

# **Book of Abstracts**

## **Core to Core Program**

**on**

**Establishment of an international research core for new  
bio-research fields with microbes from tropical areas**

## **International Symposium**

**on**

**Microbial Research and Biotechnology for Biomass  
Utilization**

**The 4<sup>th</sup> Satellite Seminar**

**4th – 5th September 2017**

**Venue: Beuth University of Applied Sciences Berlin, Haus Gauß, B 554**





BEUTH HOCHSCHULE  
FÜR TECHNIK  
BERLIN

University of Applied Sciences



The University of Manchester

## Core to Core Program

on

**Establishment of an international research core for new bio-research fields  
with microbes from tropical areas**

## International Symposium

on

**Microbial Research and Biotechnology for Biomass  
Utilization**

## The 4<sup>th</sup> Satellite Seminar

**Organized by:**

**Beuth University of Applied Sciences Berlin**

**In association with**

**Japan Society for the Promotion of Science (JSPS)**

**National Research Council of Thailand (NRCT)**

**Vietnam Ministry of Science & Technology (MOST)**

**Yamaguchi University, Kasetsart University,**

**Can Tho University, National University of Laos,**

**Brawijaya University, The University of Manchester,**

**Leibniz Institut für Agrartechnik und Bioökonomie (ATB) Potsdam,**

**Versuchs- und Lehranstalt für Brauerei (VLB) Berlin**

**4th – 5th September 2017**

**Venue: Beuth University of Applied Sciences Berlin, Haus Gauß, B 554**

## **Message from German Coordinator**

Prof. Dr.-Ing. Peter Götz



Welcome to Berlin to all participants, guests, colleagues and friends for the 4th Satellite Seminar of the Asian Core-to-core Program on “Establishment of an international research core for new biological fields with microbes from tropical area”. It is a great honour and privilege for me to host the seminar at Beuth University of Applied Sciences in Berlin.

The core to core network now exists for almost 20 years since 1998 with Germany participating since 2013, and in the current funding period, after Indonesia, Japan and Vietnam, this Satellite Seminar is the first one outside of Asia. From the German side therefore I want to thank all coordinators to make this possible.

For September 4. and 5. 2017, we compiled a demanding program to cover current research topics and to allow speakers from the network to present their newest results and insights. A total of 24 scientific lectures are presented in this seminar, 2 keynote lectures and 22 oral presentations. On behalf of the organizing committees, I want to thank the keynote lecturers and oral presenters for their contributions. Elaborating presentations from their experience and their research results is the main issue to make the seminar possible.

I fully expect that the 2017 seminar will be a great success and it will strengthen the international outreach of our core-to-core program. Looking forward, we should find new support and ways to continue this excellent platform for the exchange of scientific and practical knowledge on the latest developments and emerging challenges in our field of research. Aside from the scientific program, the CCP dinner allows for networking and discussion of all aspects of our research. Finding new ideas, new projects and new friendships will continuously strengthen our research core and prepare us for future collaborations.

Again I want to thank everybody in our core-to-core network for the great work and also the funding organizations for the financial support of the network. Special thanks go to the members of the organizing committees and the supporting staff, who made the event possible.

For the realization of the seminar in Berlin, we are especially grateful to the co:bios foundation for the financial support.

I look forward to your participation in the upcoming event and wish us all a successful and inspiring seminar.

Yours sincerely,

*Peter Götz  
German Coordinator and Chairman of the Local Organizing Committee  
Professor, Beuth University of Applied Sciences, Berlin*

**Message from Japanese Coordinator**

Prof. Dr. Mamoru Yamada



It is our great pleasure to hold the 4th Satellite Seminar in the Core-to-Core Program (Advanced Research Networks) entitled “Establishment of an international research core for new bio-research fields with microbes from tropical areas (World-class research hub of tropical microbial resources and their utilization)” in Germany. This is a milestone of our Core Programs because it is the first time to have a seminar outside Asia. I would like to express my appreciation for the enormous effort of the organizing committee members of this seminar at the German side, especially Professor Dr. Peter Götz and his colleagues, as well as the financial support of Beuth University of Applied Sciences, Berlin.

The Core-to-Core Program (2014-2018) is a post-program of the JSPS-NRCT Core University Program (1998-2007) entitled “Development of thermotolerant microbial resources and their application” and the JSPS-NRCT Asian Core Program (2008-2012) entitled “Capacity building and development of microbial potential and fermentation technology towards new era”, and is expected to create top world-class research centers that partner over the long term with other core research institutions around the world in advancing research in leading-edge fields, on issues of high international priority. A large number of scientists are participating in this program, from seven countries of Thai, Vietnam, Laos, Germany, Indonesia, United Kingdom and Japan. Thus, we should challenge the new bio-research fields with microbes from tropical areas in this program. There are five projects in this Core-to-Core Program as follows.

Project 1: Explorational Research of Useful Microbes

Project 2: Genome-based Research on Thermotolerant Microbes

Project 3: Research on Environmental Microbes Sustaining Tropical Ecosystem

Project 4: Research on Microbes Useful for Food, Packaging, Health, and Ecosystem

Project 5: Development of Next-generation Fermentation Technology for New Wave

I hope members at German side to work together with counterparts on original research topics related to these projects. During this seminar, you may obtain beneficial information or new ideas from presentation and discussion, which promote your further experiments. While, as we have the Young Scientist Seminar every year and some systems supporting a student exchange, this program is expected to contribute not only to microbial sciences but also to education to foster our successors.

Finally, I would like to thank all attendants and their contributions to this seminar, and the financial supports of National Research Council of Thailand (NRCT), Japan Society for the Promotion of Science (JSPS), MOST of Vietnam, Beuth University of Applied Sciences, University of Brawijaya and National University of Laos.

*Mamoru Yamada  
Japanese Coordinator  
Professor, Yamaguchi University*

## **Message from Vietnamese Coordinator**

Assoc. Prof. Dr. Ngo Thi Phuong Dung



With all pleasure and it is a great honor for me to preside over the message of the Vietnamese side on the occasion of the 4th Satellite Seminar of the Core to Core Program, that will be held on 4th – 5th September 2017 at Beuth University of Applied Sciences, Berlin and kindly hosted by the German counterpart of CCP.

The Vietnamese team is very happy to be available to continue our active participation in this new program of Advanced Research Networks on “Establishment of an international research core for new bio-research fields with microbes from tropical areas” – World-class research hub of tropical microbial resources and their utilization. Our team is also very delighted to have a good opportunity to join and work with many more counterparts from Japan, Thailand, Laos, Germany, Indonesia, United Kingdom and Vietnam.

May I take this occasion to express a sincere thanks to the support institutions of all partner countries, and I would like to acknowledge the excellent effort of the organizing committee and team, especially Prof. Peter Götz - the CCP German Coordinator and Beuth University of Applied Sciences.

We are also grateful to the keynote lecturers, the oral speakers and the poster presenters as well as all participants who significantly contribute to the success of this seminar event.

We strongly believe that during this event we will learn the informative research findings and useful experiences, contributing toward the success of our research collaboration program.

*Ngo Thi Phuong Dung  
Vietnamese Coordinator  
Associate Professor  
Biotechnology R & D Institute  
Can Tho University*

**Message from Thai Coordinator**

Assoc. Prof. Dr. Gunjana Theeragool



I am delighted to welcome all of the distinguished guests and participants to the 4th Satellite Seminar of the Core to Core Program A. Advanced Research Networks on “Establishment of an International Research Core for Bio-research Fields with Microbes from Tropical Areas (World-class Research Hub of Tropical Microbial Resources and Their Utilization)”.

Kasetsart University and Yamaguchi University established the Core University Program with financial support from the Japan Society for the Promotion of Science (JSPS). It took place over 10 years (1998-2007). The success of the 10 year core university program had the potential to be extended to the Asian Core Program. This program was created with financial support from JSPS and the National Research Council of Thailand (NRCT), ran for 5 years (2008-2012), and received collaboration from 4 active teams from Japan, Vietnam, Laos and Thailand respectively. Following on from this fruitful collaboration, we have established the Core to Core Program A. Advanced Research Networks. This 5 year (2014-2018) program receives financial support from JSPS, NRCT, the Vietnam Ministry of Science & Technology (MOST), the National University of Laos, The University of Brawijaya (Indonesia), Beuth University of Applied Sciences (Germany) and The University of Manchester (England).

This 4th Satellite Seminar is the third academic activity arranged after the successful Joint Seminar in FerVA-AP2017 (July 26-27, 2017) and International Joint Seminar in Thailand Research EXPO (August 26, 2017). This seminar will provide a good opportunity for the participants to meet and discuss their future areas of collaboration in order to obtain the most fruitful results. In addition, I hope that the presentations and discussions which take place during this seminar will spur the participants towards the development of new research opportunities and productive collaboration.

On behalf of Thai Coordinator, I would like to express my sincere appreciation to Beuth University of Applied Sciences especially Prof. Dr.-Ing. Peter Götz, German Coordinator, for organizing the 4th Satellite Seminar. My thanks also go out to the invited speakers and all of the oral and poster presenters for contributing their research work to this seminar. Thanks also to the Japanese, Vietnamese, Laotian, Indonesian and English coordinators for their cooperation in arranging this 4th Satellite Seminar. Last, but not least, I would like to express my sincere gratitude to JSPS, NRCT, MOST in Vietnam, the National University of Laos, The University of Brawijaya, Beuth University of Applied Sciences and The University of Manchester for their continuing financial support.

*Gunjana Theeragool*  
*Thai coordinator*

## **Message from Lao Coordinator**

Assoc. Prof. Dr. Somchanh BOUNPHANMYI



On behalf of Faculty of Natural Science, National University of Laos, it is great honor for me to deliver over the message of Lao scientist team on the occasion of the 4th Satellite Seminar of the New Core-to-Core Program A. Advanced Research Network

As we have successfully organized all three Satellite Seminars during four years of implementation of the New Core-to-Core Program, I fully expect that the 4th Seminar which will be held on 4-5 September, 2017 in Beuth University of Applied Sciences Berlin, will also be a great success and will provide as a excellent platform for further strengthen on our Advanced Research Network.

In this occasion, I would like to express our sincere appreciation and gratitude to the CCP committee members, the Japan's Society for the Promotion of Science (JSPS) and The National Research Council of Thailand (NRCT) for kind support and cooperation. We are also so grateful to key speakers, oral speaker and poster presenter for their kind sharing knowledge and experience in this seminar.

Finally, I am very pleased to convey our gratitude and most appreciation to local organizers leading by Professor Dr. Peter Götz, German Coordinator, for organizing the 4th most fruitful Satellite Seminar of CCP

*Somchanh Bounphanmy  
Lao Coordinator  
National University of Laos*



**Message from Indonesian Coordinator**

Dr. Anton Muhibuddin



Dear All,

Firstly, I express many thanks to Prof. Peter Goetz as a coordinator of CCP for Germany side and also Prof. Mamoru Yamada, as a coordinator of CCP for Japan side who has arranged and facilitated this program. I hope, participation of Indonesian scientists always support CCP programs better. It is not easy to run this program in Indonesia as well as Thailand and Japan which has run since 1987. This is due to the schedule and scheme of academic and research funding are different in Indonesia. But, this year our side got enough big support from Indonesia government. We will send 20-40 scientist to Japan for 2 months (The budget is around 400.000 US\$). We will support all of their funding need, including living cost and international-local travel expenses. As the country with around 260 million population, the number of scientists in Indonesia is also very big and potential to support development of science and technology in the world. Moreover, the quality and quantity of our natural resources is very great to support the development of world's science.

Overall, I am delighted to welcome all of the speakers and participants in this satellite seminar. On behalf of Indonesia Coordinator, I would like to express my sincere appreciation to Beuth University of Applied Science, especially to Prof. Peter Goetz for organizing the Seminar. My thanks also go out to all participants for contributing their research work to this seminar. Thanks also to the Japanese, Thailand, Vietnamese, Laotian, Germany and UK coordinators for their cooperation in arranging this seminar. I also would like to express sincere gratitude to University of Brawijaya and University of Wahab Hasbullah, Ministry of RISTEKDIKTI, JSPS, NRCT, Vietnam Ministry of Science & Technology (MOST), the National University of Laos, Beuth University of Applied Sciences (Germany) and The University of Manchester (England) for their financial support.

Kind regards,

*Anton Muhibuddin*  
*Indonesia coordinator*

## Message from UK Coordinator

Prof. Dr. Constantinos Theodoropoulos



It is my great pleasure to participate at the 4th Satellite meeting of the Core to Core Programme organised by Beuth University of Applied Sciences Berlin on the held in Berlin, Germany. It is a great opportunity for the Core to Core Programme seminar series to visit Europe for the first time.

The Core to Core Programme is a great network for collaboration between Institutions in Japan, Thailand, United Kingdom, Vietnam, Laos, Germany and Indonesia, offering a strong and dynamic platform for exchange of ideas and researchers and creating opportunities for real advances in the all important area of industrial biotechnology. I have already participated two years on a row in the seminar series first in Japan two years ago and then in Thailand last year and was very happy with the quality of presentations as well as the strong participation from all the partners.

I am looking forward to continue the fruitful discussions we started last year with a number of members of the programme, which produced a number of successful visits from program partners to my lab in Manchester, as well as to establish new dynamic interactions with other Core-to-Core network members. The UK side can offer a number of contributions in the area of Integrated Fermentation technology using a combination of state-of-the-art experimental and computational techniques.

The 4th Satellite meeting will help to disseminate the latest research advances from the programme partners and I am particularly looking forward to the generation of new exciting multi-partner collaborations that will emerge through these stimulating days of oral presentations and posters.

Finally, I would like to thank the local organisers of this 4th Satellite Meeting as well as the JSPS and NRCT for their financial and academic support of the Core to Core Program.

*Constantinos Theodoropoulos  
UK Coordinator  
Professor, University of Manchester*

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**Core-to-Core Program: 4<sup>th</sup> Satellite Seminar, 4<sup>th</sup> – 5<sup>th</sup> September 2017**  
**Beuth University of Applied Sciences Berlin**  
**Berlin, Germany**

Time	Detail	Speaker
<b>September 3, 2017 (Sunday)</b>		
Delegates arrive, check in at hotel		
<b>September 4, 2017 (Monday)</b>		
9:00 - 9:30	Registration	
9:30 - 9:45	Welcome address	Vice President Beuth HS Hans Gerber
9:45 - 10:15	<b>Introduction of the Program</b>	Mamoru Yamada <i>Yamaguchi U.</i> Peter Götz <i>Beuth HS</i>
10:15 - 11:00	<b>Keynote lecture 1</b> - Improvement of Vinegar and Bacterial Nanocellulose Fermentation at High Temperatures by Adaptive Mutation	Gunjana Theeragool <i>Kasetsart U.</i>
11:00 - 11:30	<b>Coffee break</b>	
<b>Session 1</b>	<b>Research on Environmental Microbes Sustaining Tropical Ecosystem and Genome-based Research on Thermotolerant Microbes</b> <i>Chairman: Peter Götz</i>	
11:30 - 11:50	Preliminary study of chemical investigation of hard coral-associated bacteria	Enjuro Harunari <i>Toyama Prefectural U.</i>
11:50 - 12:10	Role of Arbuscular Mycorrhiza (AM) and <i>Pseudomonas fluorescens</i> in increasing P uptake and corn growth in Andisols	Budi Prasetya <i>University of Brawijaya</i>
12:10 - 12:30	Qualification of rtPCR assays for detection of selected pathogenic microorganisms in a grass-silage biogas process	Steffen Prowe <i>Beuth HS</i>
12:30 - 12:50	Effects of Ethanol and Sugar Concentrations on Growth and Ethanol Fermentation of Thermotolerant Yeasts and Increasing of Ethanol Fermentation by Adaptation and Mutagenesis	Savitree Limtong <i>Kasetsart U.</i>
13:00 - 14:30	<b>Lunch Break</b>	
<b>Session 2</b>	<b>Explorational Research on Useful Microbes</b> <i>Chairman: Johannes Bader</i>	
14:30 - 14:50	AdvancedBioPro – Establishment of an automated laboratory for biotechnological process development	Beate Heilmann <i>Beuth HS</i>
14:50 - 15:10	Exploring the bacteriocin of Lactic Acid Bacteria isolated from Etawa goat kefir as anti-biofilm against virulent <i>Klebsiella pneumonia</i>	Tri Yudani M.Raras <i>Brawijaya U.</i>

15:10 - 15:30	Physiological Characterization and Molecular Identification of <i>Meyerozyma guilliermondii</i> and Its Potential	Anton Muhibuddin <i>University of Brawijaya</i>
15:30 - 15:50	Amylolytic Enzymes from L-lactic Acid Producing <i>Enterococcus faecium</i> K-1 and Their Effects	Chartchai Khanongnuch <i>Chiang Mai U.</i>
15:50 - 16:10	Production of 1,3-diols in engineered <i>Escherichia coli</i>	Naoya Kataoka <i>Yamaguchi U.</i>
16:30 - 19:00	Tour of Department (on demand) (CCP Coordinator Meeting)	

<b>September 5, 2017 (Tuesday)</b>		
8:45 - 9:30	<b>Keynote Lecture 2</b> - Pre-treatment of biogenic feedstocks for the lactic acid fermentation	Joachim Venus <i>ATB Potsdam</i>
9:30 - 9:45	<b>Coffee break</b>	
<b>Session 3</b>	<b>Development of Next-generation Fermentation Technology for New Wave Industry I</b> <b>Chairman: Katja Karstens</b>	
9:45 - 10:05	Impact of culture conditions on the stability properties of <i>Lactobacillus acidophilus</i> during further processing	Martin Senz <i>VLB Berlin</i>
10:05 - 10:25	Hydrogen and Methane Production from Palm Oil Mill Effluent Using Two-Stage Thermophilic Fermentation: from Lab Scale to Mobile Unit	Poonsuk Prasertsan <i>Prince of Songkla U.</i>
10:25 - 10:45	Biobutanol Production with Designed Biomass on Biorefinery	Kenji Sonomoto <i>Kyushu U.</i>
10:45 - 11:05	Systematic Feedback Isolation Applied for Meta-Fermentation Study	Yukihiro Tashiro <i>Kyushu U.</i>
11:05 - 11:20	<b>Coffee break</b>	
<b>Session 4</b>	<b>Research on Microbes Useful for Food, Food Preservation, Health and Ecosystem Preservation</b> <b>Chairman: Peter Neubauer</b>	
11:20 - 11:40	SUPPLE – Sustainable plant-based production of extremozymes	Johannes Klinger <i>Beuth HS</i>
11:40 - 12:00	The Role of Glycogen in the Diauxic Shift of <i>E. coli</i>	Hiroshi Matsuno <i>Yamaguchi U.</i>
12:00 - 12:20	The influence of ethanol extract <i>Dioscorea alata</i> L. on CD4+CD62L+ and CD8+CD62L+ profile of BALB/c mice model digestive tract allergy	Sri Nabawiyati Nurul Makiyah <i>U. Muhammadiyah Yogyakarta</i>
12:20 - 12:40	Polyhydroxyalkanoates production with <i>Ralstonia eutropha</i> from biogenic waste streams	Sebastian Riedel <i>TU Berlin</i>
12:40-13:00	Improvement of poly-histidine tag for the production and purification of functional proteins	Rinji Akada <i>Yamaguchi U.</i>
13:00 - 14:30	<b>Lunch Break</b>	
<b>Session 4</b>	<b>Development of Next-generation Fermentation Technology for New Wave Industry II</b> <b>Chairman: Steffen Prowe</b>	
14:30 - 14:50	Microalgae growth for the optimization of starch and lipid production: Combining experimental and computational tools	Constantinos Theodoropoulos <i>University of Manchester</i>
14:50 - 15:10	Monitoring tools for improved characterization of the liquid phase in anaerobic digestion	Stefan Junne <i>TU Berlin</i>
15:10 - 15:30	Statistical Optimization of Single Cell Oil Production from Starch Hydrolysate and Glycerol by Thermotolerant Oleaginous Yeast <i>Pichia sp.</i> Scj 01 using Response Surface Methodology	Vichai Leelavatcharamas <i>Khon Kaen U.</i>

15:30 - 15:50	Brewing using Mixed Cultures	Roland Pahl <i>VLB Berlin</i>
16:00 - 16:30	Closing remarks, end of seminar	
18:30	<b><i>Conference Dinner for Speakers</i></b>	



## Lectures

### **Improvement of Vinegar and Bacterial Nanocellulose Fermentation at High Temperatures by Adaptive Mutation**

Gunjana Theeragool, Kallayanee Naloka, Pornchanok Taweecheep, Minenosuke Matsutani, Toshiharu Yakushi and Kazunobu Matsushita 16

### **Preliminary study of chemical investigation of hard coral-associated bacteria**

Enjuro Harunari, Amit Raj Sharma and Yasuhiro Igarashi 17

### **Role of Arbuscular Mycorrhiza (AM) and *Pseudomonas fluorescens* in increasing P uptake and corn growth in Andisols**

Budi Prasetya, Luqman Qurata Aini 18

### **Qualification of rtPCR assays for detection of selected pathogenic microorganisms in a grass-silage biogas process**

Steffen Prowe, Katrin Kelemen, Franziska Hannemann 19

### **Effects of Ethanol and Sugar Concentrations on Growth and Ethanol Fermentation of Thermotolerant Yeasts and Increasing of Ethanol Fermentation by Adaptation and Mutagenesis**

Sornsiri Pattanakittivorakul, Noppon Lertwattanasakul, Mamoru Yamada and Savitree Limtong 20

### **AdvancedBioPro – Establishment of an automated laboratory for biotechnological process development**

Beate Heilmann, Marvin Machava, Elisabeth Grohmann, Steffen Prowe, Carsten Lübke, Wolfgang Jabs, Stephan Hinderlich, Peter Götz, Sabine Hagemann, Johannes Bader 21

### **Exploring the bacteriocin of Lactic Acid Bacteria isolated from Etawa goat kefir as anti-biofilm against virulent *Klebsiella pneumonia***

Tri Yudani M.Raras 22

### **Physiological Characterization and Molecular Identification of *Meyerozyma guilliermondii* and It's Potential**

Anton Muhibuddin, Koko Ady Sulisty, Antok Wahyu Sektiono 23

### **Amylolytic Enzymes from L-lactic Acid Producing *Enterococcus faecium* K-1 and Their Effects on Lactic Acid Production Using Starch as a Substrate**

Chartchai Khanongnuch, Kridsada Unban, Apinun Kanpiengjai, Goro Takata, Keiko Uechi and Wen Chien Lee 24

### **Production of 1,3-diols in engineered *Escherichia coli***

Naoya Kataoka, Alisa S. Vangnai, Thunyarat Pongtharangku, Toshiharu Yakushi and Kazunobu Matsushita 25

### **Pre-treatment of biogenic feedstocks for the lactic acid fermentation**

Joachim Venus 26

### **Impact of culture conditions on the stability properties of *Lactobacillus acidophilus* during further processing**

Martin Senz, Sophie Palinski, Sandra Dounia, Maximilian Schmacht, Martin Hageböck 27

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## Improvement of Vinegar and Bacterial Nanocellulose Fermentation at High Temperatures by Adaptive Mutation

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Thermotolerant acetic acid bacteria play important role in industrial vinegar and bacterial nanocellulose (BNC) fermentation. We have successfully isolated thermo-adapted strain, TH-3, from *Acetobacter pasteurianus* SKU1108 and an ethanol-adapted strain, 7E-13, from TH-3 strain for commercial rice vinegar fermentation without cooling system. Comparison of chemical compositions in vinegar prepared from various strains revealed that the lowest amount of acetoin in vinegar was obtained in vinegar prepared from TH-3. Based on sensory test TH-3 also produced the best flavor whereas 7E-13 produced the best taste. Finally, 7E-13 was selected for further fed batch fermentation and 6.84% acetic acid was obtained from 70 L jasmine rice moromi containing 5.89% ethanol and supplemented with 0.5% YPGD after 24 h cultivation in 100 L jar fermentor at 37°C, 400 rpm agitation speed and 0.75 vvm aeration rate. In addition, several thermo-and ethanol-adapted strains were isolated from *Komagataeibacter intermedius* MSKU3 and *K. xylinus* MSKU12. Two adapted strains from thermal (KWT-4) and ethanol (KWE-3) adaptation of MSKU3 as well as one thermo-adapted strain C37-10 from MSKU12 were selected. Comparison of vinegar fermentation was carried out by fed batch fermentation at 37 and 38.5°C for 14 days. The tested strains were MSKU3, KWT-4, KWE-3 and mix culture of KWT-4-KWE-3. The four strains produced nearly the same amount of acetic acid (5.70-6.48% v/v) at 37°C whereas at 38.5°C, the mix culture, KWT-4, KWE-3 and MSKU3 produced 6.00, 5.94, 5.70 and 2.82% acid respectively. However, both KWT-4 and KWE-3 lost the BNC producing ability and this ability was recovered from the four revertants obtained from only KWE-3 not KWT-4. Among the four revertants isolated from KWE-3, one of them possessed 2 bp insertion and the other three revertants showing 1 bp deletion in cellulose synthase operon protein C. SEM micrographs of BNC prepared from those revertants were observed and compared to the wild type strain. In contrast, thermo-adapted strain, C37-10, obtained from MSKU12 could enhance BNC fermentation under static culture at 37°C for 7 days. However, the maximum yield of BNC (8.05 g/L dry weight) was obtained from the adapted strain at 35°C for 7 days. In conclusion, both types of adapted strains are shown to be useful for understanding the adaptation mechanism and advantageous for vinegar and BNC fermentation at high temperatures.

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**Preliminary study of chemical investigation of hard coral-associated bacteria****Enjuro Harunari<sup>1</sup>, Amit Raj Sharma<sup>1</sup> and Yasuhiro Igarashi<sup>1</sup>**<sup>1</sup>*Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan*

A number of pharmaceutically useful natural products were isolated from marine organisms such as sponges and tunicates. Such marine invertebrates are the important source of drug screening, however the most significant concern is the supply of producing organisms. Recent investigations showed that bacteria are the true producers of natural products isolated from marine invertebrates as exemplified by bryostatin 1 and didemnin B. For this reason, bacteria residing in marine organisms are attracting much attention as a new source of drug discovery. Especially, there are few reports for secondary metabolites isolated from hard corals. To the best of our knowledge, no natural products isolated from bacterial species associated with hard corals are reported. The objective of this study is to understand the diversity and potential of secondary metabolites production in hard coral-associated bacteria. Our currently ongoing study suggests that some bacterial isolates from cultured hard corals are producing structurally new compounds. We report taxonomy, screening, and structure analysis of the isolates.

## Role of Arbuscular Mycorrhiza (AM) and *Pseudomonas fluorescens* in increasing P uptake and corn growth in Andisols

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On the Andisols, phosphorus (P) was absorbed by Al and Fe (aluminum and iron minerals) becomes unavailable to plants. The important effort to overcome this problem is using biological agents. The *Pseudomonas fluorescens* bacterium is a biological agent which was capable to dissolve unavailable soil P, since it can produce organic acids that can chelate Al and Fe, and dissolved P so available to plants. Arbuscular Mycorrhiza (AM) is a fungus that has access to the source of organic-P via its mycelia which are able to colonize the root-hair. This research aims is to know the role of AM and *P. fluorescens* bacterium in increasing P-uptake on Andisols, and also knowing the role of AM and *P. fluorescens* in increasing plant growth of corn and know which the combination of AM and *P. fluorescens* bacterium that is optimum to increase P-uptake and corn growth. The experimental methods in this research is Completely Randomized Design and P content of corn was analyzed. Analysis of variance (5%) and follow-up Duncan test was done. The results showed that inoculation of AM and *P. fluorescens* have significantly increasing P-uptake (24%) and corn growth (27.59%).

**Keywords :** Arbuscular Mycorrhiza, *P. fluorescens*, P-uptake

## Qualification of rtPCR assays for detection of selected pathogenic microorganisms in a grass-silage biogas process

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Grass and corn silage, dung, green and bio waste are used as substrates for biogas processes. Infected substrate might contaminate the whole biogas plant, including the material circuit including the use of digestate for agricultural use. In addition, biological markers are still missing as parameter for control of biogas processes [1], but were part of the network project BiogasMarker (03/2013–08/2016) using mesophilic and thermophilic, mono- and bi-phasic biogas demonstration plants.

A rapid detection and reproducible quantification using qPCR was established in order to evaluate the risk of pathogens during a biogas process. Therefore, during the project Pathogen diagnostic within biogas reactors the development of qPCR based detection systems for some selected relevant pathogens was established. In addition to available detection systems [2, 3, 4, 5], the focus was especially on phyto- and human pathogenic microorganism which had been selected by a risk priority analysis [6] such as *Xanthomonas translucens*, *Clostridium difficile*, *C. sordellii* and *L. monocytogenes*. For these assays, an appropriate plasmid vector system for the quantification was established. An inter-laboratory assay revealed the need of an optimization and validation of all protocols [7]. The associated protocols were validated and the experimental determination of LOD and LOQ were performed. An internal amplification control was also included in all single- and multiplex PCRs. Spiking experiments did check for interferences.

The validation was successfully performed using extraction and quantification of biogas samples from different biogas plants. All established single- and multiplex assays were able to comply with the requirements and might now provide a tool for the identification of key pathogens shown to be most relevant within biogas plants. This might enable the definition of biological qPCR parameters for further regulations to control safety of biogas processes.

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## Effects of Ethanol and Sugar Concentrations on Growth and Ethanol Fermentation of Thermotolerant Yeasts and Increasing of Ethanol Fermentation by Adaptation and Mutagenesis

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High sugar concentration at the beginning and high ethanol concentration during ethanol fermentation inhibit growth and ethanol fermentation of ethanol fermenting yeasts. Therefore we aimed to study the effect of ethanol and glucose concentrations on growth and ethanol fermentation of the thermotolerant yeasts, and to increase the tolerance to ethanol and sugar by adaptation and mutagenesis. Seven effective thermotolerant ethanol fermenting yeasts; including five strains of *Saccharomyces cerevisiae* i.e. M30, Sc90, TJ1, TJ3 and DMKU-S087 and two strains of *Kluyveromyces marxianus* i.e. DMKU3-1042 and DMKU-KS07; were used. Determination of growth on yeast extract peptone dextrose (YPD) agar at 15-40°C showed that all strains grew well at 15-37°C and three strains i.e. *K. marxianus* DMKU3-1042, *K. marxianus* DMKU-KS07 and *S. cerevisiae* DMKU-S087 grew at 40°C. We selected 37°C incubation temperature for further studies. Growth on YPD agar supplemented with 6-16%v/v ethanol at 37°C revealed that all strains grew on YPD agar supplemented with 6%v/v ethanol, five strains viz. *S. cerevisiae* M30, *S. cerevisiae* TJ3, *S. cerevisiae* DMKU-S087, *K. marxianus* DMKU3-1042 and *K. marxianus* KU-KS07 grew on medium supplemented with 8%v/v ethanol and three *S. cerevisiae* strains i.e. M30, TJ3 and DMKU-S087 grew on medium supplemented with 10% v/v ethanol. None of the strains grew on medium supplemented with 12-16%v/v ethanol. Determination of growth on YPD agar containing 2-30% glucose at 35-40°C revealed that all strains showed good growth in all glucose concentrations at 37°C. Ethanol production by all strains at 37°C in YPD broth containing 20-40% glucose were determined. *S. cerevisiae* DMKU-S087 produced the highest ethanol concentrations in YPD broth containing 25-40% glucose and the highest ethanol concentration of 12.7%w/v was obtained in 40% glucose. Based on these results the thermotolerant strains of *S. cerevisiae* showed better tolerance to both ethanol and sugar than *K. marxianus* strains. We attempted to increase the tolerance to ethanol and sugar of *S. cerevisiae* DMKU-S087 by adaptation. The strain DMKU-S087 was repeatedly transferred to new medium containing higher ethanol or glucose concentrations. This method improved the tolerance to ethanol and sugar from 10% to 12% and from 40% to 45%, respectively. However, the adapted strains obtained did not increase ethanol fermentation. Mutagenesis by UV and ethyl methane sulfonate (EMS) of *S. cerevisiae* DMKU-S087 was performed to increase ethanol fermentation. Ninety six mutants out of 262 colonies produced higher ethanol concentration than the wild type in 15% glucose at 37°C by 8-27%. In molasses broth containing 20% total sugar and 0.1% ammonium sulfate at 37°C and 100 rpm for 48 h, four strains out of 17 strains produced higher ethanol than the wild type by 1.45-7.8%. Mutagenesis could be appropriate mean for improvement of ethanol fermentation.

## **AdvancedBioPro – Establishment of an automated laboratory for biotechnological process development**

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With the interdisciplinary joint research project “AdvancedBioPro” we aim at generating an automated laboratory for biotechnological processes. The laboratory will eventually facilitate a complete bioprocess chain within one institution: from initiation and development, over optimization, until upscaling for industrial application. State of the art robot-based technology for high through-put screening will be combined with already established methods. Furthermore, the newly developed methods will be implemented in teaching schedules at Beuth University of Applied Sciences Berlin.

The project will be integrated thematically in the search and production of new antimicrobial agents. Since numbers of antibiotic resistance are rising inexorably, innovative approaches are needed to meet the demands of discovering new substances.

At the beginning, a broad variation of bacteria was isolated from environmental samples by applying small-scale automation using a basic liquid handling robot. Further planned steps include the implementation of a complex liquid handling platform for microbial isolation and screening. The isolated bacterial strains were identified by MALDI Biotyper Systems and screened for antimicrobial activity against human pathogenic microorganisms such as Methicillin-resistant *Staphylococcus aureus*. Afterwards, antimicrobial substances will be purified and tested for toxicity in cell culture systems. Promising candidates will be proliferated in bioreactors and the downstream process will be developed.



## Exploring the bacteriocin of Lactic Acid Bacteria isolated from Etawa goat kefir as anti-biofilm against virulent *Klebsiella pneumonia*

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*Klebsiella pneumoniae* play a big role for diverse hospital-acquired infections in immunocompetent and immunocompromised patients. The major infections caused by *K. pneumoniae* are pneumonia and complicated urinary tract infections and these infections contribute to mortality rates in all age groups, particularly in infants. Furthermore, *K. pneumonia* remains one of the major biofilm-forming nosocomial pathogens. Biofilms are the crucial factors responsible for the development of multidrug-resistant strains. Because of the increasing complexities of most microbial infections and resistance to conventional therapy, alternative therapies are required, and screening for small molecules that inhibit the attachment and biofilm formation. The present study aims to observe the effects of bacteriocine produced by lactic acid bacteria (LAB) isolated from Etawa goat kefir, a popular, healthy, and fermented dairy product consumed by the majority of people in Indonesia on biofilm formation by *K. pneumonia*.

We have successfully isolated 40 Lactic Acid Bacteria isolates from kefir: 25 from kefir supernatant and 15 from grain. We began with biofilm assay using Crystal Violet method. It was demonstrated that the spent culture supernatant extract of ten isolates of LAB posses potential anti-biofilm. The measurement of Optical Density (OD) showed a reduction of OD on *K pneumoniae* treated with supernatant extract of bacteriocin of LAB isolates. Our future work will be examine the degradation of biofilm of *K.pneumoniae* under electron microscope. When it is proved that the bacteriocin from LAB isolates could destroy biofilm, we would then purify the bacteriocin and identify the type of bacteriocin as well as other important content of the extract of LAB that potentially has ability to inhibit the development of biofilm using High Performance Liquid Chromatography. Concerning the isolates of LAB, we will identify the species of the strain based on their 16s rRNA sequence analysis.

**Key words:** immunocompetent, lactid acid, bacteria, bacteriocin

## Physiological Characterization and Molecular Identification of *Meyerozyma guilliermondii* and It's Potential

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Yeast isolates from deeper soil needs to be determine their characteristics in liquid medium, ability to live on media with different types of sugars, media that containing ethanol with different concentrations and to know their potential as agents biocontrol to *Fusarium* sp. Molecular identification was aimed to known yeast species based on DNA sequences and than its can be obtained other information related to yeast species and their role in the health of the environment.

Result showed that, yeast could grow in all types of sugars at 300C and 370C, the yeast isolates Amb 6, Amb 8, Amb 9, and Amb 17 are fermentative facultative while *Kluveromyces marxianus* Dmku 3 is fermentative obligate, the yeast isolates also tolerant to media that contain ethanol but yeast can not grow on medium more than 10% ethanol concentrations. The results of the molecular identification showed that Amb 6, Amb 8, Amb 9, and Amb 17 were *Meyerozyma guilliermondii* (*Pichia guilliermondii*) Telemorf phase (sexual), *Candida guilliermondii* Anamorf phase (asexual). Based on visual appearance showed there is antibiotic inhibition mechanism, so the isolates have a potential as agents biocontrol to *Fusarium* sp. however there is no difference between treatments.

**Key words:** Yeast, Molecular Identification, Antagonism, and Characterization.

## Amylolytic Enzymes from L-lactic Acid Producing *Enterococcus faecium* K-1 and Their Effects on Lactic Acid Production Using Starch as a Substrate

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An amylolytic lactic acid bacterium isolate K-1 was isolated from the starchy waste of cassava starch manufacturing factory and identified as *Enterococcus faecium* based on 16S rRNA gene sequence analysis. This bacterium was capable of producing 99.2% optically pure L-lactic acid from MRS broth containing 10 g/L cassava starch as the sole carbon source when cultivated at 37°C for 48 h. The main starch degrading enzyme activity from extracellular fraction produced by *E. faecium* K-1 was  $\alpha$ -amylase, meanwhile, cyclodextrinase was found to be the main starch degrading enzyme in intracellular fraction. An extracellular  $\alpha$ -amylase was purified to homogeneity by 40-60% ammonium sulfate precipitation, HiTrap Q column, Resource Q column chromatographies and finally by Superdex 200 gel filtration chromatography. The molecular weight of the purified enzyme was estimated to be approximately 112 kDa with the optimal pH value and temperature of 7.0 and 40°C, respectively. It was stable at a pH range of 6.0-7.0, but was markedly sensitive to high temperatures and low pH conditions, even at a pH value of 5. Ba<sup>2+</sup>, Al<sup>3+</sup> and Co<sup>2+</sup> activated enzyme activity, while enzyme deactivation was found with Mn<sup>2+</sup> and Zn<sup>2+</sup>. Substrate specificity of the purified  $\alpha$ -amylase was higher in amylose and no activity was detected against  $\alpha$ -cyclodextrin and pullulan. The main hydrolysis product from starch, amylose, amylopectin and glycogen was maltotriose. This bacterium was capable of producing L-lactic acid of 8.2 g/L under controlled pH at 6.5 condition in MRS broth containing 10 g/L cassava starch as a sole carbon source when cultivated 37°C for 48 h. The appropriate and cheap nitrogen source to replace the expensive organic nitrogen sources (beef extract, yeast extract and peptone) in MRS medium revealed that corn steep liquor (CSL) was the most suitable organic nitrogen source. The most significant medium compositions affecting lactic acid production evaluated by using Plackett and Burman design were CSL, cassava starch and sodium acetate. Optimum levels of these components for achieving the maximum lactic acid of 12.32 g/L predicted by central composite design (CCD) and response surface plot were consisted of 83.46 g/L CSL, 37.44 g/L cassava starch and 19.43 g/L sodium acetate. The experimental lactic acid of 13.83 g/L lactic acid was obtained and calculated to 87.7% validation. A control pH condition of 6.5 improved and stabilized the yield of L-lactic acid production directly from starch even at a high concentration of starch at up to 150 g/L. This paper is the first report describing the properties of purified  $\alpha$ -amylase from *E. faecium*. Additionally, pullulanase and cyclodextrinase activities were also firstly recorded from *E. faecium* K-1.

**Keywords:**  $\alpha$ -amylase; cyclodextrinase, amylolytic lactic acid bacterium; ALAB; L-lactic acid; starch; *Enterococcus faecium*

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## Production of 1,3-diols in engineered *Escherichia coli*

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To widen the diversity of chemical compounds produced by microbial fermentation, synthetic pathways for 1,3-diols were constructed in *Escherichia coli*. Firstly, 1,3-butanediol was targeted. 1,3-Butanediol is one of the representative 1,3-diols, which has been used as a building block for the production of industrial chemicals including pheromones, fragrances, and insecticides. Despite its large demand, the biological production has been restricted from a non-existence natural synthesis pathway. To achieve 1,3-butanediol production from renewable sources, a synthetic pathway was designed and constructed in *E. coli*. The pathway consists of phaAB from *Ralstonia eutropha*, bld from *Clostridium saccharoperbutylacetonicum*, and endogenous alcohol dehydrogenase(s) of *E. coli*. The *E. coli* strain harboring the 1,3-butanediol synthetic pathway efficiently produced 1,3-butanediol from glucose under aerobic growth conditions (1, 2). Next, to expand the product spectrum, the 1,3-butanediol synthetic pathway was modified by replacing phaA with bktB from *R. eutropha*, because BktB has specificity for longer-chain CoA molecules, and pct from *Megasphaera elsdenii* was added to convert exogenously supplied organic acids to their CoA derivatives. The *E. coli* strain harboring the modified synthetic pathway produced 1,3-pentanediol, 4-methyl-1,3-pentanediol, and 1,2,4-butanetriol from mixture of glucose and propionate, isobutyrate, and glycolate, respectively (3).

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## Pre-treatment of biogenic feedstocks for the lactic acid fermentation

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Besides increasingly important issues with regard to quantity and availability of raw materials together with their properties and quality the feedstock costs are very important for the production of bulk chemicals. Especially for biotechnological processes, in which the carbon of various substrates should be converted into microbial products, there is an increasing interest in the use of cheap raw materials, biogenic residues and wastes.

Renewable feedstocks (e.g. lignocellulosics, green biomass, agro-residues, and food waste) are being used as raw materials for the production of microbial lactic acid [1]. Lactic acid, its salts and esters have a wide range of potential uses and are extensively used in diverse fields, e.g. bioplastics. The goal is to develop a fermentation process based on the substitution of expensive nutrient supplements by cheaper materials from biomass due to their main proportion of the whole process costs.

So, there have been various attempts to provide bulk chemicals like lactic acid from inexpensive raw materials also at low costs. However, these feedstocks cannot be used normally for fermentation directly because the fermentable sugars are bound in the structure especially as cellulose and hemicellulose. They have to undergo a pre-treatment to release these sugar components. Various methods for the pre-treatment are available and widely used, e.g. chemical, physico-chemical and/or biological methods. Possible disturbing impurities and inhibitors (e.g. phenolic components from lignocellulosics, heavy metals in municipal waste or recycled paper), difficult to use components (e.g. pentoses) and partly fluctuating or relatively low concentrations of bio-available carbon sources in these materials should be considered. Special detoxification steps can help to improve the fermentability and conversion efficiency of such lignocellulosic hydrolysates. According to the difficulties mentioned in the mobilization of fermentable sugars a range of other, easy accessible substrates are suitable for subsequent fermentation processes (such as residues from fruit and vegetable processing, by-products from starch and sugar factories or from the baking industry).

Depending on the further processing of the lactic acid the separation of impurities after fermentation is a major process cost too [2]. Therefore an optimization is necessary to find a balance between the substitution of expensive nutrients and the limitation of interfering or undesirable components of natural raw materials respectively.

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## Impact of culture conditions on the stability properties of *Lactobacillus acidophilus* during further processing

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For the production of probiotic and starter cultures for the food and beverage industry, the viability and vitality of the cells is of major interest, since the declared amount of living microorganisms at the end of shelf-life is a main quality criterion. Thus, it is of fundamental interest to produce cultures which are as robust as possible to withstand the conditions during the different processing steps and storage until the time of usage or consumption. The culture media and the process conditions during the fermentation can significantly impact the stability properties of produced cultures and preparations (Senz et al., 2014).

This contribution presents current research for the production of stable *Lactobacillus acidophilus* cultures. One main object of investigation is the media alteration during heat sterilization, which can influence the growth performance, the cell morphology as well as the robustness of the cultures during further processing like freezing and drying as well as the storage of dried preparations. The influencing factors are evaluated by means of diverse process analytical technologies (PAT). The results of diverse studies are discussed in respect to improved fermentation and downstream strategies for the production of robust cultures.

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## Hydrogen and Methane Production from Palm Oil Mill Effluent Using Two-Stage Thermophilic Fermentation: from Lab Scale to Mobile Unit

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Palm oil mill effluent (POME) is commonly treated by mesophilic anaerobic digestion for biogas ( $\text{CH}_4 + \text{CO}_2$ ) production. Some mills use biogas to generate electricity for in-site consumption or off-site connecting to grid to earn revenue. The process on two-stage thermophilic fermentation for production of hydrogen followed by biomethane has been investigated. The two-stage process was developed from lab scale reactors (1 L-5 L) to two sets of mobile units (5 L-25 L then 50 L-250 L reactors). The process development included inoculum preparation, optimization studies, types of reactor (ASBR, CSTR, UASB), batch and continuous mode of fermentation, operation parameters, etc. The process was operated using the HRTs of 2 and 10 days for  $\text{H}_2$  and  $\text{CH}_4$  production in the scaled-up process to two sets of mobile units. The two-stage process in 50 L-250 L reactors exhibited the average production rates of 3.68  $\text{LH}_2/\text{L.d}$  and 23.46  $\text{LCH}_4/\text{L.d}$  with the yields of 0.101 and 0.763  $\text{L/g VS}$ , respectively. Microbial community was *Thermoanaerobacterium*-rich sludge in hydrogen reactor while the archae community was mainly consisting of *Methanoculleus* sp., *Methanosarcina* sp. and *Methanosarcina thermophila* in methane reactor.

Keywords: hydrogen, methane, palm oil mill effluent, biogas, scale-up

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## Biobutanol Production with Designed Biomass on Biorefinery

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Biorefinery is an integrated process to produce various products including green chemicals and biofuels from renewable resources with low environmental impact. Our laboratory has utilized potent microbes in biorefineries for converting designed biomass into high value chemicals such as lactic acid (1) and biobutanol (2). Inedible biomass is required for design suitable for the strains and fermentation processes. Designed biomass is also cost-competitive substrate for overall processes including fermentations, which can simplify pretreatment and hydrolysis processes for lignocellulosic biomass with less-energy and less-cost. This presentation focuses on development of butanol production bioprocess with inedible resources through the concept "Designed biomass".

We firstly used several organic acids as designed biomass and succeeded in Salvage synthetic production of butanol from butyrate (3-5), lactate (6, 7) and acetate (8, 9). We also reported efficient butanol fermentation without carbon catabolite repression using cellobiose instead of glucose in mixed sugars with xylose (10). Recently, we have achieved simultaneous utilization of hexose and pentose sugars by constructing a designed incomplete hydrolysate from rice straw with low enzyme loading. It improved not only biomass utilization but also enzyme efficiency for butanol production, resulting in a highly cost-effective butanol fermentation. But glucose-oriented hydrolysis (complete hydrolysis) with high enzyme cost showed ineffective fermentation.

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## Systematic Feedback Isolation Applied for Meta-Fermentation Study

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Recently, we have proposed a new concept of ‘meta-fermentation’, for productions of value-added substances with controlled mixed culture systems (1). Compared with a pure culture system, there are several advantages in meta-fermentation such as no requirement for media sterilization, but still challenges to overcome to decrease by-product formation, and facilitate control of the fermentation process. In addition, because of difficulties in analyzing fermentation states, there is little technical knowledge on meta-fermentation, and the theory behind it has not been well established. On the other hand, optically pure L-lactic acid (L-LA) has been used as the feedstock for the synthesis of high-quality polylactic acid as a biodegradable bio-plastic. Many studies have reported L-LA fermentation in pure culture using LA-producing bacteria, while there are fewer studies on LA production in meta-fermentation. The factors affecting LA production are poorly understood for meta-fermentation to date. In this study, we aimed at investigating the effects of several control factors (temperature and pH control strategy, etc.) on L-LA production in meta-fermentation. Furthermore, we tried to establish isolate method for predominant strains from meta-fermentation.

### 1. Investigation of effect of several factors on product formations from food waste

Meta-fermentations were performed in model kitchen refuse (MKR) medium using a compost as the inoculum (2). In fermentation by pH swing control at 7.0 every 24 h, butyric acid was main product at 30°C and 37°C, while L-LA with 100% optical purity was predominantly produced at 45°C-55°C by *Bacillus coagulans* strains (1). The pH constant control at 7.0 shortened fermentation time but decreased L-LA production drastically due to the growth stimulation of heterofermentative *Bacillus thermoamylovorans*. Deliberately switching from pH swing control to constant control exhibited the best performance for L-LA production (3). These results present a novel pH control strategy for efficient L-LA production in meta-fermentation based on a concept different from that of pure culture systems.

### 2. Development of systematic feedback isolations for major strains in meta-fermentation

We developed a systematic feedback isolation strategy for the isolation and rapid screening of multiple targeted strains from meta-fermentation by combining optimizations of culture conditions and screening by MALDI-TOF MS at colony level. As the results, 3 targeted species and 3 strains closely related to the target species were successfully isolated.

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**SUPPLE – Sustainable plant-based production of extremozymes**

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Microorganisms originating from extreme environments, such as locations with high temperatures or high osmotic pressure, express enzymes called extremozymes due to their robustness to harsh conditions. This makes them of special interest for industrial applications. The SUPPLE project focusses on the development of novel applications for extremozymes in industrial processes. Together with partners from Italy and Germany, the production of antioxidative and detoxifying enzymes from extremophiles is investigated.

The production of such enzymes in plant cells allows the expression of complex proteins with optimal glycosylation, folding and structure. Therefore, an industrially applicable, plant-based production platform for the expression of the mentioned extremozymes will be developed in this project, to provide biocatalysts for further studies in the cosmetics and food industry.

As a reference, the enzymes were first expressed in bacteria. For the development of the production process, this was done initially in small cultures in order to prepare a later "scale-up" of the expression. In addition, a purification strategy for the enzymes was developed in order to obtain pure enzymes. The cultures and extracts were examined at various stages and the activity of the enzymes obtained was examined in order to analyze the success of the expression and the purification steps. This will allow a precise control and optimization of every developed process step.

## The Role of Glycogen in the Diauxic Shift of *E. coli*

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When cultured in a two carbohydrates medium, glucose and lactose, *E. coli* may uptake them in an order of glucose first and then lactose. A short lag phase between the consumption of these two carbon sources, called diauxic lag phase, is considered as the period for *lac* operon expression (Boulineau et al. 2013). Interestingly, in a simulation result from the stochastic model in (Puchalka et al. 2004), some cells were found to be dead during the diauxic lag phase. It suggests that an uncovered mechanism might be working to support *E. coli* activities during the *lac* operon expression.

The role of glycogen for the primary carbon source in the early stage of *E. coli* has been computationally confirmed in our previous studies (Yamamotoya et al. 2012) and (Tian et al. 2013) with the hybrid functional Petri net (HFPN) simulation. In this presentation, we show another role of glycogen as a supplemental energy, that is *E. coli* utilizes glycogen to maintain its life in the diauxic phase.

A simulation result conducted by an HFPN model predicts that *E. coli* without glycogen exhibits a longer diauxic lag phase than the one with glycogen. To confirm this prediction, we have experimentally examined effects of disruption of *glgA* for glycogen synthase, *glgB* for 1,4- $\alpha$ -glucan branching enzyme, and *glgC* for glucose-1-phosphate adenylyl transferase on growth in minimal medium containing glucose and lactose. All these mutants in addition to a double mutant of *glgA* and *glgB* showed a delayed growth after consumption of glucose. The level of glycogen was found to be very low in all mutants tested. Therefore, it is likely that as predicted in the simulation model, glycogen is working in the diauxic lag phase in *E. coli*.

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## The influence of ethanol extract *Dioscorea alata* L. on CD4<sup>+</sup>CD62L<sup>+</sup> and CD8<sup>+</sup>CD62L<sup>+</sup> profile of BALB/c mice model digestive tract allergy

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Naive T cells and activated T cells express different adhesion molecules and patterns of migration. Naive T cells express CD62L. The research aims to assess the CD4<sup>+</sup>CD62L<sup>+</sup> and CD8<sup>+</sup>CD62L<sup>+</sup> profile of BALB/c mice model allergy on the sensitivity, the challenge and the gastrointestinal tract allergy phase after treatment with ethanol extract of *Dioscorea alata* L. (EEDA). An experimental study with post test only control group design using 63 BALB/c mice was divided into seven groups: control group (I) and treatment groups with the ethanol extract of *D. alata* L. tubers dose (0.00, 0.17, 2.01, 10.04) g · kg<sup>-1</sup> bw (II-V); an antihistamine drug treatment (VI) and diosgenin treatment of 200 mg · kg<sup>-1</sup>bw (VII). Treatment groups induced with ovalbumin i.p. on 15 d and 22 d, orally on 23 d to 30 d subsequently. Mice were sacrificed on (18, 25 and 31) d, the spleen was taken and isolated to measure CD4<sup>+</sup>CD62L<sup>+</sup> and CD8<sup>+</sup>CD62L<sup>+</sup> profile with flowcytometry. The results showed that EEDA dose (0.17, 10.04) g · kg<sup>-1</sup> and drug on sensitivity phase, EEDA dose 2.01, 10.04 g · kg<sup>-1</sup>; and drug on challenge phase; EEDA dose (0.17, 2.01, 10.04) g · kg<sup>-1</sup>, antihistamine drug and diosgenin on gastrointestinal tract allergy phase increased the percentage of CD4<sup>+</sup> expressing CD62L<sup>+</sup>, but not diosgenin on sensitivity and challenge phase, EEDA dose (0.17, 2.01, 10.04) g · kg<sup>-1</sup>, and antihistamine drug increased the percentage of CD8<sup>+</sup> T cells expressing CD62L<sup>+</sup> on the sensitivity, challenge, and gastrointestinal tract allergy phase. The conclusion is EEDA and antihistamine drug increase the percentage of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing CD62L<sup>+</sup> on the sensitivity, challenge, and gastrointestinal tract allergy phase and diosgenin increase the percentage of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing CD62L<sup>+</sup> on the gastrointestinal tract allergy phase of BALB/c mice digestive tract allergy model.

**Key words:** cell, adhesion, ethanol, migration

## Polyhydroxyalkanoates production with *Ralstonia eutropha* from biogenic waste streams

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Polyhydroxyalkanoate (PHA) biodegradable and biocompatible polyesters are alternatives to petroleum-based plastics. *R. eutropha* stores excess carbon as PHA polymers under stress or nutrient limitation conditions up to 90% of its cell dry weight. This efficiency of the wild type strain makes *R. eutropha* important for industrial PHA production. However, high production costs, together with cheaply available conventional plastic, are preventing their use as bulk material. Carbon feedstock costs are one of the major production costs. This study shows a closed process chain for the production and recovery of PHB and novel PHA co-polymers from low cost biogenic waste streams. In this study, we used industrial rendered waste animal fats from lowest quality, with a high content of free fatty acids, for PHA production studies [1]. Since, besides being inexpensive, the low quality makes the fat very unattractive for other applications, e.g. biodiesel production. In order to screen different waste animal fats as carbon source for PHA production, a cultivation method using shake flasks and micro-well plates was developed. Several handling issues had to be overcome, since the high melting temperatures of up to 55°C made the fats difficult to handle and *R. eutropha* was not able to emulsify the fat efficiently. Additionally, reproducibility is often poor and restricts the identification of best conditions. To overcome these issues, we applied various shake flask designs and emulsifiers and developed suitable methods for screening. Beside the wildtype for PHB production, we use a recombinant *R. eutropha* strain that produces the PHA copolymer poly(hydroxybutyrate-co-hydroxyhexanoate) (P(HB-co-HHx)) when grown on lipid-based feedstocks [2]. An increasing HHx concentration in the polymer directly correlates to an enhancement of the polymer properties as melting temperature, crystallinity and flexibility. This co-polymer has better properties than the homopolymer PHB, which is commonly produced by the wild type strain. PHA recovery was performed with non-halogenated solvents [3]. This process was scaled-up to 100 L. The usage of biogenic feedstocks will allow to reduce the current high market prizes for PHA in the future.

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## Improvement of poly-histidine tag for the production and purification of functional proteins

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In genetic engineering, highly efficient protein production and purification processes are required for the application of useful proteins, such as food processing enzymes and biopharmaceuticals. The protein purification process is often difficult even when protein production was successful in host organisms. In yeast, we have developed high-copy overexpression plasmid and an extraction method for overexpressed intracellular proteins. The last step is purification process. To purify a protein in a simple way, poly-histidine-tag affinity purification has been developed using the binding of poly-histidine peptide to nickel resin. To improve poly-histidine tag, we examined many types of poly-histidine tags attached to the red fluorescence protein (yEmRFP). The yEmRFP can be visualized by eye without fluorescence detection equipment, providing easy monitoring of protein fate. At first, histidine repeat numbers were increased to achieve strong binding to nickel resin. Although increased histidine number enhanced protein binding to the resin, it decreased RFP production in yeast. The increased ionic strength by longer poly-histidine peptide may interfere with the structural stability of RFP. Therefore, we searched amino acids that alleviate the lower RFP protein production caused by longer poly-His sequence. We found that insertion of Pro, Glu, Asp, Gly, Ala, Asn, or Gln was effective to reduce the poly-His negative effect on RFP production. Among these amino acids, Glu and Asp are acidic amino acids, which may counteract with the basic character of poly-His. Several amino acids repeats containing His and Glu or Asp were designed, and examined their effect on RFP production. Finally, we selected 6xHE sequence as a tag for stable protein production and easy purification. In 6xHE sequence, tag protein binding to nickel resin was weaker than 6xHis sequence but the binding and elution were controlled by pH change and addition of ionic chemicals. We propose that 6xHE tag will become another choice for protein production and purification from various host organisms.



## Microalgae growth for the optimization of starch and lipid production: Combining experimental and computational tools

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Microalgal biomass has been regarded as a sustainable and renewable feedstock for biofuel production due to its ability to naturally accumulate carbohydrates - mainly in the form of starch – and lipids. These carbon-based compounds can potentially be used as raw substrates for biofuel production (Brennan & Owende, 2010). Studies have shown that carbohydrate and lipid content in microalgae cells is positively influenced under conditions of nutrient limitation. However, this increase in starch and lipid content is coupled with a decrease in biomass production (Figueroa-Torres et al, 2017, Markou, Angelidaki, & Georgakakis, 2012), an adverse effect on the overall cultivation process. An efficient approach towards improving starch and lipid production by means of a cultivation strategy involves the use of kinetic models capable of predicting the main compositional elements of microalgae growth (Bernard, 2011). However, there have been very limited modelling efforts in the literature regarding the simultaneous production of starch and lipids by microalgal biomass. Thus, the aim of this work is to develop a multi-parameter kinetic model for the optimization of starch and lipid production. The proposed model considers a set of intracellular carbon flows between two main cellular compartments: a pool of active biomass and a storage pool comprising of starch and lipids. Model parameters were fitted against experimental datasets generated from lab-scale cultivation of *Chlamydomonas reinhardtii* CCAP 11/32C in standard Tris-Acetate-Phosphate (TAP) media under different concentration regimes. Fitting was carried out through an in-house developed optimization algorithm. Validation of the model was then carried out against different experimental datasets. The model can be used to predict three carbon-based cellular pools – starch, lipids, and active biomass – as well as nutrient consumption and pH evolution, useful factors for the establishment of optimal cultivation conditions.

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## Monitoring tools for improved characterization of the liquid phase in anaerobic digestion

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Monitoring tools for the liquid phase of anaerobic digestion are often restricted to a very few parameters. This might be sufficient for an operation at steady-state conditions, but not suitable at dynamic changes of feedstock and loading rate or when – in general – a poor yield is achieved and process optimization and plant retrofitting is required. Some examples of improved monitoring in the liquid phase in industrial plants, combined with suitable monitoring methods to transfer conditions of the lab into the large scale are presented. Novel techniques are introduced that allow the optimization of biogas production on site. The first method describes an automated vitality measurement of cells based on the polarizability in an electric field. The cells' polarizability is dependent on the membrane integrity and transport capacities of ions inside the cell. When different process conditions are compared, the polarizability provides a good indicator of the ability of cells to convert carbohydrate sugars and produce methane. If disturbing agents like antibiotics or other inhibitory compounds are present, or in the absence of essential trace metals, the polarizability decreases already very early before a reduction of product formation indicates an activity loss of the cells.

Another technique describes the monitoring of cell size distributions directly in culture broth based on laser-light back-reflection. This method can be used to evaluate the efficiency of pre-treatment methods. A narrow distribution of small particle sizes indicates suitable operation conditions.

While sensors are often mounted arbitrarily in the liquid phase, a multiposition probe equipped with several sensors, was applied at different positions in an industrial plant, which was fed discontinuously in order to achieve fluctuating gas provision. Using these techniques, an improved monitoring becomes feasible: the sensors are located at spots, in which process disturbances occur firstly, thus reducing the operational risk at a dynamic feedstock load, while enabling an adjustment of mixing conditions.

Improved monitoring can be integrated into model-based approaches, which are then used to predict the operation under flexible feedstock load, the environmental impact at different operating conditions if combined with a life cycle analysis approach, and as an early warning tool by recognizing the current state of the process.

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## Statistical Optimization of Single Cell Oil Production from Starch Hydrolysate and Glycerol by Thermotolerant Oleaginous Yeast *Pichia sp.* Scj 01 using Response Surface Methodology

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Thermotolerant oleaginous yeasts were isolated from agricultural wastes at 40 °C and 6 isolates of yeasts were obtained. However, only the isolate Scj 01 could also utilize glycerol as a carbon source. Statistical based experimental designs to optimize fermentation condition for single cell oil production from sweet potato hydrolysate and glycerol by this Scj01 strain were conducted by using Response Surface Methodology (RSM). Initial screening of important factors influencing single cell oil production, i.e. substrate concentrations, inoculum size (%), initial pH, agitation speed (rpm) and temperature (°C) were tested. Results indicated that only agitation speed (rpm) showed statistically significant ( $p=0.0042$ ) influence on lipid content when sweet potato hydrolysate was used. On the other hand, initial inoculum size and glycerol concentrations exhibited statistically significant ( $P\leq 0.05$ ) influences on single cell oil production when glycerol was used as the substrate. Placket-Burman design was further used to optimize the levels of these selected variables, which were agitation speed (rpm), initial pH, inoculum size (%) and glycerol concentrations, in order to maximize the lipid content and to determine the interaction effects among the tested variables by using Box-Behnken design or CCD (Central Composite Design) of RSM (Response Surface Methodology). It was found that the optimal conditions for lipid accumulation at 40 °C when sweet potato hydrolysate was used were 3% inoculum size, 200 rpm and initial pH 4.0, in which the obtained lipid content of 2.24 g/l or 42.5% (w/w) on the cell dry weight basis was estimated. On the other hand, the optimal conditions for lipid accumulation when glycerol was used were 1% inoculum size, initial pH 6.0 and 4% glycerol and the obtained lipid content of 1.19 g/l or 40.9% (w/w) on the cell dry weight basis was estimated. Analysis of fatty acid compositions of the yeast oil by GC showed that both substrates consisted of similar fatty acid profile in which oleic acid was the major fatty acids (66.4 and 55.4%) while palmitic acid (12.4 and 20.0%) and linoleic acid (7.3 and 9.8%) were also found. These fatty acid contents were similar to the plant oil. Single cell oil from this oleaginous yeast, thus, could be an alternative potential oil feedstock for biodiesel production.

### Reference:

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## **Brewing using Mixed Cultures**

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Mixed culture beers have traditionally been a niche area of brewing but nonetheless some of these beer styles have survived over the centuries. Recently this niche style of brewing has enjoyed a revival in popularity not only in Europe but worldwide. These “sour beer” mixed cultures highlight flavour and aroma profiles obtained by the use of different microorganisms specifically *Saccharomyces* yeasts, lactic bacteria as well as non-*Saccharomyces* yeast strains.

Of interest to the VLB are not specifically the traditional methods of the styles using these mixed cultured but also confronting the challenges that such cultures present and managing them with the controlled use of microorganisms as well as the use of modern production methods to increase reproducibility, production as well as microbiological safety within the brewery.

Within the framework of these challenges are a number of areas of interest. Included in these areas are the specific characteristics of the microorganisms themselves (acid production, temperature optimum, ester production etc.) and their effect on the final product as well as ease of use, safe dosing procedures as well as effective cleaning and disinfection.

In recent experiments, controlled procedures have been completed using *Saccharomyces cerevisiae*, *Lactobacillus delbrueckii*, and *Brettanomyces bruxellensis* to produce the traditional Berlin sour beer “Berliner Weisse”.

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