATION (BPB)</td>
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<td>17:20-17:30</td>
<td><strong>Comparative Study of Three Different Molecular Sizes Sericin Extracted from the Cocoon of Antheraea mylitta as Ecofriendly Antimicrobial Agent <em>Soumita Dutta and Ananta Kumar Ghosh</em></strong></td>
</tr>
<tr>
<td>17:30-17:40</td>
<td>*<em>The Fabrication of Natural Rubber for Transdermal Drug Delivery Patch <em>Apisit Banpean, Nophawan Paradee, Anuvat Sirivat and Supomonman Niamlang</em></em></td>
</tr>
<tr>
<td>17:40-17:50</td>
<td><strong>Assessment of Software for Somatic Single Nucleotide Variant Identification using Simulated Whole-Genome Sequencing Data of Cancer <em>Phongphak Khongthon, Wisut Lamlerthton, Kanthida Kusonmano, Supapon Cheevadhanarak and Weerayuth Kittichotirat</em></strong></td>
</tr>
<tr>
<td>17:50-18:00</td>
<td><strong>Development of Algorithm for Aneuploidy Detection Based on Genome Coverage 0.005X Data <em>Tantip Arigul, Verayuth Praphanphoj, Sawanee Sutheeworapong, Supapon Cheevadhanarak and Weerayuth Kittichotirat</em></strong></td>
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<tr>
<td><strong>Chairman</strong></td>
<td>Prof. Fengwu Bai</td>
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<tr>
<td>14:00-14:20</td>
<td>*<em>Application of SMB Chromatography Process in Biotechnology <em>Prof. Yoon-Mo Koo</em></em> (Inha University, Republic of Korea)</td>
</tr>
<tr>
<td>14:20-14:40</td>
<td>*<em>Bioprocessing of Agro-residues through the Application of Cross-linked Enzyme Aggregates of Cellulases <em>Prof. Virendra Swarup Bisaria</em></em> (Indian Institute of Technology-Delhi, India)</td>
</tr>
<tr>
<td>14:40-15:00</td>
<td>*<em>Optimization of Supercritical Fluid Extraction of Lipids from Gliricidia Sepium Seed Kernel <em>Prof. Joseph Auresenia</em></em> (De La Salle University, Philippines)</td>
</tr>
<tr>
<td>15:00-15:20</td>
<td>*<em>Aqueous Two-phase Extraction for Bacteriorhodopsin Purification from Halobacterium salinarum <em>Prof. Cheng-Kang Lee</em></em> (National Taiwan University of Science and Technology, Taiwan)</td>
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<p>| <strong>25 July 2017</strong>                |                                                                                                            |
| <strong>Chairman</strong>                    | Assoc.Prof. Sarote Sirisansaneeyakul                                                                    |
| <strong>Co-Chairman</strong>                 | Prof. Kyuya Nakagawa                                                                                      |
| 13:00-13:20                     | *<em>Membrane Enriching of Fermentation Broth and Sea Water Desalination by Forward Osmosis and of Δπ=0 Reverse Osmosis (VFAs, NaCl, Ethanol) <em>Prof. Ho Nam Chang</em></em> (KAIST, Korea) |
| 13:20-13:40                     | *<em>How to Operate Freeze-drying Process for Assuring Product Quality of Biological Products <em>Prof. Kyuya Nakagawa</em></em> (Kyoto University, Japan) |
| 13:50-14:00                     | <strong>Denaturation of Inactivated FMDV in Ion Exchange Chromatography: Evidence by Differential Scanning Calorimetry Analysis <em>Yanli Yang, Songping Zhang and Zhiguo Su</em></strong> |</p>
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| 14:00-14:10 | O-BBE-03 | Physicochemical Properties of Spray-dried Mango Phenolic Compounds Extracts
Francis Dave C. Siacor, Kramer Joseph A. Lim, Alden A. Cabajar, Camila Flor Y. Lobario, Evelyn B. Taboada* and Daniel J. Lacks* |
| 14:10-14:20 | O-BBE-04 | A Review on the Large-scale Production and Purification Processes for Fungal α-Amylase Production
Md. Mehei Hasan, Nasima Akter and Sheikh Md. Enayetul Babar* |
| 14:20-14:30 | O-BBE-05 | Media Optimization and Batch Kinetics Studies for Recombinant Human Interferon α2b Production by Pichia pastoris
Srikant Katla, Bappa Karmakar, Subbi Rami Reddy Tadi, Naresh Mohan, B. Anand and Senthilkumar Sivaparakasam* |

Chairman: Prof. Virendra Swarup Bisaria
Co-Chairman: Prof. Joseph Auresenia

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<th>Session</th>
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Prof. Duk Jae Oh (Sejong University, Republic of Korea) |
| 15:50-16:10 | IV-BBE-07 | Utilization of Lemongrass Biomass for Biovanillin Production by Phanerochaete chrysosporium
Prof. Madihah Md. Salleh (University Teknologi Malaysia, Malaysia) |
| 16:10-16:30 | IV-BBE-10 | Investigating Effects of pH on Microbial Growth in Continuous Stirred Tank Bioreactors
Mr. Subhashis Das (Norway's Arctic University, Norway) |
| 16:30-16:40 | O-BBE-07 | Mussel-inspired Biocatalytic Membrane for Micro-pollutant Removal
Jianquan Luo*, Xiaotong Cao and Yinhua Wan |
| 16:40-16:50 | O-BBE-08 | Heat Reflux Extraction Technique to Obtain Nicotine Compound from Nicotiana tabacum var. Virginia
Ahmad Fauzantoro, Haryuni, Mahdi Jufri, Yuswan Muharram and Misri Gozan* |
| 16:50-17:00 | O-BBE-09 | Optimization of Integrating Ethanol Production by using Jerusalem Artichoke Stalk
Kai Li, Jin-Cheng Qin, Chen-Guang Liu* and Feng-Wu Bai |
| 17:00-17:10 | O-BBE-10 | Flocculation Control by c-di-GMP Phosphodiesterase Genes in Zymomonas mobilis
Juan Xia, Chen-Guang Liu* and Feng-Wu Bai* |
| 17:10-17:20 | O-BBE-11 | Production of L-Alanyl-L-Glutamine by Recycling E. coli Expressing α-Amino Acid Ester Aciytransferase
Yi-Min Li, Wen-Jie Yuan*, Jiao-Qi Gao and Feng-Wu Bai |

SESSION | ENVIRONMENTAL BIOTECHNOLOGY (EB) |
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Chairman: Prof. Mohd Ali Hassan
Co-Chairman: Prof. Philippe F.-X.-Corvini

xviii  
July 23-27, 2017 Pullman Khon Kaen Raja Orchid Hotel, Khon Kaen, Thailand
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<tr>
<td>13:00-13:20</td>
<td>IV-EB-01</td>
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<td>Bacteria Feeding on Antibiotics – Are these of Environmental Relevance and Do They Contribute to the Pool of Antibiotic Resistant Bacteria? Prof. Philippe F.-X-Corvini (FHNW, Switzerland)</td>
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<td>13:20-13:40</td>
<td>IV-EB-02</td>
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<td>Biomethanation of POME using Anaerobic Hybrid Reactor: Its Potential and Implementation Assoc.Prof. Pawinee Chaiprasert (KMUTT, Thailand)</td>
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<tr>
<td>13:40-14:00</td>
<td>IV-EB-03</td>
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<td>Green Bio-based Products in the New Bioeconomy Prof. Mohd Ali Hassan (BioTech UPM)</td>
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<td>14:00-14:10</td>
<td>O-EB-01</td>
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<td></td>
<td>Rapid and Simple Detection of Arsenic in Water and Soil Sample using Molecular Sensor under Neutral pH Nutsara Mekjinda, Miyahara Yoshitumi, Itaru Hamachi, Akio Ojida and Jirarut Wongkongkatep*</td>
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<td>14:10-14:30</td>
<td>O-EB-02</td>
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<td>New Tools and Candidate Genes for Enhancing Nitrogen Biofertilizer Potential of the Cyanobacterium Anabaena in Stressful Environments Shree Kumar Apte*</td>
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<td>14:30-14:40</td>
<td>O-EB-04</td>
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<td>Development of in situ Petroleum Bioremediation Strategy with Biosurfactant Producing Hydrocarbon Degrading Bacteria from Refinery Waste Poulomi Sarkar, Sufia K. Kazy and Pinaki Sar*</td>
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<tr>
<td>14:40-14:50</td>
<td>O-EB-05</td>
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<tr>
<td></td>
<td>Identification of Microbiomes in Anaerobic Wastewater Treatment Sludge Fed by Different Volatile Acids using 16S rRNA Metagenomics Approach Wanna Chetruengchai, Benjaphon Suraraksa, Peerada Prommeenate, Kanthida Kusonmano, Supapon Cheevadhanarak and Weerayuth Kittichotirat*</td>
</tr>
<tr>
<td>14:50-15:00</td>
<td>O-EB-06</td>
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<tr>
<td></td>
<td>Identification of CRISPR-Cas Systems of Arthrospira platensis C1 using Bioinformatics Approach Salisa Charoensri, Kanthida Kusonmano, Weerayuth Kittichotirat, Sawanee Sutheeworapong, Thanawat Srisuk, Chineae Thammarongtham and Supapon Cheevadhanarak*</td>
</tr>
<tr>
<td>15:00-15:10</td>
<td>O-EB-07</td>
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<tr>
<td></td>
<td>Metatranscriptome Analysis Revealed Putative Causative Agents of Aggregated Transformed Microvilli (ATM) in Penaeus vannamei Prasobsook Paenkaw, Sawanee Sutheeworapong, Jeerayut Chaijarawanich, Chineae Thammarongtham, Kanthida Kusonmano, Supapon Cheevadhanarak and Anuphap Prachumwat*</td>
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<td>Chairman</td>
<td>Prof. Xian-En Zhang</td>
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<td>14:00-14:30</td>
<td>KN-NBB-01</td>
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<td>DNA Nanotechnology-enabled Organization for Biosensors Prof. Chunhai Fan (Chinese Academy of Sciences, P.R.China)</td>
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<td>14:30-14:50</td>
<td>IV-NBB-01</td>
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<td>Single-particle Tracking of Virus Entry and Uncoating in Live Cells Prof. Zongqiang Cui (Chinese Academy of Sciences, P.R.China)</td>
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## SESSION
### NANOBIOTECHNOLOGY, BIOSENSORS AND BIOCHIPS (NBB)

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<td>14:50-15:10</td>
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<td>IV-NBB-02</td>
<td>Viral Nanoparticle of Simian Virus 40 as a Multifunctional Platform for Nanobiotechnology</td>
<td>Prof. Feng Li (Chinese Academy of Sciences, P.R.China)</td>
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<td>IV-NBB-03</td>
<td>Self-assembled Protein Nanostructure for Highly Sensitive Bio-sensing</td>
<td>Prof. Dong Men (Chinese Academy of Sciences, P.R.China)</td>
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<td>16:20-16:40</td>
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<td>IV-NBB-04</td>
<td>Mycobacterium Tuberculosis Proteome Microarray for Global Studies of Protein Function and Immunogenicity</td>
<td>Prof. Jiaoyu Deng (Chinese Academy of Sciences, P.R.China)</td>
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<td>16:40-17:00</td>
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<td>IV-NBB-05</td>
<td>Photonic Crystal Based Bioassays</td>
<td>Prof. Xiangwei Zhao (Southeast University, P.R.China)</td>
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<td>17:00-17:10</td>
<td>O-NBB-01</td>
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<td>Spores for the Applications of Analytical Chemistry</td>
<td>Yuqiang Xiang, Ruihua Fei, Miaomiao Xia, Zheng Li and Yonggang Hu</td>
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<td>17:10-17:20</td>
<td>O-NBB-02</td>
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<td>Detection of Periodontal Disease Biomarker Protein for an Early Diagnosis of a Periodontitis</td>
<td>Bang Hyun Lee, Youngkyung Ko, Ju chul Park and Man Bock Gu</td>
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<td>17:20-17:30</td>
<td>O-NBB-03</td>
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<td>Synthesis and Characterization of Magnetic Nanoparticle-Graphene Oxide Composites using Coprecipitation and Solvothermal Processes for Cation Removal</td>
<td>Buddhawatchana Suwanphithak, Kittiwut Kasemwong, Pakorn Opaprakasit and Paiboon Sreearunothai</td>
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<td>17:30-17:40</td>
<td>O-NBB-04</td>
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<td>Production of Nanocellulose from Locally Isolated Gluconacetobacter sp. BCZM for Biotechnological Application</td>
<td>Mustapha Abba, Zaharah Ibrahim*, Chun Shiong Chong, Madihah Md Salleh, Adibah Yahya, Saiful Izwan Abdul Razak and Shaza Eva Mohamad</td>
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## SESSION
### SYSTEMS AND SYNTHETIC BIOTECHNOLOGY (SSB)

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<td>IV-SSB-02</td>
<td>Genome Mining and High Efficiency Production of Terpenoids by a Robust Precursor Supply Platform</td>
<td>Dr. Guangkai Bian (Wuhan University School of Pharmaceutical Sciences, P.R.China)</td>
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<td>16:10-16:20</td>
<td>O-SSB-02</td>
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<td>Proteomic Analysis Reveals the Underlying Mechanisms of Improved Acetic Acid Stress Tolerance by SET5 Overexpression</td>
<td>Mingming Zhang, Jiaxiang Li, Fengwu Bai and Xinqing Zhao*</td>
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## SESSION TISSUE ENGINEERING AND BIOMATERIALS (TEB)

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<td>IV-TEB-01</td>
<td>RNA Therapeutics and Anabolic Gene Delivery for Tissue Engineering and Regenerative Medicine Prof. Yu-Chen Hu (National Tsing Hua University, Taiwan)</td>
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<td>15:50-16:10</td>
<td>IV-TEB-02</td>
<td>Endothelial Progenitor Cells, Small Molecules, Extracellular Matrix and Polyhydroxylkanoate Scaffold for Blood Vessel Tissue Engineering Prof. Chao-Ling Yao (Yuan Ze University, Taiwan)</td>
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<td>16:10-16:30</td>
<td>IV-TEB-03</td>
<td>Incorporation of Surface-modified Hydroxyapatite into Poly(methylmethacrylate) Bone Cement for Better Functionality Prof. I-Ming Chu (National Tsing Hua University, Taiwan)</td>
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<td>16:30-16:40</td>
<td>O-TEB-01</td>
<td>Preparation of Nitric Oxide-releasing Photo-crosslinked Electrospun Chitosan Nanofibrous Scaffolds for Bone Tissue Engineering Ming-Hua Ho* and Lumapat Paul Noel Quirante</td>
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<td>16:40-16:50</td>
<td>O-TEB-02</td>
<td>CRISPR Interference (CRISPRi) System for CHO Cell Engineering and Product Yield Improvement Chih-Che Shen, Li-Yu Sung, Mei-Wei Lin, Jhang-Shun Yu and Yu-Chen Hu</td>
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## SESSION YOUNG SCIENTISTS (YS)

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<td>KN-YS-01</td>
<td>Application of Synthetic Biology Tools for Metabolite Overproduction Prof. Min-Kyu Oh (Korea University, Republic of Korea)</td>
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<td>14.30-14.50</td>
<td>IV-YS-01</td>
<td>Mitigation of Carbon Dioxide by Oleaginous Microalgae for Lipids and Pigments Production Prof. Benjamas Cheirsilp (Prince of Songkla University, Thailand)</td>
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<td>14.50-15.10</td>
<td>IV-YS-02</td>
<td>Dynamic Regulation, Synthetic Biology Devices and Product Biosynthesis Prof. Quanfeng Liang (Shandong University, P.R. China)</td>
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<td>15.10-15.30</td>
<td>IV-YS-03</td>
<td>Biocatalyst Engineering toward Biomedical Applications Prof. Noriho Kamiya (Kyushu University, Japan)</td>
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## SESSION AFOB-EBF JOINT SESSION I ON "ENZYME/CATALYSIS"

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<td>Nanobiocatalysis for Microbial Decontamination and CO\textsubscript{2} Conversion Prof. Jungbae Kim (Korea University, Republic of Korea)</td>
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<td>16.30-16.50</td>
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<td>Novel Bio-based Oligoesters by Immobilized Lipases Prof. Francisc Peter (University Politehnica Timisoara, Romania)</td>
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<td>16.50-17.10</td>
<td>IV-Joint I-02</td>
<td>Enzyme Catalysis and Engineering for Sustainable Technology Prof. Pimchai Chaiyen (Mahidol University, Thailand)</td>
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<td>AFOB-EFB JOINT SESSION I ON &quot;ENZYME/CATALYSIS&quot;</td>
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| 17.10-17.20 | O-Joint I-01 | The Sugar Oxidation of Pyranose 2-Oxidase  
*Thanyaporn Wongnate, Panida Surawatanawong, Litavadee Chuaboon and Pimchai Chaiyen*  
  |
| 17.20-17.30 | O-Joint I-02 | Adsorption and Covalent Cross Linking with Chitin:  
Immobilization of Dextranase on a Renewable Organic  
Polyaminosaccharide  
*Afseehn Aman*, Faiza Shahid and Shah Ali Ul Qader  
  |
| 17.30-17.40 | O-Joint I-03 | Construction of a New Anchoring Protein System for Yeast Cell-Surface Display by using Bioinformatic Approach  
*Apisom Phienluphon, Wuttichai Mhuangtong, Katevadee Boonyapakron, Duangdao Wichadakul, Verawat Champreda and Surisa Sawannarangsee*  
  |
| 25 July 2017 |  |
| Chairman | Prof. Francisc Peter |
| 13.00-13.30 | KN-Joint I-02 | Tuning Enzyme Promiscuity for New Pathways and Products  
Prof. Magali Remaud-Simeon (University de Toulouse, France)  
  |
| 13.30-13.50 | IV-Joint I-03 | Design of Robust Nanobiocatalysts through Protein Supramolecular Engineering  
Prof. Patrick Shahgaldian (University of Applied Sciences and Arts Northwestern Switzerland)  
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| 13.50-14.10 | IV-Joint I-04 | Turning Sugars into Electricity: Engineering of Pyranose Oxidase for Biofuel Cells  
Prof. Dietmar Haltrich (BOKU University of Natural Resources and Life Sciences, Austria)  
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| 14.10-14.20 | O-Joint I-04 | High Level Expression of Recombinant Keratinase from *Bacillus licheniformis*  
*Mukitu Nahar, Shakila Nargis Khan*, Muhammad Manjurul Karim and Md. Mozammel Hoq  
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| 14.20-14.30 | O-Joint I-05 | Structural and Enzymatic Characterization of Acetolactate Decarboxylase from *Bacillus subtilis*  
*Xangling Ji*, Mingyang Li, Yanbin Feng, Sijin Wu, Tianqi Wang, Zhongji Pu, Jingyun Wang, Yongliang Yang, Song Xue and Yongming Bao  
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| 14:30-14:40 | O-Joint I-06 | High Production of Genistein Diglucoside Derivative using Cyclodextrin Glycosyltransferase from *Paenibacillus macerans*  
*Ruizi Han*, Binbin Ge, Mingyang Jiang, Guochao Xu, Jinjun Dong, Ye Ni  
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| 14:00-14:20 | IV-Joint II-01 | New Cytokinin Derivatives - A Tool to Understand and Improve Establishment of Micropropagated Plantlets  
Prof. Karel Doležal (Palacký University & Institute of Experimental Botany ASCR, Czech Republic)  
  |
| SESSION AFOB-EFB JOINT SESSION II ON "PLANT BIOTECHNOLOGY" |
|-------------|-----------------------------------------------|
| Room        | Chat Tan 1-2                                 |
| 14:20-14:40 | IV-Joint II-02 Screening and using DNA Barcodes for Identification of *Dendrobium* Species  
*Prof. Huynh Huu Duc* (Ho Chi Minh City University of Pedagogy, Vietnam) |
| 14:40-15:00 | IV-Joint II-03 Phytohormone Metabolite Profiling in Plant Tissues  
*Prof. Ondřej Novák* (Palacký University & Institute of Experimental Botany ASCR, Czech Republic) |
| 15:00-15:20 | IV-Joint II-04 Rice Breeding for Salt Tolerance in Mekong Delta via Marker-assisted Selection  
*Prof. Bui Chi Buu* (Institute of Agricultural Sciences for Southern Vietnam) |
| 15:20-15:40 | IV-Joint II-05 Adaptation of Rice Cultivation in the Coastal Areas of Bangladesh under Changing Climate Conditions by Application of Salt-tolerant Biofertilizer  
*Prof. Muhammad Manjural Karim* (University of Dhaka, Bangladesh) |
GENERAL INFORMATION

Registration/Information Desks

All delegates will receive the name badge, the receipt, the certificate, and conference package which includes proceeding book of abstract, lunch coupon and all relevant conference information.

The Registration and Information Desks will be open at the following time:
Monday 24th July 07:30 – 17:00
Tuesday 25th July 08:00 – 15:30

Speaker Loading Files

All presenters are required to load their files in the presentation room at the break before their sessions
If the speaker would like to preview their files, please go to staff room.

Presentation Guideline: Time allocation for each presenter;

Plenary speaker 40 minutes
Keynote speaker 30 minutes
Invited speaker 20 minutes
Oral presentation 10 minutes
As time is limited, questions or comments can be made during the coffee break and lunch time.

Poster information

All posters should be posted on July 24 in the morning. Please check your poster number and collect the sticky tape at the poster desk.

Poster presenters must be at their posters at 13.00-14.00 hr on July 24, and 11.00-12.00 hr on July 25. All posters will be evaluated by the committee and awards will be given at the closing ceremony.

Excursion

For the paid participants, please go to excursion desk to confirm your attendant and get updated information about the trips.

If you would like to join the excursion, the tickets are available at Excursion Desk.
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IV-BBE-04  Aqueous Two-phase Extraction for Bacteriorhodopsin Purification from Halobacterium salinarum
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IV-BBE-08  How to Operate Freeze-drying Process for Assuring Product Quality of Biological Products
Kyuya Nakagawa*

IV-BBE-09  Current Status of International Standardization on “Bioprocessing” in ISO/TC 276, a Technical Committee of International Organization for Standardization (ISO) for “Biotechnology”
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IV-EB-02  Biomethanation of POME using Anaerobic Hybrid Reactor: Its Potential and Implementation
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IV-EB-03  Green Bio-based Products in the New Bioeconomy
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### IV-YS-02
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*Quanfeng Liang*

### IV-YS-03
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*Noriho Kamiya*

**SESSION: AFOB-EFB JOINT SESSION I ON "ENZYME/CATALYSIS"

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### IV-Joint I-02
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### IV-Joint I-03
Design of Robust Nanobiocatalysts through Protein Supramolecular Engineering

*Patrick Shahgaldian*, *Philippe F.-X. Corvini* and *M. Rita Correro*

### IV-Joint I-04
Turning Sugars into Electricity: Engineering of Pyranose Oxidase for Biofuel Cells

*Dietmar Haltrich*

**SESSION: AFOB-EFB JOINT SESSION II ON "PLANT BIOTECHNOLOGY"

### IV-Joint II-01
New Cytokinin Derivatives - A Tool to Understand and Improve Establishment of Micropropagated Plantlets

*Karel Doležal, Magdalena Bryková, Vlasta Matušková, Marek Zatloukal, Lenka Plačková, Lucie Plíhalová, Ondřej Novák* and *Miroslav Strnad*
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**Screening and using DNA Barcodes for Identification of *Dendrobium* Species**

*Duong Hoa Xo, Huynh Huu Duc, Nguyen Nhu Hoa and Nguyen Truong Giang*

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### IV-Joint II-03

**Phytohormone Metabolite Profiling in Plant Tissues**

*Ondřej Novák*, Aleš Pěnčík, Danuše Tarkowská, Jan Šimura, Veronika Turečková, Jana Oklešťková, Karel Doležal and Miroslav Sirnád

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### IV-Joint II-04

**Rice Breeding for Salt Tolerance in Mekong Delta via Marker-assisted Selection**

*Bui Chi Bua and Nguyen Thi Lang*

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### IV-Joint II-05

**Adaptation of Rice Cultivation in the Coastal Areas of Bangladesh under Changing Climate Conditions by Application of Salt-tolerant Biofertilizer**

*Sumonta Chandra Paul, Shahnaz Sultana, Samia Parveen, Shakila Nargis Khan, M Mozammel Hoq, Sirajul Hoq and M Manjurul Karim*

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P-AFB-02 In Vitro Growth and Development of Dendrobium sp. Treated with 2-Aza-8-Oxohypoxanthine Forming Lepista sordida
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P-AFB-03 The Effects of Bacterial EPS Produced by Rhizobium sp. on Rhynchostylis PLBs Micropropagation
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P-AFB-04 Development of Ornamental Dwarf Echinacea Plants using RNA Interference Technique to Down-regulate Brassinosteroid-biosynthetic Genes
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P-AFB-05 Changes in the Component Contents and Nitrile Oxide Production Inhibitory Activity of Japanese Apricot, Prunus mume, in the Fruits Maturation Stages
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P-AFB-06 Influence of Exopolysaccharide Producing Lactic Acid Bacteria on the Physical and Rheological Properties of Stirred Yogurt
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“Sustainable Biorefinery for Secondary Products”

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July 23-27, 2017 Pullman Khon Kaen Raja Orchid Hotel, Khon Kaen, Thailand
PLENARY SPEAKER ABSTRACTS
PL-01

Systems Metabolic Engineering for Bio-based Chemicals Production

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Abstract

Due to our increasing concerns on climate change and our environment, there has been increasing interest in bio-based production of chemicals and materials from renewable non-food biomass. As microorganisms when isolated from nature are not capable of producing such chemicals and materials of our interest at sufficiently high efficiencies, systems metabolic engineering is essential to improve the performance. In this lecture, I will describe general systems metabolic engineering strategies together with examples of developing strains for the production of several different chemicals and materials. It is expected that bio-based chemicals production will be driving the next generation chemical industries.

Keywords: Bio-based chemicals; Biorefineries; Systems metabolic engineering

Selected References:

PL-02

Bioeconomy towards Thailand 4.0

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Abstract

Bioeconomy is the next generation of Thailand’s agriculture based economy, moving away from a focus on feedstock supply to increase value-added products and drive the country towards Thailand 4.0. Bioeconomy will accelerate economic growth through the greater adoption of science, technology and innovations, transform agricultural based economy towards innovations based economy, generate high value added products and industrial growth opportunities from Thailand’s competitive advantage on economic crops. Bioeconomy is formed by two main factors. 1) Modern Farming or modern agriculture; focusing on development of technologies to ensure a high-yielding production and sustainable of agricultural products. Modern Farming comprises of large field farming, efficient water management, and the use of agricultural machineries and technologies to increase productivity. 2) Biorefinery; an integrated sustainable process that converts renewable feedstocks such as sugarcane, cassava, and agricultural wastes into a spectrum of high value added products such as biofuels, biochemical, bioplastics, biopharmaceuticals, food, and animal feed. Bioeconomy results from the Public-Private Collaboration initiative that fosters collaborations among government, private sectors and the civil society. With the total investment of ~ 400 billion baht for the next 10-year, Bioeconomy framework dividing into 3 phases 1) Develop bioenergy as a stepping stone for the Bioeconomy and create demands for investment in biochemical, bioplastic and biopharmaceutical sectors. 2) Develop Biorefinery complexes and Biopolis, the smart city based upon integrated innovation and Bioeconomy for value-added bio-based products. 3) Move Thailand towards the Regional Hub, establish biopharmaceutical pilot plant and commercial plant, implement domestic/ international full-scale Clinical Research. Bioeconomy is an important mechanism in driving Thailand’s economy for a sustainable future. It is the best solution driving Thailand to serve as the world’s bioindustrial hub, create investment in large-scale bioindustry, increase value added from agricultural products through science and innovation, build equality and elevate competitiveness in the agricultural sectors.

Keywords: Bioeconomy; Innovations; Modern agriculture; Modern farming
PL-03

Engineering Artificial Cell Factories for Exploring Nature Products:
Convergence Research and Disruptive Technology

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Abstract
Maybe the transition from descriptive study to mechanistic research by the end of the 19th century, marked with initiation of genetics and fermentation biochemistry, could be considered the beginning of modern biology. The introduction of concepts and tools of molecular biology in 1950’s and genomics in 1990’s are truly the two revolutionary landmarks of life science. Living organisms and biological systems have been studied based on a broader spectrum of complete nature blueprints employing recombinant technology for more efficient manipulations and better applications ever since. Meanwhile, along with the development of omics-based systems biology analyses, bottom-up forward engineering strategy and robust “standardized” technology platforms began to show its capability of enabling both the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems possible. Although Synthetic Biology is symbolic for a Scientific Dream aiming at rejuvenating life science and biotechnology via convergence research and disruptive innovation, either its true connotation or revolutionary potential is yet to be fully or precisely recognized. Our recent endeavor in engineering artificial cell factories for efficient exploring nature products indicated that via integrating dedicated research teams, Synthetic Biology will grow up and revolutionize our understanding of life and our capability of industrialization of biology for the wealth and health of human beings.

Keywords: Modern biology; Nature products; Synthetic biology
Horizons of Systems Biocatalysis and Renaissance of Metabolite Synthesis

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Abstract
Biocatalytic reaction platforms represent a key enabling framework for the synthesis of metabolites and other small molecules with excellent performance, selectivity and sustainability. The universe of biocatalysts with known functions, which are important to have easily available globally, provides thereby a tremendous knowledge base for highly selective and resource-efficient synthetic reactions. Their early integration into overall synthetic goals and rapid prototyping of the most critical steps are essential in order to obtain proof-of-principle routes. Preceding the realization of the synthetic reaction sequence is the use of analytical and design tools for route selection in all orientations. These include target-oriented synthesis, diversity-, function- or starting material-oriented synthesis, whereby the route can be modularly assembled from a large number of biocatalytic and chemical reaction platforms. As biocatalytic methodologies have become first choice for certain reaction classes in the route selection and metabolic pathways provide nature’s blueprint, biocatalysis is a preferred methodology for the synthesis of metabolites and metabolite-like compounds, which attract increased interest in research and development and are relevant for a number of industries. Systems Biocatalysis is therefore ideally suited as a bottom-up approach for designing the synthesis of metabolites and metabolite-like small molecules with high molecular economy and for the further development of sustainable chemistry by organizing enzymes in vitro to generate artificial metabolic pathways for synthetic purposes.

Keywords: Biocatalytic synthesis; Metabolites; Process design; Reaction engineering; Systems biocatalysis

Selected References:
KEYNOTE SPEAKER ABSTRACTS
Transgenic Barley: A Prospective Tool for Biotechnology and Agriculture

Ivo Frébort*, Edita Holásková, Alžběta Mičúchová, Veronika Janechová, Hana Popsíšilová and Petr Galuszka

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Abstract

Barley (Hordeum vulgare L.) is the fourth most important cereal grain worldwide, being used for human consumption, animal feed and as malt in brewery industry. Conceivable genetic transformation of barley has brought a potential for improvement of its agronomic performance or use for the production of recombinant proteins. Plant morphology, yield, and tolerance to various stresses largely depend on plant hormones, i.e. auxins and cytokinins, involved in different physiological processes. Given this knowledge, the barley cultivar Golden Promise was transformed by the insertion of cytokinin dehydrogenase gene from Arabidopsis thaliana under the control of root-specific promoter. Increase hormone degradation positively affected the number and length of lateral roots. Upon the application of severe drought stress, the transgenic genotypes maintained higher water content and showed better growth and yield parameters during revitalization. Higher tolerance to drought was caused by altered root morphology resulting in better dehydration avoidance. Field experiments with the transgenic lines showed notable increase in the biomass and grain yield. Recently, barley has been successfully used in molecular farming as a bioreactor for production of human proteins and cosmetic agents. Barley can be also used for the production of antimicrobial peptides that are key components of the innate immunity, but their production remains challenging because of peptide small length, susceptibility to proteolytic degradation and toxicity to the host. Endosperm-driven expression of chimeric genes encoding the antimicrobial peptide fused with selected domains was used to generate stable transgenic barley lines. The transgenic plants were fertile and showed normal growth and development. Fused protein tags were removed by the use of protease and the products inhibited bacterial growth. Overall, barley represents a promising tool for both agricultural and biotechnological transgenic approaches, and is considered an ancient but rediscovered crop as a model industrial platform for molecular farming.

Keywords: Barley; Cytokinin; Hormone
Overproduction of Cellulase by *Trichoderma reesei* Rut C30 through Batch-feeding of Synthesized Mixture as Substrate and Inducer

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Abstract

Cellulase for hydrolyzing cellulose in lignocellulosic biomass to release glucose is a prerequisite for producing biofuels and biobased chemicals through microbial fermentation, but high cost with cellulase presents one of the biggest challenges. Here we report the synthesis of low-cost mixture from glucose by β-glucosidase through the transglycosylation reaction as substrate for the growth of *Trichodema reesei* Rut C30 and inducer for cellulase production. Compared to commonly used soluble inducer lactose, the sugar mixture facilitated mycelial growth, and induced cellulase production more effectively due to the presence of easily assimilated glucose as carbon source for mycelial growth and β-disaccharides, in particular sophorose, for cellulase induction. As a result, cellulase activity as high as 90.3 FPU (filter paper unit)/mL was achieved at 144 h with the fed-batch strategy through which glucose was controlled between 0.05 g/L and 0.30 g/L to eliminate its inhibition in cellulase production, which was 10-20 folds of that achieved with lactose as inducer, making the crude enzyme more suitable for hydrolyzing pretreated biomass without a necessity for concentration. Moreover, cellulase productivity was consequently increased to 627.1 FPU/L/h, at least 3-5 fold higher than that achieved in cellulase production using soluble inducer lactose and insoluble inducer cellulose as well, saving energy consumption and capital investment significantly for cellulase production by *T. reesei*.

Keywords: Batch-feeding; Biorefinery; Cellulase; Synthesized soluble inducers; *Trichoderma reesei*
**KN-03**

**Metabolic Innovations to Develop Food Microbiome for Global Food Security and Health Solutions**

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**Abstract**

Traditional food systems and food diversity are essential to address global food security challenges through innovative metabolically driven functional food solutions. The current global food security challenges affecting global public health has to address both nutrient deficiency challenges as well as combat diet-linked chronic diseases from higher consumption refined processed foods in the diets. We have rationalized advances in functional food design to address global food security challenges based on metabolic responses in food ecological systems in diverse ecologies from food production and processing to food design for consumption. In integrating such ecology driven paradigms for new solutions to food security and public health challenges a more systems driven understanding and innovations built on local food culture and history has to be integrated with metabolic biology. Specifically we have integrated the role of redox biology and microbiome protection for good health from food production to food design for good health. This ecologically and metabolically influenced food security and health solutions must understand and rationalize traditional fermented foods in diverse cultures and ecologies to design functional foods and as a source of healthy microbiome. Through such systems-based metabolic innovations we can advance solutions to global food security challenges such as child and maternal nutritional deficiencies in many less developed countries and globally find functional food solutions to non-communicable chronic disease (NCD) epidemics such as type 2 diabetes and its complications.

**Keywords:** Food diversity; Food fermentations; Food microbiome; Global food security; Metabolic innovations; Public health solutions; Redox biology; Traditional food systems

**Selected Reference:**
KN-04

Nanobioelectronic Device toward Biosensor and Biocomputing

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Abstract

Based on nanobioelectroic technology a cell chip using spectroelectrochemical analysis has been developed as a valuable biosensing tool. Nanoscale bioelectronic device based on hybrid biomaterial had emerged to generate new concept and technologies for the development of electronic devices. The biomaterial, especially metalloproteins, can be used as a functional unit in an electronic device. Major challenges in bioelectronic device include the miniaturization, and the demonstration of various functions implemented in biomaterial to overcome silicon-based electronic device technology. Metalloprotein-based conceptual biomemory device was developed which demonstrated memory characteristics including ‘read’, ‘write’ and ‘erase’ function. And multi-bit memory function and nanoscale memory function were constructed. Afterwards new hybrid material composed of metalloprotein/DNA/nanoparticle has been developed to construct bioprocessing device to achieve various functions. A metalloprotein that exhibits redox property was used as a biomemory signal source, and various nanoparticles with complementary DNA and metal ions were used as input signals to acquire processed output signals. Various functions including ‘information reinforcement’, ‘information regulation’ and ‘information amplification’ were accomplished in this device due to various input signals. Hybrid material including RNA/quantum dot were developed to construct nanoscale resistive biomemory. The electrochemical property in neural cell and synthesis property of nanoparticle in human cells have been investigated. The proposed hybrid material-based bioprocessing device by the integration with neural cell should be a new type of platform for development of biomolecular-based biocomputing system. Acknowledgement: by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (no. 2014R1A2A1A10051725)

Keywords: Biosensor and biocomputing; Metalloprotein; Nanobioelectronic device
Precision Medicine – A Driving Force for Biosensor Technologies
Developments and Economics

Wen-Yih Chen
Department of Chemical and Materials Engineering, Institute of Systems Biology and Bioinformatics, National Central University, Taiwan

Abstract
Precision medicine initiative is to overcome the “averaged” medical paradigm that has been practiced for decades. To illuminate meaningful translational information out of the intrinsically complicated of a biological system created an unprecedented demand for an ultrahigh throughput biosensor with capability of providing big data information. The developments of microarray and NGS, with the help of systems biology and bioinformatics, steps closer to a more precise translational approach for diseases, including cancers. Further development of a next generation biosensor is indeed needed. With the help from semiconductor's dimension (compatible with target bio-molecules makes it possible to measure what could not be easily measured in the past), we could mitigate the gap we have to realize precision medicine and companion diagnosis. In this talk, I will try to explain what's the ecosystem and infrastructure available out there from consumer electronics companies and pharmaceutical companies, who are also working on the mobile health care and bench-to-bedside diagnostics to achieve precision medicine - as well as what's the bottlenecks limitations of the current technologies, i.e. sensitivity and resolution of conventional CMOS process. Given the above information, I wish to draw the attention from semiconductor and pharmaceutical companies and elucidate how they can work together and the economics that they can generate.

Keywords: Biosensor; Field effect transistors; Precision medicine
KN-06

Dynamic Micro-patterning and Sorting Technology for Single Cell Analysis

Teruyuki Nagamune1*, Shinya Yamahira1 and Satoshi Yamaguchi2

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Abstract
Cell micro-patterning has become an important technology for a wide variety of applications, ranging from tissue engineering, cell-based sensors and drug screening platforms to fundamental studies in cell biology. However, almost all reported dynamic micro-patterning methods are applicable to only adherent cells but not to non- or weakly adherent cells, which include blood cells (especially immunocytes), circulating tumor cells, some primary and stem cells. These cells are important as research targets in biological and medical fields, and for this reason, expansion of applicable range of current micro-patterning methods to non-adherent cells is an important challenge. To address this problem, we developed a photo-responsive universal cell membrane binding reagent (P-BAM) by conjugating poly (ethylene glycol) (PEG) and an oleyl group via photo-cleavable chemical linker. The cell-capturing ability of P-BAM was regulated by the dose of light exposure and allowed the preparation of arbitrary and fine patterns of immobilized cells on the substrate. Furthermore, a versatile single cell array could be constructed on the surface of collagen-coated substrate modified with P-BAM. On this single-cell array, quantitative imaging analyses of cellular migration, morphological changes and trafficking of sortase A-mediated fluorescence-labeled GPCR were performed in a high throughput manner. The photo-induced detachment of immobilized cell from the P-BAM modified substrate was also successfully accomplished. This result indicates that target immobilized cells of interest can be recovered and sorted by photo-irradiation at a desired point in time after quantitative imaging analyses of cells on P-BAM modified substrate.

Keywords: Cell-patterning; Cell-sorting; Single cell analysis

Selected References:
3. Tan, M. et al., Lab on a Chip, in press
NBB : Nanobiotechnology, Biosensors and Biochips
DNA Nanotechnology-enabled Organization for Biosensors

Chunhai Fan

Abstract

Proteins and nucleic acids are dynamically organized in cells to realize their physiological functions with spatial and temporal orderliness. This type of elegant supermolecular assembly has inspired researchers to create molecular/biomolecular structures with dynamic organization outside of the cells. In particular, DNA nanotechnology has proven to possess extraordinary flexibility and convenience for “bottom-up” construction of exquisite nanostructures with high controllability and precision, which holds great promise in a wide range of applications, e.g. nanofabrication and molecular electronics, in-vivo and in-vitro sensing and drug delivery. In this talk, I will present several examples of using tetrahedral DNA nanostructures (TDNs) for dynamic organization of biomolecules in vitro. TDNs are three-dimensional (3D) DNA architecture with high mechanical rigidity and structural stability, which are suitable for organization of higher-ordered nanocomplexes and nanodevices. As one of the examples, we employed single-particle tracking to visualize the internalization of TDNs, and dissect the cell entry pathways of these virus-like nanoparticles. In the second example, I hope to use the way that we employed TDNs to dynamically organize the biosensing interface, and realize macroscopic applications on diagnostics.

Keywords: Biosensors; DNA; Nanotechnology

Selected References:
YS : Young Scientists
KN-YS-01

Application of Synthetic Biology Tools for Metabolite Overproduction

Min-Kyu Oh*, Sang-Woo Lee, Min-Ji Heo and Hwi-Min Jung

Department of Chemical and Biological Engineering, Korea University, South Korea, 02841 *E-mail: mkoh@korea.ac.kr

Abstract

A riboswitch was employed to engineer *S. cerevisiae* to produce a valuable metabolite. A self-cleaving aptazyme, glmS, cleaves its own transcript in response to the intracellular glucosamine 6-phosphate (GlcN6P) concentration. The aptazyme was attached to the 3′-untranslated region of FCY1, encoding cytosine deaminase to construct a suicide circuit for evolutionary engineering. Growth of the strain harboring the suicide circuit was hampered by addition of fluorocytosine, and was recovered as the intracellular GlcN6P level increased. By using this circuit, we isolated a N-acetyl glucosamine (GlcNAc) producing *S. cerevisiae* by screening an efficient glutamine-fructose-6-phosphate transaminase (Gfa1p) and haloacid dehalogenase-like phosphatases (HAD phosphatases). The suicide circuit can be translated as a double NOT gate and be applied to a wide range of organisms for efficient and high-throughput screening of inconspicuous phenotypes. Another synthetic biology tool, CRISPR/Cas9, was applied for effectively production of n-butanol in a defined medium under micro-aerobic condition. To increase butanol production, carbon flux from acetyl-CoA to citric acid cycle should be redirected to acetoacetyl-CoA. For this purpose, the 5′-untranslated region sequence of gltA encoding citrate synthase was designed using an expression prediction program, UTR designer, and modified using the CRISPR/Cas9 genome editing method to reduce its expression level. *E. coli* strains with decreased citrate synthase expression produced more butanol and the citrate synthase activity was correlated with butanol production. These results demonstrate that redistributing carbon flux using genome editing is an efficient engineering tool for metabolite overproduction.

Keywords: CRISPR/Cas9; Metabolite; Riboswitch; Synthetic biology

Selected References:

INVITED SPEAKER ABSTRACTS
AFB : Agricultural and Food Biotechnology
IV-AFB-01

Food and Probiotics

Srirat Rengpipat*

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Abstract

Expected population in the world in 2050 is ~9.7 billion which will bring us to face food crisis and food security. Food safety is currently the most concern due to decent heath resulting from safe food. Microorganisms are ubiquitous on the human body. Our gut microbiota contains ~10^{13} microorganisms, including at least 1,000 diverse species of known bacteria with >3 million genes (150X more than human genes). One third of our gut microbiota is common to most people, while two thirds are specific to each one of us. Put differently, the microbiota in our gut represents an individual fingerprint. The species composition is highly personalized and largely determined by our environment and our diet. The composition of gut microbiota may become accustomed to dietary components, either temporarily or permanently. Characterization of the microbiome in healthy persons is an important initial step in understanding the role of the microbiome in contributing to health and disease. Gut microbiota are vital to host digestion and nutrition by generating nutrients from substrates that are indigestible. Food will be the main sources of various kind of microorganisms that exist in our gut after intake. In addition, research has long suggested that microorganisms help humans by doing things like protecting us from allergies and preventing the spread of certain pathogen. Therefore, selection for the beneficial bacteria such as probiotic(s) for consumption can lead to balance gut microbiome.

Keywords: Microbiome; Microbiota; Probiotic(s)

Selected References:
Impact of Music and Sound on *Lactobacillus amylophilus* GV6 for Lactic Acid Production

Ishan Kumar Pal and Rintu Banerjee*

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Abstract

Biopolymer is undoubtedly being considered as a unique and integral material of human civilization which has gained its distinctive identity in the modern era of biomaterials. It acts as an interphase between biology and material science. Since its conception, the biopolymer has been gradually growing and has steadily occupied a huge market. The additional attention is because of its growing public and scientific interest owing to its reusability, recyclability and reduction in pollution. Lactic acid with its polymeric forms, polylactide (PLA), which is biodegradable and bioabsorbable polyester has been extensively investigated over the last several decades and have proved to be a potential polymer. PLA consists of a family of polymers of either L(+) or D(-) form of lactic acid. In the present study, an emphasis has been given on cost effective production of lactic acid from *Lactobacillus amylophilus* GV6 by using waste starchy residues as the carbon source. During optimization studies environmental as well as physico-chemical parameters influencing lactic acid production were considered. The effect of music on the overall yield of lactic acid was also studied. Among the different types of music’s such as eastern, western, jazz and rock, it was found that there were some variations in the yield of lactic acid by *L. amylophilus* GV6. Thereafter, the effect of dB sound was also studied which resulted in the improved production of lactic acid. The present article deals with the higher production (~ 65% higher yield) of lactic acid for PLA formation and its biotechnological applications.

Keywords: Improved yield; Lactic acid; Music; Optimization; Polylactide

Selected References:
Rice Husk as a Potential Substrate for Biofuel and Biorefinery

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Abstract

Rice husk is an agricultural waste abundantly available in rice producing countries that provide approximately 400 million metric tons of rice annually, of which more than 10% is husk. The analysis of rice husk biomass reveals approximately 50% by weight is celluloses component. The recent concerns on food security make rice becomes one of the inedible target. Trying to utilize rice husk as a renewable substrate for biofuel and biochemical production is investigated. The thermotolerant yeast capable of ethanol fermentation at 45°C, Kluyveromyces marxianus CK8, was used as the fermenting yeast incorporated with a commercial cellulolytic enzyme in the Simultaneous Saccharification and Fermentation (SSF) on rice husk. Among seven factors screened through a Plackett–Burman design, four factors including substrate concentration, temperature, incubation period, and pH were found to be significantly influenced in the SSF process. Finding the optimal condition for ethanol production through the combination of central composite design (CCD) and response surface methodology (RSM), ethanol yield of 15.63 g/L was obtained from a condition of 9.44% (w/v) substrate concentration, 43°C, and pH 4.2. Ethanol production yield increased 1.44 fold when compared with Separate Hydrolysis Fermentation (SHF). The xylose related oligomers prepared from rice husk was also found to be a potent prebiotics expected to use in food industry. Mixed glucose and xylose from rice husk hydrolysate was also tried to use as a substrate for lactic acid production. However, the uses of rice husk for other purposes are the main factor limited the large scale production of various value-added products mentioned previously.

Keywords: Biofuel; Biorefinery; Ethanol; Rice husk; Utilization
Production of Recombinant Enzymes from Lactobacillus plantarum Food Grade Expression System

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Abstract

One of the attractive expression hosts for food-grade expression system is lactic acid bacteria because it is considered as safe and carry the “Generally Recognized As Safe” (GRAS) status. For industrial application, an ability to secrete or displayed of recombinant proteins are attractive. Our laboratories have sub-cloned genes encoding two Bacillus hydrolytic enzymes, i.e., chitosanse (CsnA) and beta-mannanase (ManB), into pSIP-based expression vector and over expressed in Lactobacillus plantarum WCFS1. In addition, to avoid using antibiotics, the erythromycin resistance gene was replaced on the expression plasmid with the alanine racemase (alr) gene, which led to comparable levels of protein production and secretion efficiency in an alr-deficient L. plantarum TGL02. For surface display, both enzymes were fused to different anchoring motifs of L. plantarum for attachment to the cell surface and the resulting fusion proteins were expressed in L. plantarum WCFS1. Both ManB and CsnA could be efficiently produced by secretion or surface display on L. plantarum using pSIP-based expression vectors. This approach could be applied in production processes relevant for food industry in the future.

Keywords: CsnA; Enzyme; Expression; Food-grade; Lactobacillus plantarum; Secretion, Surface display, ManB

Selected References:

AM : Applied Microbiology
Yeast Lipids for Sustainable Biodiesel Production

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Abstract

Microbial lipids, especially produced by oleaginous yeasts, consist mainly of triacylglycerols with fatty acid compositional profiles similar to those of commercial vegetable oils. Microbial lipids are potential feedstock for biodiesel business and oleochemical industry. Yet, it remains challenging to produce microbial lipids at competitive low costs. We have been working on most aspects of microbial lipid technology in order to advance this area and reduce the production costs. Specifically, different types of integrated processes were devised to explore low-value feedstock, such as raw glycerol, biomass hydrolysates, corn stalk, and cellular wastes of fermentation industry. Processes were established to recover lipid and even make fatty acid esters directly from the culture broth without the isolation of “fatty” cells. In another aspect, we accomplished multi-omic analysis of the red yeast *Rhodosporidium toruloides*, a robust and excellent lipid producer, and developed genetic tools. Thus, high-value lipids will be produced upon rational engineering oleaginous yeasts. Together, our efforts have been considerably advancing yeast lipid technology and should provide intriguing insights for biorefinery in general. We believe that yeast lipids will be further developed in near future for sustainable biodiesel production.

Keywords: Biodiesel; Microbial lipids; *Rhodosporidium toruloides*

Selected References:

IV-AM-02

Development of a CRISPR/CRISPRi Hybrid System for Metabolic Engineering of *E. coli* and Succinate Production

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Abstract

CRISPR/Cas9 system is a promising genome editing tool consisting of Cas9 nuclease and the single guide RNA (sgRNA). Guided by the sgRNA, CRISPR system can trigger DNA double strand break (DSB) at specific gene loci and promote homologous recombination. By using a catalytically dead Cas9 (dCas9), CRISPR can be adapted to simultaneously down-regulate the expression of multiple genes. Such CRISPR interference (CRISPRi) technology can be harnessed for regulation of metabolic pathways in bacteria and promotes the yield of downstream biomass or chemicals. In order to achieve stable gene regulation and product production, we aimed to develop a suitable CRISPR system to integrate large CRISPRi module (dCas9 and sgRNAs) into *E. coli* genome. We first compared multiple Cas9 derived from different organisms (SaCas9, St1Cas9, FnCpf1) and found both SaCas9 and St1Cas9 system exhibited 99% DSB in *E. coli* MG1655. By providing donor template DNA of different sizes, the editing accuracy reached 80~95% for both SaCas9 and St1Cas9 system, St1Cas9 group yield 2~5-fold more colonies than SaCas9 group. Therefore, we utilized the St1Cas9 system to integrate pyc gene and the CRISPRi module into lacZ locus and yield dC-PLP strain. The integrated CRISPRi module persistently knocked down the gene expression of *ptsG*, *ldhA* and *pflB* for 80~90% and enhanced the production titer of succinate. This system holds promise for metabolic engineering of microbial and paves a new avenue to the applications of CRISPR/CRISPRi technology.

Keywords: CRISPR/Cas9; CRISPRi; *E. coli*; Metabolic engineering; Succinate
IV-AM-03

ARTP Mutagenesis Tool for Life Science and Industry

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Abstract
Development of rapid and powerful mutagenesis tools is always of importance for effective evolution of strains or germplasm both for life science researches and industry. ARTP (atmospheric and room temperature plasma) mutagenesis system developed by our group can directly cause complex genome mutation including chain break and bases mutation via a unique mechanism. By quantification of the DNA damage strength and the subsequent mutation rate of living cells, ARTP has exhibited the strongest DNA damage, and the highest mutation rate among the physical and chemical mutagenesis methods. Further, genome sequencing of E. coli mutated by ARTP indicated that diverse breakages of DNA occurred. By omic analysis of the representative mutants of such as bacteria, yeast, fungi and microalgae generated by ARTP or followed by combination with adaptive evolution, global changes in the metabolic network pertaining to different phenotypes of growth rate, tolerance and productivity, was discovered, which enabled the new genetic functions to be explored. The ARTP mutagenesis was validated to be efficient for reverse metabolic engineering of microbial cell factories. More than 100 types of microbial strains and plants as well as animals have been successfully improved by ARTP mutagenesis so far. Taken together, ARTP can be a useful mutagenesis platform for life science and industry by combining with different high throughput screening methods. This work is supported by National Key Scientific Instrument and Equipment Project of NSFC (2162780028), the Tsinghua University Initiative Scientific Research Program (20161080108) and the JST CREST Project of Japan.

Keywords: ARTP; Breeding; Evolution; Life industry; Life science; Mutagenesis
BEB : Bioenergy and Biorefinery
“Sustainable Biorefinery for Secondary Products”
IV-BEB-01

Biological Bioproducts from Biomass Waste

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Abstract
The growing global population and its effects on world food security, energy consuming, natural resources exhausted, as well as the urgency for climate change mitigation, are issues that foster technological to increase the efficiency of use of natural resources, such as biomass among others. Especially concerns about the earth's sustainable management and the reduction of greenhouse gas emissions have become an important issue in the world. The recycle economy also attracts the scientists for upcycling of biomass based products. One of the alternatives is producing biofuels and biomaterial building blocks from biomass waste. Biomass wastes, which include solid waste of agricultural residues (rice straw, wet birch pulp), agro-industrial wastes (mushroom waste, cotton cellulose, etc.) and liquid waste of food and related industrial wastewater, are abundant feedstock for biohydrogen, biomethane, bio-cellulose nanofiber, and biochemicals, etc. This technology of waste to energy and biochemicals includes the pretreatment of biomass, subsequently converted to sugars (hydrolysate). Sugars are thereafter transformed into biofuels such as hydrogen, methane, ethanol, bio-cellulose nanofiber, and the biomaterial building blocks such as volatile fatty acids: Lactic acid, Ethanol, Acetone, Acetic acid, Propionic acid, and Butyric acid etc.

Keywords: Biomass hydrolysis; Bio-products; Bio-refinery; Fermentation

Acknowledgements:
The author gratefully acknowledge the financial support of the Ministry of Science and Technology of Taiwan (grant numbers: MOST 106-2915-I-035-501; MOST 105-2632-E-035-001), also thanks to the Precision Instrument Support Center of Feng Chia University in providing the fabrication and measurement facilities.
IV-BEB-02

Improved System for the Production of Activated Carbon from Oil Palm Kernel Shell

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Abstract
Conversion of biomass into activated carbon using pyrolysis technology has showed an increasing interest in recent years. However, most of the technologies developed (pyrolysis followed by activation) are conducted in a separate process, which require more steps and lengthy period of time. Therefore, an improvement process for the production of activated carbon was developed using simultaneous carbonisation-activation reactor equipped with steam activation unit. The reactor was double insulated with cement and jacketed heat insulation that cover the internal space of the reactor. The substrate used (oil palm kernel shell) was carbonised at 400°C, followed by steam activation at 900°C. This reactor produced activated carbon with high surface area up to 987 m²g⁻¹ and the yield of 30%. The whole process only took 7 h of retention time. This technology of simultaneous carbonisation-activation reactor system had shown a promising output to produce high quality activated carbon and therefore could attract the palm oil industry especially in the waste utilization and management practice as well as adding value to the generated wastes.

Keywords: Activated carbon; Palm kernel shell; Pyrolysis; Simultaneous; Steam
IV-BEB-03

Development of Microbial Cell Factories for Production of Aromatic Chemicals and Derivatives

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Abstract

Bio-based chemicals currently receive attention as sustainable, drop-in substitutes for petroleum-based chemicals. The feedstocks used for the production of bio-based chemicals have recently expanded from edible sugars to inedible lignocellulosic biomass. In the last decade, recent progress in metabolic engineering has expanded fermentation products from aliphatic compounds to aromatic compounds. In addition, synthetic biology enables the production of non-natural aromatics, including phenylpropanoids and chorismate derivatives. This diversity provides an opportunity to expand the development and industrial uses of bio-based chemicals to produce bio-based polymers, food additives, and pharmaceuticals, in addition to building block chemicals for biorefinery. However, most of the biomonomers are currently produced from edible sugars or starches that compete directly with food and feed uses. Technologies that will enable utilization of inedible and renewable lignocellulosic feedstocks are needed for development of a bio-based economy. To achieve the goal, we studied on the microbial production of aromatic compounds from renewable lignocellulosic feedstock, such as kraft pulp and sorghum bagasse. To utilize cellulose in lignocellulose as a fermentation substrate, an artificial pathway for the de novo production of aromatic compounds from glucose was developed in Escherichia coli cells with metabolic engineering and synthetic biology. In this presentation, we summarize recent progress in microbial production of aromatic compounds, especially from lignocellulosic biomass, based on metabolic engineering approaches. In addition, future perspectives and challenges in this research field will be discussed.

Keywords: Biorefinery; Lignocellulosic biomass; Metabolic engineering; Synthetic biology.

Selected References:

Microalgae as the Platform for Carbon Cycling and Circular Economy

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Abstract
Using photoautotrophic culture of microalgae to mitigate carbon dioxide emissions is promising and has been one of the hottest topics in carbon recycling and utilization. The microalgal biomass converted from bio-fixation of CO₂ can be used as feedstock/raw materials for biofuels and bio-based chemicals production. To facilitate the commercialization of microalgae-based industry, the NCKU microalgae team has been developing platform and commercialization technologies to improve and integrate the currently available technologies and make them more feasible for practical applications. The major research directions include (1) developing microalgal mutants with the enhancement in their growth rate, temperature tolerance and carbon dioxide fixation efficiency, (2) developing a large scale outdoor microalgae cultivation system using industrial flue gas and employing different operation strategies to further improve the CO₂ fixation efficiency, (3) developing effective methods for the production of microalgae-based biofuels and high-value products; the residual algal cells or byproducts (e.g., glycerol) are used to produce value-added products to enhance the economic feasibility of the overall process; and (4) Establishing a CO₂-absorbing wastewaters for simultaneous wastewater treatment and microalgae cultivation. Our recent research progress will be presented, and the challenges encountered during the development of microalgae-based CO₂ utilization technologies will also be discussed.

Keywords: Biofuels; Biorefinery; CO₂ fixation; Flue gas; Large-scale cultivation system; Microalgae; Wastewater treatment
An Update in World’s Largest Ocean Test-beds in Korea for Sustainable Marine Microalgal Biomass Production

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Abstract

Despite all the 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 $10 USD/kg. Bioenergy must be produced much cheaper than the most of the biologically-driven products.

One of the possible solutions for some of these challenges is mass culturing microalgal in large ocean area. A large-scale floating ocean cultures have several benefits: (i) lower CAPEX; (ii) no freshwater usage; (iii) relatively abundant seawater; (iv) ability to exploit the lower nutrient concentration in seawater; (v) no need to worry about evaporation; (vi) larger area to deploy; and so on.

All the opportunities and challenges of microalgal biofuels will be discussed based on our experience in Korea along with some photos and videos.

Keywords: Biodiesel; Economic production; Microalgae; Off-shore cultures; Sustainability

Selected References:


Fermentation Process Design for Bioethanol Production

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Abstract
Ethanol, a primary metabolite of Saccharomyces cerevisiae, is correlated to the amount of sugar present; the more sugar available, the more ethanol is expected to be produced. To design an ethanol fermentation process, one can directly measure the change of sugar content in the fermenter, indicating the progress of ethanol production. For large-scale production purposes, such a direct measurement is impractical. One can also indirectly measure the change of the fermentation pH or turbidity in the fermenter, indicating the status of fermentation and developing proper fermentation strategies accordingly. Past experience has shown that these types of indirect approaches have only been applicable under limited situations. Although regarded as indirect measurement, fermentation redox potential and dissolved carbon dioxide (DCO₂) level in a fermenter are more “physiologically relevant” to yeast during the course of ethanol fermentation. We have demonstrated that at sugar concentrations less than 250 g/L, the change of slope of fermentation redox potential to positive from negative indicates complete consumption of sugar. The characteristic of changing in fermentation redox potential was subsequently implemented to develop several redox potential-controlled ethanol fermentation processes. For sugar concentration greater than 250 g/L, complete sugar utilization could only be achieved with the incorporation of DCO₂ control into process design for ethanol production. Complete sugar utilization was not possible when the fermentation redox potential was used as a manipulating parameter. In this presentation, various redox potential-driven and DCO₂-driven fermentation processes will be discussed and the future improvement of these processes will be high-lighted.

Keywords: Dissolved carbon dioxide; Ethanol fermentation; Fermentation redox potential; Process design

Selected References:
BPB : Bioindustry Promotion and Bioeducation
Mannan EPS from Arctic Sphingobacterium sp. for the Treatment of Colitis Related Inflammation in BALB/c Mice

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Abstract
The emerging stories surrounding nutraceuticals indicate that most of our life-style related problems, including inflammatory bowel disease (IBD), could be dealt with diet itself. The nexus between diet, gut microbial composition and host immune system delicately balance the homeostasis in gut. Multiple environmental factors trigger exaggerated mucosal immune response against gut microbial components in genetically predisposed individuals. Active interplay between the immune and non-immune components of the gut tissue set the course of IBD pathogenesis. Prebiotics reach the colon intact and eventually modulates the microbial community composition, as well as directly binds to the receptors of gut immune system to modulate their response. In this study, Sphingobactan, an α-mannan exopolysaccharide isolated from an Arctic Sphingobacterium sp., has been purified and characterized through chromatographic methods, GC/MS and NMR. Being unable to be digested by amylases and promotional for Lactobacilli and Bifidobacteria growth, its prebiotic candidature was further tested in Dextran sodium sulphate mediated colitis model in BALB/c mice. Sphingobactan was able to address the inflammation in gut. Colitis induction and treatment efficacy was assayed through disease activity index and gene expression level through RT-PCR. The signature expression of pro-inflammatory genes in IBD (Cox2, iNOS, TNF-α) found to be decreased, and anti-inflammatory cytokines (IL-10) increased in colon tissue, upon oral treatment of Sphingobactan. The amelioration of IBD condition in mice was the result of multi-pronged attack on the acute inflammation by immunomodulatory prebiotic on gut tissue, systemic immunity, and enhanced probiotic bacteria over harmful commensals in gut microbial community.

Keywords: Anti-inflammatory; Colitis; Exopolysaccharide; Mannan; Prebiotic
Opportunities in Research and Manufacturing of Bio-pharmaceuticals in India

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Abstract
India has emerged as a global leader in vaccines with about one-third share of the total world market. India's bio-pharmaceutical sector is valued at $26 billion and it is one of the fastest growing knowledge based market which is growing 20 per cent annually for the last few years. India is the major supplier of basic Expanded Programme on Immunisation vaccine to the United Nations Children’s Fund (UNICEF). Around 75 to 80 per cent of vaccines procured by UN agencies are from the developing world and almost 80 per cent of these are from India. Similarly, India is now emerging as the global destination for the manufacture of Biologics, especially Biosimilars and cell-based therapeutics, including stem-cell research and regenerative medicine. Biosimilars have tremendous opportunity in India, particularly in monoclonal antibodies based therapeutics where innovator’s patents have either expired or will be expiring soon. A cost effective Biosimilar drug must have the quality, safety and efficacy comparable to that of the innovator’s product. India is globally regarded as having great potential to become a significant player in the development and commercialisation of Biosimilars due to its proven experience in generic drugs. Further, India’s new regulatory policy on Biosimilar products would fast-track its development process. Panacea Biotec is one of India's leading research-based health management companies with established capabilities in both generic formulation and vaccines. As one of the leading vaccine producers in the country it has significant presence in both institutional and private vaccines markets in India and abroad. It has a strong portfolio of vaccines against critical and life threatening diseases like Polio, Hepatitis B, Diphtheria, Tetanus, Pertussis, Haemophilus Influenza type B (Hib), pandemic flu (H1N1), and combination vaccines. Panacea Biotec has played a key role in global polio eradication program by supplying around 10 billion doses of Oral Polio Vaccines to Government of India and UN Agencies which led to polio free India since 2011. Today, it can support manufacture of up to one billion doses annually. Panacea Biotec has four distinguished research & development centers that specialize in Vaccine & Biologicals, Novel Drug Delivery Systems, Generic Formulations and Discovery Research. The Company also has state-of-the-art manufacturing facilities for Vaccines, Anti-Cancer products and other Pharmaceutical Formulations at various locations in the country. These facilities are approved by several International Regulatory Agencies such as WHO, USFDA, BfArM Germany, ANVISA Brazil, etc. and its product portfolio has expanded internationally with its products reaching out to more than 30 countries. Therefore, Indian companies like Panacea Biotec can provide huge opportunities for French innovators and start-up companies in forming alliances for a collaborative research, manufacturing and commercialization of Biopharmaceuticals.

Keywords: Bio-pharmaceuticals; Biosimilars and cell-based therapeutics; Vaccines
The Role of Mitr Phol R&D Innovation in Thailand’s Bioeconomy

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Abstract
Bioeconomy, creating a sustainable economic system with innovation and technology to increase the value of industrial drops, has been increasingly recognized as the national agenda of Thailand. Bioeconomy concept does not only solve environmental problems, but also creates business opportunities from sustainable and renewable products. Science, innovation and technology have been integrated with existing products and processes to achieve significantly higher levels of productivity under environmental pressure: population development and limited resources. Mitr Phol group is an organization that has focused on business and social consciousness for over 60 years under the concept of value creation to value the sustainable growth of Thai society. The company applies innovation and technology to develop its business in response to Thailand’s national bioeconomy strategy. Smart farming, applying internet of things into agriculture, and smart plant bleeding have been used to improve high quality and productivity of sugarcane under climate change factors. Bagasse and molasses, main sugarcane industrial byproducts, have been utilized and converted into renewable energy, electricity, wood-composite materials, and high value-added bio-based products.

Keywords: Bioeconomy; Innovation; Internet of things; Value-added products
IV-BPB-04

Probiotics Business and Technology Promotion from Inside to Outside

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Abstract
SYNBIO TECH INC. is a leading and professional probiotics company. We are a pioneer in researching and producing lactic acid bacteria. Our two unique patented technologies, “SYNTEK thorough” and “SYNPACK”, can significantly strengthen our probiotic products from inside to outside which are more special than others. “SYNTEK thorough” is a probiotics optimizing development system based on strain-level production process without gene modification to improve probiotic properties in all respects. By using “SYNPACK”, it can further enhance the storage stability of products and therefore ensure the functionality of probiotics. We will continually develop different functional probiotics in the future. “Better Probiotic Better Life” is SYNBIO TECH INC’s responsibility and mission that motivates us to keep developing better products for human well-being. Therefore, Indian companies like Panacea Biotec can provide huge opportunities for French innovators and start-up companies in forming alliances for a collaborative research, manufacturing and commercialization of Biopharmaceuticals.

Keywords: Biopharmaceuticals; Lactic acid bacteria; Probiotics business
IV-BPB-05

The Thai Association for Biotech Industries (ThaiBIO) Promotes Thai-Biotechnology Business to be Sustainable and Global Competitiveness

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Abstract

Biotechnology is a branch of science that aims to utilize the knowledge for human benefits. Nowadays, many valuable industrial businesses are from Biotechnology based. In USA, biotechnology business is in the highest growth, while in our country, Thailand, it is being seriously promoted by government agencies. The country with booming biotechnology industry is often from result of private sector cooperation and integration. The network of local government, researchers and investors is a key role in success policymaking. A specific association or organization is more efficient to stimulate the advancement of biotechnology industries and business. To base of bioeconomy and benefit of biotechnology business in Thailand, ThaiBIO Association was founded in 2010. We are a nonprofit organization with the intention to support the progress of sustainable life sciences technology and business in Thailand. We support and promote biotechnology research and development to be consistent with the country development in 5 areas; food and feed, agriculture, industry, medication, and energy and environment. The country capacity on biotechnology business is our main objective for compete in the regional and global market.

Keywords: Bioeconomy; Biotech industry; ThaiBIO association; Thailand 4.0

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IV-BPB-06

Production of Stereocomplex Poly (Lactic acid) from Microorganisms Isolated in Thailand

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Abstract
Bioeconomy is now promoted as a national agenda in Thailand to create the new S-curve economy. PTT Global Chemical Plc., (PTTGC), a leader in the integrated petrochemical and refining business in Asia Pacific, aspires to drive sustainable growth by initiating the commercial development of ‘Green chemicals’ research program to produce several bio-based products. Here we demonstrate the initiative to produce Stereocomplex-poly(lactic acid) (Sc-PLA), an improved heat stability of PLA, by utilization of non-GMO microorganisms isolated in Thailand. Our research group has screened the Bacillus sp. BC-001 and Sporolactobacillus sp. SK5-2 that are capable of producing L- and D-lactate, respectively, with high yield and high productivity comparable to those obtained from the industrial fermentation process. Glucose fermentation platform of BC-001 was considered simple and robust since the pH was controlled by the concentrated NaOH at 10M to achieve a volumetric productivity of an optically pure L-lactate at 5.6 g/L•h. This fermentation platform was successfully validated stepwise on 30-liters, 300-liters, and 3,000-liters fermenters, resulting in a repeatable range of the volumetric productivity of 6.8 g/L•h, 5.2 g/L•h, and 6.6 g/L•h, respectively. On the other hand, the fermentation of SK5-2 using low cost concentrated sugarcane juice in a bench scale fermentor obtained > 99% optical pure D-Lactate at almost 120 g/L. We also demonstrate that the purified L- and D-lactic acid from our fermentation processes can be polymerized to produce PLLA and PDLA which could be subsequently blended to produce stereo-complex PLA (Sc-PLA). The results show clearly that our microbes are promising strains for the production of highly optical purity L- and D-lactate, bio-based PLA and Sc-PLA that could have positive impact to Thailand sustainable economy.

Keywords: Bacillus; Lactate; Poly (Lactic Acid); Sporolactobacillus
BPMB : Biopharmaceutical and Medical Biotechnology
Large Scale Whole Genome Sequencing of *Mycobacterium tuberculosis* in Thailand: Implications for National End TB Strategy

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Abstract

Tuberculosis (TB) has always been a significant health problem throughout human history. Research and development for controlling tuberculosis has been promoted in the last few decades resulting in development of novel diagnostics and treatment modality. Recently there has been a global effort, coordinated by WHO, to eliminate TB in 2050. In order to do so, it requires implementing all existing useful tools as well as tools not existing yet for TB control. Whole genome sequencing is technology with great potentials in diagnosis and epidemiological studies. A project to sequence the genomes of about 1200 isolates of *M. tuberculosis* as well as densely genotyping the host genomes has been carried out for two years, by the collaboration between Medical Life Sciences Institute MOPH, Mahidol University and University of Tokyo under SATREPS program and funding from AMED and JICA. The information enable the first time detail phylogenetic analysis of the Indo-Oceanic Lineage, common in Eastern Africa Southern Asia and ASEAN. Implication in vaccine development will be discussed. It also indicates good correlation between a large array of genetic markers and phenotypic susceptibility testing of first-line anti-TB drugs. Some other genotype-phenotype analysis of the bacterial genomes will be briefly presented. The study provides a foundation for applying next-generation sequencing and single-molecule sequencing technology in TB control in Thailand.

Keywords: Drug resistance; Indo-oceanic family; Phylogenetics; Tuberculosis; Whole genome sequences
IV-BPMB-02

Intelligent Particle Adjuvants for Advanced Vaccine Formulation

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Abstract

Subunit antigen such like, virus-like particle (VLP), split antigen, protein/peptide antigen, is of interest for safety profiles, compared with conventional attenuated or inactivated vaccine, but their immunogenicity usually is lower. Furthermore, therapeutic vaccine for cancer has been paid high attention in recent years, however, immune tolerance is a big challenge. Alum adjuvant usually is added in vaccine formulation to enhance its immune-response, however, it can effectively enhance humoral immune response, but only induce limited cellular immune response because the antigen only can be processed in lysosome. As the result, immune tolerance cannot be overcome. As an intelligent vaccine delivery system instead of conventional adjuvant, we designed and prepared uniform biodegradable (chitosan, PLGA, CaCO₃) particles with high pH-sensitivity. We developed membrane emulsification technique to obtain uniform chitosan droplet. Furthermore, we developed a new method to obtain chitosan gel particle without using chemical crosslinker, it showed high pH-sensitivity. H5N1 split antigen was loaded on it by adsorption. It was found that due to the lower pH environment in lysosome and high pH-sensitivity of Gel NP, a large amount of H5N1 escaped from lysosome into cytoplasm, and induced high level of cellular immunity. In vivo study demonstrated that Gel NP induced both higher humoral and cellular immune responses, compared with crosslinked chitosan particle (GC NP) and Alum adjuvant. pH-sensitive PLGA hollow particle was obtained by encapsulation of NH₄HCO₃ as well as OVA model antigen, and was used as OVA delivery system for therapeutic vaccine of cancer. After the particle was up-taken by DC into lysosome, the particle was broken in lower pH by generation of CO₂ and NH₃ gas to release OVA rapidly to cytoplasm. Finally it enhanced both higher humoral and cellular immunity, and mouse immunization induced greater lymphocyte activation, more antigen-specific CD8+ T cells. pH-sensitive CaCO₃ nanoparticle was successfully prepared by using OVA antigen to induce crystallization. And its high pH-sensitivity induced high cellular immune response, in vivo test showed that it prevented the tumor growth and prolonged the survival of mouse apparently.

Keywords: Adjuvant; Antigen; Formulation; Intelligent; Particle; Vaccine
IV-BPMB-03

Transdermal Drug Delivery Systems for Cancer Immunotherapy using Oil-based Nanocarrier

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Abstract
Transcutaneous immunization is a novel non-invasive method as an alternative to conventional immunization with injection. Skin immunocompetence comprised of abundant antigen presenting cells in epidermal and dermal layers of skin can provide an effective tool for transcutaneous immunization, whereas the outermost hydrophobic layer of skin called stratum corneum disturbs the penetration of antigens into skin. In this study, we evaluated the applicability of the transcutaneous immunization using a surfactant-coated antigen nanocarrier to the cancer immunotherapy. To realize an effective transcutaneous delivery of antigens, we have developed a Solid-in-Oil (S/O) technique that produces an oil dispersion of hydrophilic biomolecules. In the present study, we applied the S/O nanodispersion carriers to transcutaneous immunization for the induction of cancer immunity. The topical application of S/O nanodispersions bearing TRP-2 as a melanoma antigen allowed the penetration of the cancer antigen into the deeper region of skin, dermis, by intracellular pathways. The growth inhibition of TRP-2-bearing tumor was achieved by the transcutaneous immunization with the S/O nanodispersions. An in vivo experiment revealed that the effective prevention of melanoma growth and metastasis was achieved by the S/O formulation containing the cancer antigen. It demonstrates the applicability of S/O nanocarriers to the induction of cancer immunity.

Keywords: Cancer vaccine; Drug delivery system; Immunization; Transdermal drug delivery

Selected References:
IV-BPMB-04

Preclinical Evaluation of Tumor Inhibitory Effects of Synthetic Form of Antrocin

This work was not delivered on the conference schedule.
High Level Accumulation of Soluble Diphtheria Toxin Mutants (Crm197 and Triple-mutated Toxoid) with Co-expression of Molecular Chaperones in Recombinant Escherichia coli

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Abstract

Crm197 is a diphtheria toxin (DT) mutant (G52E) which has been used as carrier protein for many conjugate vaccines. Crm197 still possesses cytotoxicity toward mammalian cells while its triple mutant (Crm197EK with K51E/G52E/E) does not. Previous studies have demonstrated that the majority of CRM197 protein expressed in Escherichia coli was present as an insoluble inclusion body with only a small amount of soluble protein. A fusion CRM197 (fCRM197) containing all the tags conferred by the pET32a vector was produced as a soluble protein in E. coli co-expressing several chaperone proteins in conjunction with low temperature cultivation. Trigger factor (Tf) enhanced formation of soluble fCRM197 (150.69 ± 8.95 µg/mL) to a greater degree than other chaperones when fCRM197 expression was induced at 25 °C for 12 h. However, prolonged cultivation resulted in a progressive reduction of fCRM197 accumulation. In contrast, at 15 °C cells, with or without Tf, fCRM197 accumulated to the highest level at 48 h (153.70 ± 13.14 µg/mL and 150.07 ± 8.13 µg/mL, respectively). Transmission electron microscopy (TEM) demonstrated that the formation of inclusion protein as well as cell lysis was reduced in cultures grown at 15 °C. CRM197EKTrxHis was another fusion mutant co-expressed with different molecular chaperones using pET48b. The soluble CRM197EKTrxHis was produced at a high concentration (97.33 ± 17.47 µg/ml) under the optimal condition (induction with 0.1 mM IPTG at 20 °C for 24 h). Cells containing pG-Tf2, expressing trigger factor and GroELGroES, accumulated the highest amount of soluble CRM197EKTrxHis at 111.24 ± 10.40 µg/ml after induction for 24 h at 20 °C. Molecular modeling of diphtheria toxin, CRM197 and CRM197EK indicated that substitutions of two amino acids (K51E/E148K) may cause poor NAD binding, consistent with the lack of toxicity. Therefore, CRM197EK might be used as a new potential carrier protein. However, further in vivo study is required to confirm its roles as functional carrier protein in conjugate vaccines.

Keywords: Carrier protein; Conjugate vaccine molecular chaperones; Crm197; Crm197EK
BBE : Bioprocess and Bioseparation Engineering
IV-BBE-01

Application of SMB Chromatography Process in Biotechnology

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Abstract
Simulated Moving Bed (SMB), a continuous chromatography technology, has been widely used in petrochemicals, fine chemicals, and sugar industries due to its high productivity, high purity, and low solvent consumption compared with conventional batch chromatography. Typical SMB consists of four zones with two inlets and two outlets, pumps and valves. During the SMB separation, proper valve operation will switch inlet and outlet ports periodically in the same direction with the fluid flow in a counter-current manner. In this presentation, applications of traditional SMB for binary mixture separation/purification of several biological products such as L-lactic acid from paper sludge saccharification and fermentation, amino acids, sugar alcohols, and continuous protein refolding will be discussed. Some modified SMB systems and SMB operation strategies, such as 2-zone SMB/chromatography hybrid system for center-cut separation in the multicomponent system, one-column SMB analogue system will be introduced. In addition, the concept of simulated moving bed reactor (SMBR), a complex but effective process which integrates reaction and to the conventional SMB will also be presented.

Keywords: Continuous chromatograph; Simulated Moving Bed (SMB)
Bioprocessing of Agro-residues through the Application of Cross-linked Enzyme Aggregates of Cellulases

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Abstract
For the conversion of plant biomass into various bioproducts, a significant bottleneck is enzymatic hydrolysis of agro-residues to soluble sugars. These sugars are then metabolized through various natural or engineered microorganisms towards products of interest. The success of projected biorefinery processes depends to a large extent on the economics of cellulase enzyme production. While a lot of emphasis has been given to produce the enzymes with high titre and productivity, the properties of these enzymes, which affect their performance in hydrolysing lignocellulosic residues, have largely been ignored. The various properties that affect the hydrolysis are catalytic efficiency, thermal stability, adsorption, end product inhibition resistance and shear inactivation. Presently, mesophilic fungal strains like Trichoderma reesei and Aspergillus niger produce cellulases at industrial scale. Various recombinant cellulase components have been successfully expressed in industrial strains which aim to improve the economics due to their high specificities for targeted bioproducts; however, these studies have mostly not attempted to improve the properties of the cellulase components. The lecture will highlight the properties which need to be improved for better cellulose hydrolysis and the attempts to make the enzymes more stable through cross-linked aggregates of cellulolytic system. In the current work, cross-linked enzyme aggregates (CLEAs) of commercial cellulase mix have been prepared and their performance as potential industrial enzymes in terms of their stability and wheat straw hydrolysis have been evaluated.

Keywords: Cellulase; Cross-linked enzyme aggregates; Trichoderma reesei

Selected References:
IV-BBE-03

Optimization of Supercritical Fluid Extraction of Lipids from
*Gliricidia Sepium* Seed Kernel

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Abstract

*Gliricidia sepium* is a fast growing multipurpose legume tree cultivated in the Philippines. It is used as shade for cocoa and coffee plantations which made it more popularly known as “Madre cacao”. Recently, oil from *Gliricidia Sepium* was used in biodiesel production and properties of its methyl esters meets the specification of biodiesel standard. This study aimed to enhance the % oil recovery using Supercritical fluid CO\(_2\) and a small quantity of nhexane as cosolvent. In order to optimize the extract yield, face centered composited design and response surface methodology were used. The following independent variables were investigated: pressure (20-40) MPa, temperature (50-70) °C and CO\(_2\) flow rate (2-4) mL/min. Experimental results were also compared using conventional methods such as Soxhlet extraction and Ultrasound Assisted Chemical Solvent Extraction. The highest % oil yield of 15. 18% was achieved using supercritical CO\(_2\) with nhexane as cosolvent after 120 minutes of extraction, 2 ml/min, 40 MPa and 60 °C.

**Keywords:** *Gliricidia sepium*; Kakawate; Supercritical CO\(_2\)*
Aqueous Two-phase Extraction for Bacteriorhodopsin Purification from *Halobacterium salinarum*

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Abstract

Purple membrane (PM), presenting as specialized patches integrated within the cytoplasmic membrane in certain extreme halophilic microorganisms, can be driven by light to generate proton gradient for ATP synthesis. Bacteriorhodopsin (BR) naturally aggregates in a highly ordered two-dimensional hexagonal array of trimers in the PM as a light-driven protein. It is consisted of a membrane integral protein bacterioopsin (BO) and a chromophore retinal covalently linked to lysine 216 in bacterioopsin structure. As a light-driven protein, BR recently has been found to have various potential photoelectric applications. The purification of the BR containing PM from halophilic microorganisms in large-scale is not an easily task because the well-established PM purification procedure is mainly based on the tedious and lengthy sucrose density gradient ultracentrifugation (SGU). Aqueous two-phase system (ATPS) consisted of two immiscible aqueous phases, polymer/polymer or polymer/salt solution, is a non-toxic, environment friendly, and easily scalable extraction process. It has been employed for many biomolecules, cells, and membrane proteins purification. We found out that PM can be easily isolated by PEG/Phosphate ATPS from the cell lysate of extreme halophilic bacteria *Halobacterium salinarum*. The purity of the isolated PM can reach the same level as that obtained by traditional SGU method. In this presentation, how to culture *H. salinarum* cells with enriched PM content and a facile ATPS for the isolation of high purity PM will be demonstrated. In addition, an aqueous three-phase system that can isolate delipidated BR in one-step from cell lysate will also be discussed.

Keywords: Aqueous two-phase extraction; Bacteriorhodopsin; *Halobacterium salinarum*; Purple membrane
Membrane Enriching of Fermentation Broth and Sea Water Desalination by Forward Osmosis and of Δπ=0 Reverse Osmosis (VFAs, NaCl, Ethanol)

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Abstract

Fermentation broth, in general, consists of more than 90% of water. Efficient separation of water from the broth plays a key role in an economical processing of small molecules. Among these are volatile fatty acids (VFAs, 3.5~7.5%) derived anaerobically from waste biomass and fuel ethanol (5% ~10%) from corn. Market prices of biodiesel and Fuel ethanol are $3.09/gallon (0.816$/L) and $2.11/gallon (0.557$/L) as of June 1, 2017. Current methods of water removal are mostly based on thermal distillation that requires a lot of energy. For instance, theoretical energy requirements of removing 1m3 of water by thermal and membranes are 706.1kwh, 0.694 kwh respectively. Considering that the former is based on reversible thermodynamics and the latter based on irreversible thermodynamics, energy can be recycled many times from the former. The reasonable numbers may be 20 kwh and 4 kwh for the comparison. The barrier of membrane process for small molecules and seawater desalination is a very high osmotic pressure of concentrated solutions. For instance, those of saturated NaCl solutions (26.47%) and fuel ethanol (99.5%) are 343.3 bars and about 6000 bars, respectively. Because of this limitation water recoveries of desalination using RO from 3.4% seawater are limited to 50%, and 30% from 4.5% seawater. The barrier may be lifted with an introduction of “Δπ=0 Reverse Osmosis” proposed by Ho Nam Chang, of KAIST, Korea in 2013, who claimed that any aqueous solution of a high osmotic pressure can be separated into water and salt (sea water), and water and ethanol. This work is being investigated “Lab-to- Market with a funding from Korea Research Foundation. Together with VFAs enrichment using forward osmosis (experimental), the authors will show experimental results of seawater desalination and a theoretical work on ethanol purification (showing that membrane energy consumption is 1/20-th of the world best ethanol purification contest).

Keywords: Membrane process; Osmosis; Sea water desalination

Selected References:

IV-BBE-07

Utilization of Lemongrass Biomass for Biovanillin Production by *Phanerochaete chrysosporium*

Huszalina H¹, Ibrahim G², Sazyani S², Madihah Md. Salleh¹*, Chong Chun Siong¹, Adibah Y¹, Shaza EM¹, Suraini A³, Nor Nadiah Mohamad Yusof³, Muhammad Abu Naser² and Amir Feisal Merican Al-Junid⁵

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Abstract

Malaysia as one of the important agricultural countries in the world produced 70 million tonnes of lignocellulosic biomass. One of the abundant agricultural wastes which have high lignocellulose content is lemongrass leaves. More than 1,150 hectares of lemongrass farm in Malaysia produced 8000 tonnes of dry leaves yearly and burned to generate steam for stripping. The usage as ruminant feedstock is however not a favor due to animal rejection against the residual sweet aroma and flavor. Although the utilization of lemongrass leaves have been widely used in various fields, the biotechnology application of lemon grass leaves using microbial bioproccesing has not been study intensively. The main purpose of this research is to investigate the potential of a one-step biovanillin production from lemon grass leaves hydrolysate by *Phanerochaete chrysosporium.* Several chemical, physical, enzymatic and combination of physicochemical pretreatments were applied in this study for ferulic acid and reducing sugar production. The highest ferulic acid recovery were obtained at 1.188 g/L during 55 minutes of boiling pretreatment within 125-249 µm as a suitable range size of lemongrass leaves. Combination of boiling and enzymatic pretreatments produced higher reducing sugar recovery which acts as co-factor in producing higher biovanillin. Optimization of ferulic acid recovery based on boiling method using composite design (CCD) enhanced ferulic acid recovery 10.5 folds and yield 58.8%. The optimization of enzymatic pretreatment of cellulast and novozyme on solid lemongrass leaves waste using CCD produced 23.7 g/L of reducing sugar which is 8 folds compare to boiling method only. The optimization of biovanillin production using 2 level factorial designs (2LFD) and central composite design (CCD) improved biovanillin production in batch culture. The consortium of enzymes related to biovanillin production was identified as Enoyl-CoA hydratase or 4-Hydroxyccinnamoyl-CoA hydratase/lyase.

Keywords: Biovanillin; Ferulic acid; Lemongrass leaves and sugar recovery; Pretreatment
IV-BBE-08

How to Operate Freeze-drying Process for Assuring Product Quality of Biological Products

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Abstract

Freeze-drying (lyophilization) has long been known as the best drying method for preserving the original properties of the resultant dried product. It favorably maintains the biological activity of pharmaceuticals, flavors, and aromas of food, viability of cell biological products, etc. However, as a matter of fact, these excellent features cannot be achieved if operation is not properly carried out. Products are subjected to freezing, and ice crystals formed in the products are sublimated during freeze-drying process. Non water components are concentrated in the freeze-concentrated phase, and this phase results in the final dry products. Sublimation removes heat from the products, so this makes product temperature down to certain levels. If product temperature is too low, lowered sublimation rate end up with a long drying operation. But if too high, products will collapse because of the melt back of the freeze-concentrated phase. Collapse may significantly diminish product quality not only the appearance of dried products but also the retention of flavor components, activity of stabilized bio-actives, etc. This lecture will give a basic concept of freeze-drying how to operate process for assuring product quality of biological products. Lecture will refer to the design space approach where safe processing conditions can be visualized on a contour diagram.

Keywords: Biological activity; Freeze-drying; Lyophilization; Process optimization
Current Status of International Standardization on “Bioprocessing” in ISO/TC 276, a Technical Committee of International Organization for Standardization (ISO) for “Biotechnology”

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Abstract
As the bio-industry grows fast and its market expands vastly in various fields of biotechnology, the needs for standardization of biotechnology has raised continuously. In order to meet the needs for standardization on biotechnology, the ISO has recently launched a technical committee, ISO/TC 276, to fulfill the activities for international standardization on Biotechnology. In the ISO/TC 276, since its foundation in 2013, five (5) working groups (WGs) have been active to cover Terminology (WG1), Biobanks and Bioresources (WG2), Analytical Methods (WG3), Bioprocessing (WG4), and Data Processing and Integration (WG5) in “Biotechnology”. In particular, WG4 is mainly looking for ‘needs and gaps’ in international standards on “Bioprocessing” to support users and producers of biotechnology products. In this presentation, after brief introduction of ISO/TC 276, current issues and projects that are being discussed in WG4 for standardization of bioprocessing will be shared for further understanding and harmonization of global standards on biotechnology.

Keywords: Bioprocessing; Biotechnology; International standards; ISO/TC276; Standardization

Selected References:
1. ISO/AWI TS20399-1/2/3, Raw materials control for bioprocessing
2. ISO/PWI20398, Methods to control bioreactor process for cell culturing
3. ISO/PWI proposal (by Korea), Guidance on process parameters for separation and purification of therapeutic cells
IV-BBE-10

Investigating Effects of pH on Microbial Growth in Continuous Stirred Tank Bioreactors

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Abstract
This paper presents the importance of pH on microbial growth and its control strategies in Continuous Stirred Tank Bioreactors (CSTBR) The CSTBR involves growth of a pure culture Pediococcus acidilactici, a lactic acid bacterium and media containing glucose as carbon and energy source whereas, a continuous flow of a base stream is fed in order to control pH. The mathematical model is developed for analyzing the parametric sensitivity of operational variables. The normalized objective sensitivity of pH-minimum with respect to various input variables, such as feed stream concentration and its dilution rate, base stream concentration and its dilution rate for pH control was calculated. A new criterion for parametric sensitivity or occurrence pH-runaway is proposed. pH-runaway condition is referred to a condition at which microbial growth is ceased beyond a certain range of pH and bioreactor becomes susceptible to destabilized. This criterion determines particular critical points where normalized objective sensitivity is maximum with respect to input parameters. The criterion is applicable to a range of similar bioreactors. From this study, it is found that the region of sensitivity determined with respect to any system input parameters is also called generalized criterion of parametric sensitivity or pH-runaway.

Keywords: CSTBR; Normalized objective sensitivity; Pediococcus acidilactici; pH-runaway

Selected References:
EB : Environmental Biotechnology
IV-EB-01

Bacteria Feeding on Antibiotics – Are these of Environmental Relevance and Do They Contribute to the Pool of Antibiotic Resistant Bacteria?

Kolvenbach B.¹, Ricken B.¹, Rapp E.², Benndorf D.², Shahgaldian P.¹, Schäffer, A.³, Kohler H.-P.E., Majewsky M.⁵,⁶, Kroll K.¹, Timm A.⁵, Peschke R.⁵ and Corvini P. F.-X.¹,⁷*

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Abstract

It is established that the presence of antibiotics in the environment contributes to the formation and spread of resistance genes among bacterial strains. Wastewater treatment plants are hubs for the emergence of resistant bacterial strains and one of the major sources for the input of bactericidal micropollutants into the environment. Among these substances, sulfonamide antibiotics are the second most used antibiotics worldwide. Sulfamethoxazole (SMX) as one representative of this chemical group is often detected in significant concentrations reaching several μg/L. We report here on bacterial strains, which are not only resistant to the sulphonamide antibiotics, but also degrade and mineralize them. One of these isolates, namely Microbacterium sp. strain BR1 is able to feed on SMX as sole carbon and energy source. The catabolism of SMX and further sulfonamides proceeds via ipso-substitution. Genes and enzymes involved in this degradation process were identified. The heterologous expression of SadA in E. Coli is sufficient to confer a sulfonamide catabolic phenotype in this bacterium. The presence of this gene might represent an additional, yet unknown resistance mechanism for bacteria against sulfonamides. Even though the classic sul1 gene is present as well in M. sp. strain BR1, its additional capacity to feed on SMX represents a superior mechanism. Current research is dedicated to the assessment of the relevance of the concomitant presence of classic sulfonamide resistance genes and sulfonamide catabolic genes cluster in bacteria of activated sludge by monitoring their distribution in selected wastewater treatment plants.

Keywords: Antibiotics; Bacteria; Catabolism
IV-EB-02

Biomethanation of POME using Anaerobic Hybrid Reactor: Its Potential and Implementation

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Abstract
An anaerobic hybrid reactor (AHR) is applied to treat and produce methane from palm oil mill effluent (POME) which containing high concentrations of COD, O & G and SS. The AHR, combination of anaerobic sludge bed and fixed film reactor, shows its potential of methane production in various situations. Reactor temperature and concentrations of SS and O&G in POME are major concerned to study their effect on the AHR process performance, stability and flexibility. According to the results, it was found that the increasing of OLR and shorten of HRT were affected to the performance, stability and microbial activity, while the operating mesophilic and thermophilic temperatures did not significant affect. In addition, the reactor was operated with various SS and O&G concentrations from 5.2 to 10.2 and 0.9 to 1.9 g/L and overall process performance in terms of COD, SS, and O&G removals was 80, 70, and 60%, respectively. When the organic concentrations were increased, the resultant methane potentials were higher, and methane yield increased to 0.30 m³CH₄/kgCOD. Microbial community and quantity was monitored in both zones of reactor. This investigation demonstrated that the AHR was occupied by the sludge and packed zones acting as acidogenesis and methanogenesis zones, respectively. The AHR technology is currently transferred to the palm oil industry. The ECoWaste biogas technology has been proven for its high rate system with high performance, good stability and flexibility in agro-industrial wastewater treatment and biogas production.

Keywords: Methane; POME; Suspended solid; Temperature

Selected References:
Green Bio-based Products in the New Bioeconomy

Mohd Ali Hassan
BioTech UPM

Abstract
This paper reports on the use and value-addition of bioresources and biomass for the production of new bio-based products in the new bioeconomy. The focus is on environmental-friendly technologies with process integration, value-addition and pollution reduction towards a more sustainable future. New biotechnology and bio-based products are developed within an integrated biorefinery to support the new bioeconomy, which could generate additional jobs in the rural areas. Overall, this green initiative addressed all the three pillars of sustainability, i.e. profit, people and planet!

Keywords: Bioeconomy; Environmental-friendly technologies; Green bio-based product
NBB : Nanobiotechnology, Biosensors and Biochips
IV-NBB-01

Single-particle Tracking of Virus Entry and Uncoating in Live Cells

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Abstract

Single-virus tracking are effective tools for studying virus entry. Here, we constructed a quantum dot (QD)-encapsulated infectious HIV-1 particle to track viral entry at a single-particle level in live human primary macrophages. QDs were encapsulated in HIV-1 virions by incorporating viral accessory protein Vpr-conjugated QDs during virus assembly. With the HIV-1 particles encapsulating QDs, we monitored the early phase of viral infection in real time and observed that, during infection, HIV-1 was endocytosed in a clathrin-mediated manner; the particles were translocated into Rab5A-positive endosomes, and the core was released into the cytoplasm by viral envelope-mediated endosomal fusion. Drug inhibition assays verified that endosome fusion contributes to HIV-1 productive infection in primary macrophages. Additionally, we observed that a dynamic actin cytoskeleton is critical for HIV-1 entry and intracellular migration in primary macrophages. HIV-1 dynamics and infection could be blocked by multiple different actin inhibitors. Our study revealed a productive entry pathway in macrophages that requires both endosomal function and actin dynamics, which may assist in the development of inhibitors to block the HIV entry in macrophages. We also observed the HIV-1 uncoating process by single particle tracking. The viral genome RNA, capsid, and matrix protein of the HIV-1 virus were labeled with a Ru(II) complex ([Ru(phen)2(dppz)]2+), the TC-FlAsH/ReAsH system, and EGFP/ECFP, respectively. Using the multicolored virus and single-particle imaging, we were able to track the sequential disassembly process of single HIV-1 virus particles in live host cells. Approximately 0.1% of viral particles were observed to undergo a sequential disassembly process at 60–120 min post infection. The timing and efficiency of the disassembly were influenced by the cellular factor CypA and reverse transcription. The findings facilitate a better understanding of the processes governing the HIV-1 lifecycle. The multicolor labeling protocol developed in this study may find many applications involving virus–host-cell interactions.

Keywords: HIV-1; Single-virus tracking; Virus-host-cell interactions

Selected References:


Viral Nanoparticle of Simian Virus 40 as a Multifunctional Platform for Nanobiotechnology

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Abstract
Biomolecular nanostructures derived from living organisms such as protein cages, fibers and layers are drawing increasing interests as natural biomaterials. We have worked with the viral nanoparticle (VNP) of simian virus 40 (SV40) to develop a platform for nanobiotechnology. SV40 VNP, assembled from its major capsid protein, has an icosahedral cage-like structure of 24 nm in diameter. Self-assembly is the principal way to integrate VNP with chemically synthesized nanomaterials. We have found that a variety of nanoparticles (NPs) of different components, surface coatings, and sizes can be encapsulated into SV40 VNP through controllable assembly. NPs can also be loaded onto the outer surface by rationally tuning the interfacial interactions between SV40 VNP and NPs. Biomineralization is another route for combining VNP with inorganic NPs, by which noble metal plasmonic NPs with tunable sizes have been fabricated in SV40 VNP. By constructing these bio-nano hybrid structures, different functionalities have been integrated: i) in virtue of encapsulated quantum dots (QDs), we have established a high-fidelity method for fluorescent tracking of viruses and protein cages in living cells and animals; ii) discrete 3D nanoarchitectures with a QD at VNP center and a tunable number of gold NPs surrounding were assembled via simultaneously using the inner and outer space of SV40 VNP, which offered valuable models for nanophotonic studies such as surface plasmon resonance coupling and fluorescence resonance energy transfer. These works have laid a foundation for development of multifunctional bio-nano materials and devices based on the SV40 VNP platform.

Keywords: Fluorescent imaging; Nanobiotechnology; Nanoparticles; Self-assembly; Viral nanoparticles
Self-assembled Protein Nanostructure for Highly Sensitive Bio-sensing

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Abstract
Proteins are great prospective materials in nanotechnology as its physical size and diversity of highly specific interactions. Aiming to build a highly sensitive biosensing system, several multi-functional protein nanostructures were built by in vitro controllable self-assembly of engineered protein that genetically modified with functional ligands. By using the yeast protein Sup35 isolated from Saccharomyces cerevisiae can be self-assembled into fibre-like nanostructure, we established a seeding-induced self-assembly strategy to construct a series of functional protein nanowires for increasing the sensitivity of biosensing (Figure 1A). The Sup35 genetically fused with protein G and an enzyme can self-assembled into protein nanowire with a high ratio of enzyme molecules to protein G, allowing a dramatic increase of the enzymatic signal when protein G was bound to an antibody target, and 100-fold enhancement of the sensitivity was obtained when applied in the detection of the Yersinia pestis F1 antigen. By using this method, several kinds of protein nanowire were constructed. These protein nanowire were applied in various types of immunoassay, greatly enhanced the sensitivity from 100- to 4000-fold over the conventional method. However, the length of protein nanowire is not easy to control, because the process of self-assembly is usually a spontaneous process until all component are exhausted. To address this problem, we have developed a controllable self-assembly strategy through rational regulation of the number of self-assembling interaction sites on each ferritin nanoparticle (Figure 1 B). As proof-of-principle, a size-controlled enzyme nanocomposite was constructed by self-assembly of streptavidin-labeled horseradish peroxidase and auto-biotinylated ferritin nanoparticles, resulting in a 10,000-fold increase in sensitivity compared to traditional immunoassays for the detection of a cardiac troponin.

Keywords: Bio-sensing; Highly sensitive; Pathogen detection; Protein nanostructure; Self-assembly

Selected References:

Figure 1 (A) Seeding-induced self-assembly strategy for the construction of functional protein nanowires. (B) Size-controlled enzyme nanocomposite for highly sensitive cTnI immunoassay.
IV-NBB-04

Mycobacterium Tuberculosis Proteome Microarray for Global Studies of Protein Function and Immunogenicity


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Abstract
Poor understanding of the basic biology of Mycobacterium tuberculosis (MTB), the etiological agent of tuberculosis, hampers development of much-needed drugs, vaccines, and diagnostic tests. Better experimental tools are needed to expedite investigations of this pathogen at the systems level. Here, we present a functional MTB proteome microarray covering most of the proteome and an ORFome library. We demonstrate the broad applicability of the microarray by investigating global protein-protein interactions, small-molecule-protein binding, and serum biomarker discovery, identifying 59 PknG-interacting proteins, 30 bis-(3’-5’)-cyclic dimeric guanosine monophosphate (c-di-GMP) binding proteins, and 14 MTB proteins that together differentiate between tuberculosis (TB) patients with active disease and recovered individuals. Results suggest that the MTB rhamnose pathway is likely regulated by both the serine/threonine kinase PknG and c-di-GMP. This resource has the potential to generate a greater understanding of key biological processes in the pathogenesis of tuberculosis, possibly leading to more effective therapies for the treatment of this ancient disease.

Keywords: Mycobacterium tuberculosis; Protein function and immunogenicity; Proteome microarray
IV-NBB-05

Photonic Crystal Based Bioassays

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Abstract
In the implement of precision medicine, it is necessary to incorporate new technological advances, such nanotechnology and nanomaterials, to realize high throughput, high sensitive and rapid bioassays. Photonic crystal (PC) has ordered nanostructures and photonic bandgaps (PBG) that can control the propagation of light and show structure colors. These properties make PC both has high surface to volume ratio and light signal enhancement, which is favored by multiplex and high sensitive analysis. Herein, in this paper we proposed to prepare kinds of PC forms by self-assembly. Then, we would like to introduce multiplex, rapid and high sensitive biodetection methods developed with PC materials in combination with microfluidic chips or plasmonic materials.

Keywords: Multiplex bioassays; Nanotag; Photonic crystal; SERS
SSB : Systems and Synthetic Biotechnology
IV-SSB-01

Engineering of *Corynebacterium glutamicum* for the Enhanced Production of Biochemicals from Biomass using Synthetic Biology Tools

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Abstract

Synthetic biology approaches can make a significant contribution to the advance of metabolic engineering of recombinant organisms. Here, in order to broaden the spectrum of host organisms, we provide a synthetic biology platform for genetic engineering of *Corynebacterium glutamicum* which have been widely used for the fermentative production of amino acids as well as for the production of economically important compounds including metabolites and recombinant proteins. We developed various gene expression systems with new synthetic constitutive promoters which are useful for tunable gene expression system in *C. glutamicum*. Also, we identified major IS-elements which can disrupt the gene expression system in *C. glutamicum* and engineered cells by removing the lethal IS-elements. In addition, using FACS-based high throughput screening, we further engineered *C. glutamicum* towards enhanced production of target products by optimization of gene expression systems. Those molecular platforms may significantly contribute to establish *C. glutamicum* as a robust and versatile microbial factory.

Keywords: Biochemicals; Biomass; *Corynebacterium glutamicum*; *Klebsiella oxytoca*; Synthetic promoter

Selected References:
Genome Mining and High Efficiency Production of Terpenoids by a Robust Precursor Supply Platform

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Abstract
Terpenoids comprise the largest family of natural products with high levels of structural diversity and play an important role in our daily life. The information provided by the in vitro reconstitution assay of mevalonate (MVA) pathway provides an ideal biochemical background for the engineering of terpenoids, which guided us to establish an efficient precursor providing platform for terpenoids. Based on this well optimized platform, a high titer of pharmaceutical precursor taxadiene was produced in E. coli and filamentous fungi quickly and efficiently. To characterize terpene cyclase and mining new terpenoids efficiently, we integrated the engineered MVA pathway and combinatorial biosynthesis to depict the comprehensive product profiles. As a result, terpenoids production ability was fully exhibited by the efficient combinatorial biosynthesis platform. 52 terpenoids were generated and 12 of them were structurally characterized with seven new terpenoids (including three new skeletons). And the rational site-directed mutagenesis was introduced to further extend the product synthesis capacity and accelerate new terpenoids discovery process. The rational combinatorial biosynthesis and protein engineering strategy enable us to fully exploit the biosynthetic potential of terpene cyclase, and accelerate the process of new terpenoids mining.

Keywords: Combinatorial biosynthesis; Genome mining; Metabolic engineering; New skeleton; Terpenoids

Selected References:
TEB : Tissue Engineering and Biomaterials
IV-TEB-01

RNA Therapeutics and Anabolic Gene Delivery for Tissue Engineering and Regenerative Medicine

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Abstract
Delayed union/non-union resulting from bone fractures or serious trauma remains a challenging problem for orthopaedic surgeons. These problems have inspired the development of tissue engineering, which combines cells, biomaterials and biological signals, to stimulate tissue regeneration. Over the past decade, gene therapy has converged with bone engineering, by which an increasing number of therapeutic genes are explored to stimulate bone repair. These genes can be administered to cells via in vivo or ex vivo approaches using either viral or nonviral vectors. This presentation will focus on the use of viral vectors for genetic engineering of mesenchymal stem cells for bone regeneration. In particular, emphasis is placed on the applications of baculovirus, an emerging nonpathogenic gene delivery vector, for the delivery of various anabolic genes and miRNA mimics/sponges to repair bone.

Keywords: Anabolic gene delivery; Bone engineering; Regenerative medicine; RNA therapeutics; Tissue engineering
Endothelial Progenitor Cells, Small Molecules, Extracellular Matrix and Polyhydroxyalkanoate Scaffold for Blood Vessel Tissue Engineering

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Abstract
Repair and regeneration of vascular tissue is the important research topic of current biomedical engineering and regenerative Medicine. Many studies indicated that cells need to grow on the suitable extracellular matrix to show the particular functionality. In this study, we tested various surface modification methods to fix fibronectin or collagen on the biodegradable polymer surface (poly(3-hydroxybutyrate, PHB and poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV). Then, the cell lines (3T3 and L929) and primary cells (mesenchymal stem cells, MSCs; endothelial progenitor cells, EPCs and human umbilical vein endothelial cells, HUVECs) were cultured on the modified surface to explore the application potential of vascular tissue engineering. In addition, Sphingosine-1-phosphate (S1P), a low molecular-weight phospholipid mediator was the first time to use it as an additive during the process of a blood vessel construction. Our data showed that the surface of alignment PHB and PHBV films can be modified successfully by chemical methods based on Ninhydrin assay and contact angle assay. XPS assay also confirmed ECM has immobilized on the film. In addition, the WST1 assay, immunocytochemistry assay and SEM showed that the surface modified films performed excellent cell compatibility. The cells cultured on the surface modified films, the cell viable assay showed that the films had good biocompatibility. We further explored the function and mechanism of S1P in promoting revascularization and protection against thrombosis in this tissue engineered vascular grafts. Taken together, our results demonstrated that PHB and PHBV films that were modified by the above chemical method and were fixed with suitable ECM can provide a potential artificial vessel for application of vascular tissue engineering.

Keywords: Blood vessel; Endothelial progenitor cells; Extracellular matrix; Polyhydroxyalkanoate; S1P
Incorporation of Surface-modified Hydroxyapatite into Poly(methylmethacrylate) Bone Cement for Better Functionality

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Abstract
Poly(methylmethacrylate) (PMMA) is the most frequently used bone void filler for vertebral augmentation in osteoporotic fracture, with strong mechanical properties needed for bone repair applications and sufficient plasticity to fit different shapes of bone defects. However, the adhesion between the PMMA-based cement and adjacent bone tissues is usually weak as PMMA bone cement is inherently bioinert. The incorporation of bioceramics to PMMA can increase cell attachment and improve bioactivity, thus can improve functionality of this material. The nano-sized hydroxyapatite (nHAP) is such a bioceramic which not only is present in natural bones but also offers a favorable environment for osteoconduction, protein adhesion, and osteoblast proliferation. However, when nHAP is blended into PMMA, the lack of affinity between nHAP and PMMA produces agglomeration of nHAP and results in heterogeneous distribution of nHAP in the polymer matrix. Thus, defects and cracks may form at the polymer/ceramics interfaces, resulting in heterogeneous distribution of stress and subsequent inferior mechanical strengths. Furthermore, the interactions between the material and bone tissue may also suffer from the uneven distribution of nHAP. In this study, we improve the affinity between polymer and ceramic interphases by grafting nHAP (gHAP) with poly(ɛ-caprolactone) (PCL) via ring opening reaction¹. The PMMA with 20 wt% gHAP showed the best osteogenic bioactivity among the composites tested without compromised mechanical properties. These results showed that the procedure making gHAP and its use in polymer/bioceramic composite has great potential to improve the functionality of PMMA cement.

Keywords: Bone cement; Hydroxyapatite; Poly(ɛ-caprolactone); Surface modification

Selected References:
YS: Young Scientists
Mitigation of Carbon Dioxide by Oleaginous Microalgae for Lipids and Pigments Production

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Abstract
Microalgae have been identified as fast-growing species whose carbon fixing rates are much higher than those of terrestrial plants. Oleaginous microalgae are the specific microalgal species that can accumulate lipids >20% of their biomass. They are considered as renewable oil sources for the 3rd generation biodiesel due to their ability to capture energy from sunlight and convert CO₂ into lipids. In this study, several oleaginous microalgae were screened for their ability to grow and produce high lipids and pigments under high CO₂ feeding strategies. The synergistic effects of light intensity and photoperiod were investigated in order to provide the adequate light energy for photosynthesis by microalgae, and the CO₂ feeding profile was optimized to enhance the CO₂ mitigation ability of the selected strain. Afterward, the selected strain was immobilized in alginate gel beads which could be easily harvested by sieving method. Moreover, the cultivation of immobilized microalgae using industrial effluent was attempted to reduce the production cost of microalgal biomass. The suitable cultivation mode for the immobilized cells was selected among three operating modes including batch, semi-continuous and repeated batch modes. The extracted microalgal lipids having similar fatty acid composition to that of plant oil suggested their high potential use as biodiesel feedstocks. This study has shown that the use of oleaginous microalgae and immobilization technique are effective not only in CO₂ mitigation and removal of pollutants but also in low-cost production of renewable energy.

Keywords: Carbon dioxide; Light illumination; Lipids; Oleaginous microalgae; Pigments

Selected References:
IV-YS-02

Dynamic Regulation, Synthetic Biology Devices and Product Biosynthesis

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Abstract
In order to improve the titer and yield of the target product, the deletion of genes responsible for by-product synthesis are often required. However, some of these genes are responsible for bacterial growth and/or cell maintenance. Therefore, these genes are rarely knocked out in metabolic engineering. To overcome this problem, our lab developed two synthetic biological devices: 1. An endogenous Type I CRISPR-Cas system for regulating metabolic flux; 2. An Auto-induced AND-gate for controlling metabolic pathway dynamically. As proof of concept, the two synthetic biological devices were applied for eco-friendly bioplastic poly-β-hydroxybutyrate (PHB) production, revealing a 2-3-fold increased production in E. coli. The synthetic devices provide tools for developing general dynamic regulation system in metabolic engineering.

Keywords: AND-gate; Poly-β-hydroxybutyrate; Synthetic biology; Type I CRISPR-Cas system

Selected References:
IV-YS-03

Biocatalyst Engineering toward Biomedical Applications

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Abstract
Designing new bioconjugates is of great interest in biological research and related applications. The combination of unique functionalities of major biological components, such as nucleic acids and proteins, has been demonstrated to be an effective approach to create novel bioconjugates. Moreover, introducing non-natural components, such as synthetic small molecules and polymers, can expand the utility of naturally occurring biomolecules. To maximize the potential of biofunctions associated with the tertiary structure of biomolecules, enzymatic conjugation has shown great interest because it offers the site-specific modification of biomolecules under physiological conditions. For the design and generation of novel bioconjugates and biomaterials, our group has focused on making proteins, nucleic acids, and synthetic polymers to be macromolecular substrates of enzymes to foster the enzymatic conjugation. For example, using microbial transglutaminase (MTG) we have succeeded in the development of mRNA detection system on tissue sections, by combining a genetically modified enzyme and a chemically modified nucleotide. A protein-grafted synthetic polymer can also be designed through a site-specific conjugation by MTG for an immunosorbent assay. Finally, we happened to find a new horseradish peroxidase (HRP)-catalyzed gelation system of thiolated polymers without exogenous H₂O₂, leading to a cell culture platform with redox-responsive hydrogels. Taken together, enzymatic manipulation holds a great promise to generate novel bioconjugates and biomaterials with designer properties.

Keywords: Bioconjugate; Biomaterial; Cell culture; Hydrogel; Immunoassay

Selected References:
ASIAN FEDERATION OF BIOTECHNOLOGY (AFOB) - EUROPEAN FEDERATION OF BIOTECHNOLOGY (EFB) JOINT SESSION
AFOB-EFB Joint Session I on “Enzyme/Catalysis”
KEYNOTE SPEAKER ABSTRACTS
KN-Joint I-01

Nanobiocatalysis for Microbial Decontamination and CO₂ Conversion

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Abstract

Enzymes are bio-catalysts, which can be employed for solving various environmental issues such as microbial contamination and atmospheric CO₂ increase. Although the high specificity, rapid reaction rate and environmentally-friendly nature of enzymes can provide a great potential in their environmental applications, their practical uses are being hampered due to their poor stability. Nanobiocatalysis, using nanostructured materials for the stabilization as well as immobilization of enzymes, has gathered a growing attention due to its unprecedented successes in stabilizing the enzyme activity. This presentation will introduce recent developments of nanobiocatalysis using acylase and carbonic anhydrase, which demonstrated the feasibility of success in microbial decontamination and CO₂ reduction, respectively. Acylase and carbonic anhydrase were immobilized on the carboxylated polyaniline nanofibers via “magnetically-separable enzyme precipitate coatings (Mag-EPC)”. Enzyme stabilization in the form of Mag-EPC and their used in microbial decontamination and CO₂ reduction will be presented in detail. It is anticipated that nanobiocatalytic materials with stabilized enzymes will be used in a variety of practical applications including microbial decontamination and CO₂ reduction.

Keywords: CO₂ reduction; Microbial decontamination; Nanobiocatalysis
**KN-Joint I-02**

**Tuning Enzyme Promiscuity for New Pathways and Products**

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**Abstract**

Protein engineering and computational design are extremely useful technologies to conceive new enzymes for enzyme-based processes, chemo-enzymatic cascades or novel metabolic pathways. An overview of our recent achievements in this field will be proposed with a first focus on computational-aided engineering of \(\alpha\)-retaining transglucosylases from glycoside-hydrolase family 13 and 70. These enzymes are sucrose-active enzymes. They naturally show a broad acceptor substrate promiscuity and transfer the glucosyl unit of sucrose onto various types of hydroxylated acceptors to yield polysaccharides, glucooligosaccharides or glucoconjugates varying in size, structure and, by consequence, physicochemical properties. To further extend their applications, we applied engineering strategies to generate novel transglucosylases working on unnatural oligosaccharide acceptors, which were chemically protected to integrate programmed chemo-enzymatic cascades. In this way, new routes for the development of various patterns of antigenic oligosaccharides could be proposed. Similar approaches were also recently applied to set up a new and artificial metabolic pathway dedicated to di-hydroxybutyrate production, a precursor of a hydroxyl-analog of methionine. The conception of this new synthetic pathway was inspired by the natural *E. coli* pathway starting from aspartate and leading to homoserine and required computer-aided engineering of three template enzymes showing no or little activity on the targetted substrates. The pathway was successfully expressed in *E. coli* to yield DHB. The strategies and approaches developed within the frame of these various engineering programmes will be described and discussed with regards to the constraints imposed by integration in either chemo-enzymatic pathways or living organisms.

**Keywords:** Metabolic pathway; Protein engineering; Sucrose-active enzymes; Tuning enzyme promiscuity

**Selected References:**


INVITED SPEAKER ABSTRACTS
IV-Joint I-01

Novel Bio-based Oligoesters by Immobilized Lipases

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Abstract
Polymer synthesis using enzymes as catalyst was focused in the recent years on the preparation of highly hydroxyl-, thiol-, or carboxyl-functionalized polyesters. The presence of functional pendant groups is of special interest, particularly in drug formulations, since they facilitate covalent anchorage of prodrugs. Such functional groups could be provided by bio-based derivatives. The developments of the last years placed the bio-based polyesters competitive for fossil based polymers. We investigated different bio-based compounds, including hydroxy-fatty acids, furoic acid, gluconic acid derivatives, as co-monomers of four- and seven-membered ring lactones for the lipase-catalyzed synthesis of novel oligoesters. The biocatalytical properties and substrate selectivity were evaluated for immobilized lipases from different microbial sources, Candida antarctica B, Pseudomonas fluorescens, Pseudomonas stutzeri, Thermomices lanuginosus, obtained in our laboratory or commercially available in immobilized form. The reactions were carried out in organic or solvent-less systems, at temperatures up to 80ºC in batch operation conditions. The formation of the reaction products, cyclic and linear oligoesters, was demonstrated by FT-IR, MALDI-TOF and 2D NMR analysis. The thermal properties of the synthesized products were evaluated by TG and DSC analysis, compared to the equivalent homopolymers. The operational stability of the most efficient biocatalyst, selected for each reaction system, was investigated during multiple reuses. This work was supported by a grant of the Romanian Authority for Scientific Research and Innovation, CNCS/CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2016-0168, within PNCDI III.

Keywords: Bio-based; Biocatalysis; Lipase; Polyesterification

Selected References:
Enzyme Catalysis and Engineering for Sustainable Technology

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Abstract
Enzymes are catalysts that are useful in industries because they can increase rates of reactions and allow processes to be carried out in green and sustainable manner. In addition, specificity and rate increment of enzymatic reactions are very useful in bioreporter applications as they can convert specific substrates into specific spectroscopic or electricity signals that are convenient for detection. We have investigated reaction mechanisms of several two-component flavin-dependent monooxygenases which use C4a-hydroperoxyflavin as a reactive intermediate for catalyzing oxygenation. These enzymes consist of a reductase component which generates reduced flavin as a product and an oxygenase component which utilizes the reduced flavin as a substrate. Many flavin-dependent hydroxylases can also catalyze additional reactions beyond the hydroxylation alone. We will discuss three examples of flavin-dependent monooxygenases. The first system is HadA which is a dechlorinase that can catalyze hydroxylation in conjunction with dechlorination. HadA can convert various chlorinated phenols, commonly used agrochemicals that cause environmental problems, into less toxic materials that can be assimilated by microbes. The enzyme is thus useful for future development in detoxification and biorefinery applications. The second system is p-hydroxyphenylacetate (HPA) 3-hydroxylase (HPAH), an enzyme that can incorporate a hydroxyl group regio-specifically into phenolic compounds. We found that HPAH can convert p-coumaric acid (lignin-derived compound) into 3,4,5-trihydroxycinnamic acid (3,4,5-THCA), a strong antioxidant that is potentially useful as medicinal agents. We also engineered HPAH and obtained enzyme variants that can be used for synthesizing dopamine from tyramine and hydroxylation of aniline derivatives. The third system is bacterial luciferase that can catalyze light emitting reaction. This enzyme can be applied in bioreporter applications.

Keywords: Dechlorinase; Flavin; Hydroxylase;luciferase; Oxygenase
IV-Joint I-03

Design of Robust Nanobiocatalysts through Protein Supramolecular Engineering

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Abstract
In nature, various organisms are endowed with the ability of producing intricately patterned and hierarchically structured inorganic matter with a meticulous proficiency. As those materials often exceed the performances of their artificial counterparts, mimicking their biosynthesis offers new opportunities to develop a wealth of novel engineered (nano) materials. Enzyme Supramolecular Engineering, in contrast to enzyme engineering, refers to a novel concept of enzyme supramolecular modification without manipulating the protein sequence by genetic engineering or covalent modification of the biomolecule. We have applied this concept to develop a novel synthetic strategy to produce nanobiocatalysts. This strategy relies on the controlled growth, at the surface of a material where an enzyme is covalently immobilized, of a soft protective layer of controlled thickness. The so-shielded enzyme displays outstanding stability vs. physico-chemical and chaotrophic stresses. In this talk, after a short introduction describing the synthetic strategy, different examples of nano-protected enzymes for different applications will be discussed.

Keywords: Enzyme; Nanoparticle; Silica; Supramolecular

Selected References:
IV-Joint I-04

Turning Sugars into Electricity: Engineering of Pyranose Oxidase for Biofuel Cells

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Abstract
Enzymatic biofuel cells (EFC) are a special type of a fuel cell, in which biocatalysts are used to oxidize substrates in order to generate an electric current. Routinely, glucose oxidase (GOx) is used in these applications to oxidize glucose, while electrons are transferred to an electrode. The flavin-dependent enzyme pyranose oxidase (POx) is a member of the glucose-methanol-choline (GMC) family of oxidoreductases. The enzyme catalyzes the specific C-2 oxidation of several pyranose sugars while transferring electrons to molecular oxygen. In addition, several electron acceptors (complexed metal ions, quinones, etc.) are used by POx as alternative substrates. POx shows several properties (lower $K_m$ value for glucose, no anomeric preference, covalently attached prosthetic group) that make it more favorable than GOx in EFC applications. Reactivity with oxygen, however, might interfere with the EBC application as it may reduce the current output and affect stability. A semi-rational approach of enzyme engineering, targeting first-shell amino acids around the active site, resulted the identification of several variants that show reduced activity with oxygen while activity with the alternative electron acceptors is maintained. Some of these variants even showed improved kinetic properties (increased $k_{cat}$ or $k_{cat}/K_m$), which was proven in bioelectrochemical experiments using a screen-printed electrode (SPE) based on carbon ink and grafted with gold-nanoparticles.

Keywords: Enzyme engineering; GMC oxidoreductases; Pyranose oxidase

Selected References:
AFOB-EFB Joint Session II on “Plant Biotechnology”
INVITED SPEAKER ABSTRACTS
New Cytokinin Derivatives - A Tool to Understand and Improve Establishment of Micropropagated Plantlets

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Abstract

Plant hormones cytokinins are naturally occurring, organic substances which influence major physiological processes in plants at extremely low concentrations. As an important and affordable cytokinin, 6-benzylaminopurine (BAP) is routinely utilized in micropropagation for its effective stimulatory properties. On the other hand, BAP and/or its endogenous metabolites may negatively influence the shoot proliferation, rooting and acclimatization processes in some plant species. Therefore, the development of other new CK derivatives exhibiting high morphogenetic activity might consequently be of a great practical importance in plant biotechnology. Based on our recent search for naturally occurring aromatic cytokinins in plants, we recently synthesized several new groups of their analogues having high activity in different CK bioassays as well as the ability to activate cytokinin receptors and/or to inhibit CKX. The best compounds have been tested as a new tool for retardation of senescence during the multiplication stage of micropropagation of selected plant species as well as to support rooting and acclimatization competence. Subsequently, wide range of endogenous plant hormones were quantified during these experiments and compared in relation to cytokinin exogenously used as well as micropropagation efficiency. Results of this quantification study have been used to design second generation of 9-substituted CK derivatives with improved metabolic properties. Moreover, these compounds also have a potential for use as antisenescent and UV photoprotective ingredients of cosmetic compositions. Research was supported by the Ministry of Education Youth and Sports, Czech Republic (grant LO1204 from the National Program of Sustainability and Agricultural Research).

Keywords: Acclimatization; Cytokinin; Micropropagation; Quantification; Senescence

Selected References:
Screening and using DNA Barcodes for Identification of *Dendrobium* Species

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**Abstract**

Vietnam belongs to tropical region, with many endemic wild orchids, especially diverse and popular in *Dendrobium* species. We have collected and conserved 137 native orchids, more than 70 of them belong to *Dendrobium* genus. However, it is difficult to discriminate at species level among closed *Dendrobium* species due to the similarity in morphology and lack of flowers of during the activities of evaluation, collection and conservation of native *Dendrobium* plants. With the purpose of effective conservation and sustainable use of the Vietnam wild orchids, in general, and *Dendrobium* orchids in particular, an efficient method should be established to identify rapid and accurate the *Dendrobium* accessions. DNA barcoding is a useful tool and method for rapid, accurate and species identification, which have been successfully in orchid. In this study, we tested 10 DNA barcodes *rbcL*, *matK*, *ITS*, *atpF-atpH*, *psbK-psbI*, *trnH-psbA*, *rpoB*, *rpoC*, *ndh*, *ycf* of 35 *Dendrobium* accessions with published primers. The results showed that eight of them were successfully amplified by 13 specific primers, only the *ycf* and *ndh* showed no result. Five of them showed high amplification as *rbcL* 100%, *matK* 95.12%, *atpF-atpH* 97.56%, *psbK-psbI* 97.56%, *ITS* 85.37%. At the beginning of the evaluation and analysis, the PCR products of *rbcL*, *matK*, and *ITS* of 35 *Dendrobium* accessions were sequenced and blasted with sequences from Genebank. Based on the blasted results, each accessions were species indentifed. In addition, genetic distance between the *Dendrobium* accessions were measured and phylogenetic relationships of them were established by using ClustalW. The results indicated that beside morphology indicator, DNA barcoding can be used as an useful and accurate marker to identify *Dendrobium* species for conservation and further purposes.

**Keywords:** *Dendrobium*; DNA barcode; Orchids; Primer
IV-Joint II-03

Phytohormone Metabolite Profiling in Plant Tissues

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Abstract
The identification and quantification of plant hormones in plant tissues are necessary for physiological studies of their metabolism and mode of action. The major problem associated with plant hormone analysis is that the amount of phytohormones present endogenously in plant tissues is very low, usually in the range of fmol to pmol/g fresh weight. We found that a combination of different sorbents, reverse phases and ion-exchange phases, was the best tool in the sample purification, giving a total extraction recovery ranging between 50-80% for all studied biologically active compounds. A fast chromatography technique, the ultra-high performance liquid chromatography (UHPLC) was coupled to triple quadrupole mass spectrometer equipped with an electrospray interface (ESI-MS/MS). In selective MRM mode, the detection limit for most of phytohormones (cytokinins, auxins, abscisic acid, jasmonates, gibberellins, brassinosteroids) was close to 1 fmol and achieved linear range was at least five orders of magnitude. Use of our procedures can allow the quantification of plant hormones and their derivatives (in total more than 100 compounds) in very limited amounts of plant material. The methods provide substantial improvements in terms of robustness, sensitivity, selectivity, convenience, through-put and cost-effectiveness over previous methods published. The new and modern analytical approaches make possible a new direction in plant hormone metabolite profiling. We believe that UHPLC-ESI-MS/MS technology can be used for fast and sensitive quantitative analysis showing reproducibility in the plant hormone profiling in different plant tissue and cell extracts.

Keywords: Liquid chromatography; Mass spectrometry; Phytohormone profiling; Targeted metabolomics
IV-Joint II-04

Rice Breeding for Salt Tolerance in Mekong Delta via Marker-assisted Selection

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Abstract
Two hundred fifty three BC₂F₂ rice lines of the OM7347/OM5629 were evaluated at seedling stage in the green house of CLRRI. Molecular markers associated with salt tolerance QTLs were identified by using 416 polymorphic SSR markers. QTLs, associated with stress tolerance at EC = 8 dS/m at seedling stage, detected from the BC₂F₂ population were located on chromosomes 1 and 3. Three QTLs were identified at the intervals of RM3252-S1-1 - RM10694, RM3740-RM5336 and RM11125-RM9 with genetic distance of 4.4, 4.5 and 18 cM on chromosome 1, respectively. Two QTLs at the intervals of RM3867-RM6959 and RM6876-RM4425 with genetic distance of 4.5 and 18.0 cM on chromosome 3, respectively. Three QTLs at the regions of RM1324-RM2412, RM1185-RM24, and RM1282-RM2560 on chromosome 1, and one QTL of RM453-RM511 on chromosome 12, were related to salt tolerance under EC = 4 dS/m at reproductive stage. Responses to salinity stress at reproductive stage such as sodium and potassium concentrations were measured. The candidate genes on chromosomes 1, 6, 10 and 12 were co-localized with the QTLs for salinity tolerance through GWAS based on the 44,100 SNP chip to identify significant SNPs. In addition, three advanced backcross populations were developed as BC₂F₂ of OM6162 / Pokkali (100 lines), BC₃F₂ of OMCS2000 / Pokkali (50 lines), BC₃F₂ population of OM1490 / Pokkali (53 lines). Their phenotypes were evaluated at seedling and reproductive stages. Marker-assisted selection was applied to identify promising lines among them through RM RM3252-S1-1 and RM223. Eleven promising progenies were selected.

Keywords: QTL; Reproductive stage; Salinity; SalTol; Seedling stage; SSR
IV-Joint II-05

**Adaptation of Rice Cultivation in the Coastal Areas of Bangladesh under Changing Climate Conditions by Application of Salt-tolerant Biofertilizer**

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**Abstract**

Salinity intrusion as a result of sea level rise in the coastal zone is one of the many effects of climate change inflicting Bangladesh, leading to reduction of normal crop production including rice. To promote the adaptation strategy for coastal agriculture under salinity stress, we focused on isolating and identifying salt-tolerant, plant-growth-promoting-rhizobacteria (PGPR) that would support plant growth by counteracting osmotic stress. Two salt-tolerant isolates were short-listed from PGPR, isolated from the rice fields of coastal and non-saline areas of Bangladesh, and were identified as *Bacillus aryabhattai* and *Ochrobactrum intermedium* that exhibited highest PGP activities under salt stress (200 mM NaCl) in vitro, viz. fixation of atmospheric nitrogen (11.1 & 6.8%), production of Indole-3- acetic acid (15 to 51 µg/ml), and solubilization of phosphorus (3,000 & 2,800 ppm/ml) respectively. A culture of 10⁹ cells combined with autoclaved charcoal powder, calcium carbonate and gum acacia was prepared to form biofertilizer for pot experiments. A 85-day long pot experiment cultivating a salt-sensitive rice variety, *Oryza sativa* BR-29 produced 85% growth in biofertilizer-added pots compared to controls (72%) under normal conditions. When challenged with 200 mM NaCl at day 65, the survival advantage of biofertilized plants was recorded greater (40%) in contrast to control pots (*E. coli*-added and without inoculants) that merely survived. Interestingly, the isolates’ resistance to salts was correlated to their resistance to drugs and heavy metals. The presence of thick exopolysaccharide as revealed by scanning electron microscopy could advocate for the resistance. Overall, their ability to promote plant growth under salt stress makes them useful as biofertilizer, hence could be taken as a preparedness program to ensure food security for vulnerable coastal agriculture under changing climate conditions.

**Keywords:** Bio-inoculant; Climate change; PGPR; Salinity
ORAL PRESENTATION ABSTRACTS
AFB : Agricultural and Food Biotechnology
Actinomycetes for Biocontrol of Crop Pathogens – Sharing Our Findings

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Abstract
Actinomycetes derived from Greek word “atkis” which means ray and “mykes” which means fungus that corresponds to their ability to produce aerial mycelium or have a fungus like-appearance are Gram-positive bacteria based on 16S ribosomal data. Among the actinomycetes, Streptomyces spp. are dominant in soil, decaying vegetation and plant rhizosphere soils. Further, Streptomyces spp. are responsible for more than 60% of biologically active compounds such as antifungal and antibacterial compounds or plant growth-promoting substances that were developed for agricultural purposes. Further, actinomycetes that have potent plant protection / biocontrol activities against pathogens have been shown to colonise root surfaces and also internal tissues of roots. In this presentation, we share our research findings - the antifungal activities of Streptomyces spp. against Fusarium oxysporum f.sp. cubense; the rhizospheric Streptomyces spp. isolated from oil palm for activities against Ganoderma boninense; the rhizospheric Streptomyces spp. for activities against Colletotrichum boninense; the rhizospheric Streptomyces spp. isolated from Hylocereus polyrhizus, for activities against Fusarium oxysporum, Fusarium decemcellulare and Fusarium semitactum.

Keywords: Biocontrol agents; Endophytic actinomycetes; Plant pathogenic fungi
Selection of Furfural Tolerant Lactic Acid Bacteria for Bioconversion of Lignocelluloses to Lactic Acid

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Abstract
Lactic acid is one of the organic acids that play an important role both in food and non-food industries. Regarding food security reason, the use of starch as substrate is not appropriate, lignocellulosic substrate has become the recent target. However, lignocellulose feed stocks require harsh chemical pre-treatment that may produce toxic compounds such as furfural and hydroxymethylfurfural (HMF) that inhibit microbial growth. The aim of this research was to select lactic acid bacteria capable of using lignocellulose substrate and have furfural tolerance capacity. Among the 407 isolated bacteria from the preliminary experiment, 25 isolates could be grown in both MRS glucose and MRS xylose as carbon sources. Identification results of these isolates indicated that 20 isolates were Lactobacillus pentosus, 3 isolates were Lactobacillus fermentum, and the remaining two were Pediococcus pentosaceus and Lactobacillus lactis. However, only 17 isolates produced high level of total acids (10–20 g/L) in MRS 20 g/L that contained glucose and xylose as carbon sources. Moreover, only 2 isolates, Y23 (L. pentosus), and CMY17 (P. pentosaceus) showed the conversion of mixed sugar higher than 70%. Additionally, both strains could grow in MRS broth containing glucose and xylose supplemented with 6 g/L and 5 g/L of furfural, respectively. Five lignocellulosic substrates including corn cob, corn stover, rice straw, rice husk and cassava pulp, were hydrolyzed by acid hydrolysis and the obtained hydrolysates were used as substrate for lactic acid production. The highest lactic acid (12.6 g/L) was produced by L. pentosus Y23 using corn stover after cultivation at 37°C for 48h, while 11.8 g/L of lactic acid was obtained from P. pentosaceus CMY17.

Keywords: Furfural tolerant; Lactic acid bacteria; Lignocellulose
Protease Producing Lactic Acid Bacteria Isolated from Dry-Fermented Catfish for Antioxidant Peptides Preparation

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Abstract
Dry-fermented catfish (Pladukra) is a Thai fermented product produced locally and consumed widely in Southern Thailand. Fermentation process occurs slowly by microorganisms in raw materials, such as lactic acid bacteria (LAB). Proteases derived from these microorganisms cause physical change of fish proteins, resulting in a semi-soft texture of this product. Therefore, dry-fermented fish may potentially be a source of LAB capable of producing protease and used for bioactive peptide preparation. In this study, we screened 258 isolates of LAB from dry-fermented catfish samples. These isolates were determined for protease activity on two substrates: casein and catfish protein. It was found that 15.43% of LAB isolates produced extracellular protease and were capable of degrading casein. Of these protease producing bacteria, 27.27% were able to utilize catfish proteins. Subsequently, identification of selected isolates was performed using 16S rDNA analysis. It was shown that the selected isolates were assigned as Enterococcus faecalis and Lactobacillus pentosus strains. E. faecalis was selected and further used to produce protease using fish broth (FB) medium. Protease was then partially purified using ammonium sulfate fractionation. These partially purified enzymes function in the range of 30-60°C, while the optimum temperature was 50 °C and pH range from 7-9. The digested catfish proteins using this enzyme demonstrated peptide with antioxidant activity. Thus, dry-fermented catfish is considered to potentially be a source of protease producer. Correspondingly, the protease from E. faecalis can be used for antioxidant peptide preparation.

Keywords: Bioactive peptide; Dry-fermented catfish; Lactic acid bacteria (LAB); Protease

Selected References:
O-AFB-04

Probiotic Fortified Seaweed Silage as Improved Supplement in Marine Fish Hatchery

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Abstract
Marine capture fisheries constitute close to 87% of Malaysia fish landing and coastal fisheries holds a major share. Effort to enhance marine aquaculture hatcheries is being intensified however, low hatching rates and high mortality is still an impending issue that needs to be solved. In an effort to solve this issue, production and utilization of probiotic fortified seaweed silage was produce using *Eucheuma denticulatum* Doty and a cocktail of probiotic microbes. Mixture was subjected to liquid fermentation for the period of 10 days, that facilitated production of protoplasmic and spheroplasmic detritus via microbial degradation of macroalgal fronds and these are called “single cell detritus” (SCD). There was a two-order increase in probiotic microbes, drop in the number of other marine bacteria to a negligible level. The silage was chemically characterized to determine their nutritive composition and used directly for rotifer production and formulated as fish feed with the addition of aquaculture binder. Presentation will include fermentation dynamics, microbial composition, silage nutritive characteristics, feed formulation and statistic on the increase in mortality of fish fries and rotifers.

Keywords: Probiotics; Seaweed; Single cell detritus

Selected References:
O-AFB-06

Influence of Starch Retrogradation on Synthesis of Resistance Starch, a Compound Vital for Diabetes Mellitus Management via Gut Microbiota Alteration

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Abstract

Gut microbiota is one of the important factors in our health. The good microbiota improves body immunity and optimizes digestion process for obtaining vital nutrients. It also reduces the risk of colorectal cancer by preventing damage to intestinal epithelium. Recently, several studies demonstrate the correlation between gut microbiota composition and the prevalence of diabetes mellitus. The consumption of prebiotics that enhance the good gut microbiota can reduce the risk of developing diabetes. One of the prebiotics is resistant starch. This carbohydrate is indigestible; however, it is fermentable by the gut microbiota to produce short chain fatty acids (SCFAs), the compounds that may reduce sugar level in blood. Starch retrogradation is known to able to increase resistant starch content. The aim of this review is to study the influence of the resistance starch produced from starch retrogradation, which induce alteration of gut microbiota, on diabetes mellitus prevalence. Its biological and chemical mechanisms are discussed comprehensively. The effect to the cell metabolic function and regulation, the ratio of which varies according to the botanical source of the starch and can significantly alter the functional properties of the starch is also explained. The review will provide beneficial information on how to design a suitable diet, particularly for the persons with diabetes.

Keywords: Diabetes mellitus; Gut microbiota; Prebiotics; Resistant starch; Starch retrogradation
Utilization of Polysaccharides Extracted from *Ficus awkeotsang* Makino in Encapsulation Applications

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**Abstract**

*Ficus awkeotsang* Mankino, also known as Aw-keo in Taiwanese, is a native plant that grows on hillsides 800–1800 m in Taiwan. The cold water extract of seeds of *Ficus awkeotsang* Makino can form jelly when divalent cations are presented in the solution. The polysaccharide is mainly composed by alpha-1,4-galacturonan and can be categorized into low methoxyl pectin. In this study, the effect of different divalent cations on the gel characteristics were evaluated based on different applications. One application is to encapsulate probiotics for improved storage and ingestion viability. Microencapsulation is suggested to be a promising approach for introducing viable probiotics through oral administration because the encapsulation matrix can provide a physical barrier against adverse environmental conditions. The aim of this study is to increase the viability of *Lactobacillus paracasei* LCW23 during exposure to simulated conditions of the gastro-intestinal tract by microencapsulation with the polysaccharide extracted from the seed of *Ficus awkeotsang* Makino. The result shows that the *Ficus awkeotsang* Makino polysaccharide has high loading capability of *L. paracasei* (98.51%) in all cases. The survivability and release rate will also be tested and reported as well as other physical properties of the microbeads. The other application is to develop a colon targeted delivery enteric coating or capsule. This part of research is aim to develop a multifunction carrier for nutraceuticals or pharmaceutics application.

**Keywords:** *Ficus awkeotsang* Makino; Microencapsulation; Polysaccharides
Role of Hemicellulose-B from *Santalum album* L. Suspension Cells in the Adherence of Lactobacilli *in Vitro*

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Abstract

Probing into the functional aspects of hemicellulose-B extracted from *Santalum album* L. suspension cells, its digestibility through gastrointestinal tract was investigated. Its role in the adherence and biofilm formation of lactobacilli was also analyzed *in vitro*. Isolated cell wall material from Sandalwood suspension culture was fractionated by sequential extraction with Imidazole, followed by Na$_2$CO$_3$, and finally with 4 M KOH. The hemicellulose-rich alkaline fraction was acidified to pH 5.0 to precipitate hemicellulose-A, while hemicellulose-B (HB), the test sample, was isolated from the supernatant fluid by precipitation with 4 volumes of chilled ethanol and subsequent centrifugation. To test its indigestibility *in vitro*, the polysaccharide was subjected to serial incubation in artificial gastric juice (low pH) and porcine pancreatic α-amylase (pH 7.0) at maintained temperature of 37°C for 8 hrs. Role of the polysaccharide in assisting biofilm formation was evaluated in 12-well plate using minimal medium with variable saccharide supplementation and visualized with BacLight Live/Dead stain. Z-stack images were collected and analyzed by Las X. The probiotic strains used in this study were *Lactobacillus rhamnosus* ATCC 7469 and *Lactobacillus acidophilus* MTCC 10307. HB remained mostly unhydrolyzed; the maximum hydrolysis obtained being <10% suggested its ability reach colon mostly unaltered. Adherence to polystyrene plate in medium supplemented with HB was indicated by thick biofilm formation as visualized in fluorescence and electron microscopy and, quantified through crystal violet staining. The results promote the heteropolymer as prebiotic candidate, thus offering an inclusive approach towards utilizing plant biomass to bring novelty in the domain of nutraceutical research.

Keywords: Biofilm; Hemicellulose B; Lactobacilli; Plant cell wall; Prebiotic
O-AFB-10

Inhibitory Effect of Anti-browning Agents on Lethal Browning in Petal Tissue Culture of Dendrobium Sonia ‘Earsakul’

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Abstract

Petal tissue culture was developed to bridge both advantages of transient and stable transformation in flower color modification of Dendrobium Sonia ‘Earsakul’. This tissue culture technique was able to induce rapidly the growth of putative shoot via direct organogenesis but it lacked the ability to regenerate due to being hampered by the effect of letharg browning. Effects of various anti-browning agents and types of subculture media on inhibition of lethal browning were evaluated. Petal tissues were cultured in different medium types including liquid and two-layered medium based on the modified half-strength Murashige and Skoog basal medium. All medium types were supplemented with or without anti-browning agents (ascorbic acid [AA], citric acid [CA], polyvinylpyrrolidone-40 [PVP], and activated charcoal [AC]). Among the treatments, the two-layered medium with 25 mg L⁻¹ PVP and 1 g L⁻¹ AC in the two-layered medium was the most effective condition that significantly inhibited lethal browning. This condition resulted in an increase of healthy explants (up to 58.33%) and prolonged browning to 12 weeks after inoculation. However, there is no significant difference among anti-browning agent treatments in terms of growth induction and numbers of organogenic tissues but PVP treatment induced bigger size of organogenic tissue (>0.5 mm) than the other treatments.

Keywords: Anti-browning agent; Browning; Dendrobium; Floral tissue; Tissue culture
O-AFB-11

Analysis of the Symptom of Grain Discoloration in Rice
(*Oryza sativa*) (var. RD-61)

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Abstract
Grain discoloration (GD) or dirty panicle disease of rice is a disease complex which is caused by various fungi such as *Alternaria* sp., *Bipolaris* sp., *Curvularia* sp., *Fusarium* sp., *Helminthosporium* sp. and *Trichoconis* sp. Two hundred rice panicles (var. RD-61) have been collected from each rice field (at Amphur Muang and Amphur Cha-Am) in Phetchaburi province. These two hundred rice panicles from each location have been assessed to determine the severity of grain discoloration using IRRI standard evaluation system for rice (IRRI, 2002). Around forty five percent of rice panicles from both Amphur Muang and Amphur Cha-Am receive score 9 (disease incidence 51-100%), the highest score for evaluating grain discoloration disease. After assessment to determine disease severity, these rice panicles have been threshed and the grains have been grouped based on the patterns of necrotic lesions. The analysis of rice panicles with dirty characteristic revealed that there were six patterns of necrotic lesions, such as distinct small spot (DSS), distinct large spot (DLS), distinct very small spot (DVSS), large black lesion (LBL), brown lesion (BL), combined BL and DSS (CBLDSS), on the grains of rice (var. RD-61) from these two rice fields. The LBL was a major pattern of necrotic lesion associating with the emptied grains from both locations. At Muang, *Curvularia* sp. was the major fungus associating with the DSS (76%) and DLS (68%), while *Fusarium* sp. was the major fungus associating with the DVSS (58%), LBL (64%), BL (60%) and CBLDSS (62%).

Keywords: Dirty panicle disease; Lowland rice variety; Plant pathogenic fungi
Mycelial Cultivation of 5 Edible Mushrooms from Khao Kra-Dong Volcano Forest Park, Thailand

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Abstract
Mushrooms are one of the many foods that are equally known for their unique taste and therapeutic properties. This super food has a myriad range of health benefits. It was the most expensive foods and have a global market measured in the world. The fruiting body of all occurs only once a year during rainy season in the month of June – August in Thailand. So, the objective of this research was study the optimal mycelial conditions in 5 edible mushrooms from Khao Kra-Dong Volcano Forest Park, Thailand. 5 edible mushrooms were as Termitomyces clypeatus KKV01, Russula emetic KKV02, Lentinus polychrous KKV03, Amanita hemibapha KKV04 and Cantharellus cibarius KKV05. Among 3 different culture medium, potato dextrose agar (PDA) was the best medium for induced the colony diameter, especially in Termitomyces clypeatus KKV01. T. clypeatus KKV01 showed the colony diameter as 87.67+1.53 mm. on potato dextrose agar (PDA) and the colony diameter on PDA+2% volcano’s soil as 47.33+0.58 mm. The optimal temperature and pH value for mycelial growth were at 30 °C and pH 7 after 7 days incubation.

Keywords: Edible mushrooms; Mushroom cultivation; Volcanic soil

Selected References:
O-AFB-13

**Angiotensin Converting Enzyme Inhibitory Activity of Enzymatic Bromelain Boletus Mushroom Protein Hydrolysate and the Membrane Ultrafiltration Fractions**

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**Abstract**

Angiotensin converting enzyme (ACE) inhibitory peptide was investigated from Boletus mushroom protein hydrolysate. Enzymatic bromelain Boletus mushroom protein hydrolysate (eb-BPH) was hydrolyzed with 0, 5, 10, 15 and 20% (w/w) bromelain at 0.5, 1, 3, 6 and 12 h. The optimum hydrolyzed condition was 15% bromelain with hydrolysis time of 6 h, which had the greatest degree of hydrolysis (66.61%). The crude eb-BPH was further fractionated into four fractions, namely, eb–BPH-1 (>10 kDa), eb–BPH-2 (10–3 kDa), eb–BPH-3 (3-1 kDa), and eb–BPH-4 (below 1 kDa) by membrane ultrafiltration. The eb–BPH-4 fraction should significantly highest (43.46%) *in vitro* inhibition of ACE, the key enzyme controlling the blood pressure regulation. Generally, eb-BPH-4 fraction had the highest of surface hydrophobicity (S\(_0\)) (1061/5 mg protein). The high activity of eb-BPH-4 in ACE inhibitory may be related to the high levels of surface hydrophobicity because ACE prefer to binding the inhibitors, containing hydrophobic amino acid in the C-terminal (Vermeirssen et al., 2004). It was suggested that Boletus mushroom protein hydrolysate could potentially use as ingredients in functional foods and nutraceuticals against hypertension.

**Keywords:** Angiotensin I-converting enzyme; Boletus mushroom; Enzymatic protein hydrolysate
O-AFB-14

The Xa21 in the Backcross Introggression Lines, BC<sub>4</sub>F<sub>2</sub>, Derived from the Thai Rice Cultivar ‘RD47’/‘IRBB21’ Cross Enhances the Bacterial Blight Resistance Against <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> Newly Isolated from Phitsanulok Province

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Abstract

Bacterial blight (BB) caused by <i>Xanthomonas oryzae</i> pv. <i>oryzae</i>. (<i>Xoo</i>) is a threatening rice disease responsible for significant yield losses. The ‘RD47’ is a popular rice cultivar in the Lower North of Thailand, but is highly BB susceptible. <i>Xa21</i>, a BB resistance gene which confers high resistance level against a broad spectrum of Asian <i>Xoo</i> strains, has been introgressed into the ‘RD47’ via a backcrossing program. BC<sub>4</sub>F<sub>2</sub> plants with heterozygous and homozygous <i>Xa21</i> were differentiated by the pTA248 marker. Meanwhile, <i>Xoo</i> was isolated from rice paddy fields in Phitsanulok province and molecularly confirmed by <i>Xoo</i> specific PCR. The <i>Xoo</i> isolate Xoo16PK001 was selected for BB resistance evaluation in the BC<sub>4</sub>F<sub>2</sub> plants carrying either heterozygous or homozygous <i>Xa21</i> genotypes using the clipping method. The BB lesion length was significantly reduced in BC<sub>4</sub>F<sub>2</sub> plants carrying the <i>Xa21</i> gene compared to the parental ‘RD47’ plants. However, there was no significant difference between heterozygous and homozygous <i>Xa21</i> BC<sub>4</sub>F<sub>2</sub> plants. This indicated that the introgressed <i>Xa21</i> gene was functional and enhanced resistance against the local <i>Xoo</i> isolate in the ‘RD47’ backcross introgression lines, BC<sub>4</sub>F<sub>2</sub>.

Keywords: Bacterial blight; Breeding; Rice; Xa21; Xanthomonas; Xoo
Development of Soybean Lines Tolerant to Aluminum through Genetic Engineering

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Abstract
Soybean is the second important food crops in Indonesia. The soybean demand is higher than the national production, so the development of high productivity soybean variety is very needed. Indonesia has 45.794 million ha of acid soil land potential for agriculture. But, development of soybean variety in acid soil meet constraints such as Al toxicity and nutrient deficiency.Liming is less economical and easily dissolved by water. Development of soybean varieties tolerant to acid soil is needed. It can be made through genetic engineering by inserting acid tolerance genes into the soybean genome. Ninety one of T0 soybean lines have been obtained through the insertion of Al tolerance genes, MaMt2 by using Agrobacterium-mediated transformation. Molecular analysis of T0 soybean plants using PCR method indicated that nine of DNA samples were positive containing MaMt2 gene. Then, PCR analysis of T1 soybean lines indicated that four lines were still carrying the MaMt2 gene, ie line GM2, GM5, GM10 and GM14. The objective of this study is to evaluate the four transgenic T2 soybean lines tolerance to acid soil in Biosafety Containment. Result of molecular analysis using PCR method showed that those four soybean lines in this study were still carrying the MaMt2 gene. Phenotypic analysis of this lines in Biosafety Containment by using four media treatments of acid soil, showing all the lines can grow on media only containing acid soil (without lime and compost). The GM2 soybean lines showed better growth than the three other lines.

Keywords: Al tolerance; Molecular analysis; Phenotypic evaluation; Transgenic soybean
AM : Applied Microbiology
Optimization of Amino Acid Decarboxylation and Sugar Fermentation to Enhance Hydrogen Sulfide Production for Rapid Screening of *Salmonella* During Selective Enrichment

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**Abstract**

To develop a rapid and high-throughput presumptive screening of *Salmonella* contamination, hydrogen sulfide (H₂S) indicator medium was proposed and tested in a microwell plate. The different three amino acids and nineteen fermentable sugars were supplemented and optimized to enhance the reaction. The black precipitates as indicator of reaction were followed by optical density (OD⁶⁵⁰) changes. All typical H₂S⁺ *Salmonella* gave copious black precipitates, correlated well with the sharp increase of the OD⁶⁵⁰ curves in the developed TFXL broth. The H₂S⁻ non-salmonellae, on the other hand, failed to form the precipitates and slowly change the broth OD⁶⁵⁰. There were situations with weak H₂S⁺ strains (*Salmonella anatum* and *S. typhi*) demonstrating slow and poor precipitation and delaying optical density development. The replacement of lysine in TFXL by other two amino acids (either ornithine or arginine) was conducted to enhance the precipitation of thiosulfate reduction. The ornithine formulation was the best all-around media to facilitate hydrogen sulfide production in all *Salmonella*, except for *S. anatum* and *S. typhi*. The arginine-based medium improved black precipitation in *S. anatum* but not in *S. typhi*. The inclusion of all three amino acids in TFLOA broth was necessary to enhance the optical density signals for all typical and atypical *Salmonella* detection. The addition of other carbon sources (i.e., dulcitol and mannitol) into TFLOA broth was also found to improve the detection of *S. typhi* and *S. anatum*, as well as other *Salmonella* serovars. The developed H₂S indicator media provided rapid presumptive results within 24 h better than conventional method; 24 h for selective enrichment followed by agar plating with additional 24-48 h for those results.

**Keywords:** Amino acid decarboxylation; Hydrogen sulfide production; Microplate assay; Presumptive screening; *Salmonella*

**Selected References:**
O-AM-02

MicroRNAs in the Chloroplast of Unicellular Alga

*Chlamydomonas reinhardtii*

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Abstract

*Chlamydomonas reinhardtii* is a microalgae that is well placed as a model organism for biotechnological exploitation. Rapid improvement of its genetic amenability provides an opportunity to expedite the development of *C. reinhardtii* as a useful industrial microorganism. *C. reinhardtii* chloroplast is a semi-autonomous organelle with its own genome of 203,395 base pairs and encodes about 80 genes. Processing of chloroplast transcripts requires the involvement of several factors, encoded either within the organelle or the nucleus. Thus, chloroplast transcripts might provide potential sites for miRNA-mediated post-transcriptional regulations. A differential analysis of small RNA derived from chloroplast genome under nitrogen deprivation was performed. The study revealed nine nitrogen-responsive intergenic chloroplast small RNAs (ccsRNAs). Four ccsRNAs were upregulated in nitrogen depleted relative to nitrogen-based conditions and three ccsRNAs were downregulated. Two ccsRNAs were specific for nitrogen-based conditions only. These microRNAs suggest their involvement in chloroplast gene regulation, which could open a new layer of interaction between nucleus and chloroplast via small non-coding RNAs. This could also pave the way towards regulation of genes in the chloroplast using small RNAs to control the metabolisms of *C. reinhardtii*.

Keywords: *Chlamydomonas reinhardtii*; Chloroplast; MicroRNAs
Screening, Isolation, and Characterization of Protease and Lipase Producing Bacteria Isolated from Fermented Shrimp Paste

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Abstract

Kapi is a fermented shrimp paste which is traditionally produced using the high content of salt and mainly consumed as a condiment by Thai people. The protease and lipase producing bacteria from Kapi were screened, isolated, characterized, and subjected to technological properties and safety assessment in order to select potential functional starter cultures. Five strains showed the highest activity of protease, as well as five strains exhibited the highest lipase activity, were selected to be examined further. Molecular identification of protease producing bacteria showed that all of them were identified as Virgibacillus sp., while all of the lipase producing bacteria were identified as Staphylococcus sp. Most of the isolated Virgibacillus and Staphylococcus could grow until the presence of 20% NaCl. One strain of Virgibacillus showed lipolytic activity, while all Staphylococcus showed no proteolytic activity. None of the Virgibacillus isolates showed hemolytic activity, while two isolates of Staphylococcus showed partial hemolytic activity as examined by blood agar plate method. All of the Virgibacillus and Staphylococcus isolates were also not a biogenic amine producer, as detected by TLC and PCR method. In addition, one Virgibacillus isolate showed no ability in biofilm formation, while others Virgibacillus and all Staphylococcus isolates showed the weak ability of biofilm formation as determined by microdilution plate method. In conclusion, all of the Virgibacillus and three of Staphylococcus isolates could be the promising starter cultures in the production of fermented shrimp paste.

Keywords: Safety attributes; Shrimp paste; Staphylococcus sp.; Starter culture; Virgibacillus sp.

Selected References:

O-AM-05

Constitutive and Methanol-Inducible Promoters from a Thermotolerant Yeast, Ogataea thermomethanolica, Suitable for Heterologous Gene Expression, Especially at Elevated Temperature

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Abstract

Using yeast as a host for expression and production of recombinant proteins offers several advantages. Newly characterized thermotolerant yeast, Ogataea thermomethanolica TBRC 656, has high potential for utilization as a protein production host due to its ability to grow at high temperature (up to 42 °C) and grow rapidly in defined media. In order to establish O. thermomethanolica as an efficient host for protein production, the identification of strong promoters for usage in the expression system is necessary for high efficiency of gene expression. Recently, two types of native promoters, the constitutive GAP promoter and the methanol-inducible AOX promoter, exhibiting strong expression of target proteins were identified. The Ot-GAP (O. thermomethanolica GAP) promoter was shown to drive gene expression at elevated temperatures up to 42 °C in both glucose and glycerol as carbon sources, which can be advantageous, especially when a large scale production in bioreactor is involved. In case of inducible promoter, the Ot-AOX (O. thermomethanolica AOX) promoter was induced by methanol and could regulate gene expression up to 45 °C. Interestingly, the promoter could initially turn on the expression of the heterologous protein at the de-repression stage in the presence of glycerol, whereas full induction of protein was observed in the presence of methanol. Thus, with Ot-AOX promoter, the target protein can be initially produced even prior to the induction phase, which would help shorten the time for protein production.

Keywords: Constitutive promoter; Inducible promoter; Methylotrophic yeast; O. thermomethanolica; Recombinant protein expression

Selected References:

Comparative Genomics and Transcriptomics Analyses Revealed the Role of Significant Genes in Thermal and Ethanol Stress Tolerance in *Saccharomyces cerevisiae* SPSC01

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Abstract

SPSC01, a self-flocculating *Saccharomyces cerevisiae* strain, is tolerant to various stresses. By comparing with the model strain S288c, 80,469 SNPs, 2,983 Insertions and 2,991 DELs of SPSC01 genome was identified, meanwhile, with the non-flocculating mutant SPSC01 Δ*FLO1* as the reference, transcriptome sequencing for SPSC01 was performed. The combined genomic and transcriptomic analyses was used to develop the pool of candidate genes with missense mutations and differential expression responsible for stress tolerance, so the *MIG1* and dubious gene *YCR049C* were selected for functional analysis. The overexpression of the mutated *MIG1* improved thermal tolerance in *S. cerevisiae* SPSC01, 6525 and other yeast strains but not in S288c, indicating that such a function is strain-specific to some extent, and molecular simulation indicated that the substitutions of amino acid mutation of Pro71Leu and Phe399Ser altered the conformation of the Mig1, and probably affected its role in regulating thermal stress response. On the other hand, the deletion of *YCR049C* enhanced ethanol tolerance with all yeast strains examined in this work, indicating that this ORF (open reading frame) might negatively regulate ethanol tolerance, providing a simple strategy for developing ethanol-tolerant strains by manipulating the potential ORFs.

**Keywords:** Comparative genomics; *Saccharomyces cerevisiae*; Self-flocculation; Stress tolerance; Transcriptomics
Screening and Characterization of High Ethanol-producing Yeast from Selected Naturally Fermenting Fruits

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Abstract
The study aimed to screen and characterize high ethanol-producing yeasts from mango, java plum, and longan for wine production. Thirteen yeast strains were isolated from overripe duhat, longan and mango. The thirteen isolates underwent initial screening for ethanol production using rice carbohydrates as substrate in a rice wine production set up. Out of thirteen isolates, only three yeast isolates produced ethanol. The isolates that produced ethanol were from java plum (DA1-4 and DA1-5) and mango (MA1-7). DA1-5, MA1-7 and positive control with baker's yeast all produced 10% v/v of ethanol while DA1-4 produced 13 percent v/v of ethanol in rice wine. The ethanol fermenting yeast strains were subjected to morphological and biochemical testing. All the three isolates have a creamy white color, circular in form, entire in margin, no Ascospores, ferments glucose, sucrose, and fructose, negative in pellicle and nitrate assimilation. Both DA1-4 and DA1-5 are characterized by smooth colony texture, reproduced by bipolar budding, and presence of pseudohyphae but positive (DA1-5) and negative (DA1-4) in acetic acid production while MA1-7 has rough colony texture, reproduced by multilateral budding, positive in acetic acid production and has true mycelium. Based on these characteristics, DA1-4 and DA1-5 were classified to belong to the genera of Kloeckera while MA1-7 to Candida.

Keywords: Candida; Ethanol; Kloeckera
A Comparative Study on Lipase Enzyme Immobilized on Acid and Glutaraldehyde Functionalized Multiwalled Carbon Nanotubes

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Abstract
Nanomaterials are being increasingly employed as support materials for enzyme immobilization owing to their biocompatibility and large surface area. Immobilization of lipase on multiwalled carbon nanotubes (MWCNT) has shown significant enhancement in the enzyme stability with respect to temperature and pH variations. Functionalization of MWCNT results into cross linking of enzyme with the MWCNT. The present study is focused on the comparative performance evaluation of the immobilized lipase on MWCNT functionalized using acid and glutaraldehyde, respectively. Acid treatment of MWCNT will cause lipase to physically adsorb on MWCNT while glutaraldehyde treatment will act as a cross linker and form a covalent bond between lipase and the functionalized MWCNT. Immobilization efficiency was assessed by comparing enzyme loading for the two differently functionalized MWCNTs. Enzyme loading is defined as the ratio of the enzyme that is attached to the MWCNT to the enzyme present in the original enzyme solution. Glutaraldehyde-MWCNT (GL-MWCNT) cross-linking showed 96% of loading efficiency while acid treatment showed 94% efficiency. The maximum enzyme activity on GA-MWCNT was obtained at 45°C and pH 7 while acid-MWCNT showed highest activity at 40°C and pH 8. Enzyme activity was higher for lipase immobilized on GA-MWCNT compared to acid-MWCNT possibly owing to greater enzyme loading in the former support. Immobilized enzyme showed higher relative activity compared to the free enzyme. Lipase on GA-MWCNT showed greater stability at higher pH and temperature compared to that of the acid-MWCNT. Further, lipase on GA-MWCNT exhibited almost 81% residual activity after 5 consecutive uses. In short, GA-MWCNT appears to be a better support material for lipase performance in terms of operational stability, enhanced activity and high residual activity upon multiple uses.

Keywords: BCA Assay; Functionalization; Immobilization; Lipase; Multiwalled-carbon-nanotube
Nutraceutical Implication of Marine Carbohydrate from *Aphanothece* sp.

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**Abstract**

Marine microalgae are rich source of structurally diverse polysaccharides that could potentially be exploited as prebiotic functional ingredients for both human and animal health applications. The exopolysaccharide from *Aphanothece* sp. was separated into two fractions by anion-exchange chromatography. The monomer composition of both fractions was determined using HPTLC and HPLC and further confirmed by using GC-MS MS. The data reflected the presence of galactose, mannos, rhamnose and xylose in fraction one, whereas glucose and mannose in fraction two. The FTIR analysis indicated the presence of major carboxylic acid group (1626 cm⁻¹), β-linkage (896 cm⁻¹) and sulphate (1062 cm⁻¹). Prebiotic activity tested using EPS as a carbon source showed significant increase in the growth of probiotic strain, *Lactobacillus rhamnosus* (MTCC 1408), *Lactobacillus acidophilus* (MTCC 10307), *Lactobacillus caesi* (MTCC 5381) and *Lactobacillus plantarum* (MTCC 2621). Survivality of pathogenic strain of *E.coli* (MTCC 443) was found to be very low in medium fortified with EPS. Effect of *Aphanothece* EPS on biofilm formation by probiotic strain (*L. rhamnosus* and *L. acidophilus*) was studied using crystal violet, Syto green 9 dye and scanning electron microscope. Role of EPS in adhesion of probiotic strain to intestinal epithelial cell and anti-adhesive property of EPS against *E.coli* adherence to intestinal epithelial cells was studied in HT 29 cell line (ATCC HTB 38). The study conducted reflects a good sign for *Aphanothece* EPS to be exploited as a promising prebiotic candidate molecule.

**Keywords:** Exopolysaccharide; Prebiotics; Probiotics
Antibacterial Efficacy of Tilapia By-products Against *Listeria monocytogenes* and *Salmonella Typhimurium* and Its Application in Fish Patties

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**Abstract**

Antimicrobial activity of fish protein hydrolysate produced from Tilapia by-products (including head-frame, fin, belly, flap meat and trimmed meat) using Protease G6 and its application in fish patties were investigated. The hydrolysate possessed some remarkable characteristics such as protein content roughly 279.058 mg/g and a degree of hydrolysis around 11.84%. An agar disk diffusion method was employed to determine the minimum inhibition concentration (MIC) of the hydrolysate on *Listeria monocytogenes* and *Salmonella typhimurium*. The MIC was found to be 80 mg extract powder/mL (nearly 22.33 mg protein/mL) for *L. monocytogenes* and *S. typhimurium*, respectively. Application of the hydrolysate (80 mg extract powder/mL) with sodium benzoate (0.1%) into fish patties, and stored this mixture at 4 °C for 7 days, could manifest the most sensitivity against both of the pathogens when compared to others treatments (separately control sample, 0.1% sodium benzoate and 80 mg extract powder/mL the hydrolysate). Under the best conditions (combination of the hydrolysate with sodium benzoate), counts of *L. monocytogenes* and *S. typhimurium* at day 7 were decreased by approximately 1.5 and 2 log CFU/g, respectively. This finding confirmed the potential application of protein hydrolysates of Tilapia by-products as antibacterial substance in fish products. Adding of the hydrolysate could cut down the excessive presence of chemical preservatives and might increase economic value for fish by-products that being often used as animal feed, fish meal, fertilizers or throw away due to low market-value identification.

**Keywords:** Antimicrobial activity; Fish patties; Fish protein hydrolysates; *Listeria monocytogenes*; *Salmonella typhimurium*
O-AM-11

Evaluation of Lipase for Its Formulation Additive in Bio-based Toothpaste and Contact Lens Solution

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Abstract:
In the present study an attempt has been made to formulate the bio-based toothpaste and contact lens solution using *Staphylococcus arlettae lipase* as a biosurfactant by replacing the existing chemical surfactants. The formulated bio-toothpaste and bio-contact solution were analyzed through different specificity tests. The results indicate that the lipase will be a better alternative for chemical based surfactants present in the usual tooth-paste and contact lens solution. The present study paves the way for utilization of lipase as a biosurfactant in different FMGC formulations like shampoo, face wash, soaps etc. as an alternative for the chemical surfactants.

Keywords: Bio-contact lens solution; Bio-toothpaste; Compatibility; Lipase
BEB : Bioenergy and Biorefinery
“Sustainable Biorefinery for Secondary Products”
O-BEB-01

Reactor Design for Levulinic Acid Production from Palm Oil Empty Fruit Bunches

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Abstract
Palm Oil Empty Fruit Bunches (POEFB) is a lignocellulose biomass that consists of cellulose, hemicellulose, and lignin. Cellulose fraction in POEFB can be hydrolyzed into levulinic acid (LA), a biodiesel platform replacing fossil fuels. LA can be produced by acid catalyst depolymerizes of POEFB into glucose, which then dehydrated to 5–hydroxymethylfurfural (HMF) and rehydration to LA. Researches about LA production, kinetic study of LA production and model simulation of LA reactor had been done. However, no studies have examined about reactor design specifications to produce LA from biomass. Therefore, the objective of this research was to investigate kinetic reaction parameters to design LA reactor from POEFB. Kinetic reaction experiments were done in 1 liter pressurized vessel with 1 M sulfuric acid as catalyst at temperature 150, 160 and 170 °C. The results showed that the activation energies of cellulose to glucose; glucose to HMF; glucose to humid; and HMF to LA were 135.66; 155.30; 112.84; and 107.81 kJ/mol respectively. LA reactor construction material was Stainless Steel 316 with steam jacket as heater. Continuous stirred tank reactor with 6-pitched blade turbine impeller was chosen as reactor type to produce LA. For 100 kg/hour POEFB in capacity, LA reactor volume was 14.5 m³ with 2.64 m in diameter and 6.17 m in height.

Keywords: Acid hydrolysis reaction; Kinetic reaction; Levulinic acid; Palm oil empty fruit bunches; Reactor design
O-BEB-02

Optimization of Sodium Hydroxide Pretreatment Enhanced Cellulose Saccharification in Napier Grass using Response Surface Methodology

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Abstract
Alkaline pretreatment of Napier grass (NP) with sodium hydroxide (NaOH) is a promising strategy to improve enzymatic conversion of NP cellulose to glucose. With aims at increasing cellulose recovery and digestibility as well as reducing process costs, a Central Composite Design (CCD) was used to obtain regression analysis and a surface response model based on the CCD, which developed to examine the interactions of pretreatment variables, i.e., NaOH concentration and a solid-to-liquid ratio, determined the optimal levels of the individual factors. Under the optimal conditions, cellulose content increased by 30% with 67% reduction of lignin from the initial contents, whereas cellulose conversion to glucose reached 80%. Analytical analyses using SEM, FTIR, and XRD were performed for biomass characterizations and the results confirmed the changes of chemical compositions and structures after NaOH pretreatment. The improved performance of enzymatic hydrolysis of cellulose in biomass was related to increased porosity and increased cellulose surface areas caused by substantial lignin removal.

Keywords: Alkaline pretreatment; Cellulase; Cellulose; Lignin; Napier grass

Selected References:
O-BEB-03

Bioethanol Production by Batch and Repeated Batch using Immobilized Yeast Cells on Sugarcane Bagasse

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Abstract
Sugarcane bagasse obtained as a waste from sugar industry was delignified to be used as a natural carrier for \textit{Saccharomyces cerevisiae} SC90 immobilization in bioethanol production. This study aimed to evaluate the efficiency of ethanol production from immobilized yeast when molasses was used as a substrate under the batch and repeated batch processes (5 cycles). Yeast immobilization on delignified bagasse represented similar manners when they were grown on molasses and yeast peptone dextrose (YPD) through the quantitative and qualitative assays using cell dry weight and visualization under a Scanned electron microscope (SEM). Therefore, molasses containing 125 g/L sugar was applied for the cell immobilization and further for fermentation process under batch and repeated batch for 5 consecutive cycles. The immobilized yeast produced the the highest yield (\(Y_{PS}\)) under repeated batch 3 at 0.435 ± 0.065 (g/g) with the productivity (\(Q_P\)) and percent theoretical yield at 0.85 g/L.h and 85.30 ± 6.51% respectively. The ethanol yields of immobilized yeasts were higher than free cell suspended from batch 2 toward batch 5.

Keywords: Ethanol; Immobilization; Molasses; Sugarcane bagasse
O-BEB-04

Hydrodeoxygenation of Bio-oil over NiMo/Al$_2$O$_3$ and CeO$_2$, ZrO$_2$ and TiO$_2$ Additives

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Abstract
This research aims to study pyrolysis of palm fruit cake at 400-500°C. The pyrolysis products consist of liquid, solid and gas. The optimum temperature to produce the highest liquid yield and highest gasoline and kerosene fractions was 450°C. The heating value of bio-oil was 31.77 MJ/kg compared to 17.02 MJ/kg of the biomass. Adding CeO$_2$, ZrO$_2$ and TiO$_2$ on NiMo/Al$_2$O$_3$ and ran catalytic pyrolysis at the same conditions, it was found that NiMo/Al$_2$O$_3$/CeO$_2$ showed inferior properties for providing low organic phase fraction. NiMo/Al$_2$O$_3$/ZrO$_2$ provided oil that has higher heating value and %C than those from NiMo/Al$_2$O$_3$/TiO$_2$, and lowest O. However, the increase in ZrO$_2$ has resulted in the decrease in liquid yield and the organic phase fraction which were lower than those from NiMo/Al$_2$O$_3$/TiO$_2$. NiMo/Al$_2$O$_3$/ZrO$_2$ was superior to NiMo/Al$_2$O$_3$, the properties of the bio-oil were significantly improved by lowering phenol and acid, increasing ester, aromatic, and hydrocarbon at the same conditions. The bio-oil from NiMo/Al$_2$O$_3$/ZrO$_2$ pyrolysis has a heating value of 35.70 MJ/kg.

Keywords: Alumina; Molybdenum; Nickel; Palm fruit cake; Pyrolysis

Selected References:
Recovering Activities of Inactivated Cellulases by the Use of Mannanase in Spruce Hydrolysis

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Abstract
Softwood materials have gained considerable attention because of their abundance and high contents of carbohydrates that can be converted into high value products, such as alternative biofuels. However, during the enzymatic conversion of softwood to biofuels, there is a decrease of cellulase activity that significantly limits the conversion efficiency. This study examined the role of mannanase in recovering lost cellulase activity by relieving the inhibition of mannan on cellulases. Kinetic experiments indicated that mannan competitively inhibited *Thermoascus aurantiacus* cellobiohydrolase (*Ta* Cel7A) activity and irreversibly inhibited *T. aurantiacus* endo-glucanase (*Ta* Cel5A) but had no inhibitory effect on *Acremonium thermophilum* β-glucosidase (*At* Cel3A). In spruce hydrolysis (100 g/L biomass) by cellulases, further supplementation of mannanase suppressed the inhibition of residual mannan on *Ta* Cel5A and *Ta* Cel7A, and the activities of *Ta* Cel5A and *Ta* Cel7A increased by 14.3 and 10.9%, respectively. The increment of *Ta* Cel5A and *Ta* Cel7A activity enhanced cellulases hydrolytic action and may benefit the subsequent cellulases recovery process. These results may help to characterize the role of mannanase in the production of alternative biofuels from softwood.

Keywords: Cellobiohydrolase; Cellulase activity; Enzymatic hydrolysis; Mannan; Spruce

Selected References:
Bioprocessing Strategies for Biobutanol Production from Biomass

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Abstract
Considering the interest of producing renewable biofuel such as biobutanol to replace demand on non-renewable petrol fuel, many researchers investigating numerous approaches in order to produce biobutanol at a low cost. Such efforts are by considering suitable feedstock material and bioprocessing technologies. Renewable materials such as starch, lignocellulosic, and algal biomass are some of the common feedstock utilized for biobutanol production, and each of them has their own advantages, yet posses several disadvantages that need improvement. Low sugar concentration generated from hydrolysis of biomass, inefficient microorganism and unsuitability of conventional batch fermentation have been noted as the main reasons for a low biobutanol yield and productivity. Therefore, several fermentation operations and integrated bioprocessing technologies have been developed to improve the biobutanol production efficiency. The challenges and the appropriateness of the technologies are being presented in this talk.

Keywords: Biobutanol; \textit{Clostridium}; Consolidated bioprocessing; Simultaneous saccharification fermentation
O-BEB-07

Effect of Pretreatment Agents on Improved Methane Recovery from Deoiled Grease Trap Waste

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Abstract
Anaerobic digestion technologies provide a promising source of energy supply to the industries and decentralized treatment systems in recent years from wastewaters to municipal solid wastes. However, anaerobic digestion limits the efficient conversion/utilization of lignocellulosic and oil containing wastewaters due to the rate-limiting pretreatment process and degradation of complex polymers. There has been reports indicated that the appropriate pretreatment steps is necessary for the enhanced bioconversion technologies from the grease trap waste (GTW). This study investigated the effects of various chemical pretreatment (Hydrothermal, fenton, H₂O₂, and alkaline peroxide) methods on solubilization of the deoiled GTW and subsequent utilization for the methane generation. The pretreatment conditions were evaluated at moderate temperatures from 80 to 121 °C with duration of 30 min. The results showed that the maximum methane production of 115 mL CH₄ obtained from alkaline peroxide pretreatment with optimal conditions of 110 °C, pH 11.5 and duration of 30 min, this value is 40% higher than the untreated deoiled-GTW. This study showed that pre-treatment step is crucial for enhanced energy recovery from deoiled grease trap waste.

Keywords: Anaerobic digestion; Biogas; Energy recovery; Municipal solid waste
Influence of Hydraulic Retention Time on Thermophilic Biohydrogen Production from Palm Oil Mill Effluent in an UASB Bioreactor

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Abstract
Palm oil mill effluent (POME), an agaro-industrial wastewater with high solids content, was subject to hydrolysis by 1% (w/v) nitric acid in order to increase its solubility and the fermentable sugar content from its cellulosic component. POME hydrolysate was then evaluated in an up-flow anaerobic sludge bioreactor (UASB) for the production of biohydrogen gas via mixed culture under thermophilic conditions. The bioreactor was fed with fresh POME hydrolysates with varied hydraulic retention time (HRT) between 48-3 hours at constant cycle length of 24 hours to test the productivity of biohydrogen and the stability of UASB. In this study, H2-producing granules (HPGs) were formed shortly after the start-up period, HPGs’ sizes were varied with the changes in the organic loading rates (OLR). The maximum H2 production rate achieved was 32.22 l H2/d at HRT 6 hr with COD removal of 56.56%. Acetic acid was found to be the dominant by-product at all HRTs, followed by butyric acid. Results suggest that UASB has a good potential for stable biohydrogen production with high digestion rate of POME.

Keywords: Biohydrogen production rate; H2-producing granules (HPGs); Hydraulic retention time (HRT); Palm oil mill effluent (POME); Up-flow anaerobic sludge bioreactor
O-BEB-09

Enhancement of Bioethanol Production via Hyper Thermal Acid Hydrolysis and Co-culture Fermentation with Optimal Yeasts Ratio using Waste Seaweed from Gwangalli, Busan, Korea

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Abstract
The Waste seaweed from Gwangalli, Busan, Korea was utilized as biomass for ethanol production. Sagassum fulvellum comprised 72% of the biomass. After hyper thermal acid hydrolysis and enzymatic saccharification, 34 g/L of monosaccharide was obtained with a low content of inhibitory compounds from 80 g/L of biomass. P. stipitis and P. angophorae were selected as optimal co-culture yeasts to convert all of the monosaccharide in the hydrolysate to ethanol. Co-fermentation was carried out with various inoculum ratios of P. stipitis and P. angophorae. The maximum ethanol concentration of 16.0 g/L was produced using P. stipitis and P. angophorae in a 3:1 with an ethanol yield of 0.47 in 72 h. Ethanol fermentation using yeast co-culture may offer an efficient disposal method for waste seaweed while enhancing the utilization of monosaccharides and production of bioethanol.

Keywords: Co-culture fermentation; Enzymatic saccharification; Hyper thermal acid hydrolysis; Waste seaweed
Improved Fermentation Performance to Produce Bioethanol from Gelidium amansii using Pichia stipitis Adapted to Galactose

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Abstract
This study employed a statistical method to ascertain optimal hyper thermal acid hydrolysis conditions using Gelidium amansii (red seaweed) as a source of biomass. The optimal hyper thermal acid hydrolysis using G. amansii as biomass was determined to have a slurry content of 12% (w/v), H2SO4 content of 358.3 mM, and temperature of 142.6°C for 11 min. After hyper thermal acid hydrolysis, enzymatic saccharification was carried out. The total monosaccharide concentration was 45.1 g/L, 72.2% of the theoretical value of the total fermentable monosaccharides of 64.2 g/L based on 120 g dry weight/L in the G. amansii slurry. To increase ethanol production, 3.8 g/L of 5-hydroxymethylfurfural (HMF) in the hydrolysate was removed by treatment with 3.5% (w/v) activated carbon for 2 min and fermented with the yeast Pichia stipitis, adapted to high galactose concentrations via separate hydrolysis and fermentation. With complete HMF removal and the use of P. stipitis adapted to high galactose concentrations, 22 g/L of ethanol was produced (yield = 0.50). Fermentation with total HMF removal and yeast adapted to high galactose concentrations increased the fermentation performance and decreased the fermentation time from 96 h to 36 h compared to traditional fermentation.

Keywords: Activated carbon; Adaptation; Bioethanol production; HMF; Hyper thermal acid hydrolysis
O-BEB-11

Toxicity Test of Tobacco Extract as Bio-larvicide Against *Aedes aegypti*

This work was not delivered on the conference schedule.
O-BEB-12

Effect of Microaeration on Kluyveromyces marxianus Fermentation with Lignocellulose Hydrolysate

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Abstract
The production of fuel ethanol by lignocellulosic materials has been paid more and more attention, due to the wide availability of raw materials, good renewability and less pollution. However, the inhibitory effect of toxic by-products mainly deriving from the pretreatment of lignocellulosic biomass on fermentative microorganisms is one of the limiting factors hindering the industrial development of bioethanol. In this paper, microaeration strategies were utilized to alleviate the adverse effects of lignocellulose-derived inhibitors on the yeasts. In the presence of multiple inhibitors including formic acid, acetic acid, furfural and 5-HMF, the effects of constant-rate aeration by pumping air at the rates of 0.1 vvm, 0.2 vvm, 0.5 vvm or 1 vvm on fermentation with glucose, xylose and their mixture as the carbon source, respectively, were studied. Aeration significantly improved fermentative performance of the strain under the stress of multiple lignocellulose-derived inhibitors, such as enhancing the rate of xylose consumption, increasing xylitol yield, and shortening the fermentation time. When the aeration rate was increased from 0.1 vvm to 0.5 vvm, the xylitol productivity and yield of xylitol increased, achieving the highest (0.22 g/L/h and 0.7 g/g) at 0.5 vvm. In spite of a slight improvement of xylose fermentation by enhancing the initial biomass concentration, it is not the main reason for the well-deserved parameters compared with aeration. Under aeration condition, glucose consumption was enhanced with a similar ethanol yield (0.41±0.01 g/g), and a significantly reduced glycerol production, which also indicated that aeration alleviated the adverse effects of inhibitors on cells. For co-fermentation of high or low concentrations of glucose and xylose, the xylose was barely consumed before glucose was exhausted under conditions without aeration. In contrast, the fermentation of xylose was not affected by the glucose under 0.5 vvm, and the residual xylose was decreased to 3.6 g/L and 11.1 g/L from 17.1 g/L and 28.5 g/L compared with the control. In addition, it was found that the transcription levels of KmTPX1, one of the key genes defending the damages by reactive oxygen species, was over three times higher than the control at 0.2 vvm, which may be the reason for the enhancement of tolerance to multiple lignocellulose-derived inhibitors. In the presence of lignocellulose-derived inhibitors, fermentation with glucose, xylose and their mixture as the carbon sources were also conducted by oxidation-reduction potential (ORP) control. And the fermentation with corn stalk hydrolysates was also investigated under different aeration strategies. The damages to cell growth and metabolism that were caused by multiple inhibitory stresses and anaerobic condition may be effectively improved under the ORP control. With the increase of default ORP values, aeration volumes increased, cell viability enhanced, and glucose and xylose metabolism accelerated. For co-fermentation of glucose and xylose, when the ORP was controlled at −150 mV and -110 mV, the stagnation of xylose consumption was effectively removed in the late fermentation stage. The xylitol yield reached the highest (0.40 g/g) at -150 mv, and the ethanol yield was as high as 0.42 g/g. In conclusion, the inhibitor-resistance and fermentative performance of yeast was improved by both constant-rate and ORP-controlled aeration, which has also been proved as the simple and feasible methods with the promising potential for industrial application.

Keywords: Aeration; Domestication; Fermentation; Inhibitor; Lignocellulose; ORP
In-situ Synthesis of Canola Biodiesel Derived Estolides via Epoxidation Route

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Abstract
Research on the formulation of estolides from plant seed oils has gained significant interest due to their promising low temperature fuel additive properties and ecofriendly nature. The current research investigation is emphasized on the formulation of canola biodiesel estolides for low temperature applications. The two steps research approach employed includes; ring opening of epoxidized canola biodiesel in the presence of oleic acid, followed by esterification of hydroxyl groups with oleic acid to produce estolides in a single reaction using HZSM-5 as heterogeneous acidic catalyst. Prepared HZSM-5 catalyst was characterized to measure the properties required for the effective catalysis. HZSM-5 demonstrated promising activity for the estolides formation, > 95% conversion was achieved at 110 °C for 6 h using 15wt% of catalyst loading. 1H-NMR technique was employed to optimize the process conditions for the complete estolides formation. Physico-chemical properties of the reaction products were determined by standard methods and characterization results revealed that the formulated estolides had improved low temperature, lubricity and rheological properties, thermo-oxidative stability. Also, biodegradability of the estolides were carried out, and their biodegradability was found to be > 90% within 28 days as per the bio-kinetic model. Overall, outcomes of the physico-chemical characterization data indicated that the prepared estolides can act as possible alternative biolubricant basestocks for various low temperature applications.

Keywords: Alkoxides; Canola biodiesel; Epoxidation; Estolide; Oleic acid
O-BEB-16

Catalytic Pyrolytic for Bio-oil Production from Palm Kernel Shell using Respond Surface Methodology

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Abstract
The appropriate conditions for bio-oil production from palm kernel shell pyrolysis with zeolite using response surface methodology were investigated. The three important factors which were reaction temperatures, nitrogen flow rates and zeolite to palm kernel shell ratios were studied. The results of analysis using response surface methodology shown in the model as $Y = 35.7954 + 0.0914X_1 + 2.1412X_2 + 1.2141X_3 - 0.5069X_1X_2 - 1.2596X_1X_3 - 2.2569X_2X_3 - 0.429X_1^2 - 0.6411X_2^2 - 1.1412X_3^2$ which had the confidential value of 0.95 showing that the appropriate conditions was reaction temperature of 466°C, nitrogen flow rate of 936.4 mL/min and zeolite to palm kernel shell ratio of 18.41wt% giving the bio-oil yield as high as 44.95%. The bio-oil yield from simulation as 41.7% with different from the experiment of 7.23%. Bio-oil yields also were investigated from the effect of using catalyst, natural zeolite catalyst gave the higher bio-oil yield comparing to thermal pyrolysis. Main functional groups in bio-oil analyzed by gas chromatography-mass spectrometer and fourier transform infrared spectroscopy were 40.97% of acetic acid and 22.09% of phenol.

Keywords: Bio-oil; Palm kernel shell; Pyrolysis; RSM; Zeolite
O-BEB-17

Oxidoreductases from Kluyveromyces marxianus Enhances Tolerance of Yeasts to Lignocellulose-derived Inhibitors

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Abstract

Bioethanol from lignocellulosic materials makes great significance to the production of renewable fuels due to its wide sources. However, multiple inhibitors generated from pretreatments represent great challenges for its industrial-scale fermentation. Despite the complex toxicity mechanisms, lignocellulose-derived inhibitors have been reported to be related to the levels of intracellular reactive oxidative species (ROS), which makes oxidoreductases a potential target for the enhancement of tolerance of yeasts to these inhibitors. Firstly, a typical 2-Cys peroxiredoxin from Kluyveromyces marxianus Y179 (KmTPX1) was identified. Saccharomyces cerevisiae with over-expressed KmTPX1 gene showed an enhanced tolerance to both oxidative stresses and multiple lignocellulose-derived inhibitors, such as formic acid, acetic acid, furfural, ethanol and salts. In particular, an enhanced fermentative performance was observed when KmTPX1-expressing S. cerevisiae was exposed to a mixture of formic acid, acetic acid and furfural (FAF). Besides, synergistic effect of thioredoxin and its reductase from K. marxianus was also proposed. Double over-expression of KmTRX2 and KmTrxR achieved a better ethanol fermentative profiles under FAF inhibitors with a shorter lag period. Finally, the mechanisms of the improved tolerance to FAF depended on a lower level of intracellular ROS for cell survival under stress. The new functional oxidoreductases from K. marxianus (KmTPX1, KmTRX2 and KmTrxR) are firstly associated with the enhanced tolerance to multiple lignocellulose-derived inhibitors in S. cerevisiae. Consequently, we provided a powerful potential for applications of the oxidoreductases in ethanol production from lignocellulosic materials.

Keywords: Ethanol fermentation; Kluyveromyces marxianus; Lignocellulose-derived inhibitors; Peroxiredoxin; Thioredoxin reductase

Selected References:
RNAi Mediated Downregulation of Lignin Biosynthetic Pathway Gene Increases Saccharification Efficiency of Sweet Pearl Millet

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Abstract
Lignocellulosic biomass has been used as a source of second generation biofuel to avoid food versus fuel conflict. The plants with high biomass and low agronomic inputs are generally used as a preferred source for biofuel production. One of such potential feedstock for bioethanol production is sweet pearl millet (*Pennisetum glaucum* L). Despite of having high amount of fermentable sugar (~16%) in its stalk, its diversified agro-industrial utilization is mostly restricted by the presence of high amount of lignin (~20%). Therefore, a strategy to reduce its lignin content by transgenic approach would be of considerable interest to overcome the problem. In the present study, we have developed transgenic *P. glaucum* plant in which expression of caffeic acid O-methyl transferase (*COMT*) gene was downregulated by RNA interference (RNAi). The most strongly repressed transgenic line exhibited 18.89 % reduction of lignin with a concomitant increase of total carbohydrate by 65.75 % and significant changes in lignin composition (S/G ratio increased by 17.24 %) compared to untransformed plant. Saccharification efficiency of the presently developed transgenic line was also found to increase by 16.93% with mild acid pretreatment and 13.08% without any pretreatment compared to untransformed control plant. Thus, it could be suggested that the presently developed sweet pearl millet variety have the prospect to become economically profitable materials for better agro-industrial implication in terms of bioethanol production as well as forage crop.

Keywords: Lignocellulosic biomass; Saccharification efficiency; Sweet pearl millet; Transgenic approach.
O-BEB-19

Consolidated Bioprocessing of Lignocellulosic Biomass for Biofuels Production using Engineered Clostridium thermocellum

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Abstract

A sustainable production of fuels and chemicals for the fast growing human population is a major challenge. Various agricultural wastes like wheat straw, sugarcane bagasse, etc are available in rural India as cellulytic biomass for bioconversion to value-added commodity bioproducts. Many microorganisms are available in nature which produces various useful bioproducts from agricultural residues but only in trace amount. For a commercially viable bioproduct synthesis, the microbes need to be engineered where the metabolic flux can be redirected towards the desired products for high yield. Clostridium thermocellum has the natural ability to convert cellulose to ethanol, making it a promising candidate for consolidated bioprocessing (CBP) of cellulosic biomass to biofuels. To further improve its CBP capabilities, a mutant strain of C. thermocellum was constructed (strain AG553; C. thermocellum Δhpt ΔhydG Δldh Δpfl Δpta-ack) to increase flux to ethanol by removing side product formation. Strain AG553 showed a two- to threefold increase in ethanol yield relative to the wild type on all substrates tested. On defined medium, strain AG553 exceeded 70% of theoretical ethanol yield on lower loadings of the model crystalline cellulose Avicel, effectively eliminating formate, acetate, and lactate production and reducing H2 production by fivefold. On 5 g/L Avicel, strain AG553 reached an ethanol yield of 63.5% of the theoretical maximum compared with 19.9% by the wild type, and it showed similar yields on pretreated switchgrass and poplar. With the elimination of the metabolic pathways to all traditional fermentation products other than ethanol, AG553 is the best ethanol-yielding CBP strain to date and will serve as a platform strain for further metabolic engineering for the bioconversion of lignocellulosic biomass.

Keywords: Biofuels; Clostridium thermocellum; Consolidated bioprocessing; Lignocellulose

Selected References:
O-BEB-20

Biogas Production from Water Lettuce in the Chao Phraya River

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Abstract
Water lettuce (Pistia stratiotes Linnaeus) is a free-floating plant aquatic weed found in the Chao Phraya River. It is a highly problematic invasive weed in water bodies. The aim of this study is to assess the potential for utilizing the water lettuce as a renewable energy source for biogas production by anaerobic co-digestion with cow dung. Samples were collected from the Chao Phraya River at Pathumthani Province. Experiment was performed in a single-stage semi-continuous anaerobic reactor. The reactor was fed with a mixture of water lettuce and cow dung of 20% (water lettuce: cow dung: water =10: 10: 80) by fresh weight and was operated at 30 Celsius by a five-day feeding. Mixed ruminal microorganisms were used as inoculum. The reactor working volume was 5 liters and the feeding rate was 625ml/5 days giving rise to the hydraulic retention time of 40 days. During operation, pH of the substrate slurry was dropped and then was adjusted to be neutral. The results showed that the average methane content obtained was 53.26 %. The methane yield from the co-digestion was 120 liters at Standard Temperature and Pressure/kg total solids added to the reactor. pH of the digested slurry in the reactor was still neutral.

Keywords: Anaerobic digestion; Bioenergy; Biogas; Co-digestion; Cow dung; Methane; Water lettuce; Weed
BPMB : Biopharmaceutical and Medical Biotechnology
Cross Resistance Mechanisms between Antibiotic, Antiseptic, and Disinfectant in Human Pathogen Pseudomonas aeruginosa

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Abstract

Pseudomonas aeruginosa is one of the multidrug-resistant pathogens persisted in the hospitals worldwide. It can adaptively resist a wide range of antibiotic, antiseptic and disinfectant. To identify its novel resistance mechanisms against these bactericidal agents, the genomic library in P. aeruginosa was constructed by using recombinant DNA technique and the modifications of the method were applied to expand the library. The constructed P. aeruginosa genomic library was used to identify the genes involved in bacterial resistance against polymyxin B (PB) as antibiotic, chlorhexidine (CHX) as antiseptic and sodium hypochlorite (NaOCl) as disinfectant. Minimum inhibitory concentration (MIC) of P. aeruginosa against PB, CHX, and NaOCl was determined and the constructed library was treated with these bactericidal agents to obtain resistance clones. Modifications of the library construction method by multiplied digestion and unpool cultivation was 10-fold increase in the number of resistance clones. Each resistance cassette from the obtained clones was retransformed into the P. aeruginosa wild type and observed the resistance level by disk diffusion and plate sensitivity assays. Interestingly, cross resistances between these agents in the resistance clones were found. DNA sequence analysis in resistance cassettes was performed by using molecular tools and bioinformatics including homologous sequence comparison and genomic annotation. Herein, both known and novel resistance gene clusters that may play role as virulent factors such as quorum sensing, efflux pump, stress response and biomolecular repair were discovered. Role of these putative genes in resistance mechanisms is being studied by gene functional and physiological analysis. Overall data showed cross resistance mechanisms through these virulence factors between these bactericidal agents in this pathogen and shaded the light in the reasons for its persistence in the hospitals with successful infections.

Keywords: Antibiotic; Antiseptic; Cross resistance; Disinfectant; Pseudomonas aeruginosa

Selected Reference:
O-BPMB-02

Protein Hydrolysates and Partial Purified Peptides on Viability and Apoptosis of Liver Cancer Cell

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Abstract
Liver cancer is a leading cause of death in Thailand and many countries around the world. Current treatments such as surgery, chemotherapy and anti-cancer drugs are limited due to stages of cancer, side effects and expensiveness of treatments. Thus, new alternative treatments are needed. Recently, researches have paid a great attention on a development of safe and effective anti-cancer drugs derived from natural products. *Acanthus ebracteatus* Vahl is a Thai herb that has been used as traditional medicine for cancers. This study therefore aimed to identify fractions that possess anticancer activities and investigate effects of these partial purified peptides on apoptosis pathway and expression of key proteins involved in cell proliferation against liver cancer cell (HepG2). To achieve the aim, the protein extraction was performed using SDS followed by pepsin digestion and collection of <3 kDa protein hydrolysate using filter membrane. The chromatogram results from a reverse phase high performance liquid chromatography (RP-HPLC) showed several peptide peaks. However, only five fractions from 19 fractions showed inhibitory effects against HepG2 cells by MTT assay. Further purification by RP-HPLC resulted in several sub-fractions. By performing the MTT assay, 1 µg of protein/mL of fractions 1.1 and 1.2 effectively inhibited HepG2 cell viability without affecting the normal cell (Vero). The results from apoptosis test showed that the fractions 1.1 and 1.2 could induce cell into early and late apoptosis. The results from Western blot analysis showed that the fraction 1.2 down-regulated NF-kB. Taken all together, these results are promising to be further analysis on purification of pure bioactive peptides and identification of other molecular targets within the cancer cells.

Keywords: *Acanthus ebracteatus* Vahl; Apoptosis; Liver cancer; Partial purified peptide
O-BPMB-03

*In vitro* Human Breathing Lung Model for Inhalation Drug Development

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**Abstract**

Drug discovery through animal testing has been proven inefficient in many instances. Even some pharmaceuticals that successfully pass clinical trials are later found to have serious side effects that can lead to unwanted suffering, costly lawsuits, or even worse, the death of patients. The current technology developed to emulate organ-level functions in miniaturized tissue-engineered models is known as “micro-physiological systems” or “organs-on-a-chip”. These models have been used to study the adsorption, distribution, metabolism, elimination, and toxicity (ADMET) of drugs *in vitro*. However, until now there are only a few such systems that can integrate both structures and flow mechanical features to recapitulate a complex lung with a physiological similar breathing motion. Here, we report a stepwise approach to engineer a multilayered microfluidic platform that integrates both branched bronchiolar and deformed alveolar features to become a full lung model. The breathing motion was mimicked in the lung model using a novel, non-pneumatic microfluidic aspiration mechanics that can stretch synthesized alveolar membranes and generate airflow in the airway. To realize the transport mechanism of inhalation drug in the breathing lung model, various sizes and charge properties of aerosolized drugs were generated and transported to the lung model through the breathing mechanism. The excessive aerosolized drugs were exhaled back to the aerosolization chamber while others remained in the *in vitro* lung model. To improve the visualization ability, the aerosols were produced from fluorescein solution and observed using a fluorescent microscope. The distribution profile of the inhaled aerosols located in the different generation of bronchi was plotted by evaluating the remained aerosols. The *in vitro* lung model that mimics complex lung organ breathing mechanism is suitable to understand the transportation mechanism of drugs. These capabilities may be particularly useful in developing and accelerating clinical translation of inhalation drugs as well.

**Keywords:** Drug delivery; Inhalation drug; Organ-on-a-chip; Respiratory
O-BPMB-04

Evaluation of a Single Use 24-well Micro Bioreactor System for CHO Cell Culture

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Abstract
Micro bioreactors are increasingly used for cell screening and early bioprocess development of mammalian cell cultures. However, many commercially available single-use, micro bioreactors systems are still restricted with regard to automated liquid addition and parallel control of pH, temperature and dissolved oxygen (DO). The micro-Matrix (Applikon Biotechnology) addresses these issues using a 24-single use mL-scale bioreactors in a micro-titre plate (MTP) format. Each well is independently monitored and controlled to mimic quantitatively laboratory and pilot scale bioreactors. This preliminary research describes the application of micro-Matrix for fed-batch CHO cell cultivation. The feeding is achieved using either standard bolus or continuous feeding strategies and the results compared to standard 24-well MTP cultivation with bolus feeding. The results are discussed in view of robustness, reproducibility and scalability of fed-batch operation.

Keywords: 24 wells; CHO cells; Fed-batch; Micro bioreactors
Stephania spp. Exerting Estrogenic and Anti-estrogenic Activities

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Abstract
Tuberous root of Stephania spp. have been used as ingredients of folk medicine in Thailand such as for blood tonic to treat anemia, rejuvenation supplement, and nervous system maintenance. Moreover, they are consumed as food in northern and north-eastern region in Thailand. There are 3 Stephania spp. wildly known in the market, i.e. S. erecta, S. suberosa, and S. venosa which local names are Bua bok pa, Boraphet pung chang, and Sabu lueat, respectively. This study was aimed to investigate estrogenic and anti-estrogenic activities of these plants by yeast estrogen screening (YES) system. Yeast harboring human estrogen receptor β with deleted SNQ2 or PDR5 gene were applied for screening of estrogenic and anti-estrogenic activities of the plant extracts, respectively. S. erecta exhibited both activities whereas S. suberosa and S. venosa shown only anti-estrogenic activity. The result suggests that S. erecta might be a new phytoestrogen source for estrogen replacement in women. Moreover, dry powder of 6 S. erecta tuberous roots (SE001-SE006) from different areas were investigated for primary metabolite profiles. The result showed that 67 compounds (21 unknown and 46 known compounds) were obtained from GC-MS result analysis using MetAlign alignment, compound identification by AMDIS and Aloutput2 and multivariate data analysis by SIMCA-P, respectively. However, the primary metabolite profiles of S. erecta implies that the contents of compounds in tuberous roots from different sources were not difference.

Keywords: Anti-estrogenic activity; Estrogenic activity; GC-MS; Yeast estrogen screening

Selected References:
Medically Important Compounds, Traditional Uses and Their Formulations for Healthcare Products

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Abstract
Nepal bears high biodiversity of natural flora which contains large number of valuable medicinal plants specifically to the Himalayan region. Traditional uses of those medicinal plants by the local healers and old aged people are helping greatly to the areas where modern medicine is yet to be reached. This research output can have a great opportunity to the community that will help them to develop and grow the specific plant species in their own area which in future can lead to support financial crisis and lack of job opportunity in respective areas. This research indicated importance of medicinal plant residing around the community which might have very high value in future if they were protected and developed in bioprospecting approach. The possibility of finding new active compounds from the traditionally used medicinal plants and development of different healthcare products from the extracts of those plants is very high in these plants. This research not only activates the possibility of finding active drug ingredients for the future lead drugs but also widens the horizons of using less purified components as healthcare products which ultimately can develop a society based research that will increase the interest to the community people for using their own plants for their business based work plan and of course, ultimate benefit will go to the government in generating money from taxation.

Keywords: Bioprospecting; Healthcare; Ingredients and drugs; Medicinal plants

Selected References:
O-BPMB-08

Bioactivity Measurement and Bioinformatics Analysis to Develop DNA Barcoding System in Himalayan Herbs of Nepal

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Abstract
Phytochemical and antimicrobial activities of five most important medicinal herbs including the National Flower, the Rhododendron arboreum of Nepal was performed. The study showed the high content of flavonoids in Berberis sp., Rhododendron, Boerhaavia diffusa and Elshotzyaia strobilifera species. Among other extracts, B. petiolaris showed 13 mm zone of inhibition in 200 mg/ml against Klebsiella pneumonia while R. arboreum showed ZOI of 8 mm in 200 mg/ml concentration to S. aureus in antimicrobial study. Based on bioinformatics analysis of cytotoxicity Rhododendron was found to be have new flavonoids (myricitrin-5-methyl ether) having the toxic effect that was reflected in the leaves with LC50 value 14064.79 which could have great potential as a source for natural health products. Furthermore, the molecular and bioinformatic analysis of R. arboreum showed a distinctly different pattern of phylogenetic tree as compared to other Rhododendron species data available in NCBI indicating the endemic property of the plant. ITS and MatK genes were isolated and optimized the protocol for the analysis of national flower (R. arboreum) that will endorse the new avenue in the molecular biology of other national priority Himalayan herbs for their conservation and database establishment.

Keywords: Antimicrobial; Antioxidant; Myricitrin; Phytochemical and DNA barcoding
 Comparative Study of Three Different Molecular Sizes Sericin Extracted from the Cocoon of Antheraea mylitta as Ecofriendly Antimicrobial Agent

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Abstract

In the present scenario of environmental consciousness emphasized is given for the development of eco-friendly antimicrobial agent. Silk sericin, a water soluble glycoprotein of silk cocoon is used to develop products for biomedical application. Sericin was extracted from the cocoon of tasar silkworm, A. mylitta by boiling with 0.02 M Na₂CO₃ and then fractionated by ultracentrifugation into three different molecular sizes; fraction 1 (F1) contains the proteins ranging 50-200 kDa, fraction 2 (F2) 30-50 kDa, and fraction 3 (F3) 10-30 kDa. Antibacterial activity of these sericin fractions were evaluated using different concentrations (400 μg/ml, 200 μg/ml, 40 μg/ml) against Gram positive (Staphylococcus aureus) and Gram negative (Escheria coli) bacteria by both qualitative (agar diffusion assay) and quantitative (broth dilution and colony counting assay) methods. At 400 μg/ml, F1 showed an inhibition zone of 6 and 8 mm for S. aureus and E. coli, respectively, by agar diffusion method and was less for F3 (5.2;7.1mm) and F2 (4;5.8 mm) than F1. Broth dilution method showed a reduction of 51% and 68% growth for S. aureus and E. coli, respectively, by F1, which was higher than F3 (43%;57%) and F2 (34%;49%). Similarly, in colony counting assay F1 showed least number of colonies than F3 and F2. Similar trends of inhibition were found in lower concentrations of sericin but at reduced level. FE-SEM analysis showed disruption of bacterial cell wall in sericin treated bacteria indicating that sericin may disrupt bacterial cell wall/membrane to exert its inhibitory activity as potential antimicrobial agent.

Keywords: Antheraea mylitta; Antimicrobial activity; Cocoon; FE-SEM; Sericin

Selected References:

The Fabrication of Natural Rubber for Transdermal Drug Delivery Patch

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Abstract
The fabrication of natural rubber latex for transdermal drug delivery patch was studied in this research work. The natural rubber latex was deproteinized and the amount of nitrogen in deproteinized natural rubber (DPNR) was characterized. The percentage of nitrogen in DPNR was 0.04713 which confirmed the successful of deproteinization process. Sulindac (Sul), an anionic drug, was selected as the model drug. The effect of plasticizer type on physical properties, cytotoxicity and permeation characteristic was investigated. The sulindac loaded DPNR (Sul – loaded DPNR) patches were fabricated via the UV irradiation method using various types of plasticizers. These included ethylene glycol (EG), propylene glycol (PG), polyethylene glycol (PEG), glycerol (GLY), dibutyl phthalate (DBP) and silicone oil (Si). The agglomeration of Sul-loaded DPNR film was observed in Sul-loaded DPNR with EG, PG, PEG, and GLY. The Sul-loaded DPNR film using DBP and Si as plasticizer showed the flexible and smooth film. The cytotoxicity of DPNR films was investigated by MTT assay. The cell viability when using DPNR, DPNR-Si and DPNR-DBP were 65 %, 90 % and 69 %, respectively. The in-vitro permeation of the drug from the films was studied using a modified Franz diffusion cell filled with a phosphate-buffered saline (PBS) at pH 7.4 and maintained at 37 °C. The amount of sulindac permeation from DPNR-Si was higher than DPNR-DBP because of the higher solubility of Sul in silicone oil. Thus DPNR-Si could be a new choice for flexible and human friendly TDD patch.

Keywords: Deprotienized natural rubber; Plasticizer and Sulindac; Transdermal drug delivery
Assessment of Software for Somatic Single Nucleotide Variant Identification using Simulated Whole-Genome Sequencing Data of Cancer

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Abstract
Next-generation sequencing (NGS) is becoming one of the most widely used methods in genetic and genomic studies. Over the past decade, it has allowed researchers to gain a better understanding of disease-causing mutations in human. Although, the multitude of software and human variation databases have been created from several research groups around the world, but the analysis of genome sequencing data still remains complicated. In many cases, it was relatively difficult to identify the majority of true somatic single nucleotide variants (sSNVs) because they may not be supported by enough sequencing reads to pass the minimal criteria. This could be caused by the contamination of normal cells, tumor heterogeneity, or sample preservation. As a result, some sSNVs calling software that performs well in one sample may perform poorly in another. Therefore, this study aimed to assess the accuracy, in term of sensitivity and specificity, of multiple sSNVs calling software using simulated genome sequencing data with known variants. Results from this study can then be used to construct a reliable sSNVs identification pipeline to support the analysis of real cancer genome sequencing data in the future.

Keywords: Analytical pipeline; Somatic single nucleotide variants; Variant allele fraction; Whole-exome sequencing

Selected References:
Development of Algorithm for Aneuploidy Detection Based on Genome Coverage 0.005X Data

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Abstract
Success IVF treatment relies on embryo quality. Pre-implantation genetics screening prior to embryonic transfer, using micro-array or next generation sequencing techniques (NGS), can help to reduce the low-quality embryos and improve the IVF success rate. We have developed an algorithm to analyze NGS data at ultra-low coverage (100,000 reads) to detect abnormal chromosome counts. The aim was to reduce repeating assay in situation where read counts are very low. The algorithm was tested against commercially available software and standard karyotyping. The results are very promising, with 100% accuracy for both autosome and sex chromosome abnormalities. The software is easy to operate and can be run on a desktop computer with minimum run time. It can be regarded as an alternative method that the users can use before consider to repeat their assays.

Keywords: Aneuploidy; Next generation sequencing; Preimplantation genetics screening; Ultra-low genome coverage

Selected References:
BBE : Bioprocess and Bioseparation Engineering
Denaturation of Inactivated FMDV in Ion Exchange Chromatography: Evidence by Differential Scanning Calorimetry Analysis

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Abstract
Quality improvement for livestock vaccines is becoming more and more important. Removing impurities by purification is important for livestock vaccines to avoid side effects and ensure sufficient immunogenic protection on animals. In this study, three anion-exchange media with similar particle size and ligand density except pore size were applied and compared for purification of inactivated foot-and-mouth disease virus (FMDV) antigen. The dynamic binding capacity for DEAE-POROS (214 nm) and DEAE-650M (106 nm) were 11.53 and 10.03 mg/mL, while that for DEAE-FF (32 nm) was less than 1/10 of the previous two. The recovery of inactivated FMDV after chromatographic process of these three media was 68.42%, 66.32% and 54.46%, respectively, showing a decrement as the decrease of pore size of media. Possible denaturation of the FMDV on the surfaces of anion exchange media was analyzed by differential scanning calorimetry (DSC). FMDV is known to be prone to dissociate into smaller subunits 12S. In solution, this process is reflected by \( T_{m1} \) of DSC, which is at about 48.52°C. When inactivated FMDV were absorbed on DEAE-FF, the \( T_{m1} \) became 41.73°C, indicating the increased possibility of dissociation. The \( T_{m1} \) for DEAE-650M and DEAE-POROS was 44.04°C and 45.37°C, showing less dissociation and improved stability than on DEAE-FF. After DEAE-POROS chromatography, 94% FMDV recovery with 7.7-fold purification was achieved, further polishing by size exclusion chromatography led totally 173-fold increase in purity with average overall recovery of 79%.

Keywords: Differential scanning calorimetry; Dissociation; Foot and mouth disease virus; Ion exchange chromatography; Stability
Physicochemical Properties of Spray-dried Mango Phenolic Compounds Extracts

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Abstract
Phenolic compounds have been reported to possess anti-oxidative, anti-inflammatory, antibacterial and antiviral properties. Due to these properties, the demand for these compounds, in food and pharmaceutical applications, has risen and their economic value continues to increase over the years. However, the production of these compounds is limited by the availability of raw materials and the unstable characteristics of the compounds. One such plant-based source that can be used for the production of phenolic compounds is mango seed kernel. It is found that kernel extracts (in water) contain about 30-35 g/L of total phenolic compounds (in terms of gallic acid equivalence) with 320-350 µmol TEAC (Trolox equivalence antioxidant capacity) per gram of extract. The objectives of this study are to recover phenolic compounds from mango seed kernel extracts using spray-drying technology and to evaluate the effects of maltodextrin concentration and initial solids concentration of the extract to the physicochemical properties of spray-dried phenolic powders. Results indicate that varying the maltodextrin concentration and the initial solids content of the extract significantly affected the water solubility and water activity indices of the powders, as well as their color properties and total phenolic compound content. The ash content of the powders is affected by the change in initial solids concentration; however, it is noted that the maltodextrin concentration did not significantly affect the parameter. Furthermore, the moisture content is found to be insignificantly affected by both maltodextrin and initial solids concentration.

Keywords: Kernel; Maltodextrin; Phenolic compounds; Spray-drying

Selected References:
O-BBE-04

A Review on the Large-scale Production and Purification Processes for Fungal α-Amylase Production

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Abstract

The world market for industrial enzymes is estimated to be US$ 8.18 billion in 2015 in which α-amylase, a class of industrial enzyme, possesses approximately 25% of the world enzyme market. This is increasing with increasing applications in detergents, pharmaceuticals, and food & beverages. This review study, a prerequisite to establish a new production process in Bangladesh, is aimed at screening of fungal amylase producing organisms, optimization of solid state fermentation conditions for maximum amylase production by the best amylase producer, and characterization of the crude and pure amylases. From the published articles, ten fungal isolates were screened for higher α-amylase production. Among these, five strains showed much higher amylase activity under solid-state fermentation (SSF) conditions and were checked for further studies. Various culture conditions such as incubation period, incubation temperature, pH of the medium, and types of substrates were reviewed for maximum α-amylase yield. Aspergillus niger showed maximum amylase production (1162.32 IU/g) for 2-day incubation period at a temperature of 30 °C and initial pH of 5.0 using wheat bran as substrate. The study indicated that Aspergillus niger might be an important source for amylase. From the literature, it is found that Thermomyces lanuginosus produced highly active amylase with second highest purity. The enzyme was purified using several purification techniques like ammonium sulfate precipitation, DEAE Sepharose and Q Sepharose chromatography, Sepharose CL-6B, and Superose 12 filtration. After purification using the above methods the enzyme activity was found to be 3384 IU/mg which was 16.7 times higher than its concentration produced in submerged fermentation. So Thermomyces lanuginosus may be an important source for the production of crude and pure amylases and can be cost effectively produced in industry.

Keywords: Alpha amylase; Enzyme activity; Fungal; Large scale production; Solid state fermentation

Selected References:
O-BBE-05

Media Optimization and Batch Kinetics Studies for Recombinant Human Interferon α2b Production by *Pichia pastoris*

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Abstract
Interferon α2b (IFN α2b), a type I interferon having multiple biological activities, such as antiviral, antiproliferative and immunomodulatory activity is being used extensively to treat chronic hepatitis B and C and some types of cancer. *Pichia pastoris* is a widely used eukaryotic system for recombinant protein production which has characteristics of easy to use, requires simple medium and can be grown at high cell density with improved volumetric productivity. Design of experiments (DoE) approach was used for media optimization for the production of recombinant human IFN α2b by *P. pastoris*. Initial screening studies elucidated that glycerol and (NH₄)₂SO₄ as elite carbon and nitrogen substrates for IFN α2b production. The Plackett-Burman design suggested that glycerol, (NH₄)₂SO₄ and methanol as the significant factors influencing IFN α2b production. The optimal concentrations of glycerol (46.06 g/L), (NH₄)₂SO₄ (10.15 g/L) and methanol (1.38 %v/v) were identified by response surface methodology (RSM) using Box-Behnken design. A maximum IFN α2b production (53.5 mg/L) was achieved with the optimized medium at shake-flask level. The RSM data obtained was used to construct artificial neural network linked genetic algorithm (ANN-GA) addressing the non-linear problems of the RSM. The predictive ability of the ANN-GA was higher than RSM and corroborated with the experimental results. Batch fermentation experiment in a bioreactor with the optimized medium resulted in 25.02 g/L biomass, yield coefficient (0.53 g biomass/ g glycerol). Monod’s model was used for fitting, simulated kinetic parameters found to be maximum specific growth rate 0.19 h⁻¹ and Monod’s saturation constant 10.84 g/L.

Keywords: Batch fermentation; DoE; Interferon α2b; *Pichia pastoris*

Selected References:
Mussel-inspired Biocatalytic Membrane for Micro-pollutant Removal

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Abstract
Though a biocatalytic membrane integrating separation, adsorption and catalysis functions, is promising for aquatic micro-pollutant removal, the trade-off between catalytic efficiency and stability limits its application. In this study, the biocatalytic membranes were prepared by ‘reverse filtration’ of laccase and subsequently various mussel-inspired coating strategies: single dopamine (DA) coating, DA/polyethyleneimine (PEI) co-deposition, and DA/Cu$^{2+}$ co-deposition, where a nanofiltration (NF) membrane was used as the matrix. The sandwich structure (skin layer-laccase-support/coating layer) of these biocatalytic membranes endowed their both sides with catalytic ability, which was used to construct a bifacial enzymatic membrane reactor (EMR) for highly efficient micro-pollutant removal (taking bisphenol A (BPA) as an example). Compared with the single DA-coated membrane, the biocatalytic membranes prepared by DA/PEI and DA/Cu$^{2+}$ co-depositions exhibited much better performances in terms of enzyme loading, activity and permeability, as well as the stability of immobilized enzyme. The BPA removal efficiency was highest for the EMR with the DA/Cu$^{2+}$-coated membrane, probably due to the enhanced electron transfer, while it was lowest for the EMR with the DA/PEI-coated membrane because of the high diffusional resistance due to the dense PDA/PEI layer (though it almost eliminated enzyme leakage). It was also found that the BPA removal efficiency decreased with recycle, especially for the EMR with higher initial BPA removal due to product accumulation (i.e. membrane fouling) in the membrane. Finally, the trade-off between BPA removal efficiency and long-term stability was broken by applying the bifacial EMR with DA/Cu$^{2+}$ co-deposition in flow-through mode, since the pressure-induced convective mass transfer improved the contact between substrate and enzyme together with removal of the product.

Keywords: Bifacial EMR; Biocatalytic membrane; Dopamine coating; Enzyme immobilization; Micro-pollutant

Selected References:
Heat Reflux Extraction Technique to Obtain Nicotine Compound from *Nicotiana tabacum* var. Virginia

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**Abstract**

Indonesia is currently the 6th largest tobacco producer countries in the world. It was supplied approximately 2.7% of tobacco in the world. In fact, more than 95% of tobacco was used only as a cigarettes raw material in Indonesia. Nicotine was known an active compound in tobacco plants. It was known also as a neurotoxin compound capable to effectively kill pest, particularly agricultural pest. Therefore, this research was focused on the development of Heat Reflux Extraction (HRE) technique to obtain optimally nicotine compound in tobacco extract. *Nicotiana tabacum* var. Virginia leaves were taken from Ponorogo (East Java, Indonesia). The yields of *N. tabacum* extract from HRE were 10.1±1.3, 16.5±2.5, 23.0±0.4 and 27.3±0.5%, at 2, 4, 6, and 12 h of extraction time, respectively. Nicotines were characterized by HPLC. They were 3.0±0.7, 3.4±1.1, 6.3±1.1 and 5.8±0.4%, respectively. The highest yield of nicotine for the range of 2 to 12 h of HRE was achieved at 6 h.

**Keywords:** Heat reflux extraction; *Nicotiana tabacum* var. Virginia leaves; *N. tabacum* extract; Nicotine compound
Optimization of Integrating Ethanol Production by using Jerusalem Artichoke Stalk

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Abstract
Jerusalem artichoke (JA) is a potential energy crop for biorefinery due to its unique agronomic traits such as resistance to environmental stresses and high biomass yield in marginal lands. Although JA tubers have been explored for inulin extraction and biofuels production, there is little concern on its stalk (JAS). In this article, the pretreatment of JAS by alkaline hydrogen peroxide was optimized using the response surface methodology to improve sugars yield and reduce chemicals usage. Scanning electron microscopy, X-ray diffraction, and thermogravimetric analysis were applied to characterize the structures of the pretreated JAS to evaluate the effectiveness of the pretreatment. Furthermore, the feeding of the pretreated JAS and cellulase was performed for high solid uploading (up to 30%) to increase ethanol titer, and simultaneous saccharification and fermentation with 55.6 g/L ethanol produced, 36.5% more than that produced through separate hydrolysis and fermentation, was validated to be more efficient.

Keywords: Enzymatic hydrolysis; Ethanol fermentation; Jerusalem artichoke stalk; Pretreatment; Response surface methodology
O-BBE-10

Flocculation Control by c-di-GMP Phosphodiesterase Genes in Zymomonas mobilis

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Abstract

Zymomonas mobilis, a facultative anaerobic Gram-negative bacterium, features ED pathway to obtain higher ethanol yield and specific productivity than Saccharomyces cerevisiae. A self-flocculent strain ZM401 mutated from wild type ZM4 exhibits excellent advantages such as cost-effective biomass recovery by sedimentation. However, the mechanism of flocculation remains unclear. A global-wide second message c-di-GMP was investigated, which is modulated by diguanylate cyclases (DGC) and phosphodiesterases (PDE). In many bacteria, it has been demonstrated that elevated level of intracellular c-di-GMP result in biofilm formation and suppressed cell motility. In Z. mobilis, five genes named ZMO1055, ZMO0401, ZMO1487, ZMO1365, and ZMO0919 are responsible for c-di-GMP biosynthesis and degradation. ZMO1055 was identified a single-point mutation in EAL domain (A525V) for PDE in ZM401. Expression of original ZMO1055 from ZM4 in ZM401 resulted in deflocculation, whereas deletion of mutant ZMO1055 in ZM401 promoted aggregation with the flocculation efficiency from 83.2% to 94.6%. Thus, PDE genes negatively contribute to the flocculation phenotype. In order to further confirm this hypothesis, ZMO0401 encoding the same enzyme with ZMO1055 was overexpressed and knocked out in ZM401, which led to deflocculation and increased flocculation, respectively. Overexpressing ZMO1487 with only PDE activity in ZM401 caused the loss of flocculating phenotype. Besides, yhjH from E. coli with the function of PDE was heterologous expressed in ZM401, which led to deflocculation as well. On the other hand, the overexpression of ZMO1365 and ZMO0919 with only DGC activity in ZM401 showed no obvious phenotype change. Based on the performance of all engineering strains and intracellular c-di-GMP quantified by HPLC-MS/MS, it can be concluded that the enhanced functional PDE decreased intracellular c-di-GMP, which consequently weakened the flocculation of ZM401. In this study, the effect of c-di-GMP-associated genes on the flocculation of Z. mobilis ZM401 was confirmed. Up to now, it is the first insight into the regulation of flocculent Z. mobilis, thus further exploration of mechanism underlying flocculation and development of engineered flocculating strains in biorefinery are expected.

Keywords: c-di-GMP; Flocculation; Phosphodiesterase; Zymomonas mobilis
Production of L-Alanyl-L-Glutamine by Recycling E. coli Expressing α-Amino Acid Ester Acyltransferase

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Abstract
L-alanyl-L-glutamine (Ala-Gln), as parenteral nutrition, represents the great application potential in clinic, and it is the high solubility, thermostability and high decomposition rate that make Ala-Gln a promising substitution for L-glutamine (Gln). However, a lack of an appropriate synthetic approach that is efficient, safe, and inexpensive limits the comprehensive applications of Ala-Gln at present. In this study, the fermentation process of producing Ala-Gln was proposed to improve its industrial scale production, which adopted an Escherichia coli strain overexpressing α-amino acid ester acyltransferase (SAET) from Sphingobacterium siyangensis AJ2458. Then, the optimum induction conditions and the optimum reaction conditions were investigated. Under such conditions, the novel recombinant E. coli strain, as an intact cell catalyst, was able to rapidly catalyze L-alanine methyl ester (Ala-OMe) and Gln to synthesize Ala-Gln. Results showed that the concentration and molar yield of Ala-Gln were 367.9 mM and 61.3% from 600 mM Ala-OMe and 600 mM Gln within 30 min, respectively. Similarly, 283.3 mM of Ala-Gln was achieved from 400 mM Ala-OMe and 400 mM Gln within 30 min, and the corresponding molar yield was 70.8%. On this basis, we attempted to develop consecutive batch reaction to produce Ala-Gln. Results indicated that the engineered E. coli strain could maintain high Ala-Gln yields and the stability of enzyme after several cell recycling. Consequently, consecutive batch Ala-Gln reaction by the recombinant E. coli strain with SAET provides the cost-efficient and environmentally friendly approach for its large-scale production, and contributes to its further applications in clinic.

Keywords: α-Amino acid ester acyltransferase; Cell recycle; Cost-efficient; Fermentation process; L-alanyl-L-glutamine

Selected References:
EB : Environmental Biotechnology
O-EB-01

Rapid and Simple Detection of Arsenic in Water and Soil Sample using Molecular Sensor under Neutral pH

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Abstract
Arsenic is often found as a result of nature and human activity especially tin mining where arsenic is associated with sulfide-rich waste. Arsenic derivatives such as arsenate (As (V)) is easily dissolve in water especially under low pH, thus contamination of arsenate in water and soil samples have serious effects on human health inducing cardiovascular disease, neurodegenerative disease and skin cancer. Hence, a simple and rapid detection of arsenate in water and soil samples is of important and urgently required. This study focuses on the fluorescent detection of arsenate compound in water and soil samples using a molecular sensor comprised of acridine moiety as a fluorophore and two dipicolylamine (Dpa) units complexed with Zn(II) as a bidentate binding site. The binding mechanism of the complex sensor to arsenate is via coordination chemistry and the binding capacity is comparatively high with an apparent binding constant ($K_{\text{app}}$) of $8.3 \times 10^5$ M$^{-1}$. The sensor demonstrated a good selectivity over several anions such as sulfate, acetate, iodide, bromide, chloride, pyrophosphate, phosphate and ATP. These results allow us to detect arsenate contamination in a water and soil samples with the detection limit of 10 µM arsenate and the sample size of only 4-30 µL. This newly developed molecular sensors method for fluorescent detection of arsenic will be a versatile and useful tool for the detection of arsenate contamination on site where the concentration of arsenic is dynamic due to the season, human and natural activities.

Keywords: Arsenic; Detection; Fluorescence; Molecular sensor; Soil; Water

Selected References:
O-EB-02

New Tools and Candidate Genes for Enhancing Nitrogen Biofertilizer Potential of the Cyanobacterium *Anabaena* in Stressful Environments

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Abstract

As a naturally abundant, photosynthetic, nitrogen-fixing microbe, the cyanobacterium *Anabaena* contributes significantly to the nitrogen and carbon economy of tropical soils, especially in cultivation of rice paddy. However, its nitrogen biofertilizer potential is sensitive to common agricultural abiotic stresses. Engineering enhanced stress tolerance capabilities in this microbe through genetic manipulation is desirable, but is seriously limited by the availability of appropriate tools and techniques and knowledge of suitable candidate genes. In recent years, our laboratory has succeeded in (a) devising an electroporation protocol for genetic transformation that achieves good frequency gene transfer and overcomes problems associated with the current practice of triparental conjugation between *E. coli* strains and *Anabaena* (b) constructing a novel integrative expression vector pFPN, that localizes desired gene at a defined locus in *Anabaena* genome and facilitates its high level expression from an eco-friendly light-inducible promoter, and (c) identified several genes responsible for enhanced heterocyst formation and nitrogen fixation (*hetR*), chaperones (*groESL, cpn60*) for protein folding and homeostasis, and several oxidative stress tolerance genes (superoxide dismutase, catalases and peroxiredoxins) which confer superior stress tolerance to *Anabaena*. The approach has proved very useful for constructing recombinant *Anabaena* strains capable of nitrogen fixation in stressful environments.

Keywords: *Anabaena*; Eco-friendly biotechnology; N$_2$ fixation; Stress tolerance

Selected References:
Development of in situ Petroleum Bioremediation Strategy with Biosurfactant Producing Hydrocarbon Degrading Bacteria from Refinery Waste

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Abstract
Natural ecosystems worldwide are threatened by petroleum hydrocarbon pollution. Sustainable remediation of these hazardous contaminants is a prime technological impediment. Ubiquity of numerous hydrocarbonoclastic microorganisms in petroleum rich environments provides scope for developing effective, economic and environment friendly bioremediation strategies. In order to understand the role of microorganisms in hydrocarbon degradation and to devise microbe based bioremediation technology the present study was undertaken. Bacterial strains belonging to genera *Burkholderia*, *Enterobacter*, *Pandoraea*, *Brevundimonas* and *Kocuria* were isolated following enrichment from Digboi petroleum refinery (IOCL, Digboi, Assam) waste and their candidature as bioaugmentation agent for refinery waste remediation was assessed through microcosms based studies. Strains showed superior growth and degradation (>65%) of different hydrocarbon substrates (dodecane, hexadecane, etc.) (100 mM) as sole carbon source and tolerance to wide ranges of pH (3.0-9.0), temperature (5-45 °C) and toxic heavy metals (As, Cd, Co, Pb, and Ni). Concomitant with hydrocarbon utilization, *Burkholderia*, *Enterobacter* and *Pandoraea* strains also produced copious amounts (E24>50%) of biosurfactant (BS). Subsequently a bacterial consortium was formulated using the strains affiliated to genera *Burkholderia*, *Enterobacter* and *Pandoraea*. Eleven microcosms containing the consortium, extracted crude biosurfactant and different combinations of other nutrient supplementation (nitrate, phosphate or yeast extract) were set up. Appreciable attenuation (>100 g/Kg) of total petroleum hydrocarbons and reduction in nitrate (40%) and phosphate (63.6%) concentrations was observed in the different amended microcosms within 30 days. The study demonstrated applicability of versatile hydrocarbon degrading bacterial strains and indigenously produced biosurfactant in formulating low cost bioremediation technologies.

Keywords: Biosurfactant; *Burkholderia*; Consortium; Hydrocarbonoclastic; *Pandoraea*
Identification of Microbiomes in Anaerobic Wastewater Treatment Sludge Fed by Different Volatile Acids using 16S rRNA Metagenomics Approach

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Abstract

Anaerobic digestion (AD) can convert organic materials to valuable biogas. However, the accumulation of volatile fatty acid, such as acetate and lactate, can cause pH to drop, destroy methanogens activity, and eventually failure of the digestion process. Therefore, it is essential to reduce acid concentrations in order to solve the problem of anaerobic digestion failure and increase methane production. Previously, we attempted to enhance methanogenic activity of wastewater treatment sludge from a cassava starch factory by feeding acetic acid and lactic acid to enrich acetic acid utilizer (AM) and lactic acid utilizer (LU). The specific methanogenic activities (SMA) of AM and LU samples are 0.43, and 0.20 g COD/g VSS/day, respectively. While changes in SMA were observed, the microbial community structures in samples are still unknown. Therefore, this study aims to characterize and compare microbial communities in different sludge enrichment samples using 16S rRNA metagenomics approach. Our result shows that AM consists of 536 Operation taxonomic units (OTUs), which can be divided into 27.28% Archaea and 72.72% bacteria with a total of 71 genera. On the other hand, LU consists of 468 OTUs making up of 10.15% Archaean and 89.85% bacteria with a total of 60 genera. A higher amount of methanogens was found in AM sample (23%) as compared to LU sample (10%), which is in agreement with our SMA measurements. AM sample is dominated by Methanothrix while Methanothrix and Methanobacterium were found to be the dominant groups in the LU sample. More studies will be carried out to better understand how the changes in microbial population can affect biogas production.

Keywords: 16S rRNA metagenomics; Acetic acid utilizer (AM); Anaerobic digestion; Lactic acid utilizer (LU)
O-EB-06

Identification of CRISPR-Cas Systems of Arthrospira platensis C1 using Bioinformatics Approach

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Abstract

Arthrospira platensis C1 is a cyanobacterium of commercial importance. It has been widely used as food and feed supplement in human and animals, including as colorant in food industry. To improve the production of A. platensis C1, the CRISPR-Cas system could be applied for editing its genome. The CRISPR-Cas system, an adaptive immune system found in bacteria and archaea, has recently been a breakthrough technique for genome editing, since it provides high targeting efficiency. In this work, a pipeline consisting of CRISPRDetect software and other bioinformatics analysis tools was developed for detecting CRISPR-Cas systems in the genome of A. platensis C1. The result reveals the minimum element necessary for the CRISPR-Cas system including CRISPR arrays, direct repeat (DR), spacer sequences, and Cas genes. For the whole genome of A. platensis C1, there are five CRISPR arrays composing 78 repeats and 73 spacers with size ranging from 33 to 37 and 34 to 57 nucleotides, respectively. The discovery of the CRISPR-Cas systems could serve as a basic knowledge of CRISPR-Cas systems in A. platensis C1 that might be a applicable for strain manipulation in the future.

Keywords: Arthrospira platensis C1; Bioinformatics; CRISPR-Cas systems; Immune systems

Selected References:

O-EB-07

Metatranscriptome Analysis Revealed Putative Causative Agents of Aggregated Transformed Microvilli (ATM) in *Penaeus vannamei*

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Abstract

Aggregated transformed microvilli (ATM) has appeared in farmed *Penaeus vannamei* since early mortality syndrome (EMS) outbreaks. Its severe formation can cause white feces syndrome and retarded shrimp growth, which lead to farm losses. However, the ATM causative agent(s) is still unknown, but likely a pathogen(s). To search for these agents, the metatranscriptome analysis of RNA-seq of hepatopancreas from ATM and normal *P. vannamei* were employed. After quality preprocessing of ~33 million RNA-Seq paired-end raw reads, ~4 million high quality reads were obtained for de novo assembly, generating 94,877 contigs (N50 = 1,788 bases), of which, 33,083 contigs were found to be non-shrimp sequences (i.e., transcriptome of the associated microbiota). The result from differentially expressed gene (DEG) analysis revealed that there were 2,010 significantly highly expressed contigs in the ATM sample, in which 912 were predicted as protein encoding genes. Among these 912 genes, several were identified as sequences of putative pathogen origins, being primary candidates for further validation in ATM samples.

Keywords: Aggregated transformed microvilli; Metatranscriptome; RNA-seq
NBB : Nanobiotechnology, Biosensors and Biochips
O-NBB-01

Spores for the Applications of Analytical Chemistry
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Abstract
Bacterial spores have a rigid core, and display remarkable resistance to malnutrition, heat, radiation, chemicals, and desiccation. These properties contribute to their attractiveness as vehicles for cell-based biosensors. We successfully demonstrated that wild-type bacterial spores could be used for the antioxidant capacity and phenol assay, based on the existence of laccase (i.e., CotA protein) on the spore surfaces of Bacillus subtilis and B. amyloliquefaciens. Moreover, a novel class of composites based on spore-based monodisperse microparticles were synthesized and characterized. The versatile composites exhibited a remarkable unique possibility as new biosensor for highly selective and sensitive immunoassay and separation science.

Keywords: Bacillus amyloliquefaciens; Bacillus subtilis; Bacterial spores; Cell-based biosensors

Selected References:

Fig. 1. Application of spore@Zr4+ microspheres for immunoassays.

Fig. 2. Enrichment factor of spore@Fe3+ toward β-casein and BSA mixture at different mass ratios.
Detection of Periodontal Disease Biomarker Protein for an Early Diagnosis of a Periodontitis

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Abstract

Over 80 percent of people in the world are suffering from periodontal diseases. Even though most of them largely preventable, the number of patients is increasing since they cannot get treatments at a proper time. It is because the present diagnostic method can only judge the occurrence of the disease, not discerning real-time activity of diseases, which makes patients’ symptom worse. To overcome limitations of current diagnostic methods for periodontal diseases, we implemented aptamers on aptasensor which can quickly estimate the disease progression by quantitatively measuring the concentration of the biomarker protein for early diagnosis of periodontal diseases. Aptamers which can specifically and sensitively bind to the periodontitis-related molecule were successfully screened by using target immobilization-free Graphene Oxide (GO)-SELEX. For analyzing the specificity and affinity of aptamers to their target, Fluorescence Resonance Energy Transfer (FRET) and Surface Plasmon Resonance (SPR) assays were done. This result suggests a possibility of developing aptamer-based biosensor as a useful point of care diagnostic kit.

Keywords: Aptamer; Periodontal diseases
Synthesis and Characterization of Magnetic Nanoparticle-Graphene Oxide Composites using Coprecipitation and Solvothermal Processes for Cation Removal

Buddhawatchana Suwanphithak\textsuperscript{1}, Kittiwut Kasemwong\textsuperscript{2}, Pakorn Opaprakasit\textsuperscript{1} and Paiboon Sreearunothai\textsuperscript{1}\textsuperscript{*}

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Abstract
In this study, ferrite nanoparticles were attached on graphene oxide (GO) to enable magnetic separation using coprecipitation and solvothermal process. X-ray diffraction (XRD), scanning electron microscope (SEM), vibrating sample magnetometer (VSM), were used to characterize the composites, and examine the attachment of ferrite nanoparticles onto the graphene oxide and the sulfonated graphene oxide (SGO). It was found that ferrite particles have been successfully attached on GO and SGO surfaces. In addition, cation adsorption experiment was carried out to evaluate the potential of using these composites in the cation removal based on contact time, pH, and the adsorption isotherm using Sr(II). The ferrite nanoparticles attached onto GO and SGO composites could be separated and recovered by magnetic separation with the highest cation adsorption capacity attained at pH 8 and contact time of about one hour. These composites show potential for cation removal with rapid separation time.

Keywords: Cation removal; Co-precipitation; Graphene oxide; Magnetic separation; Solvothermal; Sulfonated graphene oxide

Selected References:
O-NBB-04

Production of Nanocellulose from Locally Isolated *Gluconacetobacter* sp. BCZM for Biotechnological Application

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Abstract

Bacterial nanocellulose (BNC) has been characterised as highly versatile biobased renewable material that is biodegradable and nontoxic. The unique properties of BNC such as high crystalinity, water holding capacity, tensile strength and flexibility offers good potential applications in biomedical, health care and the environment. In this study, the BNC was produced by locally isolated bacterium, *Gluconacetobacter* sp. BCZM. using the modified Hestrin and Schramm (HS) medium. The BNC was formed between the surface of the liquid and air of the culture medium in the form of white gelatinous layer. An average yield of 6 g/L under static condition at 30 °C was obtained after successful purification of the BNC gel followed by oven drying at 60 °C to a constant weight. Fourier Transform infrared spectrum (FTIR) analysis of the BNC showed strong absorption peaks at 2900 cm\(^{-1}\) and 3335.36 cm\(^{-1}\) indicating C-H and O-H stretching respectively. Other peaks obtained represent crystalline and amorphous region of pure cellulose. The physical and chemical characteristics of BNC was determined using Scanning Electron microscopy (SEM), X-Ray diffraction (XRD) and Thermogravimetric analysis (TGA). The results strongly implied that *Gluconacetobacter* sp. can potentially be used to produce BNC. Further work is in progress to apply BNC for vascular tissue engineering.

Keywords: Bacteria; Biodegradable, Crystalinity; Nanocellulose; Purification

Selected References:
Proteomic Analysis Reveals the Underlying Mechanisms of Improved Acetic Acid Stress Tolerance by \textit{SET5} Overexpression

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Abstract

Improvement of inhibitor tolerance of \textit{Saccharomyces cerevisiae} benefits efficient cellulosic ethanol production. In our previous study, \textit{SET5} overexpression exerted improved acetic acid tolerance of \textit{S. cerevisiae}. Meanwhile, fermentation efficiency was increased in the \textit{SET5} overexpression mutant in the presence of acetic acid, as well as in corn stalk hydrolysates. To explore the underlying mechanisms of \textit{SET5} overexpression on acetic acid tolerance, proteomic analysis was performed. It was shown that 467 proteins were changed, among which 380 proteins were upregulated and 87 downregulated. Functional category analysis revealed that proteins involved in glycolysis and PP pathway were upregulated, which was conducive to energy supply and precursor supplementation. The upregulation of proteins involved in MAPK and TOR/ROS pathway represented a positive response to inhibitors in strain BSET5. Meanwhile, Set5p overexpression promoted synthesis, processing and transport of proteins, which enable high efficiency and stability of cell growth and metabolism. Finally, four genes, \textit{YPS1}, \textit{RCN2}, \textit{YRO2} and \textit{RTC3} which encode the upregulated proteins in the proteomic analysis were examined for their functions in acetic acid tolerance. Decreased acetic acid tolerance was revealed when these four genes were deleted in BSET5, suggesting that the function of Set5p overexpression is closely related to these four genes. Further studies are ongoing to unveil the detailed mechanisms and explore novel functional genes to improve cellulosic ethanol production using \textit{S. cerevisiae}.

Keywords: Acetic acid tolerance; Proteome; \textit{Saccharomyces cerevisiae}; Set5p

Acknowledgement

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Selected References:

O-SSB-03

Integrative Omics Approach to Studying Hybridization in Carnivorous Pitcher Plants

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Abstract

Hybridization plays a significant role in the evolution and diversification of plants. Natural hybridization in tropical pitcher plants is extensive, which evolved intricate pitcher at leaf tip for insect trapping, resulted in a species-rich carnivorous Nepenthes genus. To investigate the effects of hybridization on biomolecular compositions, we carried out an integrative omics study on pitcher tissue and pitcher fluid of *Nepenthes ampullaria*, *Nepenthes rafflesiana* and their hybrid, *Nepenthes x hookeriana*. They were chosen because of distinct feeding habits with *N. ampullaria* as a detritivore which can utilize nutrient from leaf litter, compared to true carnivores of *N. rafflesiana* and *N. x hookeriana* which only feed on insects. Proteomics informed by transcriptomics (PIT) approach was taken to identify the protein composition in the pitcher fluid, while metabolomics approach for profiling chemical composition of pitcher tissue. This is the first study applying PacBio isoform sequencing (Iso-Seq) on Nepenthes species to generate unprecedented full-length transcriptomes, as well as the first comprehensive metabolite profiling. The hybrid, *N. x hookeriana* shared more similar biomolecular profiles with *N. rafflesiana* than *N. ampullaria*. This is consistent with morphological observation and previous genetic study. Further analysis is on-going to identify novel proteins and/or bioactive compounds in the pitcher.

Keywords: Iso-Seq; Metabolomics; Nepenthes; Proteomics; Transcriptomics

Selected References:


TEB : Tissue Engineering and Biomaterials
Preparation of Nitric Oxide-releasing Photo-crosslinked Electrospun Chitosan Nanofibrous Scaffolds for Bone Tissue Engineering

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Abstract
In this work, sodium nitroprusside-releasing chitosan-based (CS/SNP) nanofibers were fabricated via electrospinning. Prepared CS/SNP nanofibers were capable of sustainably releasing 37 μg SNP/mg for up to 7 days. SNP is known to release nitric oxide (NO), a radical of interest in bone tissue engineering, upon reduction and photo-degradation. NO–releasing nanofibers have been prepared previously by other groups, however their applicability to bone tissue engineering has never been investigated. This work serves to fill this gap. To improve nanofiber stability and mechanical properties, one-step photo-crosslinking of blended CS/SNP nanofibers was carried out by addition of tetraethylene glycol diacrylate (TTEGDA) and 2,2-dimethoxy-2-phenylacetophenone (DMPA), and incorporation of UV irradiation into the electrospinning process. Photo-crosslinked nanofibers were characterized via scanning electron microscopy (SEM), Fourier transform infrared spectroscopy and swelling test. Application of photo-crosslinking was found to significantly improve nanofiber stability in aqueous environments. SEM images revealed that the porous nanofibrous structure could be maintained up to 24 hours. Biocompatibility of CS/SNP nanofibers towards mouse osteoblasts was also significantly improved. Addition of SNP into the nanofibrous scaffolds were found to improve their biocompatibility to osteoblasts and gingival fibroblasts (GF). Cell viability of 7F2 mouse osteoblasts and human GF cells were affected by SNP content in a dose- and time-dependent manner. MTT assays revealed that 7F2 cell viability increased with increasing SNP content, whereas GF cell viability peaked in CS/20% SNP nanofibers. Fluorescence microscope images also revealed that CS/SNP nanofibers improved cell attachment, spreading and proliferation. Osteogenic differentiation and mineralization were also enhanced by the nanofibers, as evidenced by elevated expressions of osteogenic differentiation markers including alkaline phosphatase (ALP), osteopontin (OPN) and calcium. Photo-crosslinked electrospun CS/SNP nanofibers are thus shown to have excellent potential as bone tissue engineering scaffolds.

Keywords: Bone tissue engineering; Chitosan; Nanofiber; Nitric oxide (NO); Sodium nitroprusside (SNP)
O-TEB-02

CRISPR Interference (CRISPRi) System for CHO Cell Engineering and Product Yield Improvement

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Abstract

CHO cell has been widely used for therapeutic protein production. Generation of stable CHO cell line typically requires co-integration of dhfr and foreign gene into chromosomes and subsequent methotrexate (MTX) selection for co-amplification of dhfr and foreign gene. CRISPR interference (CRISPRi) is an emerging system that effectively suppresses gene transcription through the coordination of dCas9 protein and guide RNA (gRNA), but CRISPRi has yet to be explored in CHO cells. Here we first demonstrated the functions of CRISPRi system and proved effective CRISPRi-mediated suppression of dhfr transcription in CHO cells. Using this approach, we generated a CHO cell line with egfp and dhfr co-integrated into the chromosome. The CRISPRi-mediated repression of dhfr, combined with MTX selection, imparted extra selective stress to force CHO cells to co-amplify more copies of dhfr and egfp genes. Compared with the traditional method relying on MTX selection (up to 250 nM), the CRISPRi approach increased the dhfr copy number for ≈3-fold, egfp copy number for ≈3.6-fold and enhanced the EGFP expression for ≈3.8-fold, without appreciable adverse effect on cell growth. We further exploited the CRISPRi approach to enhancing the productivity of granulocyte colony stimulating factor (G-CSF) for ≈2.3-fold. This study demonstrates, for the first time, the application of CRISPRi in CHO cells to enhance recombinant protein production and may pave a new avenue to CHO cell engineering.

Keywords: CHO cell; CRISPRi; Therapeutic protein
AFOB-EFB Joint Session I on “Enzyme/Catalysis”
Abstract
Pyranose 2-oxidase catalyzes the oxidation of aldopyranoses by using molecular oxygen as an electron acceptor to yield the corresponding keto-aldoses and hydrogen peroxide. This enzyme belongs to the Glucose-Methanol-Choline (GMC) oxidoreductase superfamily and contains flavin adenine dinucleotide (FAD) as a cofactor. P2O catalyzes regio-specific oxidation at the C2 position. Here, the hydride transfer reaction from glucose to oxidized flavin catalyzed by pyranose 2-oxidase (P2O) was investigated by density functional theory calculations and transient kinetics. Our findings suggest that the first step of the P2O reaction is a hydride transfer from C2 position of glucose to N5 of the flavin. Then, the proton abstraction occurs by the conserved residue, His548. In fact, the hydride transfer enhances the proton acceptor ability of His548. The computational results are consistent with kinetic studies of variant forms of P2O at residues His167, Thr169, Val546, His548, and Asn593, and kinetic isotope effects and pH-dependence studies of the wild-type enzyme. The interactions around the sugar binding site (Thr169, Gln448, Asp452, Tyr456, Phe474, Val546, His548, and Asn593) are important for dictating the formation of the carbocation intermediate. Our findings also suggest that P2O can convert not only monosaccharides (glucose, galactose, xylose, arabinose, and mannose) but also disaccharides (maltose and sucrose). Therefore, the enzyme is useful for providing a pool of keto-sugar intermediates for synthesis of rare sugars, fine chemicals and drugs. Knowledge obtained from these studies should be useful for industrial applications to produce high value sugars.

Keywords: Density functional theory; Flavin adenine dinucleotide; Glucose-Methanol-Choline (GMC) oxidoreductase superfamily; Pyranose 2-oxidase

Selected Reference:
Adsorption and Covalent Cross Linking with Chitin: Immobilization of Dextranase on a Renewable Organic Polyaminosaccharide

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Abstract

Chitin is a renewable biomaterial with noteworthy applications. It is the main constituent of the crustaceans’ exoskeleton and the second most abundant polyaminosaccharide. Its chemical structure provides multiple possibilities for it to be utilized as a raw material in several bioprocessing applications without devising detrimental effects on the environment. Biodegradable and biocompatible nature of chitin branched out several research opportunities therefore, the main objective of the current research was to utilize chitin as a matrix for immobilization of dextranase. This polyaminosaccharide is composed of β-(1→4) linked 2-acetamido-2-deoxy-β-D-glucose units and because of this unique bioactive structure, a comparative study of enzyme immobilization was designed using two different immobilization mechanism systems. The research outcome of the current study resulted in opening of new prospects for immobilization of α-(1→6)-D-glucan 6-glucanohydrolase (dextranase). This hydrolase has several applications in glycobiology as it catalyzes the endohydrolysis of (1→6)-α-glycosidic linkages in dextran. This biocatalyst was immobilized using adsorption and covalent binding methodologies. The results suggested that although, adsorption of dextranase with chitin was simple, cost effective and efficient but this system failed to increase the reusability of the enzyme multiple times. Comparatively, covalent crosslinking improved both enzyme stability along with increased recycling efficiency. The strong interaction between the active sites of chitin and dextranase also played a vital role in improving thermal stability and activation energy. The anchoring of different molecular weight of dextran at the active site of the immobilized enzyme on chitin was also successfully studied. In conclusion both methods of immobilization using chitin as a suitable matrix could be applicable for the continuous production of different types of isomaltosaccharides.

Keywords: Adsorption; Chitin; Covalent binding; Dextranase; Immobilization
O-Joint I-03

Construction of a New Anchoring Protein System for Yeast Cell-Surface Display by using Bioinformatic Approach

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Abstract
Consolidated bioprocessing (CBP) is a promising breakthrough in low-cost processing of cellulosic biomass in which cellulase and hemicellulase production, enzymatic saccharification, and ethanol fermentation are consolidated into a single process step. Such process is critical to the development of industrial production of ethanol from lignocellulosic biomass. Cell surface display systems and techniques, essential for building up CBP-enabling microorganisms have been developed in the last decade. To establish novel cell surface display system with comparable or better capability comparing with the existing system, the identifying, engineering, and validating the novel anchor proteins is indispensable. Here, based on the combining Bioinformatics and Biotechnology approaches, we have screened a number of potential anchoring proteins from yeast and fungal genomes. Ability to be used as anchoring motif for cell surface display was elucidated by fusing them with the yeast enhanced green fluorescent protein so that the surface localization was achieved by visualization under microscope and by immunofluorescence technique. Finally, the display of cellulase on yeast surface were compared to obtain the novel anchoring motifs.

Keywords: Anchor; Cell surface display; Cellulase; Consolidated bioprocessing; Yeast

Selected References:
High Level Expression of Recombinant Keratinase from Bacillus licheniformis

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Abstract
Keratinases have great importance in eco-friendly dehairing processing of leathers, production of poultry feed and prion degradation. Here, the kerA gene (1,156 bp) encoding keratinase from Bacillus licheniformis MZK05 was cloned into two commercially available vectors, pGEX-6P-2 and pET-30a(+) and expressed in E. coli BL21. Maximum expression of GST-kerA fusion protein was observed after induction with 0.3 mM IPTG for 3 hours. The 58 kDa GST-kerA fusion protein, purified using Glutathione Sepharose and cleavage by PreScission protease produced the KerA protein of about 39 kDa. A 4-fold increase (312 U/ml) in keratinase activity was obtained by recombinant protein when compared to the wild type strain. On the other hand, the activity of His-tagged keratinase, expressed from the recombinant pET-30a(+) vector with 2 mM lactose induction followed by purification by Ni$^{2+}$-NTA resin, was recorded 358 U/ml, 4.5 fold higher than that of the wild type strain. Keratinase productivity was compared between the shake flask and bioreactor cultivation under the comparable conditions in terms of media composition, inoculum volume and physico-chemical parameters. In the bioreactor, elevated productivity for GST-keratinase (48,000 U/L/h) and His-keratinase (62,000 U/L/h) were observed when compared to that of shake flask cultures. The expression of the His-tagged protein was checked using SDS-PAGE and confirmed by western blotting. The protein could therefore be subjected for large scale production in a bioreactor for its technical applications.

Keywords: Bacillus licheniformis MZK05; Bioreactor cultivation; Keratinase

Selected References:
O-Joint I-05

Structural and Enzymatic Characterization of Acetolactate Decarboxylase from *Bacillus subtilis*

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Abstract

Acetoin is an important physiological metabolite as microbial excretion, whose function mainly includes avoiding acification, participating in the regulation of NAD/NADH ratio, and storing carbon. In industry, acetoin is one of the main flavorings and also widely used in cosmetic and chemical synthesis. Acetolactate decarboxylase (ALDC) involves in the well-known anabolism of acetoin, catalyzing (R)- and (S)-enantiomers of acetolactate to generate a single product, (R)-acetoin. As yet rare atomic level structures of ALDC are present despite the enzyme is widely existing in microorganisms, except the ever-reported X-ray crystal structure of ALDC from *Bacillus brevis*. In this work, we solved and reported a 1.8 Å resolution crystal structure of ALDC from *B. subtilis* (B.s.-ALDC). Dimeric assembly is observed in the solved structure, which was consistent with the elution scenario conducted by the molecular filtration. A zinc ion is coordinated by highly conserved histidines (191, 193 and 204), together with conserved glutamic acids (62 and 251). Glycerol was used as a cryoprotectant and was also observed to coordinate to the zinc ion through one oxygen atom. Kinetic studies of B.s.-ALDC using circular dichroism, permitting the conversion of acetolactate to chiral acetoin to be followed with a real-time tracking, revealed a Km value of 20.94 mM and a kcat value of 2.2 s\(^{-1}\). We used both enantiomers of \(\alpha\)-acetolactate as substrates to further investigate the substrate bias of B.s.-ALDC by means of molecular docking and dynamic simulation in silico. The binding free energy of (S)-acetolactate with B.s.-ALDC is about 30 kcal/mol lower than that of (R)-acetolactate, indicating a more stable binding for (S)-acetolactate. We also first characterized the solution structure of B.s.-ALDC by nuclear magnetic resonance (NMR). Using residual dipolar couplings (RDCs) we could show that overall structure of B.s.-ALDC is very similar to the crystal structure.

**Keywords:** Acetoin; Acetolactate decarboxylase; *Bacillus subtilis*; Crystal structure

**Selected References:**

High Production of Genistein Diglucoside Derivative using Cyclodextrin Glycosyltransferase from Paenibacillus macerans

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Abstract

Genistein has been regarded as one important soy isoflavone with multiple health benefits, whereas its applications are limited by the low hydrophilicity. To improve the water solubility of genistein, codon optimized cyclodextrin glycosyltransferase from Paenibacillus macerans was employed for genistein transglycosylation in this study. At least four novel transglycosylation products of genistein were produced and identified by HPLC and LC-MS: genistein monoglucoside, genistein diglucoside, genistein triglucoside and genistein tetraglucoside derivatives. Obviously, the yields of genistein monoglucoside and genistein diglucoside exhibited great superiority compared with other two products. To maximize the yield of genistein diglucoside, various reaction conditions such as genistein dissolvents, glycosyl donors, substrates concentrations and ratios, enzyme concentrations were optimized as well as the reaction pH, temperature and time. Finally the yield of genistein diglucoside was enhanced by 1.5 folds under the optimum reaction system. Our study demonstrates that the production of genistein diglucoside could be specifically enhanced, which is one of the most important genistein derivatives with better water solubility and stability.

Keywords: Co-α-CGTase; Genistein; Genistein diglucoside; Optimization; Solubility

Selected References:
AFB : Agricultural and Food Biotechnology
P-AFB-01

Exploring the Effect of Carbon Sources on Growth and Cordycepin Production in *Cordyceps militaris*

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Abstract

*Cordyceps militaris* is one of *Cordyceps* species which is widely used for cordycepin (3'-deoxyadenosine) production. However, it is hard to produce large amounts of this bioactive compound for industrial production. To explore cellular responses underlying the cordycepin biosynthesis in *C. militaris* strain CMRU01, the effect of the different carbon sources i.e. glucose, sucrose and xylose on growth and cordycepin production was performed using surface liquid cultivation process. Among these three carbon sources tested, sucrose shows to be the best carbon source for both biomass and cordycepin productivities in *C. militaris* in terms of the maximum specific growth rate (0.24 day⁻¹), biomass (0.18 g L⁻¹ day⁻¹) and cordycepin productivities (5.98 mg L⁻¹ day⁻¹). This basic study provides a useful information for further optimizing the cordycepin yields and productivities.

Keywords: Biomass; Carbon sources; Cordycepin; *Cordyceps militaris*
P-AFB-02

In Vitro Growth and Development of Dendrobium sp. Treated with 2-Aza-8-Oxohypoxanthine Forming Lepista sordida

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Abstract
2-aza-8-oxohypoxanthine (AOH), a derivative compound of 2-azahypoxanthine (AHX) was purified and identified from the fairy-ring-forming fungi, Lepista sordida. AHX and its derivatives have been reported to enhance plant growth. Thus, micropropagation of Dendrobium sp. treated with AOH was investigated. Young shoots were cultured on MS medium containing 3% sucrose and 150 mL/L coconut water and kept at 25±2°C for 16/8 h (day/night) for 4 weeks. Then the shoots were transferred to new MS medium containing AOH (0, 1, 3, 5 and 10 mg/L) and commercial plant growth regulator, BAP (1 and 5 mg/L) individually and cultivated for 8 weeks. The result revealed that AOH treatment obviously induced and increased numbers of shoot comparable to control. However, AOH treatment induced the numbers of shoots less than BAP treatment. For Dendrobium plantlets cultivated in medium containing AOH showed higher and healthier shoots than plantlets of control and BAP treatment. For root induction, AOH treatment induced root formation with 100% of explants forming root. Whereas 60% of explants forming root were obtained from the control. Furthermore, reduction of explants forming root in BAP treatment (20-47%) was occurred.

Keywords: 2-aza-8-oxohypoxanthine; Dendrobium sp.; Fairy rings; Lepista sordida; Micropropagation

Selected References:
P-AFB-03

The Effects of Bacterial EPS Produced by *Rhizobium* sp. on *Rynchosystis* PLBs Micropropagation

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Abstract

The effects of extracellular polysaccharide (EPS) produced by *Rhizobium* sp. on micropropagation of *Rynchosystis* protocorm-like bodies (PLBs) were investigated. Various concentrations (0, 0.2, 0.5, 1.0 or 2.0 mg/L) of EPS were supplemented in VW liquid medium containing 10 g/L sucrose and 150 ml/L coconut water for *Rynchosystis* PLB culture. Survival rate of PLBs, dry weight, PLBs diameter, shoot induction rate and shoot length were evaluated at 4th and 8th weeks of culture. The results showed that highest survival rate (80%) was obtained when PLBs were cultured in VW medium containing 0.2 mg/L EPS after culture for 4 weeks. The highest shoot induction rate (60%) was obtained from the medium containing 0.5 mg/L of EPS. *Rhizobium* EPS promoted the shoot elongation and gave the highest shoot length (9.0 mm) when the culture was supplemented with 1.0 mg/L EPS. However, there were no statistical differences on the observation of dry weight and size of PLBs in the medium with or without EPS supplements.

Keywords: Bacterial EPS; Micropropagation; PLBs

Selected References:

P-AFB-04

Development of Ornamental Dwarf *Echinacea* Plants using RNA Interference Technique to Down-regulate Brassinosteroid-biosynthetic Genes

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Abstract

*Echinacea purpurea* is an ornamental plant that is economically important in pharmaceutical trade. Dwarf plants have many advantages in horticulture industry, including denser growth, increasing resistance to damages by wind and rain, and generating valuable ornamental plants for commercial applications. Defects in brassinosteroid (BR) biosynthesis or loss-of-function mutants of BR-biosynthetic genes have been reported to show dwarf phenotypes. In this work, we identified three BR biosynthesis-related genes (*EpDWF3* and *EpDWF4*) from *E. purpurea* and developed transgenic dwarf *Echinacea* plants using RNA interference (RNAi) technique. RNAi vectors (pFGC5941-*EpDWF3* and pFGC5941-*EpDWF4*) were constructed to express hairpin double-stranded RNA and induce sequence-specific RNA silencing. Transgenic *Echinacea* plants were generated by *Agrobacterium*-mediated transformation and regeneration experiments. The presence of RNAi construct and the suppression of *EpDWF3* or *EpDWF4* in transgenic plants were determined by genomic DNA PCR and RT-PCR. The suppression of *EpDWF3* or *EpDWF4* displayed in dwarfed phenotypes, short plant height, and small flower diameter. Our results indicate that the knock-down of BR biosynthesis-related genes by an RNAi technique enables the production of valuable dwarf plants for commercial horticultural or agricultural applications. This work was supported by Korea Institute of Planning and Evaluation for technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agri-Bio Industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (112019-5).

Keywords: Brassinosteroid; Dwarf plant; *Echinacea purpurea*; RNA interference
P-AFB-05

Changes in the Component Contents and Nitric Oxide Production Inhibitory Activity of Japanese Apricot, *Prunus mume*, in the Fruits Maturation Stages

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Abstract

Fruits of Japanese apricot, *Prunus mume*, in various maturation stages are used for traditional foods in Japan, for example pickled plum (umeboshi) and apricot liquor. Because the apricot fruit extract inhibits the excessive nitric oxide (NO) radical production in lipopolysaccharide (LPS)-stimulated RAW264 macrophage cells, it can be expected to have a potential role in preventing chronic inflammation-related diseases. The objective of this study was to determine the optimal maturation stage to show the NO production inhibitory activity during the apricot fruit maturation with 4 representative cultivars in Fukui, i.e. Benisashi, Kensaki, Shinheidayu and Fukudayu. The apricot fruits as well as purees of unripe, ripe, and full-ripe stages were extracted and investigated. As the maturation stage is advanced, the NO production inhibitory activity and total polyphenol contents of all cultivars were decreased. As for organic acid content, citrate was increased in contrast to the decrease of malic acid, and the fruit taste not only increased in acidic but also decreased in bitter along with maturation. In addition, the color values (a*, b*) of apricot puree showed increase in red and yellow and decrease in green and blue corresponding to the color change of the fruits.

Keywords: Anti-inflammatory effect; Component changes during maturation stages; Nitric oxide production; Polyphenol contents
P-AFB-06

Influence of Exopolysaccharide Producing Lactic Acid Bacteria on the Physical and Rheological Properties of Stirred Yogurt

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Abstract

Exopolysaccharides (EPS) are naturally produced by some lactic acid bacteria during fermentation process. EPS are a natural stabilizer that contributes to texture and physical properties of fermented products. The aim of the present work is to investigate the effects of EPS on physical and rheological properties of fat free stirred yogurts. Stirred yogurts made with high EPS producing culture (YF-L812) and low EPS producing culture (YC-380) were fermented at different conditions (37°C/4h, 37°C/6h, 43°C/4h and 43°C/6h). Samples with high EPS incubated at 37°C exhibited significantly higher viscosity than samples with low EPS, but samples made at 43°C did not show difference in viscosity. All yogurts made with high EPS producing culture showed significant lower syneresis. Microstructure of yogurts observed using fluorescent microscopy showed that samples made with high EPS producing culture exhibited clear protein matrix, while microstructure of samples made with low EPS producing culture could not be observed. Storage modulus (G') of YF-L812 was significantly higher than YC-380 samples for all conditions. These results indicated that EPS modified physical and rheological properties of stirred yogurts.

Keywords: Exopolysaccharide; Rheology; Stirred yogurt

Selected Reference:
Acidity and Phenolic Compounds Affecting on Viable Probiotic Cells in Pomegranate Juices

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Abstract
This work was aimed to study on acidity and green tea affecting Lactobacillus plantarum viable cells in pomegranate juices. The experiments were carried out using minimal medium (MM) alone and MM with green tea extract (GTMM) varied at pH 3.5 and 7.0. The probiotic cells, L. plantarum were treated for 3 h at 25°C in each defined medium before inoculating into commercial or fresh squeezed pomegranate juices at initial cell concentration ~10^7 cells/ml. Whereas, the untreated probiotic cells were used as control. The results showed the probiotic cell viability was best in the untreated condition. The viable probiotic cells were highly decreased in commercial pomegranate juices due to their high acidity (~pH 2.8). As compared to the untreated probiotic cells, the treated probiotic cells likely consumed higher phenolic compounds. Not only total phenolic contents were highly decreased in commercial and fresh pomegranate juices by probiotic cells treated with MM and GTMM. But also, the sugar contents in juices were reduced vastly when added with treated probiotic cells. These results indicated that the juice acidity and phenolic compounds could acclimatize favorably probiotic L. plantarum cells in pomegranate juices.

Keywords: Phenolic compounds; Pomegranate; Pretreatment; Probiotic

Selected Reference:
Physical-assisted Alkaline Extraction of Xylan from Rice Straw

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Abstract
Rice straw is an agricultural waste found abundantly in Thailand. Rice straw, a lignocellulosic material, consists of high amount of hemicellulose (26.65%) attractively as raw material for xylan source. The aim of the present work was to evaluate an efficiency of physical-assisted extraction of xylan from rice straw on the recovery yield of xylan. Rice straw was delignified by peracetic acid and treated with alkali KOH (4–18% w/v) with heating at 70 °C was optimized. Afterwards, the delignified rice straw optimally treated with alkali KOH (16% w/v), were combined with microwave irradiation and ultrasonic for xylan extraction, as compared to the conventional heating. Microwave-assisted extraction provided the highest recovery yield of xylan from rice straw at 74.34%, whereas the xylan recovery yield from the conventional and ultrasonic-assisted extractions were 65 and 63%, respectively. In addition, an effect of the microwave power (100–450 W) on the xylan extraction from delignified rice straw was studied. The recovery yield of xylan was maximized at 93.12% by 300-W microwave power for 10 min. Also, the xylan products were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and the result found the xylan characteristic. Potentially, xylan extracted from rice straw was promising as substrate for producing high-value added xylitol and xylooligosaccharides.

Keywords: Alkali potassium hydroxide extraction; Microwave-assisted; Rice straw; Ultrasonic-assisted; Xylan extraction

Selected References:
Production of Manno-oligosaccharides from Copra Meal

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Abstract
Coconut is known for its great versatility as seen in the many uses of its different parts and it is found throughout the tropical area including Thailand. Coconut is part of the daily diet of many people and is grown industrially for the edible. Copra meal is a by-product from coconut oil extract process, and Thailand produced copra meal feed wastes around 23,000 MT annually during 2009-2014. Copra meal is typically rich in galactomannan and linear mannan, which can be used as a substrate to produce manno-oligosaccharides (MOS) by enzymatic hydrolysis using 1,4-β-D-mannanase. In this work, we reported the optimized conditions for the hydrolysis of defatted copra meal using crude recombinant 1,4-β-D-mannanase from Bacillus licheniformis expressed in Lactobacillus plantarum. The bioconversion reactions containing defatted copra meal equivalent to 1, 2, and 4% of total mannan were incubated with 1, 5 and 10 U/ml of 1,4-β-D-mannanase at 50 °C. The products were analyzed by thin layer chromatography (TLC) and high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Our results indicated that the reaction mixture containing defatted copra meal equivalent to 2% total mannan and 5 U/ml of 1,4-β-D-mannanase was the best production of MOS with ~30% conversion yield. The produced MOS exhibited potential prebiotic properties as the ability to promote the growth of probiotic Lactobacillus spp. and Bifidobacterium spp.

Keywords: β-mannanase; Coconut; Copra meal; Manno-oligosaccharides

Selected Reference:
P-AFB-10

Optimization for Antifungal Production by Endophytic Fungi Isolated from Thai Orchid Species

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Abstract
A total of 97 isolates of endophytic fungi were isolated from 20 species of Thai orchids. The dual culture technique showed that isolate CKL19-3 isolated from Ascocentrum curvifolium exhibited the strongest anti-pathogenic fungal activity against Cuvularia sp. and Fusarium sp. The condition optimization including incubation time, medium composition, initial pH and temperature for the production of antifungal compounds from fungal isolate were investigated. The results showed that the optimum conditions for bioactive compound production from CKL19-3 were in potato dextrose broth (PDB) or malt extract broth (MEB) at 25 and 30 °C, pH5 for 4 days.

Keywords: Antimicrobial; Bioactive compounds; Endophytic fungi

Selected References:
P-AFB-11

Construction of One-Step Detection of Aflatoxin B₁ in Agricultural Products using scFv-EmGFP Format

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Abstract
Fluorescence-linked immunosorbent assay (FLISA) has become one of the most efficient analytical methods because it is a less time-consuming technique, with high sensitivity and reliability. This is because the secondary antibody and substrate for conjugated enzyme, necessary for conventional ELISA, can be avoided. In this research, single-chain variable fragment (scFv) antibody against aflatoxin B₁ was engineered to fuse with the Emerald Green Fluorescent Protein (EmGFP) and expressed in different E. coli expression hosts. Our results indicated that E. coli C3029 is the most suitable host, when compared with E. coli C3026 and BL21 (DE3). The IC₅₀ value of scFv-GFP from E. coli C3029 was 0.017 µg/ml. This system has potential to be used for both quantitative and qualitative analysis of aflatoxin B₁ in agricultural products.

Keywords: Aflatoxin B₁; Emerald Green Fluorescent Protein (EmGFP); Enzyme-Linked Immunosorbent Assay (ELISA); Fluorescence-Linked Immunosorbent Assay (FLISA); Single-Chain Variable Fragment (scFv) Antibody

Selected References:
P-AFB-12

Antagonism and Growth Enhancement Potential of *Trichoderma* spp. on *Brassica juncea* var. Marpha (Broad Leaf Mustard)

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Abstract

Microbial isolates from plant associated habitats are being considered as valid alternatives to synthetic pesticides. The aim of this study was to select *Trichoderma* to be added as soil inoculants, as an innovative, economic and sustainable alternative to synthetic fertilizers for plant growth promotion. The isolated *Trichoderma* were characterized by morphological and microscopic analysis. The antagonistic effects of *Trichoderma* isolates were tested against three pathogenic fungi; *Sclerotinia minor*, *Fusarium oxysporum* and *F. solani*. Dual culture technique was employed and percentage of inhibition on mycelial growth of pathogenic fungi was calculated. Isolates BC and KB showed the highest antagonistic effect against *S. minor* by 100%. Isolate DH and TH showed partial suppression of *F. solani* by 64.74% and 70.94%, respectively. Isolates HA and Y restrained *F. oxysporum* by 85.12% and 85.90%, respectively. Hence, BC, KB, DH, TH, HA and Y could be a potential bio-control agent. *In vitro* and *in vivo* growth promotion study was carried out by seed treatment method. Isolate EO exhibited the best effect on root length, shoot length, plant wet weight, plant dry weight, germination percentage and seedling vigour index in *in vitro* condition. However, the greenhouse studies indicated that GS showed the highest shoot length, TH promoted the highest root length and HA showed maximum number of leaves. Significant difference was observed in root length in the greenhouse experiment and plant wet weight in seedling assay at p≤0.05. The results presented in this study reinforce the concept of biological control and plant growth promotion by *Trichoderma*.

Keywords: *Fusarium oxysporum*; *Fusarium solani*; Plant growth promotion; *Sclerotinia minor*; *Trichoderma* spp.

Selected References:
P-AFB-13

Chemistry of Honey Protein and Studies on Its Physicochemical Properties

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Abstract

Honey is a supersaturated solution of sugar and water and is considered as a functional food. It is a high nutritive value food and is a natural food preservative. The composition of honey varies greatly depending on its floral and geographical origin and the minor constituents include enzymes, proteins, amino acids, vitamins, flavonoids, phenolic, and minerals. Though the quantity of protein present in honey is very low, it is rich in nutritional and pharmacological properties. Protein characterization is very important to know about its bioactivity and regarding bioactivity, peptides are more potent bioactive compounds than proteins. Food-derived peptides are potential natural antioxidants without marked adverse effects and are more bioavailable and absorbed as dietary nitrogen in comparison to proteins or free amino acids. Limited heed is paid on the generation of peptides from proteins and evaluation of bioactivity of Indian Monofloral honey protein. Hence the present study was mainly intended to biochemically characterize the physicochemical properties of Indian Monofloral honey (Eucalyptus globulus), and generation of peptides from it. SDS-PAGE analysis of the purified protein confirmed the molecular weight of the protein to be 55 kDa. The protein was identified to be Major Royal Jelly Protein 1. Tryptic digestion of the purified protein resulted in bioactive peptide generation with increased DPPH radical scavenging activity. The isolation of the peptides from protein can be a way for food fortification and answer to serious health problems.

Keywords: Antioxidants; Bioactivity; DPPH activity; Peptide
P-AFB-14

Improved Production of 3’-fucosyllactose in Engineered *Escherichia coli* by Enhanced Expression of α-1,3-fucosyltransferase

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**Abstract**

Fucosyllactoses (FLs) are the major components of the breast milk. FLs have been reported to benefit human health. Especially, 3’-fucosyllactose (3FL) has a lot of health benefits. 3FL is the inhibitors of bacteria and viruses in human epithelial cells. It exhibits anti-adhesive effects on respiratory viruses. It also help the construction of healthy gut ecosystem. 3FL can be produced either by enzymatically or through whole cell biocatalysts. Enzymatic synthesis is economically infeasible because the GDP-L-fucose is very expensive. Therefore, metabolic engineering of microorganism may provide feasible route to mass production of 3FL. Fucosyltransferase (FT) is the key enzyme for microbial synthesis of the 3FL from GDP-L-fucose. However, expression of the FT in *Escherichia coli* (*E. coli*) has many hurdles. For example, α-1, 3-fucosyltransferase can easily form inclusion bodies because it has membrane anchoring region. The low solubility might be the major problem of the low activity of FT. In this study, α-1, 3-fucosyltransferases (1,3FTs) from *Helicobacter pylori* (*H. pylori*) were engineered to improve soluble expression in *E. coli*. Codon usage of 1,3FTs were optimized first. Then, C-terminals of FTs were systematically truncated to induce soluble expression. Because 1,3FTs are composed of catalytic region, heptad repeat (coiled coil), and positive & hydrophobic domain (membrane anchoring). The expression of engineered 1,3FTs were increased by 6.6 times, and the production of 3FL was also enhanced by 8.9 times. This work will become a good starting point for further directed evolution of the enzyme.

**Keywords:** 3’-fucosyllactose; α-1,3-fucosyltransferase; Codon usage; Enzymatic synthesis
P-AFB-16

Extraction and Analysis of Polysaccharides from Hom-Thong Banana Peels at Different Ripening Stages by Microwave-assisted Water

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Abstract
Hom-Thong banana peels have been enormously discarded as agricultural wastes in Pathum Thani province, Thailand due to popular consumption and vast cultivation of this banana variety. Interestingly, fruit peels are technically composed of pectin and cell wall polysaccharides, which have been intensively used in foods and cosmetics as thickening/gelling agents. Thus, this research aimed to extract the polysaccharides from Hom-Thong banana peels at different ripening stages by microwave irradiation with heating banana-peel powder suspended in water. After centrifugation, the supernatants were precipitated with 95% ethanol and dried to the polysaccharide extracts (PE). The results showed that 900-W microwave operated for 3 min provided the extraction yield (EY, % wPE/wpeel) of unripe peels (UP) and ripe peels (RP) were highest at 9.66 and 6.77%, respectively. For total polysaccharide content (TPC, % wpoly/wPE) of PE from UP and RP that were analyzed by using phenol-sulfuric acid method, it revealed that the ripe peels had higher TPC than the ripe peels which both PE were observed the highest TPC as respective 115.92 and 49.97% when it was extracted for 3 min. More than 100% TPC found in UP might be the error from phenolic compound absorbance. These PE, abbreviated as respective UP3 and RP3, were chosen for being qualitatively determined by using FT-IR spectrophotometer and Brookfield laboratory viscometer. The results indicated that UP3 had chemical composition differently from RP3 and commercial orange-peel pectin, however, their apparent viscosity of 1.34, 1.34 and 1.41 cP, respectively were not statistically different. The C-N stretching vibration at 1311 cm⁻¹ was found apparently only in FT-IR spectrogram of UP3, which might be responsible for latex and protein to be degradable at banana-peel ripening stage. Nonetheless, allergenicity of PE will be carried out for applicable use in pharmaceuticals and medicals.

Keywords: Hom-Thong banana peels; Microwave-assisted extraction; Polysaccharides; Ripening stage

Selected References:
Non-digestible Oligosaccharides from Finger Millet (Eleucine coracana)

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Abstract
Non-digestible oligosaccharides (NDOs) are monomeric units (3–20) of saccharides that are setting an immense role in prevention of chronic diseases worldwide. These are obtained from plants sources such as cereal grains (millets, rice, wheat). Finger millets is one of the non-digestible oligosaccharides (NDOs), comprising about 11.5% of NDOs. Different system parameters were optimized for extraction, such as time and temperature, solvent ratio (water: ethanol) and sample concentration (w/v) were determined for extraction of NDOs. Maximum quantity of NDOs were obtained with 0.1% (w/v) sample extracted at 55 °C in 80% ethanol (solvent ratio) for 45 min. Activated charcoal and celite column (1:1) was used for purification of NDOs by varying ethanol concentration (5–50% v/v). The monomeric composition of oligosaccharides are xylose, rhamnose, glucose, galactose and mannose with a functional group of β-linkages. Prebiotic activity score of NDOs was done with probiotic and enteric microbes. Digestibility of the NDOs was also determined by mimicking the digestive system.

Keywords: Finger Millet; NDOs; Prebiotic activity score

Selected References:
In Vitro Evaluation of Prebiotic Properties in Thai Tropical Fruits

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Abstract

Tropical fruits are known having beneficial effects for human health. Non-starch polysaccharides obtained from fruit are potential source of functional prebiotic. This study aimed to evaluate prebiotic properties of Thai tropical fruits. Fourteen samples of selected fruits: banana, pomegranate, white and red dragon fruits, pea eggplant, great morinda, rambutan, tamarind, mangosteen, orange, lychee, bergamot, salak and grape were investigated. All flesh samples were prepared for measuring prebiotic properties. The prebiotic activity using 2 probiotic strains, Lactobacillus fermentum and Enterococcus faecalis, was tested with all fruit samples. The selected fruit which could enhance growths of probiotics were measured for prebiotic index (PI). Fluorescent in situ hybridization technique (FISH) was used to enumerate specific bacteria including Bifidobacterium spp., Clostridium spp., Bacteroides spp., C. coccoides, Eubacterium rectale, Atopobium spp., Lactobacillus spp. and Enterococcus spp. in the fecal culture fermentation. The results showed that three selected tropical fruits comprising of white dragon fruit, grape and great morinda, represented the high values of prebiotic activity (PA) which can stimulate the growths of two probiotics. When evaluation of PI, great morinda has the highest PI amongst all fruits tested. This fruit could stimulate the growths of bifidobacteria and lactobacilli and could suppress the growth of Atopobium spp., C. histolyticum and Bacteroides spp. in human gut model.

Keywords: Gut model; Prebiotic; Thai fruit

Selected References:
P-AFB-19

Analysis of ITS2 and psbA-trnH Sequences of *Annona muricata*

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Abstract

Sequences of ITS2 and psbA-trnH have been widely used in molecular phylogenetic studies and species identification. In this study, young leaves of *Annona muricata* were collected from Chumphon, Trang, Nakhon Ratchasima, Nakhon Si Thamarat, Burirum, Nong Khai, Udon Thani and Prachuap Khiri Khan provinces, Thailand. The ITS2 and psbA-trnH regions were amplified and sequenced. Sequences were aligned using Clustal Omega. The maximum likelihood (ML) and neighbor-joining (NJ) phylogenetic trees were constructed using MEGA 6.0. The results showed that ITS2 sequence variation was observed within and among sampling locations, while very small differences were found in psbA-trnH region. ML and NJ phylogenetic trees of ITS2 sequences showed the same branching pattern in which populations were classified into 3 main groups and 2 separate populations that did not belong to any group. Our results demonstrated that ITS2 sequences of *A. muricata* had significant sequence diversity between populations within and among each geographic location.

Keywords: *Annona muricata*; ITS2; Phylogenetic tree; psbA-trnH
P-AFB-20

Effect of Sodium Benzoate and Potassium Sorbate on the Self-Life of Fine Cut White Rice Noodle

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Abstract

The aim of this study was to determine the effects of sodium benzoate (SB) and potassium sorbate (PS) on the self-life of fine cut white rice noodles. At the beginning of storage, the bacterial count in the noodles with and without adding preservative noodles were up to 1.00-2.90 and 3.44-4.57 log CFU/g, respectively, but fungi were not detected. The noodles with PS (700 and 1,000 ppm) and the combination of PS and SB (600+400 ppm) were stored at room temperature for 8 days. Under the microbiology quality criteria of Department of Medical Sciences, the results showed that the addition of PS at 1,000 ppm prolonged self-life of product up to 4 days, while the other samples had only 2 days shelf-life. For sensory evaluation, the triangle test was performed and revealed that they no significant difference between noodles with 1,000 ppm PS and industrial produced noodle with SB (p≥0.05).

Keywords: Fine cut white rice noodle; Potassium sorbate; Self-life; Sodium benzoate
P-AFB-21

Effect of Low Pressure Plasma on Physico-Chemical and Cooking Properties of Riceberry Brown Rice

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Abstract
Low pressure plasma is one of a novel technology that used for surface modification in the field of food processing. The aim of our research was to improve physico-chemical and cooking properties of riceberry brown rice using low pressure plasma treatment. The grain samples were treated using inductively coupled plasma (ICP) system of low-pressure argon plasma at 4 mbar with 100 W input power and 13.56 MHz radio frequency in a semi-continuous downer reactor. Coil turn of copper electrode was varied at 4, 7 and 10 turns to study the effect of plasma intensity by controlling coil turns per vertical length at a constant value. Number of treatment cycles was also increased up to 7 cycles to investigate the effect of residence time during plasma treatment. After plasma treatment, the hydration behavior of riceberry brown rice had significantly increased. For the optimized condition, the results indicated that the 4 coil turns of copper electrode could reduced the cooking time of riceberry brown rice up to 3 min after being treated for only 4 cycles. Morphological aspects of the surface etching were observed by scanning electron microscope (SEM) and a decrease in contact angles of plasma-treated samples could confirm the better water absorption. This was clearly agreed with a decrease in cooking time of plasma-treated riceberry brown rice. The textural and sensory qualities of riceberry brown rice were improved by plasma treatment.

Keywords: Cooking properties; Physico-chemical properties; Plasma treatment; Riceberry brown rice

Selected References:
Reducing Cooking Time while Preserving Phytochemicals and Antioxidant Capacity of Riceberry Rice by Low Pressure Argon Plasma

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Abstract

Low pressure argon plasma has been beneficial in food areas and biotechnology. In this work, we applied low pressure argon plasma to improve cooking time of riceberry grains. The rice grains were exposed to plasma at various exposure times, while the untreated grains were used as control. Plasma exposure significantly reduced ($p < 0.05$) an optimal cooking time of rice from 29.0 to 25.5 min. Surface etching and porosities in the bran layer were observed under a scanning electron microscope (SEM), which supposed to be affected by plasma exposure. The treatment did not, however, cause any changes to the phytochemicals of riceberry grains. Total antioxidant capacity and total phenolic content of the plasma treated rice were not significantly different ($p < 0.05$) from those of untreated samples.

Keywords: Antioxidant capacity; Cooking time; Low pressure plasma; Riceberry rice; Total phenolic content

Selected References:
AM : Applied Microbiology
Production of Bacterial Cellulose from Byproduct of Sweet Corn Canning Process

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Abstract
The shortage of coconut juice, a major nutrient source for producing cellulose by bacteria, effects on cellulose production by Thai farmers. Liquid byproduct of sweet corn canning process contains fermentable sugars which could be utilized to get higher economic benefits. Therefore, this research aimed to produce bacterial cellulose (BC) from liquid byproduct of sweet corn canning process instead of coconut juice by *Gluconacetobacter xylinus*. The ratio of byproduct of sweet corn to coconut juice for cellulose production was studied. The result revealed that amount of cellulose produced from the ratio 50:50 (w/w) of byproduct of sweet corn to coconut juice with 1% sucrose (w/w) was not significant different from 100% coconut juice. Furthermore, the factors that could affect the cellulose production such as sucrose, ammonium sulfate, magnesium sulfate, acetic acid and production time were investigated using Plackett-Burman design resulting in 8 treatments. The result showed that ammonium sulfate, acetic acid and production time directly effected on the amount of cellulose and water holding capacity. However, SEM ultra-structure of cellulose obtained from the ratio 50:50 showed lower incorporation of cellulose micro-fibril in comparison to cellulose produced from 100% coconut juice. Furthermore, it had lower water holding capacity but higher tensile strength than the cellulose produced from coconut juice as a sole medium. From these findings, the byproduct from sweet corn canning process is a potential substrate to substitute of coconut juice for cellulose production. Further studies on enhancing BC productivity in byproduct of sweet corn canning process and material properties analysis will be the next challenge for production of value added product from food industrial wastes.

Keywords: Bacterial cellulose; Coconut juice; *Gluconacetobacter xylinus*

Selected References:
P-AM-02

Simple and Visual Detection of *Salmonella* using Amino-modified Magnetic Nanoparticles

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Abstract
Detection of pathogens in foods is very important for food safety and quality control. *Salmonella* is recognized as the main cause of foodborne bacterial illness in humans worldwide. Herein, potential applications of amino-modified magnetic nanoparticles (MNPs) for isolation and detection of target DNA from *S. Typhimurium* (TISTR 292) as a model microorganism were studied. Quantitative and qualitative investigations of DNA isolated were determined by spectrophotometry and polymerase chain reaction (PCR) assay using the primers designed to target the *invA* and transcriptional activator *SprB* genes. Spectrophotometric results showed that the isolation of *S. Typhimurium* DNA with amino-modified MNPs and PEG/NaCl buffer provided excellent yields, while the PCR assay gave similar results for all the methods used. For detection of *S. Typhimurium*-specific PCR amplicons, the optical assay based on the flocculation phenomenon of amino-modified MNPs was used. The results obtained can be detected by visualization with the naked eyes. The presence of target amplicons from *S. Typhimurium* as well as several serovars of *Salmonella* yielded visible positive results, while the negative results were observed in the PCR-negative reaction and blank samples. No cross-detection was found when testing with the DNA isolated from non-target bacteria. This approach was applied to detect *Salmonella* in chicken samples, and all positive results were in agreement with the results obtained from the traditional cultivation method. Thus, the use of amino-modified MNPs for the isolation and detection of DNA targeting to *Salmonella* is applicable, simple, cheap, and the results can be visible to the naked eyes without any special instrument.

Keywords: Amino-modified MNPs; Bridging flocculation; DNA isolation; PCR; *Salmonella*; Visual detection

Selected References:
P-AM-03

Screening of Soil Actinomycetes for Bioactive Compounds Against Plant Pathogens

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Abstract

A total of 474 actinobacterial strains were primarily isolated from the rhizospheres of local plants in Trang Province. In terms of primary screening, co-culture method was conducted against some phytopathogenic indicator strains, i.e., Colletotrichum gloeosporioides DoA d0762, C. gloeosporioides DoA c1060, C. capsici DoA c1511, Xanthomonas campestris pv. campestris (Xcc) and Pectobacterium carotovorum subsp. carotovorum (Pcc). Following that, crude extracts from nineteen isolates were obtained by means of organic solvent extraction and subsequently monitored via disk diffusion susceptibility assay to ascertain their inhibitory potency. High-performance liquid chromatography (HPLC) technique was also employed so as to analyze the components within the crude mixtures. Composed of certain bioactive metabolites, selected crude extracts from isolates SWW177, SWW225, SWW368 and SWW455 were purified by chromatographic approaches in accordance with bioassay-guided fractionation prior to characterization by a range of spectroscopic techniques for their chemical structures. Despite there being six isolated known compounds, 1-methoxypyrrrole-2-carboxamide produced by Streptomyces griseocarneus SWW368 was acquired and elucidated as a new antibacterial pyrrole derivative. It, however, will need further improvement and validation before being put into practice.

Keywords: 1-Methoxypyrrrole-2-carboxamide; Antibacterial; Bioassay-guided fractionation; Streptomyces

Selected Reference:
P-AM-04
Assessment of Airborne Bacteria over Bangkok Areas

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Abstract
Exposure to airborne microbes and their by-products can affect human health such as respiratory disorders and other adverse health effects. In this study, seventy samples of total suspended particle (TSP) were collected from the air over Bangkok areas including Bang Khun Thian, Yan Nawa, Huai Khwang, Thon Buri, Din Daeng, Bang Kapi, Wang Thonglang and Lat Phrao during 2012 for diversity assessment of airborne bacteria and estimating the correlation between meteorological factors and the bacterial species detected over Bangkok areas. Genomic DNA of mixed bacterial cultures was extracted and the 16S rRNA gene was amplified by the Polymerase Chain Reaction (PCR). The PCR products were cloned and screened by colony PCR method. The recombinant clones were clustered into operational taxonomic units (OTUs) by the restriction fragment length polymorphism (RFLP) technique. Then, the sequence analysis of the representatives of each OTU was performed. A total of 1,532 positive clones were identified to 34 species within 13 genera of bacteria. These consisted of Acinetobacter, Adhaeribacter, Bacillus, Brevibacillus, Clostridium, Cronobacter, Desulfovibrio, Enterobacter, Escherichia, Geobacillus, Staphylococcus, Shigella and Streptomyces. Bacillus cereus, B. antracis and E.coli were found in common among the studied locations. In addition, most of airborne bacteria detected in this study are pathogen of humans such as B. cereus, B. antracis, E. coli, E. cloacae and S. aureus. The number of bacterial species was not correlated with temperature, the relative humidity and the concentration of particulate matter <10 µm (PM_{10}).

Keywords: Airborne bacteria; Bangkok; Total suspended particle

Selected References:
P-AM-05

Heterologous Expression and Characterization of a Thermostable and pH-tolerant Laccase from Symbiotic Bacteria of Termite Gut

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Abstract
Laccases belong to the group of multicopper oxidase, and are able to oxidize an exceptionally high number of substrates, thus have broad applications in textile, pulp, food and the degradation of lignin. In a previous study, a symbiotic bacteria of Bacillus stratosphericus which exhibit obvious laccase activity against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from the digestive gut of termite. Here the cloning and expression of laccase gene (cotA) as well as characterization of the recombinant laccase were reported. The length of cotA gene was 1533 bp, encoding 510 amino acids. The cotA gene was overexpressed in Escherichia coli JM109 and the recombinant protein was purified by Ni-NTA column and further characterized. The Km and Vmax of the purified recombinant laccase (CotA) was 0.278 mM and 555.55 U/mg, respectively when using ABTS as substrate. The CotA had high optimal temperature at 90°C, and kept 50% of enzyme activity after incubation at 50°C for 180 min. The optimal pH of CotA was 4.5-5.0, but it was alkaline stable. There was still 90% residual enzyme activity after 120 min alkaline treatment at pH11. In addition, CotA has strong decolorization ability to indigo, crystal violet and malachite green, and the decolorization rate is more than 80% after 3 h, indicating the application prospect in dyes decolorization. The present study shows that the laccase gene from symbiotic bacteria of termite is overexpressed in E. coli and the recombinant laccase has multiple properties, like thermo and alkaline stability and dyes decolorization, which facilitate industrial application. This study enrich our understanding of the diversity and versatility of microbes and genes in nature.

Keywords: Bacillus stratosphericus; CotA; Dyes decolorization; Termite gut; Thermo-alkaline laccase
P-AM-06

Utilization of Pineapple Peel Waste Liquid for Production of Biosurfactants by Rhodotorula glutinis BCRC 22360

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Abstract
In this study, the surface tension and emulsification properties of the biosurfactants from the sterilized fermented supernatants (defined as SSNs) of pineapple peel waste liquid fermented by the oleaginous yeast Rhodotorula glutinis BCRC 22360 were investigated. The surface tension (ST) values of SSNs at the first 2 hrs Ft (fermentation time) decreased, and then the values of those during 2~8 hrs Ft increased and then those at last 8 hrs Ft gradually decreased as increasing Ft. The emulsification indices (EIs) of SSNs at the first 4 hrs Ft of fermentation time, respectively, decreased and then all EIs of them increased. During 8-week storage time, the EIs of the 48-hr-Ft and 0-hr-Ft SSNs (defined as 48hSSN and 0hSSN, respectively) gradually decreased as increasing storage time. The EIs of 48hSSN were higher than those of 0hSSN, but the ST values of 0hSSN were higher than those of 48hSSN during exposure to high salinity (12% NaCl). However, the ST values of 48hSSN increased as increasing NaCl concentration and the NaCl concentrations utilized have no appreciable effect on the EIs of 48hSSN. The ST values of 0hSSN were higher than those of 48hSSN within a wide pH range (2–12) except for pH 6, but the EIs of 48hSSN were higher than those of 0hSSN during exposure to the pH < 10 range. The highest EI of 48hSSN was obtained at about pH 5. This study demonstrated that the biosurfactants produced from waste liquid of pineapple peel fermented by the oleaginous yeast R. glutinis BCRC 22360 are worth of developing the impending applications in a variety of industries such as environmental, food, and cosmetic industries in the future.

Keywords: Biosurfactants; Emulsification; Pineapple peel waste liquid; Rhodotorula glutinis; Surface tension
P-AM-07

Anti-acne Activity of Water Extracts from Sanguisorba officinalis

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Abstract
The present study was conducted to evaluate antimicrobial activity of Sanguisorba officinalis L. (Rosaceae) root against an etiologic pathogen of acne vulgaris. Initially, cold water extract (CWE) and hot water extract (HWE), respectively, were evaluated against acne causing bacteria Propionibacterium acnes for their in vitro antimicrobial activity, using agar disc diffusion method. The zone inhibition (cm) against P. acnes of these extracts at 500 μg/μL was 3.2 x 3.0 (CWE) and 3.4 x 3.5 (HWE), while kanamycin (positive control) was 3.1 x 3.0 at 1 μg/μL. After filtration, each extract was re-extracted successively with hexane (fraction 1), ethyl acetate (fraction 2) and butanol (fraction 3) using separate funnel. The ethyl acetate faction in both CWE and HWE showed anti-microbial activity and the active fraction was analyzed by TLC and HPLC for the identification of the phenolic acids and flavonoids. The most abundant phenolic acids were ferulic acid and caffeic acid, while the most abundant flavonoids were quercetin and kaempferol.

Keywords: Antimicrobial activity; Cold water extract; Hot water extract; Propionibacterium acnes; Sanguisorba officinalis L. root
Chemically Induced Bacterial Ghosts as Efficient INS-1 Gene DNA Delivery Vehicle in vitro

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Abstract

In this study, we used chemically induced bacterial ghosts (BGs) from Salmonella typhimurium for the first time as a delivery carrier for murine INS-1 gene fused with red fluorescence protein (RFP) gene to murine macrophages. Real time PCR analysis confirmed that BGs loaded with pIRES2-DsRed2 plasmid carrying the INS-1 gene. In vitro transfection studies showed that the S. typhimurium ghosts (STGs) more efficiently delivered the pIRES2-DsRed2 plasmid within the macrophages. Most importantly, STGs loaded with pIRES2-DsRed2 plasmid carrying INS-1 gene showed higher expression level of INS-1 than plasmid itself. In conclusion, our findings demonstrated that chemically induced STGs could be used as a delivery vehicle in gene therapy.

Keywords: Bacterial ghosts (BGs); Gene therapy, Murine INS-1 gene; pIRES2-DsRed2 plasmid; Salmonella typhimurium
P-AM-09

Diversity of Antibiotic Resistant Diarrheagenic *Escherichia coli* of Phylogroup B1 Isolated from Communal Source Water and Household Water

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Abstract

There are six pathotypes of *Escherichia coli* causing diarrheal diseases and fall into four phylogenetic groups (A, B1, B2, and D). Strains of B1 can cause intra-intestinal infections. A total of 64 *E. coli* isolates of B1 phylogroup were obtained of which 28 were from household drinking water and 36 isolates were from communal source water from Arichpur (a low income area), Tongi, Bangladesh. We analyzed and compared the antibiotic susceptibility of isolates to 12 different antibiotics for the presence of virulence genes (*estB, eltA, vt1, vt2, eaeA, ial, pCVD, bfpA and ipaH*). Of the 36 communal source water isolates ETEC was the most prevalent pathotype 62% (*n=22*), followed by EPEC 20% (*n=7*), EHEC 6% (*n=2*) and EIEC 3% (*n=1*). Accordingly, 28 household drinking water isolates, ETEC was most prevalent 68% (*n=19*) followed by EHEC 15% (*n=4*), EPEC 8% (*n=2*), and EAEC 4% (*n=1*). Thirty one percent of the isolates (*n = 19*) were resistant to more than three classes of antibiotics of which 37% isolates (*n= 13*) were from communal source water and 22 of isolates (*n=6*) were from household drinking water. Results of fingerprint revealed that at 95% level of similarity, communal source water and household drinking water isolates were distributed in 15 genotypes by BOX-PCR, 27 genotypes by REP-PCR, 34 genotypes by ERIC-PCR and 35 genotypes by RAPD-PCR method. The finding of the study revealed that pathogenic multi-drug resistant *E. coli* may act as an important reservoir of genetic determinants of antimicrobial resistance and can easily be transferred to other microorganisms in the environment through horizontal gene transfer which has important public health implications.

Keywords: Antibiotic resistance; Drinking water; Pathogenic *E. coli*; Phylogroup B1

Selected References:


Prevalence of Diarrheagenic Escherichia coli in Case Household Environment in Bangladesh

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Abstract
Diarrheagenic Escherichia coli has been contributed a significant role to the global burden of diarrheal diseases. In this study, the prevalence of diarrheagenic E. coli was studied in households of diarrhea patients in Arichpur, Dhaka city, Bangladesh. During 4 months period, 40 rectal swabs from patients in 32 different households and swabs from 4 spots (cutting knife, latrine door knob, drinking glass and food plate swab), food and drinking water samples were collected from each household. Direct DNA samples were examined for virulence genes characteristic of enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), enteroinvasive E. coli (EIEC) and cytolethal distending toxigenic E. coli (CTEC) by PCR. The cultured E. coli strains were analyzed for virulence typing and multilocus sequence typing (MLST). The presence of the virulence genes of diarrheagenic E. coli were detected in 33% (13 of 40) rectal swab samples, 53% (74 of 140) household swab samples, 8% (3 of 37) food samples and 6% (2 of 34) water samples in PCR analysis of direct DNA. Among 50 E. coli isolates from rectal swabs and environmental samples, 20% (10 out of 50) strains were diarrheagenic (3 EAEC, 2 ETEC, 1 EPEC, 1 EHEC, 3 CTEC). MLST analysis of the toxigenic strains showed multiple STs (Sequence Type) with most dominant type was ST 10 (2 strains) and 4 strains showed various novel STs. The phylodynamic tree constructed by MLST data showed that 9 toxigenic strains clustered with the clinical diarrheagenic database strains. The data suggesting high-risk areas for diarrheagenic E. coli contamination within case household environment emphasizes designing interventions for in-house sanitation and hygiene infrastructure in Bangladesh.

Keywords: Bangladesh; Diarrhea; E. coli

Selected References:
Formulation of Lipid Production Medium for Microalgal Cultivation

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Abstract
Microalgal lipids could be commercially used as food and biofuel, since its rapid growth potentially providing high lipid productivity. As autotrophic cultivation suitably enhances microalgal biomass and lipid accumulation, however, optimal production medium for microalgal cultivation is extremely essential. Formulation of lipid production medium for microalgal cultivation was conducted by using Plackett-Burman experimental design. A model microalga, \textit{Ankistrodesmus} sp. IFRPD 1061 was cultured in BG-11 medium varied nutrient compositions, carried out in algal chamber with controlled light intensity at 12 Klux, 30 °C and light-dark cycles of 16:8 h. Air mixed 2% carbon dioxide was fed continuously at a flow rate of 0.67vvm through a PTFE membrane filter. Under optimal conditions, lipid productivity of \textit{Ankistrodesmus} sp. IFRPD 1061 was maximized at 108 mg L\textsuperscript{-1} d\textsuperscript{-1}.

Keywords: Cultivation; Lipid; Microalgae; Nutrient

Selected References:
Enhanced Cellulolytic Enzyme Production by Fungal Co-cultivation

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Abstract
Cellulolytic enzyme is important to lignocellulosic biomass processing for bioethanol and high valued biomaterials. This enzyme is a complex system composed of endoglucanase, exoglucanase and β-glucosidase, which perform synergistically to hydrolyze cellulosic substrate into glucose. However, the cost of these enzymes is considered high, resulting in a significant problem to the commercialization of bioethanol. Thus, an efficient and cost-effective enzyme system should have high titer of cellulases and contain balance of these enzymes. The aim of the present work was not only to isolate filamentous fungi isolated from soil and decaying wood in the northern and north-eastern Thailand, with the potential to have high cellulolytic enzyme activities, but also to investigate the co-cultivation of the selected promising fungal strains for an efficient production of cellulolytic enzyme. The screening results on carboxymethyl cellulose (CMC) and avicel agar plates revealed that 6 of 635 fungal isolates identified as Athelia rolfsii, Penicillium simplicissimum, Curvularia eragrostidis, Aspergillus sp., Lasiodiplodia theobromae and Mucor sp., showed the highest activity of endoglucanase and avicelase. Pairwise combinations of six fungal isolates were grown on Potato Dextrose Agar (PDA) and CMC agar plate to study the compatibility of each co-cultivation. Solid-state fermentation of rice bran supplemented with rice husk using monoculture and selected fungal co-culture was performed, and initial moisture content was optimized in culture flasks. Cellulolytic enzyme activities of each fungal co-cultivation will be analyzed and further discussed.

Keywords: Cellulolytic enzyme; Co-cultivation; Fungi; Solid-state fermentation
P-AM-13

Isolation and Identification of Actinomycete Degrading Polylactic Acid (PLA) Packaging

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Abstract

Nowadays polylactic acid (PLA) is used extensively from the viewpoint of environmental concern and good practice in solid-waste management, as food packaging abundantly used in food industry. Unfortunately, the study on biodegradation of PLA packaging is very limited. In this work, isolation and identification of actinomycetes degrading PLA packaging were carried out. These actinomycetes were intentionally isolated from various sources, i.e. liquid biofertilizers, botanical garden soils, cattle pen soils, composts and garbage dump soils. Those microbes grown in basal medium containing 1.0 g/L PLA packaging as sole carbon source were selected after 5 passages transferring in liquid broth. Among them, the isolate KKU215 gave the highest cell density after 4-week incubation. The weight loss of PLA packaging which degraded by the isolate was 2.65%. The results from scanning electron microscopy analysis indicated that the isolate KKU215 could clearly degrade PLA packaging. The degradation of pure PLA by KKU215 as clear zone formation on emulsified PLA agar plate was observed. The 16S rDNA gene sequence of the isolate KKU215 was related to the genus Streptomyces, and was shown 99.31% similarity with S. tendae and S. violaceorubidus. Thus, the isolate KKU215 was assigned as Streptomyces sp. KKU215. Up to date, there is no report of relevant Streptomyces species capable of degrading PLA. Therefore, Streptomyces sp. KKU215 will be a promising strain for use in biodegradation of PLA packaging.

Keywords: 16S rDNA; Degradation; Polylactic acid (PLA) packaging; Streptomyces

Selected References:
P-AM-14

Enzymatic Digestion of Food Waste and Lactic Acid Production from Food Waste Hydrolysate by Thermotolerant Bacillus sp. NF11

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Abstract
Lactic acid is organic acid that is important for various applications in food, pharmaceutical, and cosmetic industries. At present, lactic acid is used as a monomer for poly-lactic acid (PLA) production and used as the raw material of biodegradable plastic production. For industrial process, cost of lactic acid production depends on raw materials, fermentation and downstream process. In this study, food waste from Khon Kaen University canteen was used for decreasing raw material costs. Optimization of glucoamylase loading for food waste digestion was studied. The results show that glucoamylase loading at 50, 100 and 150 U g⁻¹ of total solid could produce approximately 90 g l⁻¹ of reducing sugars. Therefore, 50 U g⁻¹ total solid of glucoamylase was used as an optimum dosage for food waste digestion. Optimization of fermentation conditions for lactic acid production by Bacillus sp. NF11 were conducted by Central Composite Design (CCD). The initial pH and yeast extract concentration were optimized. According to the results, the optimum initial pH and yeast extract concentration for lactic acid production at 50 °C were 6.37 and 3.51%, respectively. Optimization of aeration method by agitation was also examined. It was found that shaking at 150 rpm for 1 minute every 24 hours showed the best result. The final lactic acid concentration and % yield of 90.1 g l⁻¹ and 98.4 % were obtained after incubating for 3 days when the optimum condition was used. These results suggested that the canteen food waste could be used for lactic acid production by thermotolerant Bacillus sp. NF11.

Keywords: Bacillus sp.; Food waste hydrolysate; Lactic acid production

Selected References:
Optimization of Chitosanase Production by a Newly Isolated Lentzea sp. OUR-II in Submerged Fermentation

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Abstract
Chitosanases are widely produced by various microorganisms such as bacteria, fungi and cyanobacteria. However, low enzyme activity and cost of production are challenging tasks for production of chitosanases at industrial scale. This study aimed to optimize cultivation conditions of newly isolated Lentzea sp. OUR-II, a mesophilic actinomycetes, using shrimp shell waste as an inexpensive substrate. The highest chitosanase activity was obtained using 1.5% (w/v) shrimp shell powder as carbon source supplemented with 0.05% of chitosan powder as inducer, 0.1% (NH₄)₂SO₄ as nitrogen source in medium with initial pH 5.0 and 5% inoculum size. Under this optimal condition, the production of chitosanase increased more than 17-fold after 5 days of cultivation at 30 °C. Interestingly, crude enzyme of Lentzea sp. OUR-II presented both of endo- and exo-chitosanolytic enzymes that exploited in production of chito-oligosaccharide and glucosamine.

Keywords: Chitosan; Chitosanase; Lentzea sp.; Shrimp shell waste

Selected References:
Human scFv Antibody against Propionibacterium acnes

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Abstract
The acronym scFv stands for recombinant single-chain fragments variable in which the V_H and V_L domains of an antibody are joined by a peptide linker and expressed as a single polypeptide chain of 25-30 kDa. The scFv libraries have been used to identify the antibodies of interest which are then applied to several antibody related research purposes. The aim of this research is to find scFv antibodies against Propionibacterium acnes which is acnes-associated anaerobic Gram-positive bacteria. An in-house naive human phage-displayed scFv library containing approximately 5x10^8 antibody structures was used for biopanning against boiled P. acnes preparation. The recombinant clones were affinity selected and the specific binding was confirmed by phage ELISA. Three scFv clones, designated as yPac1A8, yPac1E4 and yPac1E7, were obtained after one round of panning. After that, the soluble scFv was produced by infecting the phage into non-suppressor Escherichia coli strain HB2151. For large-scale expression, the scFv gene from phagemid vector was sub-cloned into pET21d+ expression vector and expressed in SHuffle E. coli strain C3029. The expressed scFv from cell lysate was purified by immobilized metal affinity chromatography (IMAC). The specific binding of purified scFv to whole cells of P. acnes was demonstrated by ELISA. Out of three clones, clone yPac1A8 showed the highest binding signal. This clone will be used as a model scFv to conjugate with nanoparticles in order to widen the scope of scFv application in future and the resulting conjugates are expected to be useful in anti-acnes preparations as well.

Keywords: Biopanning; ELISA; Escherichia coli; Propionibacterium acnes, scFv; SHuffle

Selected References
P-AM-17

The Development of Tubular Photobioreactor for *Spirulina Platensis* Cultivation

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Abstract
Tubular photobioreactor proposes the cultivation potential of *Spirulina platensis*. However, the drawback design from low current flow was occurred around dead zone area. Therefore, the purpose of this research was to build a model for cultivation of *S. platensis* at 105 liters, 25% of the area exhibition. The low current and swirling flows could increase the light expose area. This work was separated into 3 parts. First part was a proposed model. Then, FLUENT software was used to simulate the flow behavior inside the prototype Tubular Photobioreactor. This method confirmed that the dead zone can be reduced by diminishing the size of the reactor. The next step was to build Tubular Photobioreactor pilot scale and the final stage was the cultivation of *S. platensis* using Zarrouk Medium nutrition as sodiumbicarbon source. The air inlet velocity was carried out at 0.07 m/s, the duration between the dark and light of 16:8 hours, the light intensity of 8900 lux, ambient temperatures between 35-40°C, and pH set between 8.5 to 11. The dry weight determination method was measured the growth of *S. platensis* to find the maximum biomass by comparing between Optimal Tubular Photobioreactor and Basis Tubular Photobioreactor. The result shows that the proposed model of Optimal Tubular Photobioreactor had area of low current flow, leading to the biomass up to 25%.

Keywords: CFD; Computational Fluid Dynamics; *Spirulina platensis*; Tubular photobioreactor

Selected Reference:
Elucidation of Dimorphic Mechanism by Intracellular cAMP on *Mucor circinellides*

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**Abstract**

Dimorphism fungi changes reversely yeast-filamentous form as morphology by affected in environmental conditions such as temperature, CO₂, etc. The pathogenic fungi *Candida albicans* is the most famous dimorphism fungal, commonly grow as yeast-like cell, and however becomes filamentous form that has a pathogenic in the presence of serum. Zygomycota *Mucor circinelloides* is the nonpathogenic dimorphic fungi that grow as filamentous form aerobically or yeast-like cell anaerobically. The dimorphic mechanism is however unclarified on morphology change concerned to metabolism and gene expression. In this research, the dimorphic mechanism of *M. circinelloides* J was revealed by analyzing of gene expressions on signaling pathway related to cAMP synthesis under aerobic and anaerobic conditions. To obtain genes in the cell cultured aerobically and anaerobically (N₂:CO₂ = 7:3), cultivations were started by adding yeast-like cell. In aerobic condition, all cells became completely the filamentous forms for 24 h. In contrast, in anaerobic conditions the cell has been kept yeast-like cell form through the culture. The expression of adenylyl cyclase gene in yeast-like cell higher than that of filamentous cell. Different of phosphodiesterase gene expression was able not to detect in two conditions. In fact, intracellular cAMP amount in yeast-like cell was about 2-fold higher than that in filamentous cell. Moreover, in the addition of papaverine as a PDE inhibitor to medium, as a result that intracellular cAMP was kept at higher level, filamentous growth was inhibited through the cultures. Conclusively, it was suggested that dimorphism mechanism of *M. circinelloides* was controlled by accumulating intracellular cAMP.

**Keywords:** cAMP; Dimorphism; *Mucor circinelloides*
Lignolytic Enzyme Complex as Alternative Novel Fast-acting Skin Lightning Agent through Bleaching of Melanin

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Abstract
Melanin is a black or brown phenolic polymer present mainly in skin and hair. It has biological function, which include protecting tissues from harmful ultraviolet, thermoregulation, cation chelators and antibiotics. Skin whitening is one of most active researching agenda in cosmetic industry area through melanin decomposition. Peroxidase use hydrogen peroxide to make oxidative radicalization of phenolic compound whereas laccase use oxygen. The use of mini cellulosome (mCbpA) based enzyme complex systems from Clostridium sp. is one of advanced strategies for greater potential through a highly ordered structural organization that enables enzyme complementary effect. Laccase (CueO) from Escherichia coli and dye-decolorizing peroxidase (DyP) from Bacillus subtilis are fused with dockerin module using fusion PCR protocol these chimeric enzymes was named as cCueO and cDyP. Functional enzyme complexes containing cCueO and cDyP, which has complementary effect, degraded melanin succesfully. To confirm that an enzyme complex was formed, cellulose-binding module (CBM) was used in mCbpA was used. Dockerin attached cCueO, cDyP and scaffoldin was purified by CBM purification method and confirmed by SDS-PAGE. This assembled complex caused a significant increase in the level of melanin degradation with 18.1 mg/mL decrement and this result is approximately 1.9-fold higher than single laccase. Developed enzyme complex system may exhibits much greater degradative potential in skin whitening area. Based on this result, this recombinant enzyme complex is suitable for next whitening agent in skin cosmetics industry.

Keywords: Dye-Decolorizing Peroxidase; Enzyme Complex; Laccase; Melanin binding peptide; Melanin biodegradation; Skin lightning agent
Metabolic Engineering for Enhanced Heme Production in Corynebacterium glutamicum

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Abstract
Heme is an essential cofactor of biologically, medically and industrially important hemoproteins such as hemoglobin, myoglobin, cytochrome, catalase and peroxidase and plays an important role in an electron transfer, gas transportation and oxidation reduction reaction. Recently, a variety of studies related to heme have been reported. Heme production has been used to enhance the activity of hemoproteins in Escherichia coli, and the heme biosynthesis pathway in E. coli has been analyzed, and heme including protein has been used for the electron transfer in electrodes. In this study, Corynebacterium glutamicum was engineered for enhanced heme production. Wild-type C. glutamicum was engineered by mutating and expressing two key heterologous genes related to the rate-limiting step, called KUBE1 strain. This recombinant strain was additionally engineered by expressing the transcriptional regulator regulating the expression of heme synthesis-related genes, called KUBE2 strain. These recombinant strains represented a 4.7 and 6.1-fold increase of heme production respectively over wild type strain. To enhance the activity of transcriptional regulator, the iron concentration was optimized during the culture. The recombinant strain in the optimized iron concentration represented a 7.34-fold increase of heme production over wild type strain. We confirmed that the global regulation of heme production by a transcriptional regulator is more useful than by many enzymes, as the heme synthesis pathway is long and has several feedback inhibitions by intermediates and heme. Thus, the engineered C. glutamicum is potential for heme production, and heme can be used as the material for bioelectronics.

Keywords: Corynebacterium glutamicum; Heme; Iron concentration; Metabolic engineering; Transcriptional regulator
Understanding the Role of Lactic Acid Bacteria Derived Exopolysaccharide as Potential Prebiotic Molecule

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Abstract
Beneficial gastrointestinal microbes and their secondary metabolic products modulate immune cells that regulate host health. Exopolysaccharide (EPS), the secondary metabolite are secreted by beneficial microbes that have shown its impact towards improvement of health and increasing potency of food. The quantity and structural integrity of EPS are strain and culture dependent. In the present study the EPS was isolated from Lactobacillus fermentum and its approximate molecular weight was estimated to be 112 kDa. The functional activity of EPS was investigated through its metabolic fate driven by probiotic strain (L. acidophilus MTCC 1030, L. rhamnosus ATCC 7469, L. casei MTCC 5381 and L. plantarum MTCC 2621). EPS showed resistance towards hydrolysis when subjected to stimulated gastric and intestinal juices thus indicating its prebiotic potential. Moreover, showed the anti-pathogenic property and also promoted biofilm formation of probiotic microorganisms. Use of such EPS can certainly improve gut microflora which could lead to better health.

Keywords: Biofilm; Exopolysaccharide; Lactobacillus; Prebiotics; Probiotics

Selected References:
Bioconjugated Ferromagnetic Nanoparticles and Polymerase Chain Reaction for Rapid Detection of *Campylobacter jejuni* in Chicken

Peerapon Chaisalee\(^1\), Kooranee Tuitemwong\(^2\), Wanvisa Poonlapdecha\(^1\), Yortyot Seetang-Nun\(^3\)* and Pravate Tuitemwong\(^1,3\)*

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**Abstract**

*Campylobacter jejuni* is one of the significant causative of foodborne pathogen to human all over the world usually from the consumption of chicken meat and products. The rapid detection of *C. jejuni* at early stages leading to proper prevention is very important. In this study, the development of ferromagnetic nanoparticles (FMNs) for capture and concentration of *C. jejuni* as a target organism, followed by specific duplex PCR - for the detection of *C. jejuni* in chicken was carried out. The FMNs was synthesized using polyol technique under the oxygen free and medium heat treatment in an autoclave (121 °C for 20 min/cycle for 3 cycles). The FMNs have cubic shapes with an average size of about 43 ± 9 nm. The surfaces of FMNs were modified with glutaraldehyde (25%) as a linker, and then conjugated with monoclonal antibodies against *C. jejuni* at 5 µl/ml ratio (Ab-FMN). The duplex PCR assay employed using the 16S rRNA and *hipO* genes was used to confirm the presence of *C. jejuni*. The detection limit of Ab-FMN (at 20 µl/ml) for capturing *C. jejuni* was 10⁴ CFU/ml after PCR analysis in both pure culture and spiked chicken meat without pre-enrichment.

**Keywords:** *Campylobacter jejuni*; Chicken; Duplex PCR; Ferromagnetic nanoparticles; Immunomagnetic separation
P-AM-25

Antifungal Activity Screening and Secondary Metabolite Identification of Endophytic Fungi Isolated from *Annona* spp.

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**Abstract**

A total of 130 endophytic fungi were isolated from leaves of *Annona squamosa*, *A. reticulata* and *A. muricata* collected from Si Sa Ket, Ubon Ratchathani, Buri Ram, Saraburi, Chanthaburi, Samut Prakan, Chumphon, Nakhon Si Thammarat, Nakhon Ratchasima and Trang provinces. Among those isolates, 5 endophytes including Si-sa1-4 (*Fusarium* sp.), Ub-sa3-2 (*Xylaria* sp.), Cp-ss2-2 (sterile mycelium), Br-ss3-6 (*Pestalotiopsis* sp.) and Tr-ss1-13 (*Cladosporium* sp.) showed antifungal activities against postharvest plant pathogens. Growth of *Collectotrichum gloeosporioides* was inhibited by ethyl acetate crude extracts of isolates Si-sa1-4, Ub-sa3-2 and Cp-ss2-2, while growth of *Alternaria alternata* was inhibited by isolates Si-sa1-4, Ub-sa3-2, Cp-ss2-2, Br-ss3-6 and Tr-ss1-13. Bioactive compound identification of the isolate Si-sa1-4 and its host plant, *A. squamosa*, was carried out using GC-MS. Totally, 7 compounds were identified from mycelium crude extract and 4 compounds were identified from crude extract of culture broth. Both endophyte isolate Si-sa1-4 and the host plant produced hexadecenoic acid ethyl ester, a compound associated with antimicrobial activity.

**Keywords:** *Annona* spp.; Antifungal activity; Bioactive compound; Endophytic fungi
BEF : Bioenergy and Biorefinery
“Sustainable Biorefinery for Secondary Products”
P-BEB-01

Ethanol Production using Sugarcane Bagasse after Solid-state Fermentation by Trichoderma reesei Supplemented with Beta-glucosidase

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Abstract
Sugarcane bagasse was pre-treated by 1% H2SO4 followed by 2% NaOH. Each pre-treatment process was carried out at 50°C for 40 min. The pre-treated sugarcane bagasse (PSCB) supplemented with wheat bran in a ratio of 4:1 was used as the substrate for cellulase production by solid state cultivation using Trichoderma reesei RUT C30. The enzyme activity of 11.2 ± 1.3 FPU/g-DS was achieved by the following condition: initial substrate pH 5.0; moisture 40%, temperature 30°C; incubation time 6 days. The cellulase containing PSCB (CC-PSCB) supplemented with or without β-glucosidase was hydrolyzed at 50 °C for 24 h, and then thermotolerant yeast (Kuveromyces marxianus) was added to the solutions for ethanol production at 40 °C. The yield of ethanol was 11.3 % (w/w) from CC-PSCB supplemented without beta-glucosidase and 20.4 % (w/w) from CC-PSCB supplemented with beta-glucosidase. The beta-glucosidase was from a Taiwanese fungus, Chaetomella raphigera. The gene of beta-glucosidase from C. raphigera was isolated and expressed in Pichia pastoris (SMD 1168, developed by Academia Sinica Taiwan). PSCB can be hydrolyzed using Novozyme CTec 2 at 50 °C for 24 h., and the insoluble residue from PSCB was 24 % (w/w). The hydrolyzed PSCB was added with K. marxianus at 40 °C for ethanol production for 4 days. The yield of ethanol from PSCB was 23.3 % (w/w). Direct use of CC-PSCB would eliminate additional enzyme separation processes; thus, reduce the cost of ethanol production. This study indicated that it was possible to use lignocellulosic materials to produce ethanol without using commercial cellulase.

Keywords: Beta-glucosidase; Cellulose; Ethanol production; Kuveromyces marxianus; Sugarcane bagasse

Selected References:
P-BEB-02

Acetic Acid Degradation and Tolerance Response in Biodetoxification Fungus *Amorphotheca resinae* ZN1

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Abstract
Acetic acid is the primary inhibitor generated during lignocellulose pretreatment. Biodetoxification fungus *Amorphotheca resinae* ZN1 demonstrated the extraordinary capacity for tolerance and complete degradation of high concentrated acetic acid. Here we show the detailed tolerance mechanism and degradation pathway of *A. resinae* ZN1 on acetic acid. The substrate priority of acetic acid to glucose and xylose by *A. resinae* ZN1 was identified. We found that the low concentration of glucose and high concentration of xylose in the pretreated lignocellulose biomass is the proper condition of acetic acid degradation while reducing the sugar consumption. Acetic acid conversion by *A. resinae* ZN1 is significantly accelerated when cultured on the solid biomass. The transcriptional analysis reveals that acetic acid is first catabolized to acetyl-CoA, and then assimilated into the tricarboxylic acid (TCA) cycle when it is co-cultured with glucose or xylose, or into both the TCA and glyoxylate cycle when it is the only carbon source. Acetic acid tolerance of *A. resinae* ZN1 involves in pumping protons out of the cytoplasm, activating toxic acetate anions catabolism and efflux, modifying cell membrane compositions, activating potassium ions uptake, ATP biosynthesis, amino acids uptake and biosynthesis. The excellent acetic acid degradation performance of *A. resinae* ZN1 facilitates the detoxification of the dry dilute acid pretreated lignocellulosic materials and the consequent fermentation steps. The elucidation of acetic acid degradation and tolerance of *A. resinae* ZN1 provided the important information for rationally modification of high acetic acid tolerant fermentation strains.

Keywords: Acetic acid; *Amorphotheca resinae* ZN1; Biodegradation; RNA-Seq; Tolerance

Selected References:
Simultaneous Saccharification and Co-fermentation of Holocellulose by a Hybridized Yeast FSC Strain

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Abstract
Fermentation of xylose is currently of great concern in bioethanol production from lignocellulosic biomass. Simultaneous saccharification and co-fermentation (SSCF) of holocellulose, substrate for yeast fermentation of lignocellulose, was studied using a xylose-fermenting yeast FSC strain, hybridized by intergeneric fusion between Saccharomyces cerevisiae and Candida intermedia. In a batch type SSCF of holocellulose composed of microcrystalline cellulose and xylan, an enzyme CTec2 (novozymes) was added setting an enzyme/substrate (E/S) ratio 1/14 g-E/g-S. Before inoculating the FSC seed, a frozen stock of FSC strain was activated by repeating cultivation more than 12 times with fresh culture media containing glucose and xylose. We confirmed that the FSC strain possessed a high ability to utilize xylose in fermentation of a mixture of glucose and xylose. In the SSCF operated at 30°C, ethanol was produced at 8.5 g/L, corresponding to 0.74 as an ethanol yield to an initial mass of substrate. In process of fermentation, cellobiose, xylobiose, xylose and xylitol were accumulated as intermediate products. Effects of SSCF on enzymatic saccharification of holocellulose were evaluated by comparing with simple saccharification without fermentation. Saccharification of holocellulose was greatly improved by SSCF, almost doubled for that of simple saccharification. Products of simple saccharification were cellobiose, xylobiose and xylose as well as glucose.

Keywords: Hybridized FSC Strain; Saccharification and co-fermentation; Xylose fermentation

Selected Reference:
Production of High Concentration of L-lactic Acid from Oil Palm Empty Fruit Bunch by SSF using Thermophilic B. coagulans JI12

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Abstract

Lactic acid with both hydroxyl and carboxylic acid functional groups is an excellent platform molecule for the production of chemicals such as poly lactide, 3, 6-dimethyl-1, 4-dioxane-2,5-dione, 1, 2-propanediol, 2, 3-Pentanedione, acrylic acid and pyruvic acid. The increase in the global demand for lactic acid is expected to increase from 482.7 to 1,076.9 kilo tons from 2010 to 2016, with an annual growth rate of 14.2 %. Currently, lactic acid is produced from glucose produced from starchy materials of food origin, affecting the production cost and food supply. Indonesia and Malaysia are leading producers of palm oil. Every 1 kg of crude palm oil will generate approximately 4 kg of waste biomass and one third of it is empty fruit bunch (EFB). It has been reported that 22 million ton of EFB in Indonesia and 19 million ton of EFB in Malaysia are discarded annually. This large amount of EFB would be an ideal biomass resource for producing lactic acid, which has a huge market demand due to the rapid growth of poly lactic acid (PLA) industry. Thermophilic Bacillus coagulans JI12 was used to ferment hemicellulose hydrolysate obtained by acid hydrolysis of oil palm empty fruit bunch (EFB) at 50°C and pH6, producing 105.4 g/L of L-lactic acid with a productivity of 9.3 g/L/h by fed batch fermentation under unsterilized conditions. Simultaneous saccharification and fermentation (SSF) was performed at pH5.5 and 50 °C to convert both hemicellulose hydrolysate and cellulose-lignin complex in the presence of Cellic Ctec2 cellulases using yeast extract (20 g/L) as the nitrogen source, giving 114.0 g/L of L-lactic acid with a productivity of 5.7 g/L/h. The SSF was also conducted by replacing yeast extract with equal amount of dry Bakers’ yeast, achieving 120.0 g/L of L-lactic acid with a productivity of 4.3 g/L/h. To the best of our knowledge, these lactic acid titers and productivities are the highest ever reported from lignocellulose hydrolysates.

Keywords: Bacillus coagulans JI12; Biomass; Fermentation; L-lactic acid; Lignocellulosic; Saccharification
**P-BEB-05**

**Accelerating Effect of the Crude Drug Extracts on the Ethanol Fermentation by Saccharomyces cerevisiae**

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**Abstract**

The influences of the inhibitor furfural produced during saccharification and the specific substances containing in unknown raw plants that may inhibit fermentation are important to the bioethanol fuel production from inedible resources. In this study, we focused on the promotion / suppression effect caused by the unknown plants, and then the effect of typical crude drug extracts listed in Table 1 on the fermentation of glucose by *Saccharomyces cerevisiae* was investigated. The fermentation rate was monitored by the mass of generated CO₂ on time. As shown in Table 1, it was found that the promotion and suppression effects were varied depending on the kind of crude drugs. Especially, it was noted that the adding of about 1 g/L of *Ephedra* sp. extract accelerated the fermentation rate by about 2-fold comparing with the control. The reason may shorten the generation time of yeast cell, that is, it was shorten from 3.4 h in control to 2.6 h in about 1 g/L of Ephedra extract. However, no effect to total ethanol generation was observed. Further, to demonstrate the antagonism between promotion of Ephedra and suppression of furfural, both were added and fermented. From the result, it was found that Ephedra extract accelerates the fermentation rate in spite of co-existence of furfural. It is expected that the promotion of some crude drugs on the ethanol fermentation rate will contribute to the shorten time in bioethanol production.

**Table 1** Effect of crude drug extracts on ethanol fermentation rate.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Effect</th>
<th>Scientific name</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassia tora</td>
<td>0</td>
<td>Hypericum perforatum</td>
<td>0</td>
</tr>
<tr>
<td>Coptis sp.</td>
<td>–</td>
<td>Phellodendron sp.</td>
<td>–</td>
</tr>
<tr>
<td>Ephedra sp.</td>
<td>++</td>
<td>Poria cocos</td>
<td>+</td>
</tr>
<tr>
<td>Glycyrrhiza sp.</td>
<td>0</td>
<td>Scopolia sp.</td>
<td>+</td>
</tr>
<tr>
<td>Humulus lupulus</td>
<td>0</td>
<td>Scutellaria baicalensis</td>
<td>+</td>
</tr>
</tbody>
</table>

+, promotion; –, suppression; 0, no effect

**Keywords:** Ephedra; Ethanol fermentation; Promotion
Illumination Factors Stimulate Significantly Biomass and Fatty Acid Compositions of *Botryococcus braunii* under Mixotrophic Culture Mode

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**Abstract**

Illumination factors such as light intensity and photoperiod could influence on the growth and lipid content of microalgae. In order to optimize the light intensity and photoperiod cycle, the growth of the freshwater microalga, *Botryococcus braunii* LB572, was examined on the microalgal Jaworski’s medium mixed with oceanic sediments (6:4, v/v) under mixotrophic culture mode. *B. braunii* LB572 was grown in a 10 L photobioreactor aseptically for 14 days at different light intensity (0, 45, 95, 110, and 125 μmol photon/m²/s) and photoperiod (0:24, 4:20, 8:16, 12:12, 8:16, 4:20, and 0:24 h, light:dark) at 25 °C. Fatty acid composition (C14~C22) in cells was changed depending on the light regimes: as light intensity and duration increased up to 110 μmol photon/m²/s and 16:8 h (light/dark), the content of saturated fatty acid increased up to 43.8% (w/w), whereas those of monounsaturated and polyunsaturated fatty acids decreased. Under the same culture condition, *B. braunii* LB572 was found to have a favorable cell growth with a maximum biomass and a lipid production of 8.94 and 4.77 g/L, respectively, for 13 days cultivation. And the total lipid content was found to be 58.1% (w/w).

**Keywords:** Biomass; *Botryococcus braunii*; Fatty acid; Illumination factor; Mixotroph culture mode; Oceanic sediments

**Selected Reference:**
P-BEB-07

The Optimal Conditions for Co-immobilization of Saccharomyces cerevisiae TISTR 5339 and Pichia stipitis TISTR 5806 by Entrapment in Alginate Gel Bead

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Abstract

The optimal conditions for co-immobilization of Saccharomyces cerevisiae TISTR 5339 and Pichia stipitis TISTR 5806 by entrapment in alginate gel bead were investigated. The optimization of co-immobilization was performed using a three factors Box-Behnken design. The parameters affecting the co-immobilization including reducing sugar concentration (glucose and xylose) (3-8 g/L), temperature (30-45 °C), and pH (5-6) were thoroughly evaluated. The results revealed that a quadratic model for the optimization of co-immobilization was significant with insignificant lack of fit. The optimal condition predicted by the model was 8.0 g/L of glucose and 8.0 g/L of xylose, at 37.6 °C and pH 5.2 which 98.8% of sugar was utilized. The verification experiment at the optimal condition exhibited 96.8% of sugar utilization. Therefore, the statistical aberration value between predicted model and the experiment result was 2.0%.

Keywords: Alginate gel bead; Co-immobilization; Pichia stipitis TISTR 5806; Saccharomyces cerevisiae TISTR 5339

Selected References:
Scaling Up Batch Fermentation for Lipids Production of *Rhodococcus opacus* PD630

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Abstract

*Rhodococcus opacus* PD630 was known as its ability to accumulate lipids. Glycerol at the concentration of 100 g/L was used as an inexpensive carbon source with a modified mineral salt medium. In the shake-flask experiments, ammonium acetate, was the nitrogen source and the concentrations were varied at 1.44 and 7.70 g/L, inoculum size was 25% (v/v). The lower acetate concentration (1.44 g/L) got higher biomass and lipid concentrations. Biomass concentration was 3.69 g/L (2 times compared to the other condition, 7.70 g/L) at sixth day, the maximum biomass concentration was 6.69 g/L (2.8 times) for 14 days of the cultivation, lipid concentration was 1.28 g/L (34.6% of dry biomass, 1.9 times) at sixth day and 3.28 g/L (51.0% of dry biomass, 2.6 times) at the end of fermentation. Scaling up of the batch culture in a 5 L bioreactor, with pH control at 6.8 ± 0.1, the effect of the inoculum sizes at 15, 20 and 25% (v/v) were investigated for 6-day cultivation. The lowest inoculum size of 15% got higher biomass and lipid concentrations. Biomass concentration was 3.42 g/L (1.08 and 1.16 times compared to 20 and 25% of inoculum sizes) and lipid production was 1.44 g/L (1.5 and 1.2 times) or 41.9% of dry biomass (1.07 and 1.03 times). Biomass concentration in shake flask and bioreactor experiments were likely the same at the sixth day but the fermentation in pH controlled conditions proved to be beneficial for lipid accumulation.

Keywords: Batch fermentation; Lipids; *Rhodococcus opacus* PD630; Scaling up

Selected Reference:

Alkaline Hydrogen Peroxide Treatment of Oil Palm Trunk in Biorefinery Process

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Abstract
Oil palm trunk (OPT) is a valuable bioresource for the biorefinery industry. The biorefinery, an effective and economical process, fractionates the primary constituents (cellulose, hemicelluloses, and lignin) of lignocellulosic biomass through pretreatment, which can be future converted into value-added products. Pretreatment is a critical step in the conversion of lignocellulose to fermentable sugars. Although many pretreatment processes are currently under investigation, none of them are entirely satisfactory in regard to effectiveness, cost, or environmental impact. In this study, 27 successive pretreatment conditions of alkaline hydrogen peroxide (1%, 3%, 5% of g H2O2/g of biomass at 50°C, 70°C, and 90 °C, for 30, 60, and 90 min) with a solid loading of 10% at pH 11.5 was performed to find out the optimum condition for the removal of lignin and hemicellulose. The results obtained after AHP pretreatments indicated that using H2O2 at alkaline conditions leads to the decomposition of three major structures: lignin, hemicellulose, and cellulose. The optimum condition for the maximum delignification was observed at 70 °C for 30 min with the use of 3% of g H2O2/g biomass solution, which gave lowest 11.68 (±0.29) % dry weight of lignin; whereas, the highest % dry weight of cellulose was also observed at the same conditions i.e. 73.96 (±0.08). Our earlier experiments indicated that AHP performs well in comparison with other alkaline pretreatment due to easy handling, and less requirement of sodium hydroxide (NaOH).

Keywords: Alkaline hydrogen peroxide (AHP); Biorefinery; Lignocellulosic biomass; Oil palm trunk; Pretreatment

Selected Reference
P-BEB-10


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Abstract
In industrial ethanol production, stillage from distillation unit is transferred directly to wastewater treatment plant. Utilization of stillage as a source of biofuel can improve energy efficiency and reduce carbon footprint of ethanol production process. It has been found that high amount of nutrients remained in stillage. Therefore, this study is aimed to maximize the biomass utilization efficiency by feeding stillage back into fermentation process. Starchy biomass i.e. cassava roots as well as cellulosic biomass i.e. sugarcane bagasse (SCB) and oil palm empty fruit bunch (EFB) were selected for the study. For starchy biomass, dried-milled cassava root was subjected to liquefaction by α-amylase (93°C, 2 h), saccharification by glucoamylase (62°C, 48 h), and ethanol fermentation by Saccharomyces cerevisiae (30°C, 96 h). After distillation, the stillage was fed back into the fermentation process at recycle proportion of 10, 20 and 50% from which the maximum ethanol yields at 48 h were 0.46, 0.44 and 0.43 g ethanol/g total reducing sugar (TRS), respectively. All the ethanol yields from the first run of recycle were substantially higher than ethanol fermentation without recycle (0.31 g ethanol/g TRS). However, the ethanol yield had decreased significantly in the second run of recycling. For hot compressed water pretreated SCB and EFB, ethanol yields from conventional fermentation were 0.31 and 0.34 g ethanol/g TRS, respectively. Since the first run of recycling, ethanol yields from SCB and EFB had decreased significantly. Unlike processing of cassava, cellulosic materials were subjected to harsher pretreatment steps to disintegrate their structures. These processes result in side reaction products that could be potentially inhibitory to microbial growth.

Keywords: Ethanol fermentation; Lignocellulosic biomass; Saccharomyces cerevisiae; Starchy biomass; Stillage recycle

Selected References:
Biogas Production from Water Hyacinth by Co-digestion with Cow Dung

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Abstract
Water hyacinth (Eichhornia crassipes) is a highly problematic invasive weed in water bodies. To convert weed to energy and provide a source of fuel is the aim of this study. Anaerobic co-digestion of water hyacinth with cow dung was investigated. Water hyacinth sample was collected from the Chao Phraya River at Pathumthani Province. A five-liter reactor working volume that equipped with a gas collection system was constructed. Microorganisms from cow rumen were used to inoculate the reactor. The co-substrate was prepared as a slurry at a ratio of water hyacinth: cow dung: water 13 : 7 : 80 (by fresh weight) and was fed to the reactor. The system was operated in a batch mode at 30 °C. Over a 50-day operating period, the results showed the methane yield of 343 liter methane/kg Chemical Oxygen Demand (COD) degraded or 324 liter methane/kg of total volatile solids (TVS) degraded. The methane production was 173 liters /kg total solids added to the reactor. The average methane content was 51.44%. The COD and TVS degradation efficiency were 69.71% and 71.53 %, respectively. This study demonstrates biogas production from water hyacinth as a potential renewable energy source that not only mitigates greenhouse gas emissions but also represents a beneficial use of weed, when it was properly managed, handled and processed.

Keywords: Anaerobic digestion; Bioenergy; Biogas; Co-digestion; Cow dung, Methane; Water hyacinth
Graphene/Vegetable Oil-Based Photo-Crosslinked Polymer Networks

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Abstract
There has been renewed interest recently in developing biobased polymers from vegetable oils as they offer a renewable feedstock. The widespread use of petroleum-based polymers has raised many concerns in terms of both economic and environmental aspects. In this study, biobased crosslinked polymer networks from soybean oil with functionalized graphene (FGN) or functionalized graphene oxide (FGO) were prepared by UV photopolymerization and their mechanical properties were evaluated. The vegetable oil raw material used was acrylated epoxidized soybean oil (AESO). The results from FTIR and XPS showed that FGN and FGO were successfully synthesized. The incorporation of FGO and FGN to AESO effectively enhanced the thermal stability and mechanical properties of host polymer. By contrast, untreated GO/AESO and GN/AESO exhibited relatively low thermal stability and poor mechanical properties than their functionalized counterparts. These graphene/vegetable oil-based polymers can be used as ecofriendly renewable materials for various applications to replace the existing petroleum-based polymers currently used.

Keywords: Graphene; Photopolymerization; Vegetable oil

Selected References:
P-BEB-13

Bio-Conversion of Methane and Propane to Value-added Chemicals using *Methylomonas* sp. DH-1

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Abstract

Methane is a low-priced carbon feedstock for industrial biotechnology. Methanotrophic whole cell can be used for methane bioconversion to chemicals. In this study, a newly isolated methanotroph, *Methylomonas* sp. DH-1, was employed as a biocatalyst for methane-to-methanol and propane-to-acetone bioconversions. In order to improve the bioconversion efficiency, we examined and optimized bioconversion conditions. For methane-to-methanol conversion, methanol was accumulated up to a titer of 1.340 g/L in the presence of 40 mmol/L formate and 0.5 mmol/L EDTA as MDH inhibitor and 30% (v/v) methane. A volumetric conversion rate of 0.332 g/L/h and a specific methanol conversion rate of 0.0752 g/g cell/h were obtained. For propane-to-acetone bioconversion, the maximum accumulation of 16.62 mM, average productivity of 0.678 mM/h and specific acetone productivity of 0.141 mmol/g cell/h were obtained in the presence of 40 mM formate and 40%(v/v) propane.

Keywords: Acetone; DH-1; Methane; *Methylomonas* sp; Propane

Selected References:


P-BEB-14

Production of Isobutylene by Reverse Reaction of Oleate Hydratase Dehydrating Isobutanol

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Abstract

In the production of renewable fuels and chemicals, isobutylene is an interesting molecule. Isobutylene is used for the production of butyl rubber, specialty chemicals, and a gasoline additive known as alkylate. If isobutylene is produced cost efficiently, its applications into biofuels and products could become attractive. The well-known oleate hydratase has an activity for dehydration of isobutanol and other alcohols. The aim of this study was to develop a method that converts isobutanol to isobutylene using oleate hydratase. In this study, the examination was conducted to confirm the activity of oleate hydratase for reverse reaction that dehydrates isobutanol to isobutylene. After the optimized sequences are synthesized by oligonucleotide concatenation and cloned in a pATLIC vector, competent E. coli BW25113 cells are transformed with these vectors by TSS method. The cells were reacted at 30°C and 150 rpm for 48 hr with 25% isobutanol for 20 ml of total volume. As a result of GC analysis, isobutylene was detected for 1.23 mg in 20 ml of reaction volume and activity of oleate hydratase was 0.41 U/mg. To improve the amount of isobutylene and optimize the production, oleate hydratase from varied origin was tested to identify the activity of hydration for forward reaction. Two candidates of enzyme from particular strains were selected and plan to identify the activity of reverse reaction for conversion of isobutanol to isobutylene. This work was supported by a grant from Korea Evaluation Institute of Industrial Technology(KM-16-340).

Keywords: Isobutanol; Isobutylene; Oleate hydratase
Enzymatic Synthesis of Phenyllactate by Engineering the Substrate Specificity of D-Lactate Dehydrogenase

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Abstract

3-phenyllactic acid is an antimicrobial compound against diverse species of bacteria and fungi. D-lactate dehydrogenase (D-LDH) is capable of the synthesis of 3-phenyllactic acid from phenylpyruvic acid, but its catalytic efficiency remains low. In this study, the D-LDH from Pediococcus acidilactici was rationally designed to increase its activity by analyzing substrate-enzyme contacts. A single mutation N76A increased the catalytic efficiency by 2.8-fold. This work provides an efficient strategy to improve the activity of D-LDH for 3-phenyllactic acid, and may also be useful for activity improvement of other enzymes for unnatural substrates.

Keywords: Contact analysis; D-lactate dehydrogenase; Phenyllactate; Rational design of enzyme
Direct Production of L-Malic Acid from Lignocellulose with
S. commune Mutant Constructed by Ion-beam Irradiation

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Abstract
The bioconversion process of herbaceous and woody biomass to useful materials has been recently advanced to reduce the usage of fossil fuel and hold CO₂ emissions. With a goal of the establishment of biorefinery technology from cellulosic materials as rice straw, waste-wood etc., the effective production of L-malic acid (LMA) from lignocelluloses by consolidated bioprocessing (CBP) that is the next generation type of fermentation technology with Basidiomycete Schizophyllum commune. Therefore, two points of the possibility of CBP with S. commune and the construction of a high performance mutant by irradiating ion-beam was investigated. S. commune NBRC 4928 was cultured with 100 g/L glucose at 28°C for 12 days under aerobic condition, the glucose was completely consumed and LMA was able to produce effectively 40.1 g/L. Moreover, from some results of CBPs of cellulosic materials with the fungi, LMA was able to product directly from α-cellulose and rice hull. However, since the amount of secretion of endo-β-glucanase (EG) and cellobiohydrolase (CBH) of the mold is considerably smaller than that of β-glucosidase (BGL), LMA from cellulosic materials having crystalline structure such as Avicel, Sigmacell etc. could not be produced. Therefore, to achieve the higher cellulase-secreting fungus, C¹²⁺ ion-beam irradiation was carried out against the wild fungus and then a mutant was obtained. In some CBPs with the mutant form cellulosic materials having many crystal structures, the secretion of EG and BGL was improved and direct LMA production from Avicel could be achieved.

Keywords: CBP; Ion-beam mutation; Lignocellulose; Malic acid; Schizophyllum commune
P-BEB-17

High Temperature Ethanol Production from Rice Straw by Cellulase Secreting Fungi Stimulated by Ion-beam Mutation

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Abstract
Bioconversion has been studied as hopeful technology for effective utilization of abundant biomasses and production of valuable materials. Ethanol is the most investigated by reason of easy production and purification. The use of high cost cellulase for hydrolysis of biomass, however, is essential prior to conversion to ethanol. Whole fermentation process operated at high temperature is effective, and ethanol also can be recovered by evaporation in parallel. So that high ethanol production from rice straw can be achieved, a thermotolerant Rhizopus microsporus was selected and the mutant that stimulated cellulase secretion was constructed by ion-beam irradiation. The wild strain can produce 22.5 g/L ethanol at 42˚C at the fermentation efficiency of 0.88 and 14.8 g/L ethanol even at 45˚C. To translate the strain to high performing fungus, it was irradiated with carbon ion-beam (absorbed dose: 2,500 Gy) at The Wakasa Wan Energy Research Center. Some mutants that can grow on cellulose medium at 50˚C were obtained, of which 14 mutants were high-performing strains. Among these mutants, HTM3 was able to produce ethanol as well as wild strain (14.2 g/L at 45˚C). Moreover, cellobiohydrolase (CBH) and β-glucosidase (BGL) secreted from the mutant were enhanced. CBH activity was 7.3-fold higher at 48 h and BGL was secreted 12 h faster than these of wild strain. From these results, reduction of cellulase usage can be expected in SSF process with the mutant. Bioconversion of rice or straw to ethanol by SSF with HTM3 and a small amount of cellulase reagents is being investigated.

Keywords: Ethanol; Ion-beam mutation; SSF; Thermotolerant fungi
P-BEB-18

Bioethanol Production by Recombinant Saccharomyces cerevisiae Expressing a Mutated SPT15 Gene

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Abstract

The SPT15 gene encodes a Saccharomyces cerevisiae TATA-binding protein, which is able to globally control the transcription levels of various metabolic and regulatory genes. In this study, a SPT15 gene mutant (S42N, S78R, S163P and I212N) was expressed in S. cerevisiae BY4741 (SPT15-M3), of which effects on the yeast cell properties were evaluated in batch, fed-batch and simultaneous saccharification and fermentation (SSF) processes. Organic nitrogen sources and a microaerobic condition were more favorable for SPT15-M3 than the SPT15wt control in both cell growth and ethanol production. Fed-batch cultures of SPT15-M3 using concentrated glucose solution resulted in 9-19% higher glucose consumption rate and ethanol productivity than those for SPT15wt. In addition, SPT15-M3 showed 3.9 and 4.5% increases in ethanol productivity from cassava hydrolysates and corn starch in SSF processes, respectively. It was concluded that overexpression of mutated SPT15 gene in S. cerevisiae would be a potent strategy to enhance ethanol production from glucose-based biomass.

Keywords: Bioethanol; Fed-batch; Saccharomyces cerevisiae; SPT15; Simultaneous Saccharification and Fermentation

Selected Reference:
Effects of Light-emitting Diode (LED) with a Mixture of Wavelengths on the Growth and Lipid Content of Microalgae

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Abstract

Integrations of two-phase culture separately for cell growth and lipid accumulation using mixed LED and green LED wavelengths were evaluated with the microalgae Phaeodactylum tricornutum, Isochrysis galbana, Nannochloropsis salina, and N. oceanica. Between the single and mixed LED wavelengths, mixed LED produced the highest biomass of the four microalgae, reaching I. galbana (1.03 g DCW/L), followed by P. tricornutum (0.95 g DCW/L), N. salina (0.85 g DCW/L) and N. oceanica (0.62 g DCW/L). Binary combination of blue and red LEDs could give the higher biomass production and photosynthetic pigments in the four microalgae. The highest lipid accumulation during second phase of exposure to green LED wavelengths was 38.8–56.0% for P. tricornutum, 36.0–55.2% for I. galbana, 31.5–53.0% for N. salina and 28.5–51.0% for N. oceanica, respectively. The major fatty acid in the four microalgae was palmitic acid (C16:0), accounting for 33.8%–56.0% (w/w) of the total fatty acid content.

Keywords: Fatty acid; Green LED; Microalgae; Mixed LED; Pigment; Two-phase culture
Identification of Trctf1 as a Novel Gene Involved in Cellulase Production through Investigation of the Recombinant Trichoderma reesei Engineered with Artificial Zinc-finger Transcription Factor

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Abstract

Trichoderma reesei Rut-C30 is acknowledged as a cellulases hyper-producer, but production cost of the enzymes is still too high for economic bioconversion of lignocellulosic biomass. In this study, a mutant M2 with improved cellulase production was screened from T. reesei Rut-C30 engineered with synthetic zinc finger protein (ZFP) library. Compared to the parent strain, the filter paper activity (FPase) and endo-β-glucanase activity (CMCase) of cellulases produced by the mutant increased by 67% and 35%, respectively, but the β-glucosidase activity (pNPGase) was reduced by 50%. The insertion loci of the zfp sequence was located by TAIL-PCR, which was between Tr_4597 and Tr_67627 in the genome of the mutant, and real-time PCR analysis further revealed that the transcription of Trctf1 was significantly down-regulated by the ZFP transcription factor. Furthermore, a Trctf1 gene null mutant was developed, which produced cellulases more effectively with transcriptional activators xyr1 and ace2 up-regulated. On the other hand, when Trctf1 was constitutively expressed under the control of the pdc promoter, cellulases production was substantially compromised. These results indicated the significant role of the transcription factor TrCTF1 in the repression of cellulase production by T. reesei.

Keywords: Cellulases production; Lignocellulosic biomass; Trctf1 transcription factor; Trichoderma reesei Rut-C30; Zinc finger protein (ZFP)

Selected References:
Greener Conversion of Holocellulosic Stream of Mixed Non-edible Lignocellulosics to Ethanol through Consolidated Bioprocessing Approach

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Abstract
Eco-friendly lignocellulosic deconstruction using laccase has tremendous potential to strategically depolymerize lignin without hampering structural carbohydrates of cell wall matrix. Laccase action on lignin is highly targeted and does not deviate the process flow towards undesirable intermediates which otherwise disturb the process efficacy. The scope of 2G ethanol production through this biotechnological venture was evaluated in the present study using mixed non-edible lignocellulosics composed of Ricinus communis, Saccharum officinarum (tops) and Saccharum spontaneum as a substrate. The concept of mixture was adopted since mixed lignocellulosics can serve as a commercially feasible raw material for sustainable and uninterrupted supply of lignocellulosic ethanol compared to pure feedstocks. Another facet to the present investigation is the use of co-culture of hexose and pentose fermenting yeast strains to simultaneously convert C6 and C5 sugars to ethanol. The USP of the process is the conversion of high energy density (17.02 kJ/g) mixed biomass at high substrate loading (25% w/v) by combining pretreatment, saccharification and co-fermentation phases into a single integrated step called consolidated bioprocessing (CBP). A unique cocktail of biocatalysts consisting of laccase produced from Pleurotus djamor and a complete holocellulolytic system from Trichoderma reesei RUT C30 was employed to depolymerize mixed lignocellulosic biomass. Through this approach, ethanol yield (7.07% v/v) was found to be enhanced by 2.35 folds and 1.33 folds compared to separate hydrolysis and fermentation (SHF) and simultaneous saccharification fermentation (SSF) of pretreated biomass, respectively. Besides, the fermentation time was reduced from 36-42h to 20h signifying the vigor of the biological process.

Keywords: Consolidated bioprocessing; Holocellulase; Laccase; Mixed non-edible lignocellulosics; 2G Ethanol

Selected References:
P-BEB-22

An Eco-friendly Process Integration for Second Generation Bioethanol Production from Laccase Delignified Kans Grass

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Abstract
The fundamental aspect of biomass to biorefinery approach is depends on the crucial selection of feedstocks and on their availability. Until now research has been focused towards biofuels and biochemicals production based on the physical, chemical and physic-chemical methods that are not environment friendly. Biological processes on the other hand are eco-friendly and sustainable in nature and are gaining more attention towards biofuels and biochemicals generation. Moreover, biomass feasibility in terms of availability and sugar content is still one of the major challenges in biofuel production process. The present article emphasizes process integration for bioethanol production by utilizing laccase pretreated lignocellulosic feedstock. In the present study, we have maximized the bioethanol production by combining the different processes together and compared it with the single process. The fermentation process has been optimized through response surface methodology that resulted in 63.2gL⁻¹ of ethanol for partial simultaneous saccharification and fermentation (P-SSF) and 57.91gL⁻¹ of ethanol for simultaneous saccharification and fermentation (SSF) within 25-28h. The surface area, pore size, and pore volume of the fermented biomass is found to be decreased after SSF and P-SSF that indicates extensive action of enzymes. Microscopic study showed surface distortion of the biomass after fermentation that indicated the action of cellulolytic enzymes during saccharification. Biomass crystallinity provides a pattern of amorphous and crystalline cellulose utilization which in the initial phase increased to 8.03% and after decreased to 23.49% and thus, supports the feasibility of pretreated biomass utilization for bioethanol production.

Keywords: Biofuels; Biorefinery; Crystallinity; Fermentation
Enzymatic Hydrolysis and Fermentation for Reducing Sugar and Ethanol Production from Pineapple Leaf: An Attempt towards Waste Valorisation

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Abstract
Rapidly increasing energy scarcity due to the excessive use of fossil fuels has stimulated the search for alternative sources of energy from renewable resources. Sustainable fuels have the potential to substitute petroleum based fuel. Conversion of agro-industrial wastes to value added products such as reducing sugar and ethanol is an efficient approach for waste valorization and management that also mitigates environmental pollution. In the present study, enzymatically delignified pineapple leaf waste rich in holocelluloses such as cellulose (45.20 % ± 0.98, w/w) and hemicellulose (19.80 % ± 0.73, w/w) was utilised for the production of fermentable sugars using cellulase-xylanase concoction produced from Trichoderma reesei RUT C30. Maximum concentration of reducing sugar (508.19 mg/g) was obtained at the optimum conditions of solid loading 22.68 %(w/v), 50 °C, pH 4.5, incubation time 6.30 h and enzyme concentration 19.14 IU/mL. The saccharified broth upon being subjected to fermentation with yeast, produced ethanol with concentration of 5.6 %(v/v) in 36 h. The simultaneous saccharification and fermentation (SSF) of delignified pineapple leaf waste using saccharifying enzyme concoction and yeast resulted in bioethanol concentration of 6.8 %(v/v) in 24 h. X-Ray Diffraction analysis of SSF biomass has resulted in decrease of cellulose crystallinity by 8.15 %. Decrease in pore size and surface area of saccharified and SSF biomass demonstrated the action of saccharifying enzymes on holocellulose fraction of lignocellulosic biomass. Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy studies further corroborated the efficiency of the enzymatic saccharification and fermentation processes.

Keywords: Celluase-xylanase; Ethanol; Pineapple leaf waste; Reducing sugar; Yeast
P-BEB-24

Construction of Productive Xylose-fermenting *Saccharomyces cerevisiae* by Introducing a Xylose Reductase from *Scheffersomyces stipitis*

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Abstract

Engineered *Saccharomyces cerevisiae* has been used for ethanol production from xylose, the abundant sugar in lignocellulosic hydrolysates. Development of engineered *S. cerevisiae* able to utilize xylose effectively is crucial for economical and sustainable production of fuels. To this end, the xylose-metabolic genes (*XYL1*, *XYL2* and *XYL3*) from *Scheffersomyces stipitis* have been introduced into *S. cerevisiae*. The resulting engineered *S. cerevisiae* strains, however, often exhibit undesirable phenotypes such as slow xylose assimilation and xylitol accumulation. In this study, a synthetic isozyme system of xylose reductase (XR) was developed to construct an improved xylose-fermenting strain. The engineered strain having both wild XR and mutant XR showed low xylitol accumulation and fast xylose consumption compared to the engineered strains expressing only one type of XRs, resulting in improved ethanol yield and productivity. These results suggest that the introduction of the XR-based synthetic isozyme system is a promising strategy to develop efficient xylose-fermenting strains.

Keywords: Cellulosic ethanol; *Saccharomyces cerevisiae*; Synthetic isozyme system; Xylose; Xylose reductase

Selected References:

P-BEB-25

High Production of 2,3-butanediol by Engineered *Saccharomyces cerevisiae* through Fine-tuning of Pyruvate Decarboxylase and NADH Oxidase Activities

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Abstract

2,3-Butanediol (2,3-BD) is a promising compound for various applications. Pyruvate decarboxylase (Pdc)-deficient *Saccharomyces cerevisiae* is an attractive host strain for producing 2,3-BD because a large amount of pyruvate could be shunted to 2,3-BD production instead of ethanol synthesis. However, 2,3-BD productivity by engineered yeast was inferior to native bacterial producers. To overcome these problems, the *Candida tropicalis* PDC1 gene (CtPDC1) was used to minimize the production of ethanol but maximize cell growth and 2,3-BD productivity. As a result, productivity of the BD5_G1CtPDC1 strain expressing an optimal level of Pdc was 2.3 folds higher than that of the control strain in flask cultivation. Through a fed-batch fermentation, 121.8 g/L 2,3-BD was produced in 80 h. NADH oxidase from *Lactococcus lactis* (noxE) was additionally expressed in the engineered yeast with an optimal activity of Pdc. The fed-batch fermentation with the optimized 2-stage aeration control led to production of 154.3 g/L 2,3-BD in 78 h. The overall yield of 2,3-BD was 0.404 g 2,3-BD/g glucose. A massive metabolic shift in the BD5_G1CtPDC1_noxE strain expressing NADH oxidase was observed, suggesting redox imbalance was a major bottleneck for efficient production of 2,3-BD. Maximum 2,3-BD titer in this study was close to the highest among the reported microbial production studies. The results demonstrated that resolving both C2-compound limitation and redox imbalance is critical to increase 2,3-BD production in the Pdc-deficient *S. cerevisiae*. Our strategy to express fine-tuned PDC and noxE could be applicable not only to 2,3-BD production, but also other chemical production systems.

Keywords: 2, 3-Butanediol; NADH oxidase; Pyruvate decarboxylase; *Saccharomyces cerevisiae*

Selected References:

P-BEB-26

Biohythane the Future Fuel: Comparison the Productivity between Pure (Galactose) and Complex Substrate (Macro Algae Biomass) at Mesophilic and Thermophilic Temperature Condition

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Abstract

Biohythane, the future fuel, is combination of hydrogen and methane via two stage fermentation. It has high energy value and is an efficient way to degrade waste biomass. The experiment was conducted under the batch condition in 160 mL serum bottle with the working volume of 100 mL, 15g/L pure (galactose) and complex (red algae biomass) substrate were added. The granular and digester sludges were used as seed inoculum. Various results were observed in the single stage and two stage fermentation with pure and complex substrate. The SMP (soluble metabolic products) were analyzed using HPLC and the various kind of microbial communities were measured by qPCR.

Keywords: Biohythane; Microbial community; Red algae biomass; Seed inoculum
Biorefinery of Waste Glycerol for Repeated Batch Production of 1,3-Propanediol by Klebsiella pneumonia

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Abstract
Recently, 1,3-Propanediol (1,3-PDO) as an important immediate chemical used in polymer industry has been received high attention. Biodiesel derived waste glycerol has been employed in 1,3-PDO study with positive prospects. In this study, 1,3-propanediol production was aimed from biodiesel derived waste glycerol using Klebsiella pneumoniae ATCC 8724 under repeated batch fermentation. To achieve effective cultivation, the inhibitions of the culture conditions were investigated considering the waste glycerol components in batch fermentation. Anaerobic fermentation represented better performance than aerobic cultivation. Proper range of culture conditions that initial pH, initial substrate concentration, and salts containing (NaCl and KCl) were determined to avoid inhibits. Both pure and waste glycerol were employed as substrate in this cultivation, and the highest yield of 1,3-propanediol was achieved 0.7 and 0.6 (mol/mol), respectively. Furthermore, the proper ranges of culture conditions were applied in 5-cycle repeated batch fermentation, which was successfully demonstrated with immobilized cells using waste glycerol. Above 80% of production was obtained at final cycle of batch cultivation compared to the first cycle of cultivation. The highest yield of 1,3-propanediol were achieved 0.6 (mol/mol). In addition, the by-products performance of this repeated batch fermentation was also investigated for further application.

Keywords: 1,3-Propanediol; Inhibitory effect; Immobilized cell; Klebsiella pneumonia; Waste glycerol

Selected References:
P-BEB-28

Performance of Multi-anode Yeast Fuel Cell on Bio-electricity Production During Alcohol Fermentation by *Saccharomyces cerevisiae*

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Abstract

*Saccharomyces* yeast has been appreciated in alcohol fermentation, however now it performed favourably as the bio-catalyst for sugar power in a yeast fuel cell (YFC) for bioelectricity production as green power source. In this work, YFC with one to four anodic (iron) electrode / one cathode (copper) electrode was investigated for bioelectricity production during ethanol fermentation by *Saccharomyces cerevisiae* from sucrose medium. Using the YFC with four anodes gave the electricity values higher than the ones with three, two and one anode electrode, respectively. The ethanol concentration produced from initial sucrose concentration (220 g/L) in this YFC was 51.62 g/L and gave the 58% yield based on consumed sucrose. The YFC with four anodes gave the maximum voltage and current output (2.354 V and 0.046 mA), and the current densities and power densities (37.68 mA/m² and 88.699 mW/m²) which were higher than YFC with one anode electrode (0.32 mA/m² and 0.026 mW/m²) about 1.17 times based on current density. This work showed that YFC technology will be useful to further study for electricity generation during the food or beverage fermentation process.

Keywords: Bioelectricity; Ethanol fermentation; Microbial fuel cell; *Saccharomyces cerevisiae*; Yeast
P-BEB-29

The Controllability of Heat Exchanger Network for Downstream of Ethanol Production

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Abstract

Nowadays, fossil fuels are usually used as an energy source for vehicles and for the production of electricity. But, fossil fuels have many limitations. This problem can be solved by the use of alternative resources of energy such as water, solar energy and biofuels. Ethanol is the most popular biofuel which can be mixed with gasoline to produce E20 and E85. Therefore, this research mainly focus on the downstream of ethanol process with heat exchanger network and applied the passivity concept. Heat Exchanger Network is the process integration which can be useful tool for intensifying processes. The passivity method is implemented to guarantee the controllability and to propose the robust controller tuning. Also, an analysis of a network cost index is perform after the process improvement by using Aspen Energy Analyzer. Dynamic models are generated for all variables of the unit in the state space domain. Subsequently the state space model of heat exchanger network is formulated to study the concept of passivity, while the control structure is analyzed by linear matrix inequality. Then, these transfer functions are analyzed by the passivity index to indicate that the heat exchanger network are passive or not. Furthermore, the present of passivity behavior depended upon its possible pairing schemes. Thus, the magnitude of passivity index was used to rank the pairing schemes. Hence, the passivity based decentralized unconditional stability of PI controllers for this system were designed and verified with Aspen Dynamics Simulator.

Keywords: Controllability; Heat exchanger network; Passivity concept; State space models
Production of Biooil Derived from Waste Palm Oil and Palm Empty Fruit Bunch

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Abstract
This work is presented the comparison of three biooil production plants from biomass; empty fruit bunch (EFB) from palm tree and waste palm oil. Ryield model was used to simulate pyrolysis reaction along with the product data from the literatures. Three biooil production plants were consisted of case 1: a base case pyrolysis of palm EFB from published literature for the purpose of a reference case, case 2: pyrolysis of palm EFB, and case 3: pyrolysis of waste palm oil. The heat of combustion was used to heat up the temperature of pyrolysis reactor for the base case. However, the others were used the microwave as the heat source because microwave provided more heating rate and neglect the heat transfer of media solid to raw material in pyrolysis process. The operating conditions of pyrolysis reactor are 500 °C and atmospheric pressure. In this work, the production yields of three plants were 39.79 wt% of biooil, 13.57 wt% of gas, 22.74 wt% of water, 5.90 wt% of ash, and 18.00 wt% of char as the same value to specify raw material flow rates. The results showed that palm EFB (124.38 kg/h) as the raw material gave a product yield higher than waste palm oil (94.65 kg/h). However, the market prices of palm EFB and waste palm oil were 0.85 Baht/kg and 6.5 Baht/kg, respectively. Thus, the raw material cost of biooil production from palm EFB was cheaper than from waste palm oil.

Keywords: Empty fruit bunch; Pyrolysis; Simulation; Waste palm oil

Selected References:
BPB : Bioindustry Promotion and Bioeducation
Factors Influencing Buying Decision of Biobusiness: Case Study from Interviews of Cosmetic Entrepreneurs in Thailand

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Abstract

Biobusiness in Thailand is a growing business due to the strategic direction of the Thai government. It ultimately aims to generate the driving force to stimulate the developments and foster the growth of biotechnology industry in order to transform Thailand into the center of biotechnology in Asia. This study proposed the Product Process Development (PPD) that could possibly enhance biotechnology researchers in Thailand to be able to design the research areas to serve the market demand rather than the researchers’ expertise. Regarding the second step in PPD process, interviews were conducted with the lead and general users who had the crucial role in helping the researcher learn the factors that influenced their buying decision and behavior. This study demonstrated the case study from the interviews with 15 cosmetics entrepreneurs in Thailand. Based on the interviews, this study received the factors that influenced the business buying decision, the insights of user’s need, problems, suggestions for solutions and the marketing mix (4Ps) that can support the researcher in terms of designing the biotechnology research or product that better satisfies the user’s need. Interviewing the business investors before planning on the biotechnology research design is not the only the way to increase value contributions of the research outcomes, but also it needs to make the best use of the previous and current research findings for commercialization, in which to successfully drive the biobusiness growth in Thailand.

Keywords: 4P; Biobusiness; Cosmeceutical; Marketing mix; Product planning development process

Selected References:
BPMB : Biopharmaceutical and Medical Biotechnology
**P-BPMB-01**

**Preparation of a Relatively Hydrophobic Peptide Originating from β-casein to Enhance the Water Dispersibility of Paclitaxel**

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**Abstract**

A peptide mixture, which was obtained as casein hydrolysate, was found to be effective as a dispersant for poorly water-soluble drugs such as paclitaxel (Ptx) and nutraceuticals such as curcumin. From the results of MALDI LIFT-TOF/TOF MS analysis, a major peptide that enhances the dispersibility of Ptx was identified and was found to be relatively hydrophobic peptides originating from the C-terminal of β-casein. However, casein hydrolysate is a mixture of peptides and is hard to confirm the complexation mechanism between peptides and poorly water-soluble ingredients. In the present study, the 17 residues peptide (YQEPVLGPVRGPFPIIV; PepY) was synthesized by the solid-phase methodology to use as a dispersant for Ptx. PepY was synthesized on a Wang resin by incorporation of Fmoc amino acids. After purification using HPLC, the peptide was identified using MALDI-TOF/MS. The complex between Ptx and PepY was prepared by mixing an ethanol solution of Ptx (30 mg/L, 500 μL) and an aqueous solution of PepY (1.0 g/L, 500 μL), followed by lyophilization. To the complex was added 500 μL of 10 mM sodium phosphate buffer. The aqueous mixture was shaken at 30 °C for 30 min. After filtration using a Φ 0.45 μm membrane filter, the concentration of Ptx in the filtrate was determined using HPLC. The apparent solubility of Ptx complexed with PepY increased with the increase of PepY quantity. The pH dependency for the apparent water solubility of Ptx complexed with PepY is different to that complexed with casein hydrolysate. This result suggests that another peptides in casein hydrolysate also contribute the enhancement of water-dispersibility of Ptx.

**Keywords:** Casein; Dispersibility; Paclitaxel; Peptides; Solubility

**Selected References:**

The Inhibitory Effects of Manassantin B, a Neolignan Isolated from the Roots of Saururus Chinensis on VEGF-A-induced Lymphangiogenesis and Lymph Node Metastasis

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Abstract

The lymphatic system is very important in metastasis of oral cancer. And metastasis via the lymphatic system is promoted by lymphangiogenesis. In this study, we investigated the effects of manassantin B, a neolignan isolated from the roots of Saururus Chinensis, on VEGF-A-induced lymphangiogenesis and lymph node metastasis both in vitro and in vivo. Manassantin B inhibited the proliferation, tube formation, and migration of recombinant human VEGF-A (rhVEGF-A) -treated human lymphatic microvascular endothelial cells (HLMECs). Manassantin B suppressed the VEGF-A-induced phosphorylation of VEGFR-1 and VEGFR-2. In addition, manassantin B reduced the activation of signaling factors such as FAK, PI3K, AKT, ERK1/2 and p38, involved in VEGF-A/VEGFR-1 and VEGFR-2 signaling pathway. Manassatin B reduced in vivo lymphatic vessel formation in VEGF-A-stimulated Matrigel plug. To investigate the in vivo effects of manassantin B, we established an oral sentinel lymph node animal model using BALB/c mice and oral squamous cell carcinoma SCCVII cells. We confirmed the inhibitory effects of manassantin B on VEGF-A-induced lymphangiogenesis and sentinel lymph node metastasis in the animal model. Our results indicate that manassantin B has the inhibitory effect on VEGF-A-induced lymphangiogenesis and lymph node metastasis and these suggest that manassantin B can be a useful anti-tumor agent to restrict the metastatic spread of oral cancer. This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2016R1A6A3A11933134).

Keywords: Lymph node metastasis; Lymphangiogenesis; Manassantin B; VEGF-A
Development of Recombinant Small Antibodies for siRNA Delivery

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Abstract
RNA interference, which suppresses gene expression by small interfering RNA (siRNA), has been utilized in the medical field, but specific delivery of siRNA to cells or tissues remains challenging. In contrast, antibodies have high specificity to targeted cells and tissues, and recombinant small antibodies, such as single-chain fragment of variable region (scFv) and diabody consisting of the variable regions of antibody, can more highly permeate tissues than full-size antibodies. In this study, we aim to design the small antibodies bearing the siRNA to construct a drug delivery system (DDS) of siRNA. First, we prepared 20 recombinant small antibodies (10 scFvs and 10 diabodies) with cationic peptide fragments as RNA carrier at C-terminus via 2 types of linkers by means of E.coli expression system. The charged peptides interact electrostatically with negatively-charged siRNAs. As a result, even the yield of purified small antibody-RNA carrier complex with the most expression was 4 µg/L. Next, we tried to prepare recombinant small antibody with RNA carrier by chemical conjugation using amine coupling. As a result, many small antibodies after chemical conjugation remained non-conjugation and caused multimerization. In conclusion, the fusion of RNA carrier to small antibody decreased the expressed yield in E.coli. We are in progress of preparing small antibody-RNA carrier complex by means of site-specific chemical conjugation using enzyme with small antibody and RNA carrier.

Keywords: Antibody; DDS; siRNA
The Effects of 6,8-Diprenylgenistein, an Isoflavonoid Isolated from Cudrania tricuspidata Fruit on VEGF-A-induced Lymphangiogenesis and Lymph Node Metastasis

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Abstract
The spread of tumor cells to lymph nodes commonly occurs in tumors and is an early event in metastatic disease. Metastasis via the lymphatic system is promoted by lymphangiogenesis. In this study, we investigated the effects of 6,8-diprenylgenistein, an isoflavonoid isolated from Cudrania tricuspidata fruit, on VEGF-A-induced lymphangiogenesis and lymph node metastasis both in vitro and in vivo. 6,8-Diprenylgenistein inhibited the proliferation, migration, and tube formation of human lymphatic endothelial cells (HLECs). We performed the VEGF-A-induced in vivo Matrigel plug assay. 6,8-Diprenylgenistein inhibited lymphangiogenesis in VEGF-A-induced Matrigel plug. 6,8-Diprenylgenistein suppressed the activation of vascular endothelial growth factor receptor (VEGFR) -1 and -2 stimulated by VEGF-A. Also, 6,8-diprenylgenistein suppressed the activation of signaling factors such as FAK, PI3K, AKT, ERK and p38 involved in VEGF-A induced lymphangiogenesis related signaling pathway. To investigate the in vivo effect of 6,8-diprenylgenistein on VEGF-A-induced lymphangiogenesis and lymph node metastasis, we used an oral cancer sentinel lymph node animal model. 6,8-Diprenylgenistein inhibited VEGF-A-induced lymphangiogenesis and sentinel lymph node metastasis in the animal model. Taken together, these results indicate that 6,8-diprenylgenistein has the inhibitory effects on VEGF-A-induced lymphangiogenesis and lymph node metastasis. And these results suggest that 6,8-diprenylgenistein can be a useful agent for developing new anti-cancer therapeutics.

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2016R1A6A3A11933134).

Keywords: 6,8-Diprenylgenistein; Lymph node metastasis; Lymphangiogenesis; VEGF-A
P-BPMB-05

Corosolic Acid Exhibits Anti-angiogenic and Anti-lymphangiogenic Effects to Inhibit Tumor-induced Angiogenesis and Lymphangiogenesis

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Abstract
Tumor-induced angiogenesis and lymphangiogenesis are required for cancer cell growth and spread to other tissues. These are key targets for developing new cancer chemotherapeutics. In this work, we investigated the anti-angiogenic and anti-lymphangiogenic effects of corosolic acid, a pentacyclic triterpenoid isolated from Cornus kousa Burg. A mouse colon carcinoma CT-26 animal model was employed to determine the in vivo anti-angiogenic and anti-lymphangiogenic effects of corosolic acid. Corosolic acid reduced the final volume and the blood and lymphatic vessel densities of CT-26-induced tumors, indicating that it suppresses in vivo angiogenesis and lymphangiogenesis. Corosolic acid inhibited the proliferation and tube formation of human umbilical vein endothelial cells (HUVECs) and human dermal lymphatic microvascular endothelial cells (HDLMECs). In addition, corosolic acid inhibited the proliferation, migration and tube formation of HUVECs and HDLMECs stimulated by angiopoietin-1. Pretreatment with corosolic acid decreased the phosphorylation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase 1/2, suggesting that corosolic acid contains anti-angiogenic and anti-lymphangiogenic activities to suppress FAK signaling stimulated by angiopoietin-1. Taken together, these findings suggest that corosolic acid exhibiting anti-angiogenic and anti-lymphangiogenic effects can be a useful agent for developing new cancer chemotherapeutics. This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2015R1D1A1A01059824).

Keywords: Angiogenesis; Angiopoietin-1; Colon carcinoma CT-26; Corosolic acid; Lymphangiogenesis
What are the Rules for Designing Small Antibodies with High Cytotoxicity

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Abstract
Small bispecific antibodies with T-cell-inducing cytotoxicity have high potential for making significant damages on late-stage tumor mass, and the cytotoxicity is critically dependent on structural and functional properties of antibodies. In this study, we made a variety of small bispecific T-cell recruiting antibodies constructed from a series of the antibodies against EGFR family and T-cell receptors to draw critical rules of high cytotoxic antibodies. A set of rapid operations for expression vectors construction and protein preparation enabled to screen the cytotoxicity of 100 kinds of small bispecific antibodies with diabody format, and the diabodies with $10^3$-fold high cytotoxicity of existing highly active one ($\text{IC}_{50} = 1$ fM) were identified. Correlation diagram of the cytotoxicity of diabodies with parent antibodies used and domain arrangement in diabody demonstrated not just independent influence of each factor (target, epitope, domain arrangement) on cytotoxicity but synergistic effect to enhance cytotoxicity: the diabodies retargeting CD3 in the domain order from light chain to heavy chain (LH-type) had high cytotoxicity, and the use of anti-EGFR fragment with high affinity for domain 3 in the LH-type diabody further enhanced the cytotoxicity. Here, we show the potential of this optimizing approach by means of rapid screening and correlation diagram methodology for constructing bispecific antibodies with expected cytotoxicity.

Keywords: Bispecific antibody; Immune therapy; Protein engineering; Tumor; T-lymphocyte
Identification of Specific Monoclonal Antibody Against NS1 Protein of Dengue Virus

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Abstract
The dengue nonstructural protein 1 (NS1) is a secreted glycoprotein that accumulated at high levels in the plasma of dengue viruses (DENV)-infected patients and on the surface of infected cells, but not in the viral particles. This protein has been shown to be useful as a tool for the diagnosis of acute dengue infections since it can be detected in the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to 18 DPO. Despite the global health problems associated with DENV infection, ranging from dengue fever (DF) to a severe, life-threatening symptoms, termed dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS); molecular mechanisms of viral pathogenesis are still poorly understood. Anti-NS1 antibodies recognition of NS1 on endothelial cell surface has been suggested to play a role in severe vascular leakage during severe secondary infection. Recent findings have indicated a role of the complement system in both anti-DENV protection and disease pathogenesis. Therefore, obtaining specific human recombinant antibodies against NS1 will be beneficial for the study of viral pathogenesis, which is necessary for the successful development of therapeutic antibody as well as for the diagnostic purpose.

In this study, human scFv antibodies against recombinant NS1 of Dengue virus serotype 2 were isolated from a naïve human phage display scFv antibody library (YamoI library). A total of 32 phage clones were identified and their bindings were confirmed by Phage ELISA. From these 32 clones, 10 clones were selected for soluble expression of free scFv antibodies by superinfection into non-suppressor E. coli strain HB2151. The binding of free scFv antibodies against NS1 was demonstrated by ELISA. Amino acid sequences and 3D structures of these selected recombinant scFv antibodies were analyzed by automated DNA sequencing and online-bioinformatics tools. These antibodies will be further engineered and produced for different applications in the next step.

Keywords: Antibody; Bio-panning; Dengue; NS1; Phage display; Recombinant, scFv

Selected Reference:
Differentiation of HL-60 AML Cells Enhanced Their Sensitivity to Doxorubicin

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Abstract
Acute myeloid leukemia (AML) is a malignant disease of the bone marrow, in which hematopoietic precursors are arrested in the early stage of the development. Conventional chemotherapy is still ineffective, and relapsing is commonly found. Differentiation therapy has been suggested as an alternative way to treat. The aim of this research is to investigate whether the differentiated AML cells could preferentially sensitive to an anticancer drug, doxorubicin. To induce the differentiation of HL-60 AML cells, 1.5% DMSO was incubated with HL-60 cells for 48 hours. Cell proliferation assay and cell viability test were investigated by MTT assay and Trypan blue staining, respectively. CD11b antibody conjugated with FITC was used for investigating cell differentiation by flow cytometer. The differentiated HL-60 cells were treated with various concentrations of doxorubicin. Cytotoxicity of treated cells was evaluated, and compared to undifferentiated cells, based on Anexin V and 7-AAD staining for apoptotic cells using flow cytometry. The result showed that after induction, most of the cells were alive but no proliferation was observed. The percentage of CD11b positive cells was increased after 48 hours of induction. Apoptosis analysis indicated that the differentiated cells were more sensitive to doxorubicin than undifferentiated cells. These results suggested that differentiation of AML cells could be an alternative way to treat AML patients.

Keywords: AML; Cell differentiation; Chemo drug sensitivity

Selected Reference:
Chitooligosaccharide (CHOS) Modulates Autophagy and Prevents Apoptosis in Human Neuroblastoma SH-SY5Y Cells

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Abstract
Chitooligosaccharide (CHOS) is an oligomer of D-glucosamine and N-acetyl-glucosamine, derived from the degradation of chitosan or deacetylation and cleavage of chitin. CHOS has been shown to possess diverse biological activities including anti-oxidative stress, anti-inflammation, neuroprotection and anti-obesity. However, the effects of CHOS on cellular autophagy have never been reported. The aim of this study was to investigate the biological activities of CHOS on neuroblastoma SH-SY5Y cells. CHOS with 85% degree of deacetylation was prepared by enzymatic hydrolysis using recombinant Bacillus subtilis chitosanase, and characterized by ¹H-NMR, size exclusion chromatography, and mass spectrometry. CHOS at the concentrations ranging from 100-1,000 µg/ml was not cytotoxic to the SH-SY5Y cells. Interestingly, the expression of autophagy-associated genes was altered upon the CHOS treatment. SH-SY5Y treated with CHOS showed increase in the expression of ATG5. This result indicated that CHOS could activate autophagy within SH-SY5Y cells. Moreover, the influence of CHOS on oxidative stress and apoptosis was also examined. SH-SY5Y cells were pretreated with CHOS for 24 hours prior to challenging with paraquat (neurotoxic agent) for another 12 hours. When the cells were pretreated with 1,000 µg/ml of CHOS, the number of apoptotic cells was reduced from 9.5 to 3.4%. Besides, the expression of antioxidant genes, including SOD, GPX and CATALASE, were also upregulated after pretreated SH-SY5Y cells with CHOS for 6 hours. In conclusion, this study suggested that CHOS could upregulate autophagy and effectively protect human neuroblastoma SH-SY5Y cells from a neurotoxic agent. This highlights the potential use of CHOS as a neuroprotective agent in the future.

Keywords: Antioxidant; Autophagy; Chitooligosaccharides; Neuroblastoma

Selected Reference:
Prevalence of Neutralizing Antibodies Against 4 Serotypes of Dengue Virus in Certain ASEAN Volunteers

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Abstract
Four dengue virus serotypes (DENV1-4) circulate globally, causing more human illness than any other arthropod-borne virus. Dengue can present as a range of clinical manifestation from asymptomatic, undifferentiated fever to Dengue Fever, and severe, life-threatening syndromes of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Exposure to a homotypic dengue virus infection resulted to humoral immune response by generating specific neutralizing antibodies (NAbs). NAbs are thought to provide long-lived protection against symptomatic infection and severe dengue. However, the homotypic NAb does not provide the immunity to subsequence heterotypic infection. This study was conducted to determine the prevalence of neutralizing antibody of 4 serotypes in endemic localities, i.e., Nakhon Ratchasima province in Northeastern Thailand. The overall healthy participants (n= 62) are from ASEAN countries, Thailand (n=49), Vietnam (n=4), Indonesia (n=2), Cambodia (n=2), Myanmar (n=3) and Laos (n=2) as well as 3 Austrians. Plaque Reduction Neutralization Test (PRNT) was performed in 65 serum samples. The participants who have neutralizing antibodies against 4 serotypes were found at 63.1% (95% confidence interval at ±0.12). Whereas, the prevalence of neutralizing antibodies was 67.7%, 81.5%, 70.8% and 67.7% for DENV1, DENV2, DENV3 and DENV4, respectively. The seroprevalence of IgG antibodies against DENVs of Thai and Non-Thai; ASEAN were 87.8% and 46.1%, indicating that most of the population from Thailand and ASEAN countries had already been exposed to DENVs infection. Whereas, the 3 Austrians showed no neutralization activity against 4 serotypes. The outcome of this study confirms previous observation which help contributes to the understanding the immune response of dengue serotype circulating and provides essential information for the evaluation of vaccine candidates under development.

Keywords: Dengue virus; Neutralizing antibody; Plaque reduction neutralization test (PRNT)

Selected Reference:
Screening and Bioactivity Measurement of High Altitude Plants of Nepal

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Abstract

Five different high altitude medicinal plants obtained from the area of Mardi Himalaya Base Camp – *Swertia sp.*, *Picrorhiza kurroa*, *Rheum nobile sp.*, *Dactylorhiza hatagirea*, and *Acotinum gammiei* were selected. Cold extraction was performed using methanol and ethyl acetate as solvents. Ethyl acetate extracts of most plants found to contain glycosides, terpenes and sterols. *Rheum nobile* showed the presence of alkaloids, saponins, glycosides, tannins, flavonoids and coumarins while *Picrorhiza kurroa* showed their absence. Methanolic extract of most of the plants contained glycosides, tannin, flavonoids and coumarins. Methanolic extract of *Rheum nobile* also showed positive for all the phytochemicals that were tested in ethyl acetate. The same extracts were tested on seven different pathogenic microbes in which ethyl acetate extracts showed better result than the methanolic extracts. The ethyl acetate extracts showed good antimicrobial activity when compared with the methanolic extracts whereas both extracts showed better R² values calculated by damped diffusion equations. Linear regression analysis for antioxidant DPPH and IC50 assay revealed the lowest IC50 for *P.kurroa* leaf extract with 29.61 μg/ml whereas *Swertia chirata*, *Rheum nobile* and *D.hatagirea* was found to be 39.57, 40.34 and 61.12 μg/ml respectively. This result indicates the value of high altitude herbal plants in medicinal purpose. Rigorous scientific studies may pave the new horizon of this research type in future.

Keywords: Antimicrobial; Antioxidant; IC50; Phytochemical; R²

Selected References:
Bioactivity, Cytotoxicity and Antioxidant Measurement of Himalayan Herbs of Nepal

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Abstract
Bioactivity and potent medicinal value of herbs from Himalayan region of Nepal was explored with their cytotoxicity and antioxidant property along with isolation of novel compounds, high antimicrobial activity in Himalayan exotic plants. Five different high altitude medicinal plants obtained from the area of Mardi Himal Base Camp were isolated Swertia, Picrorhiza kurroa, Rheum sp., Dactylorhiza hatagirea, and Acotinum gammiei were selected. Crude extract was extracted by methanol as solvent and tested for the presence of phytochemicals such as alkaloids, saponins, glycosides, tannins and flavonoids. Rheum sp. and A. gammiei showed the best result for antimicrobial assay in Free Model MIC (μg/ml) 52.03 and 16.52 respectively. Among the five analyzed plants, the P.kurroa leaf extract showed lowest IC50 with value of 29.61 μg/ml. Similarly, IC50 of Swertia, Rheum and D. hatagirea was found to be 39.57, 40.34 and 61.12 μg/ml respectively. Bioinformatic analysis with KEGG database provides the potential biochemical and gene regulation pathway for the active compound structural and docking motifs for drug discovery.

Keywords: Antimicrobial; Antioxidant; Docking; Phytochemical and motifs
P-BPMB-13

Finding Cancer Cell Targeted Nontoxic Peptide Therapeutics from UMP Kinase Like Protein of Nonhemolytic *Bacillus thuringiensis*

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Abstract

Biological peptides that directly target cancer cell lines cannot proceed further in clinical trials due to their inherent toxicity. Therefore, a study was conducted to predict nontoxic anticancer peptides from UMP kinase like protein of *Bacillus thuringiensis* (*Bt*). 54 nonhemolytic *Bt* strains were checked by PCR using cancer cell killing parasporin gene specific primers. Four different sizes PCR amplicons were found from nine *Bt* isolates. Sequence analysis of full length gene amplicon of *Bt*-MyIa2 strain showed significant similarities with parasporin genes but had a conserved domain similar to UMP kinase like protein with two transmembrane helixes. SVM and Benchmark method based two webserver AntiCP and iACP respectively were used to predict anticancer peptides. Twelve anticancer peptides were found high scoring from both servers but only two were exhibited as stable and nontoxic from *Ex-Passy ProtParam* tool and *ToxinPred*. Besides, we revealed that both of these peptides possess significant similarities with existing anticancer peptides of CancerPPD and TumorHoPe database by web exploration. These peptides also have a good number of positively charged amino acids and transmembrane regions which denotes their possibility to bind and penetrate negatively charged cancer cell membrane. Furthermore, apparent binding affinities of these peptides within pockets of ER, HER2, EGFR and Eph receptor of breast, lung, gastric and prostate cancer were confirmed by protein-peptide docking. So, the present study shall be a useful basis for working further on developing nontoxic anticancer peptides as therapeutics from *B. thuringiensis* strain.

Keywords: Anticancer; *Bacillus thuringiensis*; Transmembrane protein; Therapeutics

Selected References:
P-BPMB-14

Generation of Intact, Human IgG Format Antibodies that Penetrate into the Cytosol of Living Cells

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Abstract

Human IgG format antibodies cannot cross plasma membrane of living cell, which limits its use on targeting cytosolic proteins. Here, we describe full-length human IgG format antibodies, called cytotransmabs, which internalize into living cells and localize in the cytosol. We first developed a humanized light chain variable domain (VL) that penetrate into the cytosol of living cells and was engineered for association with human heavy chain variable domains (VHs). When light chains with humanized VL were co-expressed with 3 heavy chains, including clinically approved adalimumab and bevacizumab, all IgG format antibodies were internalized into the cytosol. Cytotransmabs internalized by clathrin-mediated endocytosis via the interaction with heparan sulfate proteoglycan (HSPG) and escaped into cytosol from early endosome without being further transported into other cellular compartments, such as lysosome, endoplasmic reticulum (ER), golgi, nucleus. Furthermore, human lysyl-tRNA synthetase (KRS) targeting cytotransmab, called KT4, co-localized with the cytosolic KRS protein when it was incubated with living cells, demonstrating that cytotransmab can directly bind cytosolic proteins. These results suggest that cytotransmabs, which efficiently penetrate into cytosolic space of living cells, will find widespread uses as research, diagnostic, and therapeutic agents.

Keywords: Cytosol; Cytotransmabs; Human IgG; Living cells

Selected Reference:

Physical and Biological Evaluation of the CEL-BIC™, a Newly Developed Single-use Bioreactor System for Cultures of Animal Cells

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Abstract
The usage of single-use bioreactors for cell culture has been increasing continuously, as the bio-industry of biopharmaceuticals (proteins, antibodies, cell therapies and gene therapies products) has expanded dramatically during recent decades. The single-use bioreactors (SUBs) have several advantages compared with the conventional stainless steel based bioreactors, such as flexibility in scale, low risk of contamination, reduced labors for cleaning/sterilization, and validation/regulation issues. In this study, a newly developed cell culture system, CEL-BIC™, driven by multi-directional rocking movement is introduced and evaluated its physical and biological performances as a cell culture device. The volumetric mass transfer coefficient, $k_{La}$, and mixing time were measured as physical properties of the bioreactor at working volume of 1, 5, 10 L. Recombinant CHO cells were cultivated to evaluate its biological performance at working volume of 5 L. From these experiments, the measured values of the $k_{La}$ and mixing time were competitive or superior results compared with other commercial/conventional bioreactors. And the biological evaluation data showed its comparability in the aspect of viable cell density and metabolites, particularly in CHO cell cultures. In summary, since the new SUB cell culture system driven by rocking movement provides sufficient and excellent conditions for cell cultures, it can be an attractive candidate for cell culture device to produce biopharmaceutics, in particular, antibodies or therapeutic proteins from recombinant CHO cells.

Keywords: Animal cell culture; Single-use bioreactor
P-BPMB-16

Optimization of Signal Peptide for Enhanced Production of Recombinant Interferon-beta from Animal Cells

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Abstract
Signal peptide plays important role as a modulator for protein synthesis and secretion. Recently, recombinant DNA research has been used to study signal peptide and understood that the native signal peptides are not optimal for production of corresponding target proteins. In this study, the native signal peptide of interferon-beta has been modified for production of interferon-beta, and then compared with the usage of original native interferon-beta signal peptide. Additionally, the signal peptide of IL-2 and its modified forms, in particular, modified in hydrophobic region were evaluated for enhanced production of interferon-beta. The productivity of interferon-beta that has augmented hydrophobicity was slightly higher than that from wild-type one. Besides, Extracellular levels of interferon-beta mediated by modified human IL-2 signal peptide were higher than interferon-beta levels mediated by native signal peptide of interferon-beta. These observations also showed that interferon-beta production could be increased by modifying the wild-type human IL-2 signal peptide in the hydrophobic region from animal cells. When the hydrophobicity of signal peptide increased by replacing non- or weak- hydrophobic amino acids with strong hydrophobic amino acid, leucine, productivity of human interferon-beta from animal cells could be enhanced. These findings indicate that increased hydrophobicity in their respective domain augments productivity of recombinant proteins, and this can be applied to develop cell lines producing recombinant therapeutic proteins which are important in biopharmaceutical industries.

Keywords: IFNB; IL-2; Interferon-beta; Signal peptide

Selected References:
Abstract

Hepatitis B virus (HBV) infection can cause chronic infection and puts people at high risk of death from hepatocellular carcinoma and liver cirrhosis. Recent researches have paid a great attention on a development of anti-HBV and anti-liver cancer drugs derived from natural products. In Thai traditional medicine, fruits of *Brucea amarissima* Desv have been consumed for the treatment of dysentery, malaria and cancers. The objective of this study was to investigate anti-HBV and anti-liver cancer activities of the protein hydrolysate extracted from *B. amarissima* fruits. By performing MTT assay, the results indicated that the protein hydrolysate of *B. amarissima* fruits significantly inhibited cell viability of HepG2.2.15 in dose dependent manner with the IC$_{50}$ of 10.54 µg of protein/mL. In addition, the results from a quantitative PCR showed that the protein hydrolysate extracted from *B. amarissima* fruits had no effect on the HBV gene expression ($X$, $S$ and $C$). To partially purified peptides, a reverse phase high performance liquid chromatography (RP-HPLC) was performed. Five pooled fractions were then collected and labelled F1 to F5. Each fraction was tested for the anti-liver cancer activity using MTT assay. The results showed that all pooled fractions except F4 significantly inhibited the cell viability of HepG2.2.15 with approximately 60% inhibition. Conclusion, this study firstly reported that the partially purified protein hydrolysate extracted from *B. amarissima* fruits possesses anti-liver cancer activity. The results are promising for further purification of bioactive peptides.

**Keywords:** Anti-hepatitis B virus; Anti-liver cancer; *Brucea amarissima* Desv; Protein hydrolysate
BBE : Bioprocess and Bioseparation Engineering
Expert System for Sugar Process Improvement

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Abstract
The sugar production in Thailand has a great differences in production yield due to the variation in raw material quality, levels of contamination of sand and soil as well as the sugar production operation. Several key performance indexes (KPI) were established for production control to help monitoring the sugar production. To enhance the factory operation, the intelligent computer system (expert system) for raw sugar production was constructed from material balancing incorporated with existing real time measurement and rate equations. The differences between system prediction and real process value are used for process evaluation and adjustment, if necessary. This could be incorporated to the process as a tool for process evaluation and process control in the later stage. The initial phase for monitoring system was constructed based on the 2015/2016 milling season at Mitrphol Kalasin Sugar factory in Thailand. Also, the clarification unit and evaporation section were used as the demonstration for the application of the proposed system. Based on the result comparison, the deviation of the process prediction still needs further improvement to be more efficient for system utilization.

Keywords: Expert system; Material balance; Raw sugar production; Sugar process

Selected References:
Ethanol Fermentation of Glucose/Xylose Mixtures using Sequential-co-culture System of \textit{Pichia stipitis} TISTR 5806 and \textit{Saccharomyces cerevisiae} TISTR 5606

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Abstract

Lignocellulosic biomass from agriculture is one of the most abundant and very attractive renewable resources. Many researchers have been interested in developing an economical production of bioethanol from lignocellulosic materials. Glucose and xylose are two major fermentable sugars in lignocellulosic hydrolysates. However, an optimal condition for co-fermentation of two microorganisms to simultaneously utilize these two sugars is a very important factor for high efficient and economical process for ethanol production from lignocellulosic hydrolysates. The experiments in this study were performed to preliminarily investigate an efficient ethanol fermentation process using co-culture system of \textit{Pichia stipitis} TISTR 5806 and \textit{Saccharomyces cerevisiae} TISTR 5606. Three fermentation schemes of ethanol production from glucose/xylose mixtures were evaluated including; co-culture system, sequential-co-culture system, and also sequential-co-culture system with altering glucose supplementation. The kinetic parameters of ethanol fermentation showed that ethanol yield ($Y_{\text{P/S}}$) of co-culture and sequential-co-culture technique were 0.23 g ethanol/g substrates and 0.32 g ethanol/g substrate, which were 45.61% and 62.12% of theoretical yield, respectively. Finally, this study demonstrated that sequential-co-culture system of \textit{P. stipitis} TISTR 5806 and \textit{S. cerevisiae} TISTR 5606 provided higher efficiency of ethanol production than co-culture system.

Keywords: Ethanol; \textit{Pichia stipites}; \textit{Saccharomyces cerevisiae}; Sequential-co-culture

Selected References:

P-BBE-03

Study on Characteristics and Skin Efficacy of Low-molecular-weight Peptide Derived from Glycine max L.

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Abstract

Recently, there have been numerous reports on the enzymatic decomposition of proteins, which are classified as polymers, in order to increase their value. In particular, soybean (Glycine max L.) in the form of a low-molecular-weight peptide (LP) shows increased solubility and absorption rate in humans but decreased viscosity and possibility of allergy generation, as compared to the intact protein. This property enhances its applicability in cosmetics, healthy foods, infant foods, etc. In this study, LPs (< 3,000 Da) were prepared by treatment with Bacillus protease and ultra-filtration, using soybeans as the raw material; the peptides were characterized, and their applicability as cosmetic materials was confirmed. Gel permeation chromatography showed that soybean peptides (SP) were LPs with an average molecular weight of ~ 1,200 Da. The total crude protein content in SP was 45.4%, with the total amino acid content being 6.8%. LC-MS analysis revealed that dipeptides of the Leu-Val family were abundant in SP; Val-Leu, Val-Ile, and Ile-Val had the highest concentrations (106, 57, and 25 ppm, respectively). A skin efficacy test was performed to determine the anti-aging activity of SP and thus confirm its suitability as a raw material for cosmetics. The results showed that the treatment of keratinocyte with SP (> 100 ppm) significantly increased cell proliferation over a control group (1% fetal bovine serum). In addition, for treatment with 0.1 ppm SP, the gene expression rate of col1a1 (encodes Type I collagen) decreased by UVB was exceeded that of 2µM retinoic acid (positive control). Thus, low-molecular-weight peptides derived from soybeans can be used as raw materials in new anti-aging cosmetics based on skin efficacy evaluation.

Keywords: Low molecular weight peptide; Skin efficacy; Soybean
P-BBE-04

Synthesis of Immobilized Functional Ionic Liquid Silica and Its Application in Deoximation Reaction under Mild Conditions

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Abstract

Oximes are frequently used for protecting carbonyl group, which serves as purification and characterization of carbonyl compounds. Oxime can be prepared from non-carbonyl compounds, and the regeneration of carbonyl compounds from oximes represents a potential route for synthesis of ketones and aldehydes. However, the existing methods use a lot of chemicals, thus causing environmental pollution. To overcome the disadvantages of existing methods, deoximation using ionic liquid has been studied. In general, deoximation reactions require acidic catalyst. In this work, immobilized acidic ionic liquid silica was prepared, in which functional ionic liquids were covalently bound to silica resins, and used for deoximation of cyclohexanone oxime. The immobilized functional ionic liquid silica was characterized by NMR, FT-IR, SEM and TGA. The results showed that the yield of deoximation reaction from immobilized ionic liquid silica was similar to that of amberyst-15 as catalyst (65%). However, the deoximation reaction rate by immobilized ionic liquid silica was at least 3 times higher than that of amberlyst-15.

Keywords: Deoximation; Immobilization; Ionic liquid
Enhanced Curdlan Production with Nitrogen Feeding During Polysaccharide Synthesis by *Rhizobium radiobacter*

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**Abstract**
Curdlan is a secondary metabolite synthesized by *Agrobacterium* sp. and some other bacteria. Although nitrogen source is necessary for cell reproduction, curdlan production is largely dependent on nitrogen limitation, as well as cell vitality. Here, a nitrogen feeding strategy was investigated to elevate the curdlan production by *Rhizobium radiobacter* CGMCC12099. The optimal concentration and addition time of (NH$_4$)$_2$HPO$_4$ were investigated. The results show that the enhanced cell density is correlated to the amount of (NH$_4$)$_2$HPO$_4$ added. Also, nitrogen addition in earlier fermentation stage is beneficial to the cell growth and curdlan production. Furthermore, continuously feeding strategy was employed by feeding (NH$_4$)$_2$HPO$_4$ at a constant rate of 0.62 g/L/h at 35$^{th}$ h of fermentation for 9 h, achieving a final curdlan production of 65.27 g/L, productivity of 0.544 g/L/h and glucose conversion rate of 38.89%. The curdlan production was improved by 2.1 times compared with that without nitrogen addition. This study provides a feasible and cheap nitrogen feeding strategy to enhance curdlan production.

**Keywords:** Curdlan; Nitrogen feeding; (NH$_4$)$_2$HPO$_4$; *Rhizobium radiobacter*

**Selected References:**
Online Estimation of Overall Heat Transfer Coefficient in Sugar Evaporators

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Abstract
Evaporators are important unit operations in the sugar factories. Heat transfer coefficient is an essential parameter to represent an evaporator efficiency. The decreasing efficiency was used to determine the suitable period of cleaning. An estimation of heat transfer coefficient was carried out by evaluating all measurement inputs and outputs coming into and out of the evaporator. The model based on mass and energy balances was used to infer the heat transfer coefficient. Following the available online measurement, the heat transfer coefficient was then estimated online. This study was investigated at Singburi sugar factory in Thailand during the 2015-2016 milling season. Principle factors such as juice temperature, latent heat, steam consumption, heat transfer area, juice flow rate as well as brix raw syrup were used to estimate the heat transfer coefficient. Based on the current milling rate at 15,000 tons cane per day, the evaporators were operated normally up to 30 days before cleaning. The profile/trend of decreasing heat transfer coefficient was correlated with evaporators operation and cleaning. The online estimation of heat transfer coefficient would also be used for maintaining syrup concentration at the desired value (60-65 °Bx) in the feedback control manner. This is however needed for evaluation of the economic analysis prior to the next milling season.

Keywords: Efficiency; Evaporator; Heat transfer coefficient; Mass and energy balances

Selected References:
EB : Environmental Biotechnology
P-EB-01

Capability of Progression and Lipid Accumulation of Oleaginous Yeast *Lipomyces Starkeyi* with Glucose and Xylose

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Abstract

Triacylglycerol – TAG is the major produced lipid of fatty acid composition, which is accumulated by the oleaginous yeast and it can be transesterified to fatty acid methyl ester to meet the criteria of the biodiesel standard. *Lipomyces starkeyi* is yeast that can accumulate oil up to 70% (dry mass basis) of their biomass. In this study, effect of glucose concentrations was in the range (40, 60, 80 and 100 g/L) on growth of *L. starkeyi* was determined. Mixed simple sugar of glucose and xylose was also investigated. The results showed that 40-80 g/L glucose concentration gave similar biomass concentration of 14.1 g/L, whereas 13.0 g/L dry cell weight was obtained in 100 g/L glucose cultivation. This was due to a high concentration of glucose could cause high osmotic pressure which restrict the cell metabolism. Mixed sugar of 50:50 and 80:20 glucose to xylose at the initial concentration of 60 g/L gave the highest biomass concentration of 17.5 (28.6 % oil content) and 16.8 (36.0 % oil content) g/L, with the lipid content of 5.2 and 6.8 g/L, respectively. In addition, no evidence of diauxic growth behavior was observed. As a result, *L. starkeyi* could uptake glucose and xylose simultaneously, which would be favorable for feeding the hydrolysates derived from lignocellulosic materials of agro-industrial waste.

Keywords: *Lipomyces starkeyi*; Microbial lipids; Oleaginous yeast; Triacylglycerol

Selected References:

P-EB-02

Survival and Environmental Adaptability of *Sphingomonas* sp. under Multiple Selective Pressures in Drinking Water Supply Systems

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Abstract

In this study, the survival and environmental adaptability of *Sphingomonas* sp. in water supply systems were investigated by genomics analyses. Six new strains of *Sphingomonas* were isolated and identified from the effluent of six different water treatment plants located in China and Japan. Their environmental adaptabilities, including chlorine, UV and multidrug resistances, were tested. The correlation between *Sphingomonas* and DNA phosphorothioate modification was analyzed by detecting DNA degradation (Dnd) genes using qPCR. The results show that *Sphingomonas* sp. can survive all currently used water treatment processes including ozone oxidation and chlorine disinfection and that biological activated carbon filtration can actually increase the relative abundance of *Sphingomonas* sp. After the chlorine disinfection process, *Sphingomonas* sp. becomes the dominant species in the bacterial community. Six new isolated strains of *Sphingomonas* sp. exhibit strong chlorine and UV resistances. Some strains of *Sphingomonas* sp. even exhibit high multidrug resistances. No obvious correlation was found between chlorine-tolerant bacteria and antibiotic resistance. Dnd gene clusters were identified in the isolated *Sphingomonas* sp. and were positively correlated with *Sphingomonas* sp. in a drinking water supply system. Our results suggest that DNA phosphorothioation may help *Sphingomonas* sp. survive under multiple selective pressures in water supply systems.

Keywords: Antibiotic resistance; Disinfectant resistance; DNA phosphorothioate modification; Drinking water; *Sphingomonas* sp.
Enhancing Enzymatic Saccharification in a Two-step System of High Pressure Steam Pretreated Rice Husk

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Abstract
The high cost of pretreatment, enzymatic saccharification process and enzyme dosage remains a major impediment to lignocellulosic bioconversion. Thus, a study was conducted to investigate a two–step saccharification process of rice husk pretreated at a developed operational condition of high pressure steam. High pressure steam pretreatment at 160-200 °C under 0.3-2.8 MPa and 2-10 min was applied and the efficiency of this method was justified by XRD and enzymatic hydrolysis. Optimum enzyme concentration of 30 FPU/g from B. licheniformis 2D55, temperature at 60 °C after 48 h was effective in producing reducing sugar (21.1 g/L = 0.422 g/g dry substrate) at a saccharification degree of 53.87%. Furthermore, conducting a second-step enzymatic saccharification resulted in an additional reducing sugar (7.9 g/L = 0.158 g/g substrate) and 20.44 % degree saccharification. On the other hand, the two-step saccharification process (36h and 24h) resulted in a significant increase sugar and saccharification yield compared to one-step continuous process. An optimum sugar yield of 0.581 g/g substrate with saccharification degree of 73.5% was achieved from the two-step process. On top of that, the process has improved the yield of monomeric sugars of glucose (0.465 g/g), xylose (0.010g/g) and cellobiose (0.063g/g). Therefore, it can be stated that the combination of high pressure steam pretreatment with crude thermostable cellulase from B. licheniformis 2D55 in a two-step enzymatic saccharification process could be considered an economic method for rice husk bioprocessing to produce sugar in industrial applications.

Keywords: Characterisation; High pressure steam pretreatment; Rice husk; Two-stage enzymatic saccharification
Development of Hydrothermal Liquefaction Process using Microalgae for Improvement of Bio-crude Yield and Phosphorous Contents

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Abstract
Microalgae are regarded as potential sources of third generation biofuels and have a lot of advantages including fast growth rate, ability to CO₂ capture, and high lipid contents. Hydrothermal liquefaction (HTL) is a thermal depolymerization process used to convert wet biomass such as microalgae into bio-crude oil, and performed in a subcritical condition of water corresponding to high pressure and specific temperature (300–375°C). Four main products including gas, bio-crude oil, aqueous phase, and solid residue are generated through HTL reaction. Among them, bio-crude oil can be directly converted to biodiesel via fractionation process, and post-HTL aqueous phase can be used as nutrients for microalgae growth during recultivation. In this study, we investigated the HTL condition to increase the phosphorous contents in aqueous phase, as well as the yield of bio-crude oil. Tetraselmis sp. was used as a microalgal feedstock, and HTL reaction was performed in a small batch reactor with a salt bath either at 300 °C or 350 °C. For this purpose, acetic acid was used as a catalyst in HTL reaction and the product yields were investigated according to the acetic acid concentration (0.2–2.0 M). As a result, phosphate ion (PO₄³⁻) content in the aqueous phase was increased over three times when acetic acid concentration was adjusted to 1.5 M. In addition, we found that the ammonium ion content in the aqueous phase was continuously accumulated, while the phosphate ion was not, which is expected to precipitation, when aqueous phase is recycled.

Keywords: Acid catalyst; Aqueous phase; Hydrothermal liquefaction (HTL); Microalgae; Phosphorous content

Selected References:
NBB : Nanobiotechnology, Biosensors and Biochips
Fiber-optic Based on the Localized Surface Plasmon Resonance for Detecting Human Papillomavirus

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Abstract
A reflection-based localized surface plasmon resonance fiber optic sensor has been developed to detect the 6, 11, 16, and 18 genotypes of Human papillomavirus (HPV) associated with cervical cancer. The detecting probe combines with single strand DNA 50 mers and thiolated 20 mers and annealing up to 95 ºC, leading to the double-stranded formation in the terminal and designing 5’ end 20 mer which has specificity DNA sequence. The optimal immobilizing conditions of HPV-11 on to the modified optical fiber are immersed in 200 nM DNA with 20 mM MgCl₂ and 20 mM KCl solution for 10 h. As hybridizing with HPV-11 PCR product at 42 ºC, the relative intensity change of HPV-11 DNA probe-functionalized sensor was 3.8% with a 0.86 ng/ml limit of detection. The responses of HPV-6, -16, and -18 DNA probes to particular PCR products are 4.78%, 4.81%, and 5.13% and have the 0.37 ng/mL, 0.37ng/mL and 0.695 ng/mL limit of detection, respectively. The HPV-11 probe has a good specificity except the HPV-18 PCR product and other three types of HPV probes have good specificity. The kinetic parameters of HPV-6, kₐ, k₅ and Kₐ, are 9.278x10⁵ M⁻¹s⁻¹, 1.206x10⁻⁴ s⁻¹ and 7.693x10⁹ M⁻¹, respectively. The result shows that the four types of HPV double strand DNA probes have a good affinity and response with PCR product.

Keywords: Fiber optic sensor; HPV probes; Human papillomavirus; Localized surface plasmon resonance

Selected Reference:
Easy Design of Nano-oriented Interface Molecule on Material Chip: Application of using Substrate Material Binding Protein for Biosensing

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Abstract
Many biosensors use antibodies for recognizing targets and the antibodies should be immobilized on sensor chips that transduce capturing action to optical or electrical signals. High density and homogeneous orientation of immobilized antibody increases sensitivity biosensor. In general, antibodies are chemically immobilized on sensor chips; however, the chemical method can’t control the orientation of antibody on the chips. Recently, we devised a single variable domain of the heavy chain camel antibody (VHH) with high affinity for material surface. Use of the VHH for an interface between sensor chip and recognition molecule is expected for highly sensitive detection, because it can immobilize antibody with high density on the surface of material chip and it also needs no chemical modification. In this study, we constructed a fusion protein by fusing various antibodies to C-terminal of material binding VHH and tried to make the wide use biosensor which can detect various antigens sensitivity. Consequently, sensitivity of target detection increases by using fusion protein compared with physical adsorption.

Keywords: Antibody; Biosensor; Immobilization
A Biosensor Based on Graphene Modified Ultramicroelectrode Array for Rapid Detection of Biochemical Oxygen Demand

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Abstract

Biochemical oxygen demand (BOD) is an international regulatory index for assessing organic water pollution, which needs to be rapidly detected in water quality monitoring. Aerobic microorganisms utilize organics for respiration, during which dissolved oxygen is consumed and measured as the BOD response. In this paper, based on the fabrication of ultramicroelectrode array (UMEA) and the electrodeposition of carboxyl graphene (GN-COOH), a biosensor has been developed for rapid and sensitive detection of BOD. The electrodeposition of GN-COOH was done in the electrolyte containing 2 mg/mL GN-COOH, 0.1M LiClO\textsubscript{4}, 0.073 mM HAuCl\textsubscript{4} and 5 mM PBS with N\textsubscript{2} bubbling. The electrodeposition process was carried out by chronoamperometry at -0.9V for 120s. Cells of \textit{B. subtilis} have been directly immobilized on the electrode by covalent bonding. During electrodeposition, LiClO\textsubscript{4} is added to form a three-dimensional porous network. Owing to UMEA’s high diffusion rate and graphene’s good electrochemical activity, mass transfer has been facilitated around the electrode’s surface and reduction of dissolved oxygen has been catalyzed, which leads to rapid and sensitive sensing. Under the optimized conditions, the proposed biosensor shows a linear range from 2 to 15 mg/L, with the correlation coefficient of 0.990. The response time of the detection has been shortened to 3 min. Compared with the traditional BOD electrode including immobilized microbial film and oxygen permselective membrane, the designed \textit{B. subtilis/ rGN-COOH/ UMEA} electrode facilitates electron transfer and simplifies the structure for more effective mass transfer, indicating its potential in BOD rapid detection.

Keywords: Biochemical oxygen demand; Carboxyl graphene; Immobilized cells; Mass transfer; Microbial electrode
P-NBB-05

Shorten Aptamers Binding to a Pesticide

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Abstract
Pesticides are widely used to protect crops from insects, microorganisms, fungi, weeds, and pests. However, the residue of pesticides in agricultural products can be harmful since it is known to be carcinogenic and mutagenic molecules or hormone mimickers. Thus, to detect those pesticide residues, aptamer which specifically binds to pesticides can be used as a receptor in the platform of an aptamer-based biosensor. In this study, we focused on the target-binding ability of the aptamer regarding its structure. Based on a software program (M-fold), we truncated few specific regions in an original aptamer sequence. To compare the target-binding affinity of original and truncated aptamer, a gold nanoparticle-based colorimetric assay was done. Results showed the higher sensitivity to the target when the original aptamer was truncated at some regions. More efficient aptasensor, especially in terms of its sensitivity, can be developed through this truncation study.

Keywords: Aptamer truncation; Gold nanoparticle-based colorimetric assay; Pesticide
Single Stranded DNA Aptamers Targeting Avian Influenza Virus Generated from Graphene-Oxide SELEX

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Abstract
Avian Influenza virus causes an infectious disease mainly in birds and some mammals. This contagious disease, Avian Influenza, has almost 100 percent mortality rate and ability to spread out easily by travel routes, transportation and migratory birds. There have been also some rare cases of human infection from when it was first identified in 1997. Therefore, early detection of Avian Influenza virus has great importance in terms of economics and public health by suppressing outbreak of pandemic disease. Aptamer is a nucleic acids chain which can specifically bind to target molecules such as proteins, small molecules, nucleic acids, and even whole cells. To detect viruses, aptamers have been developed as a good substitution for antibody. In this study, we successfully developed ssDNA aptamers which can recognize a subtype of an Avian Influenza virus specifically. The aptamer sequences were selected by Graphene-Oxide SELEX(Systematic evolution of ligands by exponential enrichment). These ssDNA aptamers would be further used to develop numerous types of aptasensors.

Keywords: Aptamer; Avian Influenza virus, Graphene-Oxide SELEX

Selected References:
P-NBB-07

Surface Topographic Analysis via Atomic Force Microscopy for the Evidence of Glutathione-s-transferase Immobilization on Chitosan Modified SPCE

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Abstract
Currently, enzymatic biosensors have been realized as the most promising tool for detection of pesticides level to control their toxicity and environmental contamination. Immobilization of enzyme specific to the target toxicant onto suitable substrate surface is an essential step for the biosensor development. In this study, the enzyme glutathione-s-transferase (GST) constitutea protein superfamily that is involved in cellular detoxification against harmful xenobiotics and endobiotics was introduced in this critical function. A natural biopolymer chitosan was used to modify a screen printed carbon electrode (SPCE) working surface by self-assembling method to facilitate efficient ionic and covalent bonding binding of the enzyme molecules with the aid of glutaraldehyde as a crosslinking and surface activating agent. Accomplishment in chitosan modification and GST immobilization were explored using atomic force microscopy (AFM). A layer by layer deposition was clearly resolved in both steps by nano-imaging and quantitative surface topographic determination. Differentiation among surfaces of the screen printed carbon basement, the deposited chitosan and the immobilized GST was clearly verified in all parameters measured such as a surface roughness, a height difference, skewness and kurtosis. In addition, a spatial distribution of their frequency components to the total roughness of each surface could be mapped by power spectral density (PSD) function. Simultaneously, an aggregation of the GST in vertical direction on the basal chitosan surface was also revealed.

Keywords: Atomic force microscopy; Chitosan; Glutathione-s-transferase; Immobilization; Screen printed carbon electrode

Selected References:
SSB : Systems and Synthetic Biotechnology
P-SSB-01

Comparative Genomics Reveals Conserved Genes and Common Functional Modules in *Pasteurella multocida*

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Abstract

*Pasteurella multocida* is a Gram-negative pathogen that causes a wide range of diseases in domestic animals and human via canine and feline scratches and bites. Advancing in high-throughput DNA sequencing technology and bioinformatics, the eighteen genomes of *P. multocida* obtained from various animal hosts are available, these allow to gain a better understanding of cellular processes and functions in *P. multocida* and their pathogenicity. Here, this study aims to identify conserved genes and common functional modules from the eighteen genomes of *P. multocida* using comparative genomics. Identification of the conserved genes was conducted by Prodigal and Pancoreplot programs in CMG-biotools. As a result, the predicted conserved genes were 1,399 genes. After the KEGG Mapper used, interestingly 6 common functional modules were identified which involved in the systems of iron (III) transport, iron complex transport, heme transport, molybdate transport, and ribose transport. This study serves as a scaffold to identify core functional targets for preventing *P. multocida* infection and pathogenicity.

Keywords: Comparative genomics; Functional modules; *Pasteurella multocida*

Selected References:

P-SSB-02

CRISPRi-induced Effective Gene Repression to Enhance the Production of Lipid in Microalgae

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Abstract

In recent years, the clustered regularly interspaced short palindromic repeats-associated protein (CRISPR-Cas) is an adaptive defense system existed in bacteria. It is emerging as a rapid tool that widely operates genetic modification for targeted genes from mammals, humans, plants and other cells and opens a new era of genomic editing. However, metabolic engineering of microalgae to improve their useful phenotypes by CRISPR interfering system are still not reported. The CRISPRi applied for red fluorescent protein (RFP) in C. reinhardtii showed 94% in the efficiency of repression and stable over 7 generations. Afterwards, we show successfully in obtaining CRISPRi guided recombinant strains in C. reinhardtii at the loci on phosphoenolpyruvate carboxylase isoform 1 (CrPEPC1) gene. All of CrPEPC1 silenced strains have lower Chlorophyll adsorption values at OD_{680}, but high-stability of passage cultivation based on biomass concentration. The CrPEPC1 repression increased the expression level of type 2 diacylglycerol acyltransferase (DGAT) gene CrDGTT1, which is directly related to lipid biosynthesis and show an increase of 74% and 94% for lipid content and lipid productivity, respectively. Further verification of PEPC1 down-regulation and DGTT1 up-regulation are accomplished by qRT-PCR. Finally, we successfully established CRISPRi-Cas9 platform for enhanced lipid production by genetic modification in microalgae.

Keywords: CRISPRi; Lipid production; Microalgae; PEPC1

Selected References:

P-SSB-03

Systems Approach to Characterize the Metabolism of Liver Cancer Stem Cells

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Abstract
Liver cancer stem cells (LCSCs) have attracted attention because they cause therapeutic resistance in hepatocellular carcinoma (HCC). Understanding the metabolism of LCSCs can be a key to developing therapeutic strategy, but metabolic characteristics have not yet been studied. Here, we systematically analyzed and compared the global metabolic phenotype between LCSCs and non-LCSCs using transcriptome and metabolome data. We also reconstructed genome-scale metabolic models (GEMs) for LCSC and non-LCSC to comparatively examine differences in their metabolism at genome-scale. We demonstrated that LCSCs exhibited an increased proliferation rate through enhancing glycolysis compared with non-LCSCs. We also confirmed that MYC, a central point of regulation in cancer metabolism, was significantly up-regulated in LCSCs compared with non-LCSCs. Moreover, LCSCs tend to have less active fatty acid oxidation. In this study, the metabolic characteristics of LCSCs were identified using integrative systems analysis, and these characteristics could be potential cures for the resistance of liver cancer cells to anticancer treatments.

Keywords: Cancer metabolism; Genome-scale metabolic models; Hepatocellular carcinoma; Liver cancer stem cells; Systems biology

Selected References:
P-SSB-04

Metabolic Engineering of *Mannheimia succiniciproducens* for Succinic Acid Production using Elementary Mode Analysis with Clustering

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**Abstract**

*Mannheimia succiniciproducens* has been known as efficient succinic acid producer. Although a metabolic network of *M. succiniciproducens* was previously studied using a genome-scale metabolic model, it still has more to be explored for improved succinic acid production. Here, we applied elementary mode analysis with clustering (‘EMC’ analysis) to gain further insights and increase production of succinic acid. Elementary modes (EMs) were generated from the metabolic network of *M. succiniciproducens* and clustered to investigate networks of succinic acid production. Based on EMC analysis, the *zwf* and *mdh* genes were identified as overexpression targets for the enhanced succinic acid production. Overexpression of these genes was performed in a previously developed succinic acid-overproducing *M. succiniciproducens* LPK7 strain, in which *ldhA*, *pta/ackA* and *pflB* genes encoding lactate dehydrogenase, phosphotransacetylase and acetate kinase and pyruvate formate lyase, respectively, were inactivated. Despite simple network analysis, EMC analysis provides biological information complementary to other existing computational tools, which are useful for fundamental metabolic analysis and metabolic engineering. [This work was supported by the C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2016M3D3A1A01913250)]

**Keywords:** EMC analysis; Metabolic engineering; Succinic acid
P-SSB-05

Synthetic Control of Mammalian Cell Signaling by Engineering Receptor Tyrosine Kinase

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Abstract
Phosphorylation is the most fundamental post-translational modification that relates many intracellular cell signaling, which controls basic activities of cells. Artificially regulating phosphorylation of signaling molecules will lead us to efficiently control cell fates or elucidate complex signaling pathways. Recently, many researchers develop methods that can control cell signaling by regulating phosphorylation. However, a methods that can activate only one on-target signaling molecule have not been reported. In this study, we developed a novel method based on a receptor tyrosine kinase, c-Kit. C-Kit was newly engineered in order to sustain its kinase activity while other signaling molecules will not be recruited. Then, a tyrosine motif, which can specifically bind to its correspondence on-target signaling molecule, was tethered to the engineered c-Kit. When the engineered receptor was triggered by its ligand, dimerization of the engineered c-Kit induced its kinase activity, consequently only the on-target signaling molecule was recruited to the phosphorylated tyrosine motif and activated. In this study, we chose STAT1, STAT3 and STAT5 as model signaling molecules of this method and phosphorylation of the signaling molecule was detected corresponding to its tyrosine motif. In conclusion, we established a novel method for controlling cell signaling by using an engineered receptor tyrosine kinase with a tyrosine motif, and succeeded in activating on-target signaling molecules.

Keywords: Chimeric receptor; Phosphorylation; Receptor; Signaling molecules; Tyrosine kinase; Tyrosine motif

Selected References:
P-SSB-06

The Cellular Response to H$_2$O$_2$ in \textit{Pseudomonas aeruginosa} is Altered by a Loss of a Transfer RNA (C/U/A-2'-O-)-Methyltransferase

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Abstract

To overcome the damage caused by oxidative stress, cells have evolved systems for neutralizing toxicants and repairing the cellular damage. Here, we employed bioinformatics, phenotypic assays, and biochemical studies coupled with mass spectrometry to identify and investigate the function of tRNA methyltransferases that play a role in cellular response to oxidative stress in \textit{Pseudomonas aeruginosa}. Disruption of \textit{trmJ} causes a reduced level of Cm, Um, and Am in total cellular tRNA and is directly correlated with the increased cell sensitivity to hydrogen peroxide (H$_2$O$_2$), reduced catalase activity, and reduced the expression of \textit{oxyR-recG}, \textit{katB-ankB}, and \textit{katE} in \textit{P. aeruginosa}. Studies with purified \textit{P. aeruginosa} TrmJ and seven synthetic tRNAs demonstrated that TrmJ catalyzes the formation of Cm32 in tRNA$^{\text{Met(CAU)}}$, tRNA$^{\text{Trp(CCA)}}$, Um32 in tRNA$^{\text{Gln(UUG)}}$, tRNA$^{\text{Pro(UUG)}}$, tRNA$^{\text{Pro(CGG)}}$ and tRNA$^{\text{His(GUG)}}$, and Am32 in tRNA$^{\text{Pro(GGG)}}$. These results reveal that TrmJ is a tRNA-Cm32/Um32/Am32 methyltransferase and is involved in translational fidelity and the oxidative stress response.

Keywords: Oxidative stress; Methylation; \textit{Pseudomonas aeruginosa}; Transfer RNA

Selected Reference:
P-SSB-07

Biological Synthesis of Various Nanomaterials by Recombinant Escherichia coli

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Abstract

We investigated a recombinant Escherichia coli strain expressing PCS and/or MT for the microbial biosynthesis of nanoparticles (NPs). Based on this research, various metals, including semiconducting (Cd, Se, Zn, Te), alkali-earth (Cs, Sr), magnetic (Fe, Co, Ni, Mn), and noble (Au, Ag) metals and rare-earth (Pr, Gd), were incubated in assorted combinations with the recombinant E. coli cells for the synthesis of the corresponding diverse NPs. The size of the various NPs could be controlled by adjusting the concentrations of the supplied metal ions. As the high-cell-density culture of E. coli has been well established, the efficient and cost-effective production of various metal NPs would not be a difficult task. The engineered E. coli strain reported herein should be widely applicable to biological synthesis of various NPs of interest with tailored optical, electronic, chemical, and magnetic properties. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)].

Keywords: Biosynthesis; Escherichia coli; Nanomaterials

Selected References:

P-SSB-08

Microbial Production of Four-, Five- and Six-Carbon Lactams via Novel Synthetic Metabolic Pathway

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Abstract
We report construction of a new and efficient platform metabolic pathway for the microbial production of four-carbon (butyrolactam), five-carbon (valerolactam) and six-carbon (caprolactam) lactams. This pathway uses ω-amino acids as precursors and comprises two steps. Activation of ω-amino acids catalyzed by the Clostridium propionicum β-alanine CoA transferase (Act) followed by spontaneous cyclization. The pathway operation was validated both in vitro and in vivo. Three metabolically engineered Escherichia coli strains were developed by introducing the newly constructed metabolic pathway followed by systems-level optimization, which resulted in the production of butyrolactam, valerolactam and caprolactam from renewable carbon source. In particular, fed-batch fermentation of the final engineered E. coli strain produced 54.14 g/L of butyrolactam in a glucose minimal medium. These results demonstrate the high efficiency of the novel lactam pathway developed in this study.

Keywords: β-alanine CoA transferase; Butyrolactam; Caprolactam; Lactams; Metabolic engineering; Novel metabolic pathway; Valerolactam
Bio-production of Poly(lactate-co-glycolate) by Systems Metabolically Engineered *Escherichia coli*

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Abstract
The biorefinery technologies which transform biomass into fuel, power, chemicals, and materials have received a great deal of attention as a sustainable alternative to decreasing the reliance on fossil fuels. Here, we developed recombinant *Escherichia coli* producing poly(lactate-co-glycolate) (PLGA) using renewable biomass to substitute the current chemical production process. PLGA is biodegradable, biocompatible, FDA-approved, and has been widely used in biomedical and therapeutic applications such as drug delivery and tissue engineering. To produce PLGA, we engineered *E. coli* to efficiently produce the two monomer, lactate and glycolate by employing the heterologous Dahms pathway of *Caulobacter crescentus* and by optimizing the metabolic flux based on the genome-wide *E. coli* model simulation. Then, the two engineered heterologous enzymes, propionyl-CoA transferase and polyhydroxyalkanoate synthase were expressed in *E. coli* to convert lactate and glycolate to lactyl-CoA and glycolyl-CoA, respectively and finally PLGA. However, the small fraction of unwanted monomer, 2-hydroxybutyrate was detected in produced polymers. The incorporation of 2-hydroxybutyrate generated from the *E. coli* inherent amino acid biosynthesis pathway was prevented by deletion of *ilvA* gene or supplementation of L-isoleucine, finally PLGA free of 2-hydroxybutyrate was produced.

Keywords: Biopolymer; PLGA; Poly(lactate-co-glycolate); Polyhydroxyalkanoate; Systems metabolic engineering

Selected References:
P-SSB-10

**Biosynthesis of Astaxanthin in *Escherichia coli* using Metabolic Engineering**

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**Abstract**

Astaxanthin belongs to keto-carotenoid which provides red color in microalgae, yeast, salmon and in other kinds of marine organisms. Its chemical structure makes astaxanthin a very powerful antioxidant compared to other well-known carotenoids such as lutein and lycopene. Because of many useful properties, astaxanthin has been used in diverse area including food, health supplement and cosmetic industry. However, despite its versatile uses, its productivity from natural sources is low to meet increasing demand, thus alternative production method has been required. Here, astaxanthin was produced in metabolically engineered *Escherichia coli* as one of the alternatives. Firstly, the heterologous astaxanthin pathway was introduced for construction of base strain. After that, the production amount was enhanced by metabolic engineering and following optimization of culture process. The strategies for increasing precursor pools of carotenoids, expression level of heterologous enzyme and finding optimal culture conditions also could be applied for other carotenoid research.

**Keywords:** Astaxanthin; Carotenoid; *Escherichia coli*; Metabolic engineering
TEB : Tissue Engineering and Biomaterials
Fabrication and Characterization of Antibiotic-loaded Biopolymers Core-Sheath Nanofibers for Tissue Engineering Applications

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Abstract
Biopolymers-based fibers with loading bioactive cues have gained interest for tissue engineering and drug release. Here, core-sheath alginate/soy protein isolated-polycaprolactone (SA/SPI-PCL) fibers encapsulated with tetracycline hydrochloride were fabricated via co-axial electrospinning. Morphological result of as-spun fibers showed submicron-sized smooth and uniform fibers with average fibers diameter of 0.2 – 2.8 μm. Investigation of release characteristic of tetracycline-loaded SA/SPI-PCL electrospun fibers exhibited initial burst release (50 %) after 4 h of immersion, followed by prolonged release (up to 70 %) for 14 days of immersion. Consequently, the drug-loaded fibers enabled to inhibit bacterial growth against *Staphylococcus aureus* and *Escherichia coli* investigated by disk diffusion method. The cytotoxicity test from extracts of the core-sheath fibers using human fibroblasts confirmed non-toxicity and so is compatibility with the cells, indicated by high cell viability up to 100%. This study suggests that tetracycline-loaded SA/SPI-PCL core-sheath fibers could be a promising nanomaterial to be used as a tissue engineering scaffold with drug releasing function.

Keywords: Alginate; Core-sheath nanofibers; Drug release; Electrospinning; Soy protein isolated; Tissue engineering

Selected References:
Rice Bran Mineral Extract Increases the Expression of Anagen-Related Molecules in Human Dermal Papilla

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Abstract

Even though the inducing effect of electromagnetic fields (EMF) on the neural differentiation of human bone marrow mesenchymal stem cells (hBM-MSCs) is a distinctive, the underlying mechanism of differentiation remains unclear. To find out the signaling pathways involved in the neural differentiation of BM-MSCs by EMF, we examined the CREB phosphorylation and Akt or ERK activation as an upstream of CREB. In hBM-MSCs treated with ELF-EMF (50 Hz, 1 mT), the expression of neural markers such as NF-L, MAP2, and NeuroD1 increased at 6 days and phosphorylation of Akt and CREB but not ERK increased at 90 min in BM-MSCs. Moreover, EMF increased phosphorylation of epidermal growth factor receptor (EGFR) as an upstream receptor tyrosine kinase of PI3K/Akt at 90 min. It has been well documented that ELF-MF exposure may alter cellular processes by increasing intracellular reactive oxygen species (ROS) concentrations. Thus, we examined EMF-induced ROS production in BM-MSCs. Moreover, pretreatment with a ROS scavenger, N-acetylcystein, and an EGFR inhibitor, AG-1478, prevented the phosphorylation of EGFR and downstream molecules. These results suggest that EMF induce neural differentiation through activation of EGFR signaling and mild generation of ROS.

Keywords: Bone-marrow mesenchymal stem cells; Epidermal growth factor receptor, Extremely low frequency electromagnetic fields; Neural differentiation; Reactive oxygen species
P-TEB-03

Effects of Plasma Solute Distribution in Glycocalyx Layer on the Electro-Osmosis Phenomenon

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Abstract
The objective of this study was to determine the effects of plasma distribution in the glycocalyx layer, which patches of transmembrane integrin molecules, mechanically linking cell to the extracellular matrix and the external environment. These adhesion sites are focal complex or focal adhesions or contact depending on their size, state of development, and characteristic participating proteins. At the same time, actomyosin contraction within the cell can result in stress exerted against the substratum. Either equal or opposite stress at the cell adhesion sites may deform cell and substratum. Under mechanical compression of the layer, such as might occur on the passing of stiff leukocytes through capillaries, the model predicts that gradients in the electrochemical potential of the compressed layer cause a redistribution of mobile ions within the glycocalyx and a rehydration and restoration of the layer to its equilibrium dimensions. In this paper, we specifically address the problem of plasma solute distribution in the endothelial-cell glycocalyx layers. We build upon the electrochemical model and extend the analysis to address transient mechanical deformation of the glycocalyx surface layer. The results from the finite element discretization are solved monolithically for all global unknowns by linearization of the analysis. Thus the results agree well with the nonlinear solution in the limit of small deformations of the surface layer during radial compression and recovery.

Keywords: Endothelial cell; Finite deformation; Glycocalyx surface; Plasma solute
YS : Young Scientists
The Performance Analysis of a Tesla Turbine Based-pump

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Abstract
In this modern age in quest of renewable, ecofriendly sources of energy, Tesla turbines have aroused a sudden interested among many researchers as it remains a rather an unsolved puzzle. This study focuses on utilizing the reverse Tesla Turbine principle to utilize it as an air pump intending to applications such as a turbocharger replacement in an automobile. A prototype Tesla Pump was analyzed and yielded rather promising results with volumetric flowrate of 66.12 (L/s) at 5500 RPM, paving way for further development. The experimental results were analyzed to identifying the factors effecting the performance with special focus on relationship between angular velocity and output flow-rate. As Tesla pumps are reversible continuous research has potential to lead to a large array of new applications both as a pump and a turbine especially in operating with unconventional fluids such biomass which produces solid particles.

Keywords: Efficiency; Energy; Renewable; Tesla Pump; Turbine
AFOB-EFB Joint Session I on “Enzyme/Catalysis”
P-Joint I-01

Solubilization and Functional Evaluation of Silica-Polymerizing Enzyme

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Abstract
Silica-polymerizing enzyme (silicatein) found in the glass skeleton of sponges can potentially be utilized for the immobilization of enzymes and microbial cells in bioprocess because the enzyme can catalyze the polymerization of silica under mild condition, i.e., at room temperature and neutral pH. However, aggregation and enzyme inactivation would occur when silicatein is expressed in *Escherichia coli*. In the present work, we examined aggregation property and solubilization of silicatein. The gene encoding silicatein (Sil) was inserted into pCold and pCold ProS2 vectors to produce Sil and ProS2-Sil fusion protein, respectively. These genes were expressed in *E. coli*, and the aggregation and the activity of the fusion protein were evaluated. Sil and ProS2-Sil were successfully expressed using cold shock vector. The SDS-PAGE analysis of expressed proteins indicated that the proteins were overexpressed as an inclusion body, which would be insoluble and inactive protein. The proteins could be solubilized by refolding using glutathione-containing buffer at 4 °C. The refolded proteins were used for the self-assembly test under several conditions. This experiment showed that the aggregation of the fusion protein ProS2-Sil was suppressed compared to Sil. Furthermore, we found that the refolded silicateins exhibited silica formation activity using tetraethyl orthosilicate (TEOS) as a precursor.

Keywords: Biomineralization; Biosilica; Self-assembly; Soluble protein
P-Joint I-02

Oxidase and Monooxygenase Activities of L-Amino Acid Oxidase and Monooxygenase

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Abstract
L-Amino acid oxidase/monooxyganase (L-AAO/MOG) from Pseudomonas sp. AIU 813 is a non-covalent FAD-bound enzyme that catalyzes mixed activities of an oxidative decarboxylation as well as an oxidative deamination of L-amino acids. The enzyme uses L-lysine as a native substrate and oxygen is used as an electron acceptor. L-AAO/MOG has potential to be used as enzyme sensor for detection of L-lysine by measuring the amount of H₂O₂ formed. The reaction of the oxidase (deaminase) path yields α-keto acid, hydrogen peroxide and ammonia as products while amide and carbon dioxide are formed by the monooxygenase (decarboxylase) path. The wild-type enzyme can only use L-lysine, L-arginine and L-ornithine, not other L- or D-amino acids as substrates. We have investigated the catalytic features of L-AAO/MOG that control these two activities of the enzyme. Transient kinetics of the L-AAO/MOG reactions was studied using stopped-flow spectrophotometry. For flavin reduction, rates of the flavin reduction depend on L-lysine concentration. Charge-transfer complex of the enzyme with L-Lysine is formed during the flavin reduction. For flavin oxidation, the reactions showed two and three phases kinetics when dithionite and L-lysine were used for preparing the reduced enzyme, respectively. No C4a-hydroperoxyflavin was detected and rates of the flavin oxidation depend on O₂ concentration. However, variation of L-lysine concentration does not affect the rate of the third phase. Product analysis of multiple turnover reactions of L-lysine and L-ornithine were carried out by HPLC/MS. Results show that for L-ornithine reaction, no amide product was formed in monooxygenase path. On the other hand, it shows a decarboxylated product from α-keto acid that is different from L-lysine reaction.

Keywords: Flavin monooxygenase; Flavin oxidase; L-Amino acid oxidase/monooxygenase; L-Lysine α-oxidase; L-Lysine monooxygenase

Selected References:
Isolation of Specific scFv Antibody Against Bradyrhizobium from Non-Immunized Human scFv Library

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Abstract
The objectives of this research were to isolate human monoclonal scFv antibodies against two related strains of Bradyrhizobium sp. and determine their limit of detections by phage ELISA. Two strains of specific human monoclonal scFv antibodies, i.e., clone yDOA9-RD62 and ySUTN9/2-E10, were successfully affinity selected from non-immunized human scFv library (YAMO-I library). Phage scFv monoclonal antibodies showed the specific binding to their targets in ELISA when using both pure culture bacteria and bacteriod in plant nodule as targets. Phage scFv clone yDOA9-RD6/2 specifically bound to Bradyrhizobium strain DOA9, while phage scFv clone ySUTN9/2-E10 strongly bound to Bradyrhizobium strain SUTN9-2, no cross-reactivity could be detected. The amino acid sequence analysis of the two clones were analyzed. These two isolated clones have the kappa type of the light chain (V_L) from family V_K3, whereas the variable heavy chains (V_H) were from family V_H3 and V_H4 for ySUTN9/2-E10 and yDOA9-RD6/2, respectively. These antibodies could be further developed and used as tools for the detection and monitoring rhizobial biofertilizer in the agricultural field in the future.

Keywords: Bradyrhizobium; Monoclonal antibody; Phage display technology

Selected References:
Expression of Serine Proteases from *Bacillus halodurans* Showing the Keratinolytic Activity

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Abstract
The alkaliphilic gram-positive bacterium *Bacillus halodurans* can secrete many enzymes for industrial application. In addition to thermophilic and alkaliphilic xylanases, this microorganism is also able to synthesize a variety of proteolytic enzymes according to its chromosomal DNA sequences. Two genes, *aprX* and BH0855, respectively encoding for intracellular and extracellular alkaline serine proteases from *B. halodurans* Thonburi were separately over-expressed in *Escherichia coli* BL21 (DE3). The recombinant gene product of *aprX* was found in the intracellular space without the formation of inclusion body, while the recombinant protein encoded by BH0855 was found mostly in the culture medium. As the keratinolytic activity was assayed on the recombinant proteolytic enzyme encoding by BH0855, the proteins in the medium contributed about 90% of total enzymatic activity obtained from the recombinant cell. Results suggested that the original signal peptide worked well for protein secretion in the *E. coli* system. In addition, using soluble keratin as the substrate, the enzymatic activity in the medium was determined as 1,125 U/mL with a specific activity of 330.85 U/mg, suggesting that the extracellular proteolytic enzyme is a keratinase and very potential for application.

Keywords: Alkaline serine proteases; *Bacillus halodurans*; Keratinase; Keratinolytic activity; Secretion

Selected References:
Identification and Characterizations of the Estrogen Degradation
*Pseudomonas putida SJTE-1*

Pingping Wang¹, Weiliang Xiong¹, Wanli Peng¹, Jing Xu¹, Daning Zheng¹, Xiuli Wang¹, Yicheng Wang¹ and Rubing Liang¹*

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Abstract
Environmental estrogens are one predominant type of environmental contaminants, difficult to be removed. *Pseudomonas putida* SJTE-1 was isolated and found with the capability of utilizing different estrogens as its sole carbon source including 17β-estradiol, estrone, estriol, testosterone, naphthalene and phenanthrene. UPLC analysis showed it could degrade 1 mg/L 17β-estradiol completely within 24 h; 90% of 50 mg/L 17β-estradiol or 75% of 100 mg/L 17β-estradiol was utilized in seven days. Absorption of 17β-estradiol to cell membranes was the premise for its transportation and cellular transformation. 17β-estradiol was firstly converted into estrone and then degraded into non-estrogenic chemicals. Whole genome sequence analysis indicated several putative enzymes involved in steroid degradation were found in SJTE-1 genome like hydroxysteroid dehydrogenase, 3-ketosteroid-delta-dehydrogenase, Rieske dioxygenase and catechol 2, 3-dioxygenase. Furthermore, its global responses to 17β-estradiol and glucose were analyzed and compared using the iTRAQ strategy combined with LC-MS/MS. 78 proteins were identified with significant changes in expression; 45 proteins and 33 proteins were up-regulated and down-regulated, respectively. These proteins were mainly involved in the processes of stress response, energy metabolism, transportation, chemotaxis and cell motility, and carbon metabolism, considered probably responding to 17β-estradiol and being involved in its metabolism. The up-regulated proteins in electron transfer, energy generation and transport systems were thought crucial for efficient uptake, translocation and transformation of 17β-estradiol. The over-expression of carbon metabolism proteins indicated cells may activate related pathway members to utilize 17β-estradiol. These findings provide important clues to reveal degradation mechanism of 17β-estradiol in *P. putida* and promote its bioremediation applications.

Keywords: Biodegradation; Environmental estrogens; Metabolism network; *Pseudomonas putida* SJTE-1

Selected References:
Halotolerant Fungi Catalyzed Synthesis of Short Chain Flavor Ester in Solvent Free Media using Ionic Liquids as Additive

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Abstract
Spores of halotolerant Aspergillus niger EXF 4321 were used as biocatalyst for the synthesis of flavor esters in solvent free medium. The spores of A. niger EXF 4321 were grown in plate containing minimal medium with olive oil as lipase inducer and immobilized by encapsulation with calcium alginate. The immobilized spore of A. niger EXF 4321 was used as biocatalyst for the synthesis of isoamyl acetate from acetic acid and isoamyl alcohol. In order to improve the catalytic efficiency and simplify the down streaming processing for food grade flavor esters, ionic liquids of minimal quantity was employed as additive. Several ionic liquids were screened and the reaction conditions including substrate molar ratio, catalyst amount and ionic liquids concentration were optimized. The optimal synthesis of isoamyl acetate yield of 86% was achieved with 5% [Bmim][TfO], substrate molar ratio 1:1, 20mg immobilized A. niger EXF 4321 at 40ºC for 24 hours. This study will encourage the use of fungal spore as a promising and cheap biocatalyst for production of useful chemicals.

Keywords: Aspergillus niger; Flavor ester; Ionic liquids; Solvent free medium; Spore
P-Joint I-07

Cloning and Expression of *Streptomyces* sp. CP01 Inulinase in *Escherichia coli*

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Abstract

Inulin is the carbohydrate and soluble fiber consisting of β-2, 1 linked D-fructofuranose and terminated with glucose residue. It can be digested by endo-inulinase which generates fructose and FOS as products. In this study, inulinase gene from *Streptomyces* sp. CP01 was cloned and expressed the recombinant protein in *Escherichia coli*. The results showed that the inulinase gene has 2,892 bp and encoded 964 amino acids. The nucleotide and amino acid sequences of inulinase were similar to those of *S. ambofaciens* ATCC 23877. The molecular weight of recombinant inulinase was estimated to be 107 kDa. The specific activity of crude protein was 0.0164 U/mg.

Keywords: Inulin; Inulinase; *Streptomyces* sp. CP01

Selected References:
P-Joint I-08

Generation of Recombinant Human scFv Antibody Against Zearalenone using Phage Display Technology

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Abstract

Zearalenone (ZEN) is a nonsteroidal, estrogenic mycotoxin, produced as a secondary metabolite by \textit{Fusarium} spp. It enters the food and feed chain from contaminated cereals and infiltrates into sewage or natural waters, posing potential threat to exposed livestock, wildlife and humans. Therefore, establishing sensitive and specific methods to detect ZEN become very important for food safety reasons. The aim of this study was to select a ZEN specific single chain variable fragment (scFv) monoclonal antibody from a compact phage display naïve human scFv library. The biopanning was performed by switching the conjugated proteins, i.e., bovine serum albumin (BSA)-ZEN and ovalbumin (OVA)-ZEN to increase the chance of obtaining clones that can bind to free toxin. The phage scFv antibodies were then expressed in soluble form using \textit{E. coli} HB2151 as an expression host. One clone, designated as yZA8B2, could bind to all three conjugated ZEN; BSA-ZEN, OVA-ZEN, and KLH-ZEN. Amino acid sequence analysis revealed that the variable heavy chain (VH) belonged to family 5, while the variable light chain (VL) was from family 1. Competitive ELISA results indicated that this scFv antibody clone yZA8B2 could be inhibited by soluble ZEN at a linear range of 60–5,000 ng/ml, with the limit detection (LOD) of approximately 60 ng/ml. In conclusion, a naïve phage display human scFv library could be used as a source to isolate human scFv antibody that can bind to free ZEN. This antibody will be further engineered for the detection of ZEN contamination in agricultural products in the future.

Keywords: A compact phage display naïve human scFv library (Yamo) library; Phage scFv antibody; Zearalenone
Expression of Feedback Resistant \( \text{lysC} \) Gene for L-Aminoadipic acid Production in \textit{Escherichia coli}

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Abstract

L-Aminoadipic acid (L-AAA), a non-protein structure amino acid, is an important intermediate for many medicinal compounds such as antirheumatic, psoriasis and carcinostatic drugs as well as a precursor in the production of \( \beta \)-lactam antibiotics. To increase L-AAA production in \textit{Escherichia coli}, releasing of allosteric inhibition of the enzymes in L-lysine biosynthesis pathway should be performed. The gene encoding for L-lysine feedback resistance aspartokinase III (\( \text{lysC} \)) was co-expressed with dipicolinate synthase, lysine dehydrogenase and piperideine-6-carboxylate dehydrogenase genes under T7 promoter of pRSF-Duet1. The activity of aspartokinase III from the recombinant clone was 1.4 fold higher than that of \textit{E. coli} clone containing pRSFDuet-1 (control) after induction with IPTG for 8 hours.

Keywords: Aspartokinase III; L-aminoacidipic acid (L-AAA); \( \text{lysC} \) gene

Selected References:


AFOB-EFB Joint Session II on “Plant Biotechnology”
Development of PCR-base Diagnosis Protocols for Pathogenic Virus on Common Crops in Vietnam

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Abstract

Viral disease is one of the major factors affecting crop quality and productivity. In Vietnam, a number of crops have been infected by pathogenic viruses leading to the reduction of yield and product quality. At the moment, there are no efficient treatments available for viral infection in plants; current solutions of the problem are only prevention of disease and destroying infected plants. Therefore, early and sensitive detection of these viruses is important for viral disease management. This report introduces the diagnostic protocols of 6 pathogenic viruses infecting common crops in Vietnam, including orchids (CyMV, ORSV), potato (PVX, PVY), cucumber (CMV), tomato (ToMV) and black pepper (PYMoV, CMV) using PCR, RT-PCR and real-time RT-PCR. The PCR reactions were designed for detection of RdRp/CP/ORF genes of CYMV, ORSV/PVX, PVY, ToMV, CMV/PYMoV. The amplified products were cloned and sequenced to evaluate the specification of PCR. The PCR threshold detection limit was determined using 10-times serial dilution of DNA templates. The results indicated that the PCR-based diagnosis protocols were successfully developed and able to make detection at $10^2$ virus copies/µl. The sequences of amplified products were identical with the published virus sequences. Furthermore, a highly sensitive real-time PCR was designed for detection of CyMV and ORSV on orchid at low copy number, 10 copies/ µl. In conclusion, these achievements make an important contribution to the control of viral diseases on crops in Vietnam.

Keywords: Black peper; Cucumber; Orchid; Potato; Tomato; Viral disease; Virus detection
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ศูนย์วิจัยและพัฒนา เครือเบทากรอ
Research & Development Center (RDC)

เน้นสร้างความรุ่นใหม่กับการสร้างอนาคตจากวิทยาศาสตร์
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