11th International Symposium on Biocatalysis and Agricultural Biotechnology
The Banff Centre – Banff, Alberta, Canada
September 13 to 16, 2015

Meeting Program

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Welcome Message

Welcome to the 11th International Symposium on Biocatalysis and Agricultural Biotechnology (ISBAB) in the beautiful Banff National Park. The ISBAB symposia originated in 2005 as an initiative of Dr. Jei-Fu Shaw, who had recently been appointed President of National Chung Hsing University in Taichung, Taiwan, and Dr. Ching T. Hou of the United States Department of Agriculture (USDA). The theme for the symposium was selected to highlight research in biocatalysis and biotechnology. Ching Hou invited agricultural scientists outside of Taiwan who were mainly from Pacific Rim countries. Many of these scientists were from the USDA, the Ministry of Agriculture, Forestry and Fisheries of Japan, and institutes from Korea. The first symposium proved so well received that the founders decided to continue the next year with increased international participation. In the earlier years, ISBAB was known as the International Symposium on Biocatalysis and Biotechnology (ISBB), but later the name was modified to ISBAB. The founders of the symposium also established the International Society of Biocatalysis and Biotechnology whose name shared the same abbreviation as ISBB, eventually becoming ISBAB. The first five annual symposia were held in Taiwan, the 6th in Korea, the 7th in Japan, the 8th in the USA, the 9th in Slovakia, the 10th back in Taiwan and now we are here in Canada. ISBAB members also established the peer-reviewed journal, Biocatalysis and Agricultural Biotechnology (BAB), which is published by Elsevier.

This year's symposium features participation by delegates from 11 countries! The 11th ISBAB includes the following sessions: 1) bioactive and anti-microbial agents; 2) biocatalysis; 3) biofuels, bioproducts and bioconversions; 4) epigenetics; 5) general biotechnology; 6) nutraceuticals and food biotechnology; and 7) plant biotechnology. We are pleased to have keynote speakers who provide an excellent focal point for some of these research areas. We extend our sincere thanks to our many sponsors who are listed in this program booklet. This symposium would not have been possible without their strong support.

Banff National Park, which covers 6,641 square kilometers, was established in 1885 as Canada's First National Park. It features breath-taking scenery, interesting wildlife and a range of outdoor activities including hiking. The town of Banff (1,383 meters above sea level) is a must to visit for its atmosphere, shopping, restaurants and entertainment. You are sure to enjoy the amenities at The Banff Centre which include a swimming pool and athletic facility. Our Wednesday afternoon study tour will feature an excursion to two of Canada's most famous glacial-fed lakes in the Rocky Mountains, Lake Louise (elevation 1,750 meters) and Moraine Lake (elevation 1,885 meters). Study tour buses will then deliver ISBAB delegates to a country and western style BBQ with entertainment at the "Donut". While you are here at the ISBAB, we wish you a memorable experience!

Jei-Fu Shaw
Preident, ISBAB

Ching Hou
Past President, ISBAB

Randall Weselake
Chair, 11th ISBAB
Vice President, ISBAB

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Biocatalysis and Agricultural Biotechnology (Society journal)

Journal website: www.elsevier.com/locate/bab

2015 ISBAB Fellow/WABAB Academician

Casmir Akoh

Distinguished Research Professor, University of Georgia, Athens, Georgia, USA
Past President of the American Oil Chemists' Society

In recognition of his outstanding and innovative contributions to lipid biotechnology, structured lipids, functional and healthful lipids research

Previous ISBAB Fellows/WABAB Academicians

John Harwood
Professor and Deputy Director, Molecular Sciences, Cardiff University, Wales, UK
In recognition of outstanding contributions in plant lipid biosynthesis and medical research on lipids

Kiyoshi Hayashi
Vice President, National Agriculture and Food Research Organization (NARO) Director General, National Food Research Institute (NFRI), Tsukuba, Japan
In recognition of outstanding contributions to the development on innovative enzyme utilization technology

Ching T. Hou
Senior Scientist, NCAUR, ARS, USDA. Peoria, IL, USA
President, ISBAB
In recognition of outstanding contributions to the field of biocatalysis and biotechnology, and to the establishment of ISBAB

Yung-Sheng Huang
Vice President, I-Shou University, Kaohsiung, Taiwan; Secretary-General, ISBAB
In recognition of his outstanding contributions in the areas of lipid biochemistry, metabolism and nutrition

Ramesh N. Patel
President, SLRP Associates, Bridgewater, NJ, USA
In recognition of his outstanding contributions in the areas of biocatalysis, biotechnology and new drug development

Jei-Fu Shaw
President, Chair Professor, I-Shou University, Kaohsiung, Taiwan
Vice President and President-elect, ISBAB
In recognition of outstanding contributions to the field of enzyme biochemistry and biotechnology, and to the establishment of ISBAB

Andrew H.-J. Wang
Vice President, Academia Sinica, Taipei, Taiwan
In recognition of outstanding contributions to the field of structural proteomics, drug discovery, synchrotron crystallography, structure-function relationship of enzymes and DNA

Randall J. Weselake
Professor and Canada Research Chair in Agricultural Lipid Biotechnology, University of Alberta, Edmonton, Canada
In recognition of outstanding contributions to the metabolic engineering of oilseeds and crop genomics

Suk Hoo Yoon
Professor, Department of Food Science and Biotechnology, Woosuk University, Samnye, Jeonbuk, Korea
In recognition of outstanding contributions to the food sciences and technology

11th ISBAB Organizing Committee

Committee

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Chris Kazala (Canada) – Coordinator

Christopher Schieman (Canada) – Communications Associate

Annie Wong (Canada) – Administrative Assistant

List of Sponsors

Corporate and institutional sponsorship is necessary to offer a high-quality program at reasonable cost to the delegates. The organizing committee for the 11th International Symposium on Biocatalysis and Agricultural Biotechnology would like to extend its sincere gratitude to the following organizations for their generous sponsorship of the meeting.

Alberta Epigenetics Network

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Program at a Glance

Sunday, September 13

5:00 p.m. to 9:30 p.m.	Registration – The Banff Centre
6:30 p.m. to 9:30 p.m.	Opening Mixer – Max Bell Foyer / Elder Tom Crane Bear Room

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Monday, September 14

7:00 a.m. to 8:00 a.m.	Registration
8:10 a.m. to 8:30 a.m.	Opening Remarks and Fellow Award (MB Auditorium)
8:30 a.m. to 10:00 a.m.	Keynote Presentations (MB Auditorium)
10:00 a.m. to 10:30 a.m.	Networking Break
10:30 a.m. to 12:10 p.m.	Biocatalysis I (MB 251) Nutraceuticals and Food Biotechnology I (MB 252) Plant Biotechnology I (MB Auditorium)
12:10 p.m. to 1:30 p.m.	Lunch (Vistas Restaurant)
1:30 p.m. to 3:00 p.m.	Keynote Presentations (MB Auditorium)
3:00 p.m. to 3:40 p.m.	Group Photograph and Networking Break
3:40 p.m. to 5:40 p.m.	Biofuels, Bioproducts and Bioconversions I (MB 251) Nutraceuticals and Food Biotechnology II (MB 252) Plant Biotechnology II (MB Auditorium)

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Tuesday, September 15

8:30 a.m. to 10:30 a.m.	Bioactive and Anti-microbial Agents I (MB 252) General Biotechnology (MB 251) Plant Biotechnology III (MB Auditorium)

Tuesday, September 15, continued

10:30 a.m. to 1:30 p.m.	Networking Break, Free Time and Lunch
1:30 p.m. to 3:00 p.m.	Keynote Presentations (MB Auditorium)
3:00 p.m. to 3:30 p.m.	Networking Break
3:30 p.m. to 5:30 p.m.	Biocatalysis II (MB 251) Epigenetics Workshop (MB 252) Plant Biotechnology IV (MB Auditorium)

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Wednesday, September 16

8:30 a.m. to 10:30 a.m.	Bioactive and Anti-microbial Agents II (MB 252) Biofuels, Bioproducts and Bioconversions II (MB 251) Plant Biotechnology V (MB Auditorium)
10:30 a.m. to 11:00 a.m.	Networking Break
11:00 a.m. to 11:20 a.m.	Closing Remarks Presentation on the 12th ISBAB (MB Auditorium)
11:20 a.m. to 1:20 p.m.	Free time and Lunch
1:20 p.m.	Board buses (in front of Professional Development Centre)
1:30 p.m. to 5:30 p.m.	Study Tour - Mountain Lakes
5:30 p.m. to 9:30 p.m.	Closing Dinner – Brewsters MountView Barbecue (Combined event with Phytola Science Meeting)

Keynote Presentations

FROM THE OCEANS TO THE FIELDS - PRODUCING WAX ESTERS IN PLANTS

Tim Iven[1], Ellen Hornung[1], Sofia Marmon[1, 2], Dan Yu[1], Mareike Heilmann[1], Cornelia Herrfurth[1], and Ivo Feussner[1]*

[1]Department of Plant Biochemistry, Georg-August-University, Goettingen, Germany
[2]Department of Plant Breeding, Swedish University of Agriculture, Alnarp, Sweden

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Lipids are currently in higher demand than ever due to their increased need for food, feed as well as for biofuel and applications in the chemical industry due to the growing population of our world. Therefore, there are many efforts to produce increased amounts of lipids, as well as specific lipid profile *in planta*. *Camelina sativa* has recently been recognized as an emerging low input oilseed crop for marginal land and we foresee a future where even more research on oilseed is done in this plant instead of *Arabidopsis thaliana*. Furthermore, we aim to establish it as production platform to produce feed stocks from renewable resources for the chemical industry. To be able to obtain a tailor made fatty acid profile in the oil, an increased knowledge about what controls the lipid content as well as the lipid profile of oil seeds is of great importance. Therefore, we started a systematic overview on the central steps of the lipid production in *Camelina* seeds, including characterization of the lipidomic complement of the developing seeds. In addition, the impact on the fatty acid profile of some of the major enzymes involved in triacylglycerol synthesis have been investigated by gene overexpression and silencing, sometimes in combination to investigate possible additional effects. These results are giving a deepened understanding of the lipid production in seeds of this plant, and provide the basis for future targeted approaches to modify the lipid profile and content of *Camelina* seeds.

Wax esters are neutral lipids exhibiting desirable properties for lubrication. Natural sources have traditionally been whales. Additionally some plants, bacteria and insects produce wax esters. Currently there is no biological source available for long chain-monounsaturated wax esters, which are most suited for industrial applications. To this end, we explored enzymatic activities from bacteria, insects and plants for the desired properties and analyzed their suitability and additional requirements enabling their production in *Camelina.* By expressing different enzyme combinations, we have been able to replace so far up to one third of the triacylglycerol fraction by wax esters yielding wax ester species with very high content in monounsaturated acyl and alcohol moieties. These results suggest that *Camelina* will be indeed in the future a very suitable production platform for monounsaturated-long chain wax esters.

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Dr. Ivo Feussner is a Professor for Plant Biochemistry and the Scientific Vice-Director of the Center of Molecular Biosciences at the Georg-August-University in Goettingen, Germany. He is a chemist by training and received his PhD in plant biochemistry from the Philipps-University in Marburg.

After working as a teaching assistant in pharmaceutical biology at the Martin-Luther-University in Halle/Saale, he ran his own research group at the Institute of Plant

Biochemistry (IPB) in Halle/Saale, Germany and the Institute for Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany. His research work was recognized by the “Butenandt-Habilitation Award” of the German Society for Biochemistry and Molecular Biology (GBM) in 2001 and in 2012 he received the Terry Galliard medal of the International Symposium on Plant Lipids.

Dr. Feussner is a Fellow of the Saxonian Academy of Sciences in Leipzig, Germany, and the Academy of Sciences in Goettingen, Germany. He has made major contributions to the biochemistry and molecular biology of storage and 14ignaling lipids in oil crops and model plants as well as in fungi, mosses and algae. He has published more than 200 papers and 20 patens. Currently one of his research focuses is on the biotechnological optimization of the emerging oil crop Camelina sativa for bio-based lubricants and biofuels.

ARABIDOPSIS ACYL-COA-BINDING PROTEINS CONFER PROTECTION AGAINST BIOTIC AND ABIOTIC STRESSES

Mee-Len Chye

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Sessile organisms such as plants have evolved to survive adverse conditions imposed by biotic and abiotic stresses, including pathogen invasion, extremes in temperature and drought conditions. The identification of proteins that are switched on during stress treatments represents a first step towards understanding their functions and their potential in genetic engineering. In the model plant, *Arabidopsis thaliana*, some acyl-CoA-binding proteins (ACBPs) were observed to be upregulated by various stresses. When these ACBPs were expressed ectopically in transgenic plants, the plants were showed protection against the corresponding stresses. The six Arabidopsis ACBPs of the AtACBP family (designated AtACBP1 to AtACBP6), are conserved at the acyl-CoA-binding domain and are distributed across four classes: Class I is represented by the 10-kDa AtACBP6, Class II ACBPs contain ankyrin repeats (AtACBP1 and AtACBP2), Class III is larger (39.4-kDa AtACBP3) and Class IV members are the largest and contain kelch motifs (AtACBP4 and AtACBP5). The ankyrin repeats and kelch motifs in AtACBPs can potentially mediate interaction with protein partners. Interestingly, several protein partners of ACBPs were also stress-induced proteins. Investigations using Arabidopsis T-DNA insertional mutants and transgenic Arabidopsis AtACBP-overexpressors further support AtACBP function in protection against stress treatments. AtACBP1- and AtACBP2-overexpressors were more resistant to heavy metal/oxidative stress, AtACBP2-overexpressors were drought tolerant, AtACBP3-overexpressors were protected against biotic stress and AtACBP6-overexpressors were freezing tolerant. *In vitro* assays have revealed that (His)-tagged AtACBPs bind acyl-CoA esters. Thus, the lipid-binding properties and lipid-trafficking abilities of AtACBPs likely enhance plant membranes, which consist of lipid components, to better withstand stress treatments.

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Mee-Len Chye, the Wilson and Amelia Wong Professor in Plant Biotechnology at the University of Hong Kong, completed her PhD on a Commonwealth Scholarship at the University of Melbourne and received postdoctoral training in Plant Molecular Biology at the Rockefeller University (New York) and the Institute of Molecular and Cell Biology (Singapore). She joined the University of Hong Kong in 1993 and was promoted to Professor in 2005.

She has been awarded an Edward Clarence Dyason Universitas 21 Fellowship (2004/05), an Outstanding University Researcher Award (2006/07), a Croucher Senior Research Fellowship (2007/08), and an Eileen Mary Harris Scholarship (2013). She serves on the editorial boards of Planta (Springer), Frontiers in Plant Metabolism & Chemodiversity and

Frontiers in Plant Physiology, and was Chair of the 4th Asian Symposium on Plant Lipids (2011).

The main focus of her laboratory is to understand the function and mechanism of action of stress-induced plant proteins such as acyl-CoA-binding proteins (ACBPs) and 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS). ACBPs are lipid-binding proteins that can affect plant development and stress responses. Her research team intends to use ACBPs to generate transformed plants that can better withstand the adverse effects of biotic and abiotic stresses. The enzyme HMGS of the mevalonate pathway is not only stress-responsive but can promote growth and seed yield. Ultimately, these findings will be applicable to agriculture to boost food productivity.

THINKING SMALL TO DEFINE A BIG FUTURE

Carlo D. Montemagno, PhD

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The ability to use machines to manipulate matter a single molecule at a time renders many things possible that were impossible before. Living systems do this on a regular basis. The core challenge is how to transform a labile molecule that exists in a fragile living organism and to transfer that functionality into a stable system that is economically scalable. The most significant difficulties revolve around environmental stability and the inherent structural limitations of the molecule.

Presented is the generic solution methodology used to solve these limiting challenges to produce a new class of materials and devices. Elements of the discussion will include the genetic engineering of active biological molecules into engineering building blocks and their assembly to introduce “metabolism” into engineered devices and materials. Ultimately synthesizing new classes of materials with advanced functionality that incorporates new intrinsic properties into the matter.

Two exemplars will be presented. First we will elucidate the design, engineering and assembly of a complex closed system that uses a highly modified photosynthetic process to transform carbon waste into valuable drop-in specialty chemicals without any living organisms with commercially competitive economics. Secondly we will present a new technology that stabilizes biological molecules maintaining their function for months at application relevant environmental conditions transitioning additive manufacturing from 3D space to a four-dimensional, functional space. Enabling the synthesis of a new class of printable “inks” that have stabilized and active biological molecules as integrated elements of synthesized polymer constructs to create a new class of materials that now includes biologic function as an intrinsic property.

The next wave of technological progress will enable the manufacturing of a unique class of devices and materials that embeds complex functional behavior as an intrinsic property enabling emergent functionality at multiple length scales. These systems will actively interact with their local environment establishing a new capability that will impact solution generation across multiple societal sectors including health care, resource recovery, food production and environmental restoration.

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Dr. Carlo Montemagno received a Bachelor of Science degree in Agriculture and Bio Engineering from Cornell University; a Master’s Degree in Petroleum and Natural Gas Engineering from Penn State and a Ph.D. in Civil Engineering and Geological Sciences from Notre Dame. He is a Professor in the Department of Chemical and Materials Engineering at

the University of Alberta, AITF Strategic Chair of Bionanotechnology, Program Lead of the Biomaterials Program at the National Institute for Nanotechnology, Director of Ingenuity Lab and Canada Research Chair in Intelligent Nanosystems.

His current research looks at biofunctional next-generation materials that can, for example, sense the environment, amplify information, generate energy, and change physical characteristics. These molecular-level controls have potential applications in resource extraction, environment management, healthcare, information processing and the production of high-value industrial products.

A world-renowned expert in nanotechnology, Dr. Montemagno was Founding Dean of the College of Engineering and Applied Sciences at University of Cincinnati. He has been recognized with prestigious awards including the Feynman Prize, the Earth Award Grand Prize, the CNBC Business Top 10 Green Innovator award and named a Bill & Melinda Gates Grand Challenge Winner

TOWARDS ADVANCED BIO-MANUFACTURING – DESIGNER MCROBES AND SYSTEMS FOR BIOSYNTHETIC CASCADE REACTIONS

Claudia Schmidt-Dannert

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Advances in genome sequencing, inexpensive DNA synthesis and in computational biology have let to the emergence of the field of synthetic biology which aims to reengineer and synthesize biological systems at much larger scale than manipulating one gene at a time. Reprogramming and rewiring biological systems by introducing new functionalities offers great promise for the design of cells for the production of new chemicals. In this lecture I will discuss and show examples of our efforts on engineering metabolic pathways and other complex properties into microbial cells and for *in vitro* biomanufacturing.

In several examples, I will illustrate our efforts in engineering pathways for the microbial production of valuable compounds including carotenoids and phenylpropanoids. More recently, we have taken advantage of low-cost DNA sequencing to map the uncharted territory of secondary metabolism in mushrooms (Basidiomycota) which are widely used in traditional medicine. Our studies show that Basidiomycota synthesize a variety of different sesquiterpenoid structures. We have initiated genome-wide surveys and characterization of sesquiterpene synthase diversity in mushrooms, suggesting an enormous potential for mining and refactoring of biosynthetic pathways for novel bioactive compounds.

Finally, I will outline our recent efforts in engineering protein nano-bioreactors for targeted localization of enzymes into these nano-containers. Spatial organization is central to the function of all biological systems. Consequently, application of similar principles to the organization of synthetic pathways has become an emerging strategy to increase the efficiencies of designed systems. Furthermore, the self-assembling properties of the nano-container protein subunits hold great promise for the design of robust and cost-efficient protein scaffolds for the orchestration and co-localization of *in vitro* enzymatic multi-step reaction cascades.

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Dr. Claudia Schmidt-Dannert is a Distinguished McKnight Professor in the Department of Biochemistry, Molecular Biology and Biophysics at the University of Minnesota. After obtaining her PhD at the National Research Center for Biotechnology (GBF) in Braunschweig, she went to the University of Stuttgart to lead the Molecular Biotechnology Group in the Institute of Technical Biochemistry.

She received a habilitation-fellowship from the German Science Foundation for research on in vitro pathway evolution and joined Frances Arnold's group at the California Institute of Technology. After accepting a faculty position at the University of Minnesota, she continued to engineer microbial cells with new pathways and properties for biocatalysis and biosynthesis.

Current research efforts in her group focus on harnessing the biosynthetic potential of higher fungi and engineering biological systems for more efficient multi-enzyme biocatalysis and biosynthesis. During her tenure at the University of Minnesota, Dr. Schmidt-Dannert has published numerous manuscripts, patents and book chapters; serves as Editor and board member of several journals and received several awards such as a David and Lucile Packard Fellowship and McKnight Fellow- and Professorships.

ENHANCING CROP YIELD AND VALUE THROUGH TRANSCRIPTOME METABOLIC ENGINEERING

Oliver P. Peoples*, Kristi Snell, and Meghna Malik

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The expected growth in global population form the current 7 billion people to 9 billion by 2050 together with changes in diet in developing nations requires that agriculture increase crop productivity by 70% in the at time period. This growing population and the infrastructure required to support them will place further pressure on land and scarce water resources. To meet these challenges, new approaches for enhancing food crop yield will be necessary. Metabolix is a pioneer in the field of metabolic engineering in both microbial systems and in the deployment of microbial metabolic pathways in crops. The companies crop activities were targeted at producing a microbial polymer which has a wide range of commercial applications including as a plastic, source of chemicals, denitrification and in animal feed. The key technical hurdle to be overcome is to shift 10-20% of the fixed carbon in the plant to this new molecule. In addressing these challenges the company initiated a series of research activities to enhance plant photosynthesis and improve the efficiency of carbon utilization in the plant through metabolic engineering. The company uses a combination of microbial genes to debottleneck key steps in plant photosynthesis and carbon conversion pathways in combination with global transcription factor genes to address these challenges. Recently Metabolix announced its intentions to spinout its crop science programs into a new company to be called Yield10 Bioscience. Yield10's mission is to enhance global food security by enabling step changes in food crop yield. Aspects of the new companies approach and objectives will be discussed.

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Dr. Oliver Peoples is Chief Scientific Officer and one of the co-founders of Metabolix. Actively involved in bioplastics research for more than 30 years, Dr. Peoples holds more than 90 patents and patent applications, frequently contributes to peer reviewed academic papers and is a recognized speaker at biotechnology, biopolymer, bioplastic and chemical conferences around the world.

Recognizing the longer term potential to produce bioplastics directly in crops and link renewable chemical and bioplastics production with the costs and scale available from agriculture Dr. Peoples has sustained an innovative R&D effort in this area for the last 18 years. This challenging program has resulted in steady progress toward this goal and provided Metabolix with unique capabilities in complex metabolic engineering in crops.

Leveraging these capabilities the company in collaboration with a number of partners has recently developed exciting technologies directly relevant to crop yield and global food security. Dr. Peoples received a Ph.D. in Molecular Biology from the University of Aberdeen, Scotland.

RECENT DEVELOPMENTS IN REGENERATING TRANSGENIC OIL PALM

G.K.A. Parveez[1*], M.Y. Abdul Masani[1], A.M. Dayang Izawati[1], B.Bahariah[1], M. Siti Masura[1], A. Nur Hanin[1], W.S. Wan Nur Syuhada[1], A.R. Nurfahisza[1] and I. Nor Fakhrana[1], F.H. Lim[1], S.Ravigadevi[1], Gundula Noll[2], and Dirk Pruefer[2]

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[2]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schlossplatz 8, 48143 Muenster, Germany

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Production of transgenic oil palm using microprojectile bombardment and *Agrobacterium tumefaciens*-mediated methods has been reported using embryogenic calli and Basta as selection agent. Later, other selection agents, such as green fluorescence protein (GFP), mannose and 2-dioxyglucose were also evaluated for oil palm. At the same time, efforts to produce transgenic oil palm that are free from marker genes and free from backbone sequences, were investigated. One possible way to achieve the above is through transformation of single cells such as protoplasts or pollen. Since the established of oil palm tissue culture as early as 1984, regeneration of oil palm from fine suspension culture and from protoplast has never been reported. After evaluating almost 20 different combinations of plant growth regulators, successful regeneration of plantlets from protoplasts was obtained. Later, transfer of foreign genes into protoplasts using microinjection and PEG mediated transformation was reported. At the same time also, continuous improvement on the oil palm transformation using microprojectile bombardment and *Agrobacterium tumefaciens*, are ongoing and will be discussed.

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Dr. Ahmad Parveez Ghulam Kadir, is Director of Advanced Biotechnology and Breeding Centre at the Malaysian Palm Oil Board (MPOB). He completed his PhD on Plant Genetic Engineering at the Universiti Putra Malaysia under Asian Development Bank's Scholarship. He was also appointed by the Honourable Minister of Natural Resources and Environment of Malaysia to Chair the Genetic Modification Advisory Committee (GMAC) under the National Biosafety Board in May 2010. He has 26 years of experience in plant molecular biology and genetic engineering. His main interest is in genetic modification of oil palm for novel value added products such as increasing the content of oleic acid, palmitoleic acid and ricinoleic acid besides synthesizing bioplastics in oil palm. He holds 12 patents and patent applications. He has authored and co-authored more than 60 articles in refereed journals and serves on the editorial board of Frontiers in Plant Biotechnology. His award recognitions include national level Employee of the Year for Executive Group (2007); The Outstanding Young Malaysian Award (2006); national level Prophet's Muhammad S.A.W. Birthday Award (2006); Malaysian Young Scientist Award (2001); and, Wilton R. Earle Award (1998) by The Society of In Vitro Biology.

Technical Sessions

HOW STRUCTURAL BIOLOGY HELPS UNDERSTAND THE FUNCTIONS OF PROTEINS FROM TOMATO, CORAL AND ARABIDOPSIS Andrew H.-J. Wang*

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Synchrotron biological crystallography is a powerful method to elucidate the 3D structures of proteins very efficiently. We have been using this technology to analyze a number of proteins relevant for agricultural applications. Three examples are shown.

(1). *Z,Z*-farnesyl pyrophosphate synthase (zFPS) from wild tomato *Solanum habrochaites* is responsible for the formation of *Z,Z*-FPP, a substrate for class II sesquiterpene synthesis. Here, we determined the first crystal structure of *z*FPS from *S. habrochaites* which revealed the *cis*-type prenyltransferase fold. The structure indicated Histidine103 may play important role in the enzyme function. We showed *z*FPS-H103Y retains *Z,Z*-FPP and neryl diphosphate (NPP) synthesis activity. In addition, four unexpected products had been detected, including limonene, *α*-terpineol, lavandulyl diphosphate (LDPP) and menthol. We propose the H103Y mutation lead to synthesis of LPP from two DMAPP molecules, and catalyze cyclization of NPP to form limonene, *α*-terpineol, and LDPP to menthol, respectively. (2). Chromoproteins (CPs) have unique colors and can be used in biological applications. In this work, a novel blue CP with a maximum absorption peak (λmax) at 608 nm was identified from the carpet anemone *Stichodactyla gigantea* (sgBP). In vivo expression of sgBP in zebrafish would change the appearance of the fishes to have a blue color. Structure-based enhancement of the color properties was tested and among the mutations conducted a S157C mutation shifted the λmax to 604 nm and darkened the blue color expression. Our results provide a structural basis for the blue color enhancement of the biomarker development. (3). S1/P1 nuclease AtBFN2 (EC 3.1.30.1) encoded by the *Arabidopsis thaliana* At1g68290 gene is a glycoprotein that digests RNA, ssDNA, and dsDNA. AtBFN2 depends on three zinc ions for cleaving DNA and RNA at 3'-OH to yield 5'-nucleotides. In addition, AtBFN2's enzymatic activity is strongly glycan dependent. Plant Zn^{2+}-dependent endonucleases present a unique fold, and belong to the Phospholipase C (PLC)/P1 nuclease superfamily. In this work, we present the first complete, ligand-free, AtBFN2 crystal structure, along with sulfate, phosphate and ssDNA co-crystal structures. With these, we were able to provide better insight into the glycan structure and possible enzymatic mechanism. Based on these findings, we propose a rational ssDNA binding model, in which the ssDNA wraps itself around the protein and the attached surface glycan, in turn, reinforces the binding complex.

NOVEL RECOMBINANT CHLOROPHYLLASES AND BIOTECHNOLOGICAL APPLICATIONS Yi-Li Chou[1] and Jei-Fu Shaw[1,2,*]

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Chlorophyllase (Chlase) is the key catabolic enzyme to catalyze chlorophyll breakdown to produce chlorophyllide and phytol. In our previous studies, the recombinant *Brassica oleracea* chlorophyllase 1 (BoCLH1) with poly(His)-tagged at the C-terminal was used to catalyze chlorophyll hydrolysis for the production of chlorophyllide and phytol. In this study, immobilized metal ion affinity chromatography technique were used for simultaneous enzyme purification and immobilization. Biochemical analysis of the immobilized enzyme showed higher chlorophyllase activity for chlorophyll a hydrolysis in a weak base environment and high-temperature environment compared with the free enzyme. In addition, the immobilized enzyme can effectively improve enzyme thermostability at 60 °C and can retain 60% activity after reused for 17 cycles. Therefore, the immobilized enzyme can be repeatedly reused to lower costs and is potentially useful in the industrial production of chlorophyllide and phytol.

Four novel recombinant Chlases from higher plant (*Pachira macrocarpa,* PmCLH1 and PmCLH2), algae (*Chlamydomonas reinhardtii,* CrCLH1), and photosynthetic bacterium (*Cyanothece sp.* ATCC 51142, CyanoCLH) were expressed in *Escherichia coli* (DE3) and purified for the biochemical characterization. The recombinant PmCLH1, PmCLH2 and CrCLH1 contained a conserved GHSRG lipase motif and catalytic triad Ser-Asp-His, and the recombinant CyanoCLH comprised a conserved GHSLG lipase motif and a catalytic dyad Ser-Asp. The PredictProtein server predicted all four Chlases as belonging to the α/β hydrolase superfamily. The biochemical characterization of the recombinant PmCLH1, PmCLH2 and CrCLH1 revealed higher activity at pH 6 and 40 °C, whereas the recombinant CyanoCLH exhibited higher activity at pH 7 and 60 °C. Kinetic analysis revealed that the recombinant CrCLH1 exhibited higher activity for chlorophyll a and chlorophyll b hydrolysis than did the recombinant PmCLH1 and PmCLH2. The recombinant CrCLH1 hydrolyzes chlorophyll to produce chlorophyllide, which has been reported to have anticancer and antiviral activities. In addition, enzyme kinetic analyses revealed the higher activity of the recombinant CyanoCLH for bacteriochlorophyll a hydrolysis to produce bacteriochlorophyllide a, which is a bacteriochlorin a precursor. Bacteriochlorin a has been reported to be an effective photodynamic therapy to cancer treatment. Therefore, the recombinant CrCLH1 and CyanoCLH can be used as biocatalysts to produce chlorophyllide and bacteriochlorin a precursor for medical and pharmaceutical applications.

IS PHYTASE A FROM *MYXOCOCCUS STIPITATUS* INVOLVED IN SCAVENGING PHOSPHATE? Peter Van Herk[1], Neena Rossburger[2], Adam Smith[2], Steven Mosimann[1], and Brent Selinger[2*]

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Inositol phosphates (IP) play a significant role in regulating cellular processes in eukaryotic organisms. *Myo*-inositol-1,4,5-triphosphate is an important second messenger involved in signal transduction and lipid signaling. Phytic acid (*myo*-inositol hexa*kis*phosphate, IP_6), the most abundant cellular IP, is prevalent in grains, largely indigestible by monogastric animals and has been implicated in phosphorus pollution associated with intensive livestock operations. IP_6 degrading enzymes or phytases (*myo*-inositol hexa*kis*phosphate phosphohydrolase) catalyze the step-wise release of orthophosphoric acid from IP_6. Interest in phytases arises from their use in reducing IP_6 content in livestock feed and the production of bioactive less phosphorylated IPs. Four distinct classes of phosphatases have been characterized as having phytase activity and our research group has worked on characterizing a class of phytases related to protein tyrosine phosphatases (PTPs). PTP-like phytases (PTPLPs) have a PTP-like active site (HCXXGXGRT) and lack primary sequence identity with other known phytases (histidine acid phosphatases, β-propeller phytases, and purple acid phosphatases) and PTPs. We have identified over 200 members in this family through bioinformatic studies. A major focus of our research has been the establishment of a panel of characterized PTPLPs in order to determine the range of biochemical characteristics, deliver insights into enzyme structure and function, and provide enzymes for the production of interesting IPs. The objective of this study was to characterize a divergent PTPLP from myxobacteria. IPs have been implicated in myxobacterial differentiation. Unlike previously characterized PTPLPs, PhyAms from *M. stipitatus* does not have a signal peptide (SP) as part of its 300 amino acid polypeptide. PhyAms also has the highest pH optimum (7.5) reported to date for PTPLP and a relatively low temperature optimum (45°C). The enzyme is also extremely tolerant of high salt concentrations, retaining 90% activity at 1.2 M NaCl. The first 3 steps of the PhyAms dephosphorylation pathway (3-4-5) are the same as PhyAdm from *Desulfovibrio magneticus*, a close relative to PhyAms. Similar to 8 other characterized PTPLPs, PhyAms has a very strong preference for IP_6 with little or no activity on other phosphorylated substrates including pNPP and *O*-phosphotyrosine. The lack of a SP, a neutral pH optimum, high substrate specificity, and low specific activity (2.1 µmol/min/mg at 37°C and 0.8 mM IP_6) may support the hypothesis that the primary function of PhyAms is not scavenging phosphate. Further studies are underway to examine the function of PhyAms as well as characterize other PTPLPs.

ENGINEERING OF FATTY ACID DESATURASES Aleš Bucek[1], Petra Matousková[1], Aleš Svatos[2] and Iva Pichova[1*]

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Polyunsaturated fatty acids attract a great attention due to their beneficial efect on human healts and and potential use as biofuel. Fatty acid desaturases (FADs) play a prominent role in biosynthesis of unsaturated fatty acids (FA). The large variety of FA unsaturation patterns is enabled by the high diversity of substrate, regio-, and stereo-specificities of FADs. Recently, the 3D structures of human and mouse FADs were solved (Wang et al., 2015; Bai et al., 2015) and showed that fatty acids are bound in a long sharply kinked hydrophobic tunnel that holds the acyl chain of the substrate and contains histidine-coordinating catalytic center adjacent to a king. We have employed two insect FADs that share high amino acid sequence identity but display diverse specificities for identification of key structure elements contributing to desaturase specificity regulation. Construction and characterization of chimeras of *Z*11-desaturase/conjugase (*Msex*D2), producing monounsaturated and 2-polyunsaturated FAs (2UFA), and *E*/*Z*14-desaturase (*Msex*D3), involved in biosynthesis of 3-polyunsaturated FA (3UFA) have enabled to identify one amino acid residue in position 224 from the fourth transmembrane domain that is critical for 3UFA biosynthesis by *Msex*D3. The homology model of *Msex*D3 with mammalian FADs indicated that the residue 224 is contributing to the formation of the kink in fatty acyl substrate binding tunnel. Our results represent a step toward understanding the mechanism of desaturation of fatty acids and toward engineering of membrane desaturases with new specificities.

Wang H, Klein MG, Zou H, Lane W, Snell G, Levin I, Li K, Sang B-C: Crystal structure of human stearoyl–coenzyme A desaturase in complex with substrate. *Nat Struct Mol Biol* 2015.

Bai Y, McCoy JG, Levin EJ, Sobrado P, Rajashankar KR, Fox BG, Zhou M: X-ray structure of a mammalian stearoyl-CoA desaturase. *Nature* 2015.

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PLANT ACYL-LIPID THIOESTERASES PRODUCING MEDIUM CHAIN (C6-C16) FATTY ACIDS AND BETA-KETO FATTY ACIDS Ian. P. Pulsifer[1], Rebecca S. Kalinger[1], Danielle M. Williams[1], Frédéric Domergue[2] and Owen Rowland[1*]

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Medium-chain (C6-C14) fatty acids and β-keto fatty acids have industrial value. These acids or their derivatives (e.g. alcohols, esters, and methylketones) can be used in a variety of commercial applications, such as fragrances and flavourings, insect repellants, lubricants, plasticizers, antimicrobials, and biofuels. Some of these compounds are currently extracted from natural sources, but the yield is often low. Therefore, there is interest in the metabolic engineering of oilseeds or microbes to produce these compounds in high amounts given the relevant enzyme activities. Following fatty acid synthesis in plant plastids, fatty acyl-acyl carrier protein (ACP) thioesterases hydrolyze fatty acyl thioester bonds to produce free fatty acids. Cleavage of the ACP group allows the free FA to leave the plastid for subsequent metabolism. This hydrolysis is generally carried out by FATA/FATB-type acyl-ACP thioesterases. We report here on an alternate family of plastid-localized non-FATA/B-type thioesterases called ACYL LIPID THIOESTERASEs (ALTs). The model plant *Arabidopsis thaliana* contains four ALT genes (*ALT1-4*), each with a unique expression pattern. Heterologous expression of the *Arabidopsis* ALTs in *Escherichia coli* yielded a range of C6-C16 fatty acids and β-keto fatty acids. Despite their high sequence similarities, each ALT protein generated a distinct profile of products. We also examined the *in planta* substrate specificities of ALT1-4 by overexpressing them in *Arabidopsis* seeds and then examining seed oil content. Seeds overexpressing ALT1 or ALT2 mostly generated 12:0 and 14:0 fatty acids and seeds overexpressing ALT4 mostly generated 6:0 and 8:0 fatty acids. *ALT*-like homologs were identified in the genomes of widely divergent plant and microalgae species. The products of these uncharacterized thioesterases are likely to be highly varied, making this family a rich source of enzymatic diversity of potential industrial value.

BIOACTIVE LIPIDS AND THE PREVENTION OF FATTY LIVER AND INFLAMMATION

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Numerous studies on dietary phospholipids have mainly focused on their beneficial effects on lipid metabolism in animals and humans. Our previous study demonstrated that each phospholipid has different influence and phosphatidylinositol prevents fatty liver by inducing adiponectin. Recent studies have also showed that phospholipids supplementation can modulate the function of cultured-immune cells. Furthermore, dietary phospholipids have been shown to ameliorate inflammatory processes and immune responses in arthritic and diabetic murine models, respectively. Thus, the aim of this study was to examine the immune-modulating activities of dietary phospholipids in mice. C57BL/6 (B6) mice were fed semisynthetic diets for 6 weeks, which contained either 7% soybean oil or 5% soybean oil plus 2% of either PL: phosphatidylcholine (PC), phosphatidylinositol (PI), or phosphatidylserine (PS). The cytokine production profiles of splenocytes, and liver damages in mice were evaluated after injection of concanavalin A (Con A). Production of Con A -induced pro-inflammatory cytokines (interferon-r, tumor necrosis factor-a, mononocyte chemoattractant protein-1) was significantly decreased in the splenocytes isolated from mice fed PI compared to other lipids. Supplementation of the diet with PI, but not with the other lipids, significantly suppressed the proinflammatory cytokine serum levels and the development of Con A -induced liver damages. Thus, we have showed, for the first time, that dietary soy PI supplementation affects the cytokine production profiles of splenocytes, and suppresses liver damages in B6 mice injected with Con A. A comparison of anti-inflammatory actions of highly unsaturated fatty acids, docosapentaenoic acid (DPA, 22:5n-3) and tetracosahexaenoic acid (THA, 24:6n-3), will be shown.

Teruyoshi Yanagita, Functional lipids and the prevention of metabolic syndrome, *J. Lipid Nutr. 24*:61-68 (2015)

MICROENCAPSULATION OF STEARIDONIC ACID SOYBEAN OIL IN COMPLEX COACERVATES AND STABILITY IN YOGURT Casimir C. Akoh[1*] and Ebenezer A. Ifeduba[1]

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Stearidonic acid soybean oil (SDASO) was microencapsulated within gelatin-gum arabic complex coacervates, cross-linked by transglutaminase (TG) or Maillard reaction (MR). The encapsulated SDASO was used to formulate yogurt. Oxidative stability was assessed by measuring peroxide (PV) and *p*-anisidine (*p*-AV) values of SDASO in control, TG- and, MR-modified microcapsules over 28 days storage at 4°C. Heat stability of microcapsules was determined from the percentage of microencapsulated oil released in the yogurt milk base after the heat treatment step (85°C for 30 min). SDS-polyacrylamide gel electrophoresis was used to confirm that covalent modification of encapsulants in TG- and MR-modified microcapsules occurred. The MR-modified microcapsules displayed colloidal stability while the control and TG-modified microcapsules were highly flocculated. Based on changes in PV and *p*-AV with time, the MR-modified microcapsules had better oxidative stability during storage compared to the control, while the oxidative stability of TG-modified microcapsules was the lowest. MR-modified microcapsules displayed the highest thermal stability based on the amount of oil released from microcapsules during the heat treatment step of the yogurt making process. Yogurt formulated with MR-modified microcapsules had the best oxidative stability during 14-day storage at 4 °C demonstrating that the antioxidant components of MR-modified microcapsules had good carry-through properties.

THE ANTI-CANCER POTENTIAL OF THE LESSER CONSUMED FATTY ACIDS

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The n-3 long chain polyunsaturated fatty acids (LCPUFA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid have been demonstrated to reduce the growth of human breast cancer cells and tumors. Less is known about the intermediates synthesized from the dietary 18 carbon n-3 fatty acids. Using pre-clinical models both *in vitro* and *in vivo* we have demonstrated that n-3 intermediates, stearidonic acid (SDA) and eicosatetraenoic acid (ETA) and n-6 intermediates, γ-linolenic acid (GLA) and dihomo γ-linolenic acid (DGLA) reduced ($P<0.05$) the growth of tumorigenic but not non-tumorigenic MCF-12A cells. Our studies suggest that their anti-cancer effects may only be partially due to their serving as a precursor for EPA. Working with Phytola we have extended these findings to demonstrate that an SDA-enriched oil (SO) can reduce ($P<0.05$) the growth of human breast cancer cells both *in vitro* and when fed to *Nu*/*nu* mice bearing MDA-MB-231 human breast cancer tumors. Supplementing SO increased the membrane content of n-3 LCPUFA and lowered arachidonic acid (*in vivo*), and this was associated with increased CD95 mediated apoptosis, thereby suggesting a possible mechanism for reduce tumor survival. Current studies have extended these studies to demonstrate that punicic acid (PA), a conjugated linolenic acid naturally found in pomegranate seed oil, inhibited growth of human breast cancer cells at concentrations much lower than other conjugated fatty acids (c9,t11 and t10,c12) that have known anti-cancer activity. Our findings suggest that the punicic acid and n-3 LCPUFA intermediates have the potential to serve as alternative dietary sources of bioactive lipids targeted at breast cancer. (Funding, Alberta Canola Producer Commission and CIHR)

INTAKE OF N-3 POLYUNSATURATED FATTY ACID PREVENTS ISCHEMIC INJURIES THROUGH NEOVASCULOGENESIS Feng-Yao Tang[1*], En-Pei. I. Chiang[2], and Jia-Ning Syu[1]

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Human endothelial progenitor cells (hEPCs) derived from bone marrow play a crucial role in the prevention of ischemic injuries in the course of postnatal neovasculogenesis. Frequent fish oil (FO) consumption is reportedly associated with a significantly lower incidence of coronary artery disease (CAD). However, the molecular mechanisms of eicosapentaenoic acid (EPA) / docosahexaenoic acid (DHA) are not well elucidated, and the beneficial effect of FO consumption on neovasculogenesis has not been demonstrated yet. In the current study, we investigated the effects of EPA/DHA and FO consumption on neovasculogenesis both *in vitro* and *in vivo*. The results demonstrate that EPA /DHA dose-dependently enhance the neovasculogenesis and cell migration of hEPCs *in vitro*. The mechanisms of action included upregulation of the c-kit protein as well as the phosphorylation of the ERK1/2, Akt and endothelial nitric oxide synthase (eNOS) signaling molecules in hEPCs. Furthermore, EPA significantly suppressed the expression of microRNA (miR) 221 *in vitro*. In experimental animal models, FO consumption significantly induced the formation of new blood vessels (neovasculogenesis) and prevented ischemic injuries. Taken together, it is suggested that FO consumption enhances neovasculogenesis mainly through the effects of EPA in hEPCs, thereby exerting a preventive effect against ischemic injuries.

EMPLOYMENT OF FUNGAL SOLID-STATE FERMENTION PRODUCTS IN BROILER CHICKEN FEED Tatiana Klempová[1*], Janka Janštová[1], Slavomír Marcinčák[2], Dana Marcinčáková[2], Ján Mačanga[2], Peter Popelka[2] and Milan Čertík[1]

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Increased interest in feeds and foods containing a sufficient amount of polyunsaturated fatty acids (PUFAs) has been observed. PUFAs serve as precursors for a number of biologically active molecules such as eicosanoids like prostaglandins, leukotrienes, and thromboxanes that mediate fever, inflammations, vasodilatation, blood pressure, clotting, pain, neurotransmission, and modulation of cholesterol metabolism. Therefore, the research is focused on finding new alternative sources of PUFAs. Zygomycetous fungi have been described as potential producers of commercially and nutraceutically interesting PUFAs such as gamma-linolenic (GLA), dihomo-gamma-linolenic (DGLA) or arachidonic (ARA) acid. The fungal solid-state fermentation resulted in successful enrichment of various cereals substrates with PUFAs [1]. Fermented cereals showed significant positive functional alternations such as lower starch content, elevated fiber and content protein, increased digestibility and metabolized energy. Obtained fermented bioproducts were directly supplemented into chicken feed. Application of fermented feed led to increased final body weight of animals, improved feed conversion ratio and also increased content of n-3 PUFAs in muscle tissues. Therefore, the broiler production could be consider as potential target for usage of value-added feed.

[1] M. Čertík, T. Klempová, L. Guothová, D. Mihálik, J. Kraic. Biotechnology for the functional improvement of cereal-based materials enriched with polyunsaturated fatty acids and pigments, Eur. J. Lipid. Sci. Tech. 115 (2013) 1247-1256

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EICOSAPOLYENOIC ACIDS AND β-GLUCANS IN PATTERN-TRIGGERED IMMUNITY IN PLANT-OOMYCETE INTERACTIONS Richard M. Bostock[*1], Sara M. Robinson[1], and Katayoon Dehesh[2]

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Oomycete pathogens, such as species of *Phytophthora* and *Pythium*, are among the most important plant pathogens, responsible for diseases that devastate crops, ornamentals, and tree species worldwide. Oomycetes contain the eicosapolyenoic fatty acids (EP), arachidonic acid and eicosapentaenoic acid, and branched β-1,3-glucans that are abundant in cell structures and are among the best characterized oomycete elicitors that trigger innate immune responses in plants. These elicitors were identified over three decades ago and were useful in the study of the sequence of physiological, biochemical and molecular responses preceding and coincident with induction of disease resistance. In spite of the cross-kingdom parallels where these molecules are well-characterized as immune system modulators in animals, their perception and modes of action in plants remain obscure. With the recent interest and advances in our understanding of innate immunity in plants, and the redefining of many of the classic elicitors as microbe-associated molecular patterns (MAMPs), it seems timely and important to reexamine EP and β-glucans using contemporary approaches. During attack of the plant, oomycete pathogens release EP and β-glucans; however, for the infection to be successful there must mechanisms to overcome any induced immune responses. In this talk, we will highlight some of the early studies of β-glucans and EP, discuss their roles as evolutionarily conserved signals, consider their action in relation to current models of MAMP-triggered immunity, and overview recent studies with several plant species that indicate an important role for oxylipin metabolism in EP action. Their potential application in crop protection and implications for stress physiology of modified-oil plants will be discussed.

EICOSAPOLYENOIC FATTY ACIDS INDUCE PLANT DEFENSE RESPONSES TO *PHYTOPHTHORA CAPSICI* AND ALTER OXYLIPIN METABOLISM IN TOMATO AND PEPPER

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Arachidonic (AA) and eicosapentaenoic (EPA) acids, collectively referred to as eicosapolyenoic fatty acids (EP), are 20-carbon, polyunsaturated fatty acids. EP are absent from higher plants, but are prevalent in oomycete plant pathogens such as *Phytophthora* and *Pythium* spp. EP function as microbe associated molecular patterns (MAMPs); exposure of plants to EP elicits defense signaling and responses that help protect the plants against oomycete pathogens. Solanaceous plants, such as tomato and pepper, display significantly reduced crown rot and collapse when treated with EP prior to infection with *Phytopthora capsici*. One prominent defense response induced by EP is lignification. EP treatment of tomato roots induces lignification in the roots and primes the crowns for rapid lignification following inoculation with *P. capsici.* In both tomato and pepper, EP exposure alters gene expression in oxylipin pathways in roots. Expression of genes encoding enzymes involved in 9-oxylipin production, including 9-lipoxygenases (9-LOX) and 9-divinyl ether synthase (9-DES) are increased following exposure of tomato and pepper roots to EP. In particular, expression of 9-DES is intensely induced after exposure of tomato and pepper roots to EP for different lengths of time. In tomato roots, expression of 13-allene oxide synthase (13-AOS) and 13-lipoxygenase (13-LOX) are also induced following EP exposure. Investigation into expression of these genes in pepper is ongoing. Tomato roots infected with *P. capsici* display similar changes in oxylipin pathway gene expression. These studies are contributing to a mechanistic understanding of EP action in pattern triggered immunity in plants.

HOST RESISTANCE TO *PLASMODIOPHORA BRASSICAE* AND VIRULENCE IN THE PATHOGEN Sheau-Fang Hwang[1*] and Stephen E. Strelkov[2]

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Clubroot has emerged as a major constraint to canola (*Brassica napus*) production in Alberta, Canada. To date, canola producers have switched to clubroot-resistant canola cultivars as the most effective and widespread means of managing this disease. Currently, most of the identified clubroot (*Plasmodiophora brassicae*) resistance genes are derived from turnip (*Brassica rapa* ssp. *rapifera*). Twelve markers linked to known resistance genes in the A genome were screened and a marker GC1680 linked to *CRa* was found to be polymorphic between susceptible and resistant parents, indicating that the resistance gene is *CRa* or is tightly linked to *CRa*. After inoculating susceptible x resistant crosses with isolates representing pathotypes 2, 3 and 5 of *P. brassicae*, 175 simple sequence repeat (SSR) markers were used to identify six QTL on two chromosomes that confer resistance to the pathogen. One QTL on N03 conferred resistance to all three isolates and another on N06 had a minor effect, but also contributed to resistance to all three isolates. To better understand virulence in the pathogen, non-housekeeping *P. brassicae* genes (118 in total) were assessed by PCR analysis in five *P. brassicae* pathotypes (2, 3, 5, 6 and 8). Pathotypes may differ at a few loci because of host-pathogen coevolution, and the functional alleles of these loci should be related to the pathogenicity of the corresponding pathotypes. One gene designated CR811 was present exclusively in pathotype 5. CR811 is highly expressed during canola infection, with a 22-fold up-regulation in secondary zoospores relative to primary zoospores. These findings suggest that CR811 is a possible pathogenicity factor and that it also could serve as a molecular marker for differentiation of pathotype 5 from other pathotypes. Collectively, this work is increasing knowledge of the genetics of resistance and mechanisms of virulence in the canola/*P. brassicae* interaction. Such knowledge will be increasingly important as novel virulence phenotypes of the pathogen emerge, in response to the selection pressure imposed by the cropping of clubroot resistant canola.

ANTIBIOTIC ACTIVITY OF THE STONE FRUIT PATHOGEN *MONILINIA FRUCTICOLA*: CHARACTERIZATION AND IDENTIFICATION Fang-Yi Yu[1,4], Chiu-Min Chiu[1,4], Shiow-Ju Lee[2], Maw-Rong Lee[3] and Miin-Huey Lee*[,1,4]

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Monilinia fructicola is a fungal pathogen which causes blossom blight and fruit rot of *Prunnus* species. Among our *M. fructicola* collections from peach orchards in Taiwan, strain TW5-4 grows slowly, forms dark colony in agar media and has strong antimicrobial activity against phytopathogenic fungi and bacteria. A spontaneous albino mutant of TW5-4 (TW5-4WM) with normal growth and less antimicrobial activity is isolated, while Muk1 was isolated from infected peach fruit in USA, which has been well studied in our previous researches in fungal development and pathogenicity. The differences of the three strains (TW5-4, TW5-4MW and M1) on fungal growth, sporulation, appressorial formation, melanin accumulation, pathogenicity and antimicrobial activity were investigated. Antimicrobial compounds were extracted by ethyl acetate and analyzed by thin layer chromatography (TLC). The compounds with antimicrobial activity were further analyzed and identified with LC-ESI-MS/MS. Our data showed that compared to strain TW5-4MW and M1, strain TW5-4 has less ability on growth, sporulation, appressorium formation and pathogenicity but has stronger abilities on melanin accumulation and fungal growth inhibition. The ethyl acetate extracts from mycelia of TW5-4 showed inhibitory activity against mycelial growth and spore germination of two plant pathogens, *Penicillium digitatum* and *Botrytis cinerea*. Thin layer chromatographic analysis combined with bioassay revealed that antimicrobial compounds were localized at R_f=0.45, which also could react with $FeCl_3$ and ninhydrin solution, indicating that the antimicrobial compounds might be phenolics and/or contain amine(s). Two compounds with molecular weight 351 and 347 were further identified by LC-ESI-MS/MS. After large scale preparation and purification, the antifungal compound was further identified. The compound is a polyketide. Therefore, twelve polyketide synthase genes were cloned and characterized.

ENHANCING QUALITY, AND PROBING REGULATION OF ESSENTIAL OIL METABOLISM IN LAVANDINS Soheil Mahmoud

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Lavandins (*Lavandula x intermedia*) are sterile lavender hybrids that result from natural crosses between *L. angustifolia* (English lavender) and *L. latifolia* (spike lavender). They are widely grown for their essential oils (EO), which are extensively used in personal care and hygiene products, among others. Although EO yield in lavandins is significantly (more than 10 X) higher than either parent, these plants produce substantial amounts of the monoterpene camphor which negatively influences EO quality. In order to reduce camphor production in Grosso lavender (a popular *L. x intermedia* variety), we used chemical mutagenesis to produce EO mutants, and developed several unique mutant chemotypes in which camphor production has been significantly reduced. These plants represent potential new cultivars, and provide valuable tools for studying the regulation of monoterpene biosynthesis. RNA-Seq mediated transcriptome profiling in these plants has identified several candidates for regulatory proteins involved in EO biosynthesis and secretion.

AN INTRODUCTION TO CANADA'S BIOFUELNET AND AN OVERVIEW OF NATIONAL BIOMASS FEEDSTOCK POTENTIALS J. Kevin Vessey[1*] and Warren E. Mabee[2]

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BioFuelNet Canada is a network that brings together the Canadian biofuels research community to aggressively address the challenges impeding the growth of an advanced biofuels industry, while focusing on non-food biomass as biofuel feedstocks. BioFuelNet includes renowned, multi-disciplinary experts from academia, government, industry and investment working together in a concerted and synergistic way. This group is working to develop and apply novel and innovative science, engineering and socio-economic strategies that will enhance environmental sustainability for future generations. The network benefits from a $25 million grant over 5 years (2012 to 2017) through the Government of Canada's Network of Centres of Excellence program.

Canada has a wealth of biomass, with the greatest capacity in the forestry and agricultural sectors. Potential biomass for utilization in the biofuel/bioproducts industry from the forestry sector falls into three categories: (1) residues from forest harvest operations; (2) wood made from natural disturbances (e.g. insect infestations); and (3) wood becoming available due to declines in in Canadian wood processing. Recent estimates of the potential biomass from these three categories equal 20 Million dry tonnes per year (Mdt/yr), 51 Mdt/yr and 24 Mdt/yr, respectively. From the agricultural sector, crop production data over a 10-year period estimated that an average of 48 Mdt/year, with lows of 24.5 Mdt/year in low-yielding years. The biomass potential from purpose grown agricultural crops (e.g. switch grass, hybrid poplar) is unknown at this point in time. Although many municipalities in Canada have excellent garbage sorting systems in place, due to Canada's relatively low population density, it is estimated that only up to 7 Mdt/year of municipal solid waste might be available from all municipalities in Canada.

HIGH TEMPERATURE CONVERSION OF UNUSUAL LIPID SOURCES TO HYDROCARBONS David C. Bressler*, Medhi Omidghane, Ehsan Jenab, Lin Xia, Olga Mameeva, Isabel Espinosa, and Michael Chae

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Research over the past decade at the University of Alberta has resulted in the development and scale-up of a novel pyrolysis-based lipid-to-hydrocarbon technology. This technology was licensed out to create Forge Hydrocarbons, a new Alberta-based company currently operating a 20L/hour pre-commercial pilot plant in the City of Edmonton. This presentation will focus on the research post commercialization seeking opportunities and pathways for conversion and valorization of low value lipid feedstocks including algal feedstocks, forestry tall oil, and biosolids derived from municipal waste treatment sources. The research has clearly demonstrated high potential for several of the feedstocks and an ability to create solvents, diesel and gasoline equivalent cuts. Additionally, processing some of the feedstocks not only creates valuable products, but also serves to remediate and decontaminate otherwise environmentally deleterious materials.

CHARACTERIZATION AND ENGINEERING OF MULTI-FUNCTIONAL ENZYMES FOR CELLULOSE/HEMICELLULOSE DEGRADATION Shuo-Fu Yuan[2], Tzu-Hui Wu[3], Hsiao-Lin Lee[1], Han-Yu Hsieh[2], Wen-Ling Lin[1], Barbara Yang[2], Chih-Kang Chang[1], Qian Li[4], Jian Gao[4], Chun-Hsiang Huang[4], Meng-Chiao Ho[1,2], Rey-Ting Guo[4], and Po-Huang Liang[1,2*]

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Abstract: Cellulose and hemicelluloses (xylan, mannan etc.) from plant biomass can be used to produce biofuel after enzymatic processing. We expressed an active form of CtCel5E (a bifunctional cellulase/xylanase from *Clostridium thermocellum*), performed biochemical characterization, and determined its apo and ligand-bound crystal structures. Compared with the structures of TmCel5A, a bi-functional cellulase/mannanase homolog from *Thermotoga maritima*, a flexible loop region in CtCel5E is the key for discriminating substrates. Near or located in this loop region, Tyr270, His277 and a missing Trp make no contact with the bound ligands, whereas the corresponding Y198, His205 and Trp210 of TmCel5A form H-bonds with the β-mannosyl moiety. By replacing this flexible loop with the corresponding TmCel5A loop (Tmloop), we have successfully engineered CtCel5E into a tri-functional cellulase/xylanase/mannanase. Crystal structures of CtCel5E-Tmloop/F267A reveals that the swapped loop brings back the interactions required for binding with mannobiose, as also confirmed by site-directed mutagenesis data. Our studies provide the mechanisms of substrate recognition and a blueprint for engineering CtCel5E.

POTENTIAL OF DGAT ENZYMES FOR ENGINEERING AND APPLICATION OF MICROBIAL OILS Milan Čertík[1*], Peter Gajdoš[1], and Jean-Marc Nicaud[2]

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Acyl-CoA:Diacylglycerol acyltransferase (DGAT) is considered to be one of the rate limiting enzymes for accumulation of storage lipids in cells in the form of triacylglycerols (TAG) in lipid bodies (LB). Therefore engineering of *DGAT* gene/s should have a potential for tailor-made regulation of lipid accumulation in cells with several biotechnological applications. In *Yarrowia lipolytica*, which served as a studying model, Dga1p belongs to DGAT2 acyltransferase family while Dga2p is categorized into DGAT1 acyltransferase family. To determine contribution of individual DGAT enzymes in storage lipid formation in the yeast, *DGA1yl,* and *DGA2yl* genes from *Y. lipolytica* and *DGAT2mm* from mouse (*Mus musculus*) were overexpressed into the Q4 (Δ*dga1* Δ*dga2* Δ*lro1* Δ*are1*) mutant strain, which is unable to form LB. Expression of *DGA1yl* gene resulted in the formation of many small clustered LB, whereas introduction of *DGA2yl* or *DGAT2mm* genes led to random distribution of fewer but larger LB. In addition, insertion of several gene copies rapidly increased LB formation and TAG amount in cells with unchanged qualitative fatty acid profile. However, strain bearing *DGA1* gene accumulated more stearic acid in TAG compared to strain with *DGA2* gene which synthesized TAG rich in palmitic acid. Further increase in TAG accumulation in DGAT-overexpressing yeasts was accomplished by blocking the β-oxidation pathway through *MFE1* gene deletion. In order to utilize sucrose containing waste substrates by *Y. lipolytica*, *SUC2* gene (invertase) was also introduced into the DGAT-overexpressing strains. These strains were tested for single cell oil production on molasses and glycerol as substrates. Maximal yield of lipid accumulation in the yeast reached almost 60% during fed-batch cultivations. Thus, genetically engineered *Y. lipolytica* DGAT-overexpressing strains are suitable tools for direct conversion of industrial byproducts into oil-based value added products.

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BIOCONVERSION OF MARINE BIOMASS FOR PRODUCTION OF VALUABLE LIPIDS

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Marine biomass, especially macroalgae, has been recognized as a sustainable resource for industrial production of bioenergy and value-added materials. We have aimed at producing valuable lipids such as polyunsaturated fatty acids, xanthophylls and terpenoids from macroalgal carbohydrates using marine protists, thraustochytrids, as a biocatalyst. However, thraustochytrid strains of the genus *Aurantiochytrium* do not have ability to assimilate such algal specific carbohydrates as alginate, fucoidan, laminaran and mannitol. Degradation of the polymeric carbohydrates by heat treatment generates organic acids that are often toxic to the thraustochytrids. Enzyme treatment may be costly at large scale. Thus, we have explored the other microorganisms that convert those carbohydrates to saccharides, which the thraustochytrids can utilize to grow. An acetic acid bacterium, *Gluconobacter oxydans*, that converts algal mannitol into assimilable fructose has been the best example to successfully establish a two-stage fermentation system. *Gluconobacter* was employed as a biocatalyst in the first stage prior to the lipid fermentation by *Aurantiochytrium* in the second stage. Similarly, we have obtained some microorganisms to use alginate, laminaran and some other polymers as substrates for lipid fermentation by thraustochytrids. These approaches will contribute to develop new system for efficient production of value-added lipids as well as biofuels.

METHANOTROPHIC BACTERIA FOR BIOFILTRATION AND BIOCONVERSION APPLICATIONS IN ALBERTA Peter F Dunfield[1*], Angela Smirnova[1], JoongJae Kim[1], Roshan Khadka[1], and Joseph Patrick Hettiaratchi[2]

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Methane is a clean-burning fuel and a cheap raw material for chemical biotransformations. However it is also a potent greenhouse gas 34 times worse than CO_2. Technologies that reduce methane emissions to the atmosphere, or convert these unused methane sources into economic resources, are therefore desirable. We are using methanotrophic (methane-eating) bacteria in biofiltration strategies to reduce methane emissions from remote, low-flow, and sour point sources such as abandoned oil wells, where flaring or chemical recovery strategies are impractical or impossible. Optimized biofilter design parameters (e.g. packing materials and aeration systems) are being tested, and monitoring methods developed for long-term tracking of biofilter effectiveness. The use of high-throughput DNA sequencing methods for characterizing microbial communities in the biofilters, combined with quantification of methanotrophic populations via real-time PCR of methanotroph-specific *pmoA* genes shows promise as a rapid screening method for biofilter function. Certain methanotrophic species are better indicators than others because they display boom-and-bust growth patterns and do not produce long-lived resting stages. In addition we are testing whether the methanotrophs used in biofiltration have added value in bioconversions. For example, methanotrophs used in floating biofilters on oilsands tailings ponds may contribute to detoxification of the water through the co-oxidation of cyclic and aromatic compounds by their monooxygenase enzymes. We have observed co-oxidation of some compounds of interest in model methanotroph cultures.

AUTOOXIDATION AND PHOTOOXIDATION OF TRIACYLGLYCEROLS CONTAINING CONJUGATED LINOLEIC ACIDS Suk Hoo Yoon

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Soybean oil, cottonseed oil, and corn oil were hydrogenated to obtain triacyglycerols containing conjugated linoleic acids. The oxidative stability of soybean oil, cottonseed oil, and corn oil containing CLA were less stable than oils without CLA during autooxidation of oils, and, however, the autooxidative stability of oils increased as CLA content increased. During photooxidation of oils, the oxidative stability of oils containing CLA were more stable than oils without CLA, and the stability of oils increased as CLA content increased. The mechanism of autooxidation and photooxidation of oils was probably due to the content, and anti- and pro-oxidant activity of individual conjugated linoleic acid in bulk oil and minor compounds included in the oils.

COCOA BUTTER EQUIVALENTS PREPARED FROM FRACTIONATED PALM STEARIN AND SHEA STEARIN Byung Hee Kim*

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Cocoa butter is the main component of chocolate. It is composed mainly of symmetric monounsaturated triacylglycerols (SMUTs), such as 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-*rac*-glycerol (POS), and 1,3-distearoyl-2-oleoyl-glycerol (SOS). Due to high cost and fluctuations in the supply and demand for cocoa butter, cocoa butter equivalents (CBEs), which have similar triacylglycerol composition to cocoa butter but are produced from low-cost fats/oils, are used as an alternative. CBEs can be blended with cocoa butter in any proportion without altering the physical properties of cocoa butter. The aims of this study were to produce a fractionated palm stearin enriched in POP, and to prepare CBEs by blending the fractionated palm stearin with shea stearin, SOS-rich fats. Palm stearin, containing 27.2 w/w% total SMUTs and 33.8 w/w% tripalmitin was used as the starting material for the fractionation. A liquid fraction produced from palm stearin under the best conditions (temperature, 17 °C; volume-to-weight ratio of acetone to palm stearin, 8; crystallization time, 8 h) established in this study was free of tripalmitin (<0.5 w/w%) and contained 44.5 w/w% total SMUTs (~37.3 w/w% POP, ~6.4 w/w% POS, and ~0.8 w/w% SOS). The yield of the liquid fraction was 61.3 w/w% of the initial palm stearin weight. The liquid fraction was crystallized to further increase SMUT content. After fractionation at 4 °C for 24 h, a solid fraction with total SMUT content of 63.2 w/w% (~53.0 w/w% POP, ~9.1 w/w% POS, and ~1.1 w/w% SOS) was obtained and its yield was 30.8 w/w% of initial palm stearin weight. The CBEs were prepared by blending the fractionated palm stearin and shea stearin in a weight ratio of 40:60 and contained 81.9 w/w% total SMUTs. The CBEs were blended with cocoa butter in weight ratios (CBEs:cocoa butter) of 5:95, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10. The blends were evaluated for their fatty acid and triacylglycerol compositions, thermal melting/crystallization behaviors, and solid fat content. The 5:95, 10:90, 20:80, and 30:70 blends showed similar melting/crystallization temperature ranges and enthalpies to those of cocoa butter. Furthermore, they showed similar changes in solid fat content to those of cocoa butter as a function of temperature. These results indicate that the CBEs can be blended with cocoa butter at 30 w/w% for the manufacture of chocolate products without significantly altering their physical properties.

UNDERSTANDING THE BIOAVAILABILITY OF GLYCATED COLLAGEN PEPTIDES IN HUMAN INTESTINAL CACO-2 CELL CULTURE Abhishek Bhattacherjee, Maurice Ndagijimana, and Mirko Betti*

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The meat industries in Alberta produce considerable amount of low value by-products (bovine hides, poultry skin and cartilages) which contain high amount of collagenous material. Collagen peptides are useful in skin regeneration and alleviating joint pain. However, commercially available collagen peptides have poor solubility and enzymatic instability which negatively affect their bioavailability. Glycation (the Maillard reaction) has the potential to solve this problem by increasing their ability to cross intestinal and blood barriers. The objective of this study was to evaluate how glycation with glucose influences the ability of collagen peptides to be transported in Caco-2 cell lines. For this purpose, a specific collagen peptide, Lys-Pro-Hyp, was synthesized and glycated with radioactive ^{14}C glucose at 90˚C for 7 h. The resulted glycopeptides were characterized by using HPLC and mass spectrometry techniques and subjected to transportation studies in Caco-2 cell culture model. Specific inhibitors were used to evaluate the role of major intestinal transport receptors (PEPT1, SGLT1, GLUT) and processes (transcytosis, gap junction). Radioactivity studies showed a 8.6 ± 1.2 % increase in the glycopeptide transport rate compared to control peptide ($p<0.001$). A decrease in glycopeptide transport (5.2 ± 1 %) was observed when GLUT receptor was blocked by a specific inhibitor. In case of other receptor/process no significant difference was observed in glycopeptide transport rate. These findings suggested that GLUT receptor might play an important role in glycopeptide transport in Caco-2 cell model. In conclusion, glycation of collagen peptides can be a strategy to increase their bioavailability. However, animal trials are necessary to confirm whether this approach (glycation) can be useful in living systems.

HERB-BASED FUNCTIONAL FOODS: FROM LABORATORIES TO THE MARKET

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Consumption of alternative herbal folk medicine has had a tremendous increase in the last decade. A number of medicinal plants contain secondary metabolites which have many biologically active compounds. They are used against hepatic fibrosis and heart ischemia-reperfusion and proved to have antioxidant, antithrombosis, antihypertension, antistress, antivirus, antitumbor, antiulcer, antidiabetic, antiaging and antiinflammatory activities. Non-availability of quality planting materials, low germination, slow plant growth, disease and pest incidence are the major obstacles in conventional medicinal plant cultivation. In Taiwan, many economically important medicinal plants and herbs are produced using various explant materials by tissue culture technique to meet the increasing demand for their medicinal properties. Rapid multiplication through *in vitro* tissue culture can be advantageous for the continuous supply throughout the year. We have developed and standardized efficient, simple and rapid tissue culture regeneration protocols of many medicinal plants, optimized the conditions in green house and successfully established the regenerated plantlets in the field for the large scale commercial production. Availability of tissue culture protocol is the first step towards the development of the genetic transformation.

R&D OF NATURAL MEDICINES FOR RESOLUTION OF INFLAMMATORY DISORDERS

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Herbal medicines have been used for treating or preventing diseases throughout human history. We have been conducting in exploration of traditional or folk herbal medicines, aiming to identify novel phytocompounds for treating or preventing inflammation related diseases, such as cancer, sepsis, and acute liver hepatitis, as an attempt to further development the identified natural medicinal components into botanical supplement or drug, or as a new drug lead. Comparative “OMICS” technology platforms in combination with various *in vitro* and *in vivo* cell- and gene-based bioassays, mouse skin inflammatory, murine syngeneic and xenograft mammary tumor and melanoma, endotoxin induced sepsis, and fulminant hepatitis models are employed to validate the pharmacological effects and the underlying mechanistic insights of the identified bioactive phytocompounds. How phytoagents modulate pro- or anti-inflammatory lipid mediators, *i.e.*, oxylipins, and/or other proinflammatory mediators attributed to their preventive or therapeutic effects against inflammatory disorders are investigated to shed light on the modes of action of the identified phytoagents. Moreover, how phytoagent, alone or in combination, in sensitizing the chemotherapeutic drugs efficacy and/or reduction of their side effects in tumor-bearing mice are to be addressed, as a means to evaluating the natural compound or its derivatives to be used as an option in adjuvant therapy or prevention of cancers.

EUKARYOTIC TRANSLATION INITIATION FACTOR 5A, A PROMISING TARGET FOR ENGINEERING ENHANCEMENT OF AGRONOMIC TRAITS John E. Thompson* and Tzann-Wei Wang

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There is growing evidence that eukaryotic translation initiation factor 5A (eIF5A) functions as a shuttle protein, selectively translocating mRNA species from the nucleus to the cytosol. However, this seemingly modest mechanistic role belies the prominent impact of this protein on phenotype. For example, depending on how it is achieved, modulation of eIF5A expression can delay leaf senescence and increase tolerance to environmental and pathogen stress or enhance growth leading to increased biomass and seed yield. This dual role arises from the fact that eIF5A undergoes a unique post-translational modification whereby two enzymes, deoxyhypusine synthase and deoxyhypusine hydroxylase, mediate the transfer of butylamine from spermidine to a conserved lysine on eIF5A giving rise to the unusual amino acid, hypusine. There are, therefore, two molecular species of this protein, unmodified eIF5A and hypusinated-eIF5A, and they have quite distinct functions. Separation of the two forms of the protein in *Arabidopsis* rosettes followed by MS sequencing has indicated that unmodified eIF5A is dominant in vegetative rosettes and hypusine-eIF5A in senescing rosettes, suggesting that the former regulates growth and the latter, senescence. In support of this, transgenic *Arabidopsis* plants over-expressing heterologous eIF5A have higher biomass (up to 200%), higher seed yield (up to 300%), and enhanced levels of unmodified eIF5A. As well, antisense suppression of deoxyhypusine synthase results in delayed leaf senescence that correlates with a reduction in hypusinated eIF5A. Thus eIF5A appears to function much like a biological switch, regulating growth in one position (unmodified eIF5A) and cell death in the other position (hypusinated eIF5A). This notion is supported by field studies with transgenic plants of agronomic importance. For example, transgenic banana plants with suppressed deoxyhypusine synthase, which leads to a reduction in hypusinated eIF5A, exhibit enhanced tolerance to the fungal disease, Black Sigatoka, in field studies. Over-expression of heterologous eIF5A in transgenic alfalfa, which leads to higher levels of unmodified eIF5A, resulted in a 20-45% increase in biomass in field studies.Thus eIF5A appears to be a promising target for genetically engineering improvements in key agronomic traits.

SOYBEAN SOMATIC EMBRYOS AND PLASTIDS AS A MODEL SYSTEM FOR GREEN SEED LIPID METABOLISM Salvatore A. Sparace*, Karen R. Clark[1] and Yan He[1]

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Soybean (*Glycine max* L.) somatic embryos and their plastids are being used as a model to study the metabolic interactions in plastid function. Here we present our results on the effects of light on somatic embryo growth, plastid function, and the role of these plastids in nitrogen assimilation. Soybean somatic embryos and their plastids are pigmented green with chlorophyll, suggesting a role for photosynthesis in the development of these seeds. Low light (20 µE/m^2·sec) improves embryo growth by about 15 – 30% over dark-grown embryos, but has no effect on the relative amounts of protein, lipid, starch and soluble sugars accumulated in the embryos. Unlike their spinach or pea chloroplast counterparts, fatty acid biosynthesis by isolated somatic embryo plastids is not light dependent. However, these embryo plastids exhibit light-dependent O_2 evolution under high (500 µE/m^2·sec) light intensities, but Rubisco activity (although present) is not light-dependent. Electron microscopy of isolated embryo plastids reveals that plastids vary in size from 1 – 2 µ and contain variously sized starch grains with little stromal thylakoids and essentially no granal thylakoids. Soybean somatic embryos and their plastids also exhibit activities of key enzymes of nitrogen assimilation, including aspartate amino transferase, glutamate synthase (NADH & FD-GOGAT) and especially glutamine synthetase (150 µmoles/h·mg protein). Collectively, these observations suggest that lipid metabolism and nitrogen assimilation likely occur at the same time in soybean somatic embryos and their plastids, and that these metabolic activities may be facilitated by nominal photosynthetic activities of these plastids. However, the extent to which all of these metabolic processes may impact each other remains to be determined. This research was supported by projects 8233 and 1233 from the United Soybean Board.

PARTIALLY SUPPRESSED, MITOCHONDRIAL PYRUVATE DEHYDROGENASE KINASE EXPRESSION CAN ENHANCE PHOTOSYNTHESIS OF THE INFLORESCENCE CANOPY AND INCREASE SEED OIL PRODUCTION IN *ARABIDOPSIS THALIANA* AT AMBIENT AND ELEVATED CO_2

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Growth, fatty acid (FA) and oil biosynthesis need to be determined at elevated CO_2 (EC) since current varieties may not possess the capability of allocating the additional fixed-C because sink limitation can reduce photosynthesis (Pn) at EC. Our studies mark the first attempts to harness the anabolic properties of dark respiration (Rd) to improve FA and oil biosynthesis. *A. thaliana L*. with partially suppressed, mtPDH kinase, (mtPDHK) expression showed an enhancement in key harvest indices (i.e., seed and oil). The mtPDH complex links glycolysis with the TCA cycle and mtPDHK is a negative regulator of mtPDH. Interestingly, a constitutively-directed partial silencing of PDCK, resulted in up-regulated activity of mtPDH, and may be an effective strategy for improving productivity. In order to understand why constitutively silenced lines responded so positively to both AC and EC we measured daily, gas-exchange profiles throughout their life-cycle.
Whole-plant analyses confirm that the inflorescence can contribute over 90% of daily-C-gain (dC) under ambient (AC) and EC. Rates of Pn, Rd and dC were 800-1000% higher when the inflorescence was developed. Although Pn, Rd and dC expressed on a dry-matter were 50% of those at the rosette stage when expressed on a total surface-area-basis, canopy Pn, Rd & dC remained remarkably constant demonstrating the importance of the inflorescence to carbon-use-efficiency (CUE). Water-use-efficiency (WUE) during inflorescence development was double that when the rosette-leaf canopy functioned alone. The wild-type (WT), plasmid control (pBI121) and two mutant lines ,10'4 and 3'1, having constitutive partial suppression all demonstrated increases in Pn, Rd, dC & WUE at EC. However, differences among lines were observed at particular stages of canopy development. At the rosette stage when laminar structures contributed mainly to canopy Pn, the WT and PBI121 controls were more responsive to EC than were the mutants; however, during a late inflorescence stage, 10'4 and 3'1 responded more. Our studies underscore 1) the need for profiling key phenotypic traits such as canopy, Pn and Rd throughout development as the relative balance among photosynthetic source and sink organs change, and, 2) the importance of mtPDH and dR in anabolic processes such as recapture of CO_2 and oil synthesis.

GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE 9 (*GPAT9*) CONTRIBUTES TO GLYCEROLIPID BIOSYNTHESIS IN VARIOUS TISSUES OF *ARABIDOPSIS THALIANA*

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The demand for plant-derived oils has grown substantially over the last few decades, and with our steadily growing population, it is sure to continue expanding. Unfortunately, our supply of these oils is limited by the availability of arable land, and as a result of this, there has been a surge in research efforts to generate plants with improved oil contents. While many examples of such enhancements exist, in most cases the improvements have only been modest; results that have been attributed in large part to the complexity of the lipid biosynthetic pathway. Although much progress has been made in recent years to decipher this pathway in plants, there remain gaps in our knowledge concerning the precise roles of several important genes. One such gene comprises the particular *GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE* (*GPAT*) encoding the enzyme responsible for the first acylation step of the ER-localized Kennedy pathway, which remains unidentified in plants. In this presentation, our in depth characterization of the ER-localized AtGPAT9 in the model oilseed species, *Arabidopsis thaliana*, will be discussed. Using *in vitro* assay analyses, we found that AtGPAT9 exhibited GPAT activity, preferentially acylating the *sn*-1 position of the glycerol backbone and showing a preference for acyl-coenzyme A as its substrate, which is consistent with a role in the Kennedy pathway. Over-expression of *AtGPAT9* in transgenic plants resulted in significant increases in both triacylglycerol and polar lipids in developing leaves, as well as increased lipid droplet production in pollen grains. In addition, over-expression and down-regulation of *AtGPAT9* in transgenic plants led to significant enhancements and reductions, respectively, in both seed size and seed oil content. Taken together, our results suggest that *AtGPAT9* plays a role in the Kennedy pathway of glycerolipid biosynthesis in various tissues, which brings us one step closer to a full understanding of lipid biosynthesis in plants.

DEVELOPMENT OF BIOACTIVE OILS ENRICHED IN PUNICIC ACID Elzbieta Mietkiewska[1], Robin Miles[1], Aruna Wickramarathna[1], Annette Schieck[2], Saleh Shah[1], Ariff Firman Sahibollah[1], Michael S. Greer[1], and Randall J. Weselake[1*]

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Punicic acid (18:3$\Delta^{9cis,11trans,13cis}$) accounts up to 65% of total fatty acids found in pomegranate (*Punica granatum*) seed oil. It has been shown to exhibit strong anti-cancer, anti-inflammatory and anti-obesity properties. In addition, oils with conjugated double bonds, such as punicic acid, have a potential to be used as industrial drying agents due to the increased rate of oxidation. Therefore, there is a growing interest to develop genetically engineered oilseeds enriched in punicic acid. It has been previously demonstrated that, the conjugated double bonds in punicic acid are synthesized by the catalytic action of a fatty acid conjugase which is a divergent form of FAD2 fatty acid desaturase (FADX; AY178446). This enzyme catalyzes the conversion of the Δ^{12} double bond of linoleic acid (18:2$\Delta^{9cis,12cis}$) into two conjugated double bonds at the positions $\Delta^{11trans}$ and Δ^{13cis}. Earlier attempts to produce punicic acid in *Arabidopsis thaliana* resulted in limited accumulation, which was accompanied by increased level of oleic acid (18:1Δ^{9cis}). This data indicate that over-expression of *PgFADX* in *A. thaliana* seeds was accompanied by a reduced activity of the *native* FAD2 desaturase. In the current study, a new approach for increased production of punicic acid in *A. thaliana* was developed using a combined over-expression of *P. granatum FADX* and *FAD2* (AY178447) in a high linoleic acid *A. thaliana fad3fae1* mutant background. This approach resulted in increased accumulation of punicic acid up to 21.2% of total fatty acids in the seed oil and helped to restore the natural level of oleic acid found in *A. thaliana fad3/fae1* mutant seeds. New insights into the nature of the high-oleic acid phenotype caused by the reduced *A. thaliana FAD2* transcript level and other factors limiting accumulation of punicic acid in genetically engineered *A. thaliana* will be discussed. In addition progress on development of canola (*Brassica napus*) oil enriched in punicic acid will be discussed.

UNLOCKING THE MYSTERY OF NATURAL RUBBER BIOSYNTHESIS IN LETTUCE (*LACTUCA SATIVA*)

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Natural rubber (NR) is a biopolymer, chemically defined as *cis*-1,4-polyisoprene, and it is known to be synthesized in thousands of plant species. Despite the widespread occurrence of NR in a plant kingdom, the Brazilian rubber tree is a single dominant species of current NR supply, while other plants produce either low quantity or significantly shorter NR polymers. However, the Brazilian rubber tree is a tropical, perennial woody species, to which modern molecular genetics cannot be effectively applied. To overcome this, an annual, genetically amenable lettuce (*Lactuca sativa*), known to produce NR of more than a million Dalton in size, has been investigated to unravel NR biosynthetic mechanism. A proteomics study of lettuce latex identified an unusual *cis*-prenyltransferase-like (CPTL) enzyme, which does not possess the conserved *cis*-prenyltransferase catalytic residues. *CPTL* is exclusively expressed in the lettuce latex. Silencing the *CPTL* transcript markedly decreased the NR content in multiple transgenic lines, indicating that *CPTL* is a necessary component of NR biosynthesis. CPTL interacts with a traditional *cis*-prenyltransferase (CPT), and the CPTL helps tether CPT enzyme on the endoplasmic reticulum, as supported by yeast two-hybrid and cellular localization studies in yeast and tobacco. However, microsomes isolated from the yeast expressing the latex-specific gene pair of *CPT* and *CPTL* could not synthesize high molecular weight NR observed in lettuce latex. Intriguingly, a separate, homologous CPTL/CPT protein pair, which is ubiquitously present in all cell-types could efficiently synthesize small *cis*-1,4-polyisoprenes (a known primary metabolic product, dolichol). Either CPTL or CPT recombinant enzyme alone cannot catalyze *cis*-polymerization reactions. Based on these results, we proposed a model which involves both CPT and its scaffolding protein CPTL on the endoplasmic reticulum in plant. Similarity of this model to eukaryotic lipid-droplets was further envisioned, and the significance of this finding will be discussed.

A UNIVERSAL EXPRESSION SYSTEM FOR THE BIOSYNTHETIC PRODUCTION OF ANTIMICROBIAL PEPTIDES Hans J. Vogel*, Gopal Ramamourthy, Leo Nguyen, Tomo Aizawa and Hiroaki Ishida

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The term ‘superbugs’, is often used to describe a group of pathogenic bacteria that are resistant to many commonly used antibiotics. Naturally occurring antimicrobial peptides (AMPs) offer a potential alternative for the treatment of these disease-causing bacteria (Nguyen et al, 2011, Trends Biotechnol 29(9): 464-472). However, relatively high concentrations of AMPs are usually needed to achieve effective bacterial killing. Normally AMPs are produced through standard chemical synthesis methods, but this is an expensive option. Many research groups have therefore evaluated the potential of biosynthetic production. A major stumbling block is that the expression of recombinant AMPs in overproducing *Escherichia coli* strains often kills the host, leading to poor yields. For some AMPs fusion protein systems and targeting of the expression towards inclusion bodies has proven fruitful. We have designed a new *E. coli* expression vector that relies on the protein calmodulin as a soluble fusion protein. Calmodulin can bind widely diverse amphipathic cationic peptides, including numerous AMPs, and in doing so it prevents the killing of the bacteria. To date we have successfully expressed some 15 different AMPs, belonging to distinct structural classes. After expression and purification, the AMPs are cleaved from calmodulin with TEV protease. All peptides were obtained in their full-length form, as determined by mass spectrometry, indicating that the binding to calmodulin also minimizes the proteolysis that sometimes occurs. Several related anticancer and antibiofilm peptides can also be produced with our system. The system is also very useful for stable isotope labeling that is required for NMR studies of AMPs. Moreover it can also be used to produce recombinant AMPs with novel properties, by introducing unnatural amino acids, such as fluoro-tryptophan. This work sets the stage for the future cost-effective production of many ribosomally synthesized host-defense peptides.

THE REGULATORY ROLE OF GNMT IN HUMAN CANCER PREVENTION En-Pei Chiang*[1,2], Hsin-An Ko[1], Yu-Hsiung Huang[1], Yee-Ling Tan, Yi-Cheng Wang[1], Yan-Jun Lin[1], Yi-Ming Chen[3] and Feng-Yao Tang[4]

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GNMT is a folate binding protein commonly diminished in human hepatoma. We have demonstrated that GNMT assists cellular methyl group homeostasis *in vitro.* In the present study we postulated that GNMT promote tumor prevention by assisting cellular 1-carbon metabolism and helps maintain genome integrity. To test the hypothesis, GNMT was over-expressed in GNMT-null cell lines cultured in conditions of folate abundance or restriction. The partitioning of folate dependent 1-carbon groups was investigated using stable isotopic tracers and GC/MS. DNA damage was assessed as uracil content in cell models, and in Gnmt wildtype (Gnmt(+/+)), heterozygote (Gnmt(+/-)) and knockout (Gnmt(-/-)) mice under folate deplete, replete, or supplementation conditions. We discovered that GNMT- cells utilized more exogenous formate in purine synthesis, suggesting that GNMT may promote endogenous formate generation. Furthermore, compared to GNMT-, GNMT+ cells had increased percentage of the thymidine specie derived from the mitochondria as formate, supporting our hypothesis. In conclusion, GMMT assists cellular 1-carbon metabolism in numerous ways. GNMT helps methyl group homeostasis, assists formate generation; supports methylene-folate dependent pyrimidine synthesis and formylfolate dependent purine syntheses; and minimizes uracil incorporation into DNA in folate depletion. Loss of GNMT impairs nucleotide biosynthesis. Over-expression of GNMT improves DNA integrity by reducing uracil misincorporation in DNA both in vitro and in vivo. The present study gives new insights into the role of GNMT on cellular 1-carbon metabolic kinetic. (supported by NSC102-2320-B-005-006-MY3;MOST104-2320-B-005-010-MY3;and MOST104-2911-I005-301)

RECOMBINANT VP1 OF FMDV DIFFERENTIALLY REGULATES MOTILITY OF CANCER CELLS AND MACROPHAGES Chi-Ming Liang[1*], ChiaoChun Liao[1] and Shu-Mei Liang[1,2]

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Recombinant capsid protein VP1 (rVP1) of foot-and-mouth disease virus (FMDV) is an anti-cancer protein capable of decreasing cancer cell motility on the one hand, and increasing macrophages motility on the other. The molecular mechanism for such paradoxical effects of rVP1 on cancer and immune cells are still largely unclear. Here, we showed that the miRNA expression patterns in ovarian cancer SKOV-3 cells and RAW264.7 macrophage cells were distinctively different. rVP1 upregulated more let-7a in SKOV-3 cells than in macrophages. Examination of the trajectories and migration velocity of the cells with time-lapse microscopy showed that let-7a was effective in inhibiting SKOV-3 cells migration. Pre-treatment with antisense sequence of let-7a not only blocked the inhibitory effects of let-7a but also reversed the effects of VP1 on the migration of SKOV-3 cells. In comparison, RAW264.7 cells which contain more constitutive let-7a than SKOV-3 was not as sensitive as SKOV-3 to the inhibitory effect of synthetic let-7a. RAW264.7 cells were selectively induced by rVP1 to secrete MMP-9 to facilitate its migration, whereas SKOV-3 cells were not. These results thus demonstrated that rVP1 might differentially regulate cell motility by inducing more let-7a or MMP-9 in cancer and immune cells.

LACTOFERRIN PREVENTS DIMETHYLNITROSAMINEL-INDUCED CHEMICAL INJURY OF LIVER FIBROSIS BY INHIBITING STELLATE CELL ACTIVATION Chuan-Mu Chen[1,*], Yu-Tang Tung[1], Ting-Yu Tang[1], and Hsiao-Ling Chen[2]

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Lactoferrin (LF), a siderophilic protein derived from milk with two iron-binding sites, has been demonstrated to possess a multitude of biological functions, including anti-inflammation, anti-cancer, and anti-microbial effects and immunomodulatory-enhancing functions. Liver diseases, which can be caused by alcohol abuse, chemical intoxication, viral hepatitis infection, and autoimmune disorders, are a significant health issue, because they can develop into liver fibrosis and cirrhosis. In this study, we induced hepatotoxicity in rats with dimethylnitrosamine (DMN) to establish a situation that would enable us to evaluate the hepatoprotective effects of LF against hepatic injury. Our results showed that DMN-induced hepatic pathological damage significantly decreased the body weight and liver index, increased the mRNA and protein levels of collagen alpha-1(I) (Collα-1(I)) and alpha-smooth muscle actin (alpha-SMA), and increased the hydroxyproline content. However, treatment with LF significantly increased the body weight and liver index, decreased the mRNA and protein levels of Collα-1(I) and alpha-SMA, and suppressed the hydroxyproline content when compared with DMN-treated group. Liver histopathology also showed that low-dose LF (LF-L; 100 mg/kg BW lactoferrin) or high-dose LF (LF-H; 300 mg/kg BW lactoferrin) could significantly reduce the incidences of liver lesions induced by DMN. These results suggest that the LF exhibits potent hepatoprotection against DMN-induced liver damage in rats and that the hepatoprotective effects of LF may be due to the inhibition of collagen production and to stellate cell activation.

HIGH PURITY CEREBROSIDE PREPARATION FROM STARFISH AND PREPARATION OF LIPOSOMES FROM THE REST COMPLEX LIPIDS Takaaki Abe, Hideyuki Kurihara, and Koretaro Takahashi*

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Starfish (*Asterias amurensis*) makes harm in fisheries. We must turn starfish from eliminated waste to a resource by finding their way of applications. It has been realized that starfish internal organs are rich in cerebroside (glucosylceramide) and eicosapentaenoic acid bound phospholipids. For this reason, this work was firstly conducted to obtain a high purity cerebroside in a practically viable manner and to utilize the rest remaining complex lipids (RCL) as a material for producing liposomes. From the internal organs of the starfish, 6.2% total lipid was obtained and there was 6.8% cerebroside/total lipids. High purity cerebroside from starfish was successfully and easily obtained by using a sphere silica gel packed open bed column with single elution solvent (using only acetone). 87% of cerebroside was recovered with 95% purity using a fraction collector. Then the RCL were subjected to a so called LibMec method (http://www.libmec.com/contents/technology/innovative/) that can easily prepare liposomes of uniform size. The optimum condition for the LibMec method was 3% lipid content and 20% 1-propanol. The prepared liposomes from RCL demonstrated that they have the most uniformed size among the liposomes prepared which are the intact starfish internal organ complex lipid liposomes, the RCL liposomes, and the liposomes prepared from starfish internal organ phosphatidylcholine. The liposomes prepared from the RCL showed the most negatively charged zeta electric potential that would obviously contribute to the stability, and also showed comparable antileakage property as well as antioxidative stability with other liposomes. Thus it was concluded that both the intact starfish internal organ complex lipids and the cerebroside separated RCL are both applicable to prepare liposomes maybe for cosmetic or functional drinks applications.

PLANT PROTEINS FOR NUTRACEUTICAL AND FUNCTIONAL FOOD APPLICATIONS

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High protein food formulations are increasingly becoming an important means of meeting the global challenges of primary nutritional deficiencies in the developing world. More than this though, they can also contribute to heart health, weight management and controlling the onset of diabetes. In recent years, market trends are shifting towards proteins of plant origin due to health considerations, and also complex cost and agricultural sustainability issues. This presentation introduces our recent effort to understand how plant protein structural properties (molecular weight, hydrodynamic size, surface charge and hydrophobicity, and conformation) impact their functionalities with a special emphasis on their gel and nano/micro-capsule forming properties. Their applications as nutraceutical delivery systems are also demonstrated.

ENZYMATIC SYNTHESIS OF MEDIUM- AND LONG- CHAIN TRIACYLGLYCEROLS IN PACKED-BED COLUMN REACTOR Yinglai Teng[1,2*], Manman Liu[1,2], Ning Zhang[1,2], Yong Wang[1,2] and Aijun Li[1,2]

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Medium- and long-Chain triglycerides (MLCTs) are a category of new structured triglycerides which integrate both the middle- and long-chain fatty acids on the glycerol backbone. The most typical MLCTs are MLM-type structural triglycerides (MLMs). They have middle-chain fatty acids on Sn-1 and Sn-3 positions as well as a long-chain fatty acid on the Sn-2 position. MLMs can supply the human body with energy and essential fatty acids. They have already been applied for clinical injection. In this research, commercialized structured lipid product for clinical injection was used as the reference. The enzymatic synthesis of MLMs was achieved by the interesterification of soybean oil with glycerides of octanic acid and decanoic acid. Lipozyme RM IM which is a commercial immobilized lipase was employed to catalyze the reaction for its superior stability and 1,3-specific feature. The enzyme was added in a column with a heat jacket to build a packed-bed column reactor. This can further reduce the production cost by allowing the continuous production, the reuse of the enzyme and the recycling of residual reactants. The reaction parameters were improved by single factor experiments, and the reusability of the enzyme was studied. To identify the ester composition and the distribution of fatty acids, the analysis of MLMs by GC-MS has also been performed.

LIPID DERIVED AMPHILIC SYSTEMS AND MONOMERS Aman Ullah[1*], Muhammad Arshad[1], and Shimiao Zhang[1,2]

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Amphiphilic ABA type PEG-Lipid conjugated macromolecules and different monomers have been synthesized using the copper-catalyzed azide-alkyne cycloaddition commonly termed as “click chemistry” and one pot rapid conversion technique. Characterization of the conjugates and monomers has been carried out with the help of 1H-NMR, FTIR, GPC and GCMS. The conjugates were evaluated for the encapsulation and release of an anticonvulsant drug (carbamazepine) as a hydrophobic drug model in the study. The micellization, drug encapsulation and release behavior of macromolecules was investigated by dynamic light scattering (DLS), transmission electron microscope (TEM) and fluorescence spectroscopy. From the results, it has been concluded that the nanoparticles had different average sizes due to different ratio of hydrophilic contents in the conjugate backbone and a rapid conversion technique led to the preparation of various monomers within minutes. The Amphiphilic particle size and structure could be altered by changing the ratio of hydrophilic and hydrophobic contents. The in vitro drug encapsulations highlighted that all the drug-loaded micelles had spherical or near-spherical morphology. In vitro drug release study showed the controlled release of hydrophobic drug over a period of max. 50 hours. The results indicate that there is great potential of renewable lipid-based micelle nanoparticles to be used as hydrophobic drug carriers and rapid conversions into various monomers.

PEROXISOMAL ATG37 BINDS ATG30 OR PALMITOYL-COA TO REGULATE PHAGOPHORE FORMATION DURING PEXOPHAGY Taras Y. Nazarko[1], Katharine O[1], Andreas T[1,2], Geetha Ramakrishnan[1], Pouya L[1], Mingda Y[1], and Suresh S[1,2]

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The selective autophagy of proteins, organelles and microorganisms is orchestrated by the receptor protein complexes (RPCs). The receptors play a central role in the RPCs by bringing together their components: cargo ligands, autophagic scaffolds and the phagophore protein, Atg8. Similarly, the pexophagy receptor Atg30 binds the peroxisomal ligands, Pex3 and Pex14, autophagic scaffolds, Atg11 and Atg17, and Atg8. However, how such pexophagic RPC is assembled is mostly unknown. We identified a new autophagy-related protein, Atg37 the peroxisomal membrane associated acyl-CoA binding protein and positive regulator of the RPC which is required for phagophore formation during pexophagy. Atg37 has a functional acyl-CoA binding domain which faces the cytosolic side of the peroxisomal membrane and specifically binds palmitoyl-CoA by acyl-CoA and protein in vitro binding assays. Intriguingly, the acyl-CoA binding site of Atg37 overlaps with its Atg30 binding site causing a competition between Atg30 and palmitoyl-CoA for Atg37. Therefore, palmitoyl-CoA might play an important regulatory role in the assembly and/or disassembly of the pexophagic RPC. Our group suggests that Atg37 is a new important component of the pexophagic RPC.

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METABOLOMICS: A NEW BIOTECHNOLOGY FOR IMPROVING DAIRY CATTLE HEALTH Burim N. Ametaj

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The incidence rate of major periparturient diseases of dairy cows is increasing over the years. The average productive life of a cow is less than 2 years. Several diseases like infertility, metritis, mastitis, laminitis, milk fever, displaced abomasum, ketosis and retained placenta cost dairy industry billions of dollars each year. Although much is known about the etiopathogenesis of most of the aforementioned diseases the underlying mechanisms are not fully understood. Until recently the dominant approach to diagnosis, therapy, and prevention of periparturient diseases of dairy cows has been the reductionist approach, which has tried to relate 'each disease with one altered metabolite'. This approach also has used the same philosophy for treatment of diseases and their prevention. A new metabolomics science, as part of the system biology approach, has emerged during the last decade. Metabolomics is quantitative analysis of all metabolites in an organism under a given set of conditions. Metabolome, on the other hand, is the sum of all metabolites in an organism, which is a measure of an organism's phenotype. Most teams involved in metabolomics research have used blood, rumen fluid, urine, and milk to identify biomarkers of diagnosis of various diseases. Our team at University of Alberta and in collaboration with Dr. David Wishart has been working to develop a bovine metabolome database and also identify biomarkers of disease for 6 different periparturient diseases of dairy cows. This is the first comprehensive research to undertake identification of screening, diagnostic, and predictive biomarkers in dairy cattle. We are using blood, urine, and milk samples and multiple instruments to identify screening biomarkers of diseases. The main diseases included in our study are metritis, subclinical mastitis, lameness, milk fever, ketosis and retained placenta. Preliminary data indicate that innate immunity reactants as well as carbohydrate and lipid metabolism and pathways are altered and precede occurrence of the aforementioned diseases. Research conducted by our team also has contributed in identifying multiple biomarkers of health and disease in the rumen fluid of dairy cows fed different amounts of barley grain. Grain feeding is related to multiple diseases in dairy cows like rumen acidosis, lameness, displaced abomasum, and milk fat depression syndrome. We have identified multiple metabolites that might be involved in pathogenesis of periparturient diseases of dairy cows.

USE OF GENOMIC TOOLS IN BREEDING FOR COMPLEX TRAITS - THE COLD TOLERANCE IN WINTER CEREALS EXPERIENCE D. Brian Fowler

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Level of cold tolerance is a primary factor determining the regions of adaptation and distribution of commercial cereal species. Cold tolerance is a complex, developmentally regulated, and environmentally induced quantitative character that is expressed in anticipation of and during exposure of plants to freezing temperatures and its manipulation in breeding programs has presented difficult challenges. The transition from the vegetative to the reproductive growth stage is a critical switch that initiates the down regulation of the cold response, presenting an additional complicating factor for selection programs. In this system, the developmental genes (vernalization, photoperiod, etc) determine the duration of expression of cold tolerance conferring genes while the rate of acclimation is determined by genotype dependent expression levels of these genes. Recognition of the interactions between growth stage and cold tolerance gene expression has allowed us to design strategies to minimize the risk of cold damage in different stages of phenological development. Conventional and molecular studies have identified chromosomal regions associated with cold tolerance and we now have molecular markers for a few major genes and a number of small effect candidates. However, a large amount of unexplained heritability remains. Consequently, screening for survival under field conditions is still required to identify lines adapted to specific target regions and we have been unable to produce super-hardy cultivars in most cereal species. For example, while the structural genes within the Triticeae have a high degree of homology and the regulation of cold tolerance is operational across genomes, we have not been able to successfully exploit the superior cold tolerance of rye (*Secale cereale* L.) for improvement of wheat (*Triticum aestivum* L.). Progress in this area will have to wait for a more in depth understanding of the complex signal transduction and the genetic cascade controlling low temperature gene expression.

TRANSCRIPTOME CHANGES IN TWO *VIGNA RADIATA* VARIETIES IN RESPONSE TO COLD STRESS Li-Ru Chen[1] and Tsai-Yun Lin[2*]

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Many tropical and subtropical plants are susceptible to chilling/cold in exposure to temperature of 0-15°C which limits plant growth and causes significant yield losses. Mungbean (*Vigna radiata* L. Wilczek), an important pulse crop in tropical regions of Asia, is used as a high-protein meat substitute and cereal supplement due to its high lysine content. Mungbean varieties NM94 and VC1973A were bred for high yield but differ in susceptibility to chilling/cold stress at seedling stage. Chilling stress reduces relative growth rate and damages the photosynthetic apparatus more severely in VC1973A than in NM94, revealing different levels of mesophyll cell shrinkage, vacuole rupture and the fractured plasma membranes. We compared the temporal gene expression profiles in 4°C-treated NM94 and VC1973A seedlings with a cDNA microarray containing 735 mungbean early developmental and stress responsive uniESTs to identify chilling/cold regulated genes (*COR*s) conferring differential chilling/cold responses. A common set of *COR*s was involved in cell wall assembly, biosynthesis of cryoprotectants, molecular chaperones, pathogen resistance and ubiquitin/proteasome system which are required for mungbean seedlings to tolerate cold/chilling stress. The transcriptional activation of *COR*s in NM94 seedlings may facilitate early maintenance of photosynthetic capacity, spliceosome disassembly, mRNA polyadenylation processes, translational function and detoxification of methylglyoxal levels for protection against chilling stress in NM94 seedlings. Transcripts of dehydrin, LTP and PDFs in NM94 seedlings were significantly increased by chilling in NM94 seedlings but not VC1973A, which may contribute to greater chilling tolerance. These genes are likely determinants of chilling/cold stress susceptibility of mungbean and can be used in improving the breeding stock.

SUPPRESSION OF BIOSYNTHESIS OF VOLATILE COMPOUNDS BY A PHYTOPLASMA EFFECTOR Choon Meng Tan[1], Chia-Hua Li[1], Nai-Wen Tsao[2], and Jun-Yi Yang[1,3*]

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In nature, plants interact with a range of organisms, serving as important mediators for complex communities, including pathogens, insect herbivores, and natural enemies of herbivores. In this study, we report that *Nicotiana benthamiana* expressing a secreted effector from *Candidatus Phytoplasma mali* causes a dramatic reduction in tobacco odors. This altered aroma phenotype is correlated with glandular trichome modification and the biosynthesis suppression of 3-isobutyl-2-methoxypyrazine (IBMP*),* a volatile organic compound. We further show that the absence of *NbOMT1* expression, which encodes an O-methyltransferase with a methoxypyrazine affinity, is responsible for IBMP accumulation loss in the transgenic plants. Given the importance of IBMP as an aggregation pheromone in ladybird beetles, we showed that the transgenic plants lost the ability to attract the ladybird beetles, *Oenopia sauzeti* and *Lemnia biplagiata*. As ladybird beetles are one of the natural enemies of psyllids, we propose that psyllids, phytoplasma insect vectors, may take advantage of reduced predation and increase their fitness on phytoplasma-infected plants, and therefore phytoplasmas get more chance to spread and sustain their infection.

EFFECTS OF NANOMATERIALS ON FUSARIUM OXYSPORUM F. SP. NIVEUM AND VEGETABLE SEEDLING GROWTH Pi-Fang Linda Chang[1,2,3*], Yong-Wen Liu[2], Jiang-Jhen Lin[4], and Jenn-Wen Huang[1,2]

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Materials could have new characteristics when their sizes are reduced to 1-100 nm. Materials of this size are defined as nanomaterials. Some nanomaterials have been reported to pose antimicrobial activity. Nanomaterials have potential to be applied in disease control in agriculture. So far, little is known about the effects of nanomaterials on microorganisms. In this study, silver nanoparticles in forms of nano-scale silica platelets (AgNP/NSP) and nano-scale silica platelets (NSP) were tested for their inhibitory effects on *Fusarium oxysporum* f. sp. *niveum* (E. F. Smith) Snyder & Hansen (Fon). Understanding the effects of nanomaterials on Fon and their underlying mechanisms could facilitate application of nanomaterials in plant disease management. The results showed that AgNP/NSP had stronger inhibitory effect on Fon than NSP. AgNP/NSP inhibited hyphal growth, and reduced cell activity and spore bioactivity of Fon. Application of cysteine reduced their inhibitory effect of Fon, as cysteine could bind silver ions, NSP and silver nitrate, but not AgNP/NSP. Results of scanning electron microscopy revealed that both AgNP/NSP and NSP, but not silver nitrate, attached on the cell surface of Fon and caused cell shrinkage. It indicates that AgNP/NSP display inhibitory effect on Fon different from its bulk materials, silver nitrate. In addition, Fon treated with AgNP/NSP or NSP had less secreted proteins and displayed reduced cellulose activity. AgNP/NSP induced expression of the *gas1* and *fks1* genes involved in fungal cell wall synthesis in *F. oxysporum*. The results suggested that AgNP/NSP and NSP may have multiple inhibitory mechanisms on Fon. In this study, we also tested the effects of AgNP/NSP and NSP on growth of lettuce, tomato, and watermelon. AgNP/NSP at 100 ppm reduced seed germination, root elongation and seedling growth of test plants indicating that AgNP/NSP could impact normal plant growth. Thus, application of AgNP/NSP in plant disease management shall be carefully re-evaluated, particularly their negative effects on plant growth in the future. This research was supported by the Ministry of Economic Affairs under grant No. 101-EC-17-A-21-S1-229.

FUNCTIONAL ANALYSIS OF BIOCONTROL BACTERIUM *PSEUDOMONAS TAIWANENSIS* Wen-Jen Chen[1,2] , Yu-Liang Yang[2], Tzu-Yen Kuo, Je-Ruei Liu[1,2], and Ming-Che Shih[1,2*]

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Pseudomonas taiwanensis is a board host range Gram-negative bacterium. Recently, we found that *P. taiwanensis* displayed strong antagonistic activity against rice pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Rice bacterial blight caused by *Xoo* is one of the most destructive diseases of rice worldwide. Here we combined whole genome sequencing and Tn5-transponson mutagenesis to identify anti-Xoo toxin factors and related regulatory pathway. A high quality complete sequencing was accomplished by a combination of Roche 454, Illumina Solexa, Sanger sequencing and Optical mapping. The complete sequence of the 5.08-Mb genome sequence and 4666 CDS were determined. We used transposon random insertion to identify genes involved in the production and regulation of anti-*Xoo* activity based on analyses of 6000 individual insertion-strains. Our results show that the siderophore pyoverdine biosynthetic gene (*pvd*), Type VI secretion system (T6SS), and EnvZ/OmpR two- component system have important roles in antagonistic activity against *Xoo*. On the other hand, the toxicity of *P. taiwanensis* was negatively regulated by the RpoS sigma factor. We further used MALDI-imaging mass spectrometry (MALDI-IMS) to track pyoverdine in *P. taiwanensis* and mutants. The results showed that pyoverdine was positively regulated by EnvZ/OmpR two-component system and secreted by T6SS. In contrast, pyoverdine was negatively regulated by RpoS. To the best of our knowledge, this is the first report that Pyoverdine has toxicity toward *Xoo* and T6SS can secrete small compounds.

GIBBERELLIN INCREASES SEED SINK STRENGTH IN *PISUM SATIVUM L.*

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Gibberellins (GAs) are an important class of diterpenoid plant hormones that control various aspects of plant development including seed growth and development. The involvement of GAs during pea (*Pisum sativum*) seed development was studied by comparing a GA deficient mutant *lh*-2 and a GA-overexpresser line (TG1) with their respective isogenic controls. *lh*-2 is a mutation in the LH gene (codes for ent-kaurene oxidase that converts ent-kaurene to ent-kaurenoic acid) that reduces the GA_1 levels in young developing pea seeds by 54% (Swain at al., 1993, Planta 191:482). *lh*-2 largely affects pea seed growth and development and results in seed abortion (Swain et al, 1997). The GA-overexpresser line constitutively expresses *PsGA3ox1* (*LE*; a fully functional wild-type gene that encodes a GA 3β-hydroxylase that converts GA_{20} to bioactive GA_1) in a semi-dwarf *lele* pea line ('Carneval'; *le-1*; single base-pair mutation in *PsGA3ox1*) (Reinecke et al., 2013, Plant Physiol 163:929). In our study, we observed marked delay in cell expansion in the seed coats and embryos of the *lh-2* mutants compared to the LH line. Seed coats of *lh-2* exhibited delayed hypodermal and reduced epidermal and ground parenchyma cell expansion compared to LH seed coats. *lh-2* cotyledonary storage parenchyma cell expansion was also reduced compared to the LH line. With respect to photoassimilate partitioning, starch accumulation (8-12 days after anthesis; DAA) in the seed coat and mobilization of seed coat starch to the embryo (16-20 DAA), as well as starch accumulation in the cotyledons, were dramatically reduced in the *lh-2* mutant compared to LH. In the GA-overexpresser line TG1, enhanced hypodermal, epidermal, ground parenchyma and branched parenchyma cell expansion were observed in the seed coat compared to the null control. Starch accumulation in the seed coat cells was greater at 10 DAA and mobilization of seed coat starch to the embryo was enhanced from 16 to 20 DAA in TG1 compared to the null. Greater cell expansion and starch accumulation in the cotyledonary storage parenchyma cells was correlated with an 11 to 34% larger seed size at maturity in TG1 when compared to the null control. These data support that GAs regulate specific aspects of seed coat and embryo development, and as a result, can modify photoassimilate partitioning into the developing embryo.

BIOCATALYSIS: SYNTHESIS OF CHIRAL INTERMEDIATES FOR DEVELOPMENT OF DRUGS Ramesh N. Patel

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Biocatalytic processes for producing enantiomerically pure pharmaceutical intermediates or active pharmaceutical ingredients (API's) are of growing importance. Biocatalysis is rapidly evolving into a key technology for production of fine chemicals and chiral intermediates, especially in the pharmaceutical industry, where high yielding chemo-, regio-, and enantioselective reactions are critical.

Advances in high cell density fermentation processes for production of biocatalysts along with immobilization/ reusability biocatalysts makes biocatalytic processes economically very attractive. Development and advances of molecular biology methods such as over expression of enzymes and directed evolution technologies have now lead to improvement in activity, selectivity and stability of biocatalyst under process conditions further make biocatalysis a viable option for producing single enantiomers.

Selectivity which is the main advantage of biocatalysts is highly desirable in chemical synthesis, offering benefits such as higher yields, fewer side reactions, and elimination of protection and de-protection steps, purer products, easier recovery, and reduced environmental waste. There are also operational advantages in biocatalytic processes, including the ability to carry out reactions under mild operational conditions, avoiding extremes of pH, temperature, and pressure that often require the use of expensive equipment or energy intensive processing in chemical processes. Furthermore this minimizes problems of undesired side-reactions such as decomposition, isomerization, racemization and rearrangement. In this presentation biocatalytic process will be described for the preparation of key chiral intermediates for development of drugs.

AMINO ACID NETWORKS IN ENZYME CATALYSIS David D. Boehr[1*], Jennifer M. Axe[1], Kathleen F. O'Rourke[1], Eric M. Yezdimer[1], and Nicole E. Kerstetter[1]

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Proteins can be viewed as small-world networks of amino acid residues connected through noncovalent interactions. Nuclear magnetic resonance chemical shift covariance analyses were used to identify long-range amino acid networks in the alpha subunit of tryptophan synthase both for the resting state (in the absence of substrate and product) and for the working state (during catalytic turnover). The amino acid networks observed stretch from the surface of the protein into the active site and are different between the resting and working states. Modification of surface residues on the network alters the structural dynamics of active-site residues over 25 Å away and leads to changes in catalytic rates. These findings demonstrate that amino acid networks, similar to those studied here, are likely important for coordinating structural changes necessary for enzyme function and regulation. The ability to re-wire such networks would provide new opportunities for engineering stimulus-responsive materials that may find applications in nanotechnology and/or synthetic biology.

ENZYME PRODUCTION AND USE FOR ENRICHING SOY MEAL PROTEIN AND REMOVING INDIGESTIBILITY Lu-Kwang Ju*, Qian Li, Abdullah A. Loman, and S. M. Mahfuzul Islam

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Soy meal contains predominantly protein (~53%) and carbohydrate (~32%). It is a good protein source for feed and food uses. Soy carbohydrate includes soluble and insoluble components of various molecular weights and compositions, many of which can pose indigestibility issue. There has been strong interest in further increasing the protein content, for example, to ~70% for aquaculture, similar to that in fishmeal, and in removing the carbohydrate indigestibility. We have developed an enzyme-based process and produced good enzyme mixtures for this purpose. Enriched soy protein remains as intact solids that are easily separable from the liquid hydrolysate. The hydrolysate contains monomerized carbohydrate that can be used as fermentation substrate for producing other value-added products. We have selected strains and studied effects of medium and fermentation conditions on enzyme productivity and blend of enzyme activities. We have also evaluated the process designs and conditions for the enzymatic treatment of soy meal and have developed kinetic models for describing and predicting the treatment outcomes. High protein recovery and enrichment are achieved and use of hydrolysate for making fermentation bioproducts is demonstrated. Results and further developments will be presented.

ENZYMATIC PRODUCTION OF (*2S, 3R, 4S*)-4-HYDROXY-ISOLEUCINE USING Fe(II)/ α-KETOGLUTARATE-DEPENDENT L-ISOLEUCINE DIOXYGENASE

Masakazu Sugiyama[1*], Sergey V. Smirnov[2], Tomohiro Kodera[1], Hiroyuki Nozaki[1], Shunichi Suzuki[1,] Makoto Hibi[3], Kenzo Yokozeki[1, 3], Sakayu Shimizu[3], and Jun Ogawa[3]

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The unique function of 4-hydroxyisoleucine (4-HIL), which was initially extracted from fenugreek seeds (*Trigonella foenum-graecum*), is to stimulate glucose-induced insulin secretion in a glucose-dependent manner. The L-isolucine-4-hydroxylase (L-isoleucine dioxygense, IDO) was discovered in *Bacillus thuringiensis*. IDO catalyzes hydroxylation of L-isoleucine at its C4-position in a highly *regio*- and *stereo*-specific manner [1-4].

Since the IDO requires α-ketoglutarate (α-KG) as a cosubstrate to activate oxygen using nonhem iron and form succinate and CO_2 as by-products, sufficient supply of α-KG was needed to establish an enzymatic conversion system at an industrial scale. For this purpose, we constructed a metabolically engineered *E. coli* strain. The α-KG dehydrogenase gene, isocitrate ligase gene, and isocitrate dehydrogenase/kinase phosphatase gene were disrupted in *E. coli*. Thus obtained *E. coli* Δ2 strain lacks pathway from α-KG to succinate both in the TCA cycle and glyoxalate cycle. The IDO activity in the *E. coli* Δ2 strain was able to shunt the destroyed TCA cycle, thereby coupling L-isoleucine hydroxylation and metabolism from glucose [5, 6]. Using this strain, we performed the direct biotransformation of L-isoleucine into 4-HIL over 85% molar yield at 10 kg scale.

[1] T. Kodera et al., *Biochem., Biophys., Res., Commun*., 390, 506-510 (2009); [2] J. Ogawa et al., *Appl. Microbiol. Biotechnol*., 89, 1929-1938 (2011); [3] M. Hibi et al., *Appl., Environ., Microbiol.*, 77, 6926-6930 (2011); [4] US 8273562B; [5] S. V. Smirnov et al., *Appl. Microbiol. Biotechnol*., 88, 719-726 (2010); [6] WO2009/082029

REFORMING OF SUCROSE FATTY ACID ESTERS BY LIPASE REACTIONS

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Sucrose fatty acid ester (SE) has various features depending on its degree of esterification. In particular, diester has a good property that lamellar liquid crystal can be easily formed and O/W emulsion can be made stable. Generally, SE available in the market is made by chemical synthesis methods, and it is known that the number of fatty acids esterified to a sucrose molecule varies around some central value. Actually, marketed SE has only 35 percent of diester at the most. Therefore, we tried to get a diester-rich SE by reforming of marketed SE through the use of lipase reactions.

First, we studied various kinds of reaction systems, and then we found that when using marketed monoester-rich SE and fatty acids in tert-pentyl alcohol, *Candida antarctica* lipase was able to increase the composition ratio of diester up to about 60 percent. Moreover, we found that a removal of water from the reaction mixture before and during the reaction produced an increase in the reaction rate and a further increase in the composition ratio of diester. Consequently, we were able to acquire the diester-rich SE which contained more than 70 percent of diester.

EPIGENETICS APPROACHES TO AGRICULTURAL BIOTECHNOLOGY

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Modern agricultural biotechnology relies strongly on the knowledge in the areas of genetics, epigenetics, biochemistry, molecular biology and many others. Epigenetic approaches to improvement of plant performance become more and more popular as we learn about mechanisms of regulation of gene expression that do not involve permanent alteration to DNA. Although direct use of epigenetics in agrobiotechnology is not the main stream yet, many approaches have been suggested that help to achieve the desired phenotype without altering genetic material. Epigenetics can also be used for improving the rate of plant transformation. In this talk we will discuss several epigenetics techniques that can be used either for the detection of desired traits in plants or for generation of such traits. We will also present some data demonstrating the utility of epigenetic approaches in agricultural biotechnology.

EPIGENETICS OF CANCER TREATMENT RESPONSES Olga Kovalchuk

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While modern cancer therapy has led to increased patient survival rates, the risk of treatment-related complications is becoming a growing problem. Both radiation and chemotherapy present a threat to the exposed individuals and their progeny. Furthermore, acquired therapeutic resistance often leads to treatment failure and relapse. The mechanisms of these serious treatment side effects are not well understood and may be epigenetic by nature. Epigenetic changes comprise cytosine DNA methylation, histone modifications and small RNA-mediated events.
We propose an epigenetic theory of cancer treatment side effects suggesting that mechanisms underlying treatment side effects are epigenetically regulated and associated with altered gene expression. Here, I will present new and compelling evidence to support this hypothesis, and well as discuss novel strategies for prevention and mitigation of side effects. I will also present a new method to conduct personalized treatment planning based on in-depth (epi)genomic and transcriptomic profiling.

EPIGENETIC REGULATION OF CANCER STEM CELL ASSOCIATED PHENOTYPES

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Tumours contain populations of cells with stem cell like properties, and it is believed that these phenotypically plastic cells are responsible for cancer progression and metastatic potential. Stem cell-like populations are regulated by dynamic niches, characterized by specific growth factors and extracellular matrices, as well as biophysical features such low oxygen tensions. Moreover, a growing body of evidence suggests that cancer cells co-opt stem cell-associated regulatory networks in order to sustain plasticity. We have discovered that an embryonic-associated protein called Nodal maintains stem cell phenotypes in cancer, and that it promotes classical hallmarks of cancer such as angiogenesis, invasion and metastasis. We have also found that biophysical features of a growing tumour, in particular hypoxia, can promote tumour cell plasticity by up-regulating embryonic proteins like Nodal via a combinatorial mechanism. Moreover, using quantitative SILAC-based proteomics together with developmental and cancer model system, we have identified potential anti-tumourigenic proteins in stem cell-derived extracellular matrices, that can reprogram cancer cells toward a less aggressive, more differentiated phenotype. Finally, we have determined that alterations in the expression of stem cell associated genes are at least partially driven by changes in DNA methylation as well as alterations in repressive (H3K27Me3) and active (H3K4Me3) histone marks. By studying the mechanisms by which cancer cells acquire and sustain phenotypic plasticity, we may uncover novel targets for the prediction and prevention of tumour progression.

TRANSGENERATIONAL EPIGENETIC PROGRAMMING OF PRETERM BIRTH RISK AND ADVERSE HEALTH OUTCOMES Gerlinde A.S. Metz[1*], Olena Babenko[1,2], Youli Yao[1,2], Fabiola C.R. Zucchi[1], Mirela Ambeskovic[1], Olga Kovalchuk[2], David M. Olson[3], and Igor Kovalchuk[2]

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In most cases of human preterm birth (PTB), the causes remain unknown in spite of 60 years of investigation into the leading cause of perinatal mortality and morbidity. Here we show that stress across generations has downstream effects on endocrine, metabolic and behavioural manifestations of PTB via microRNA regulation. Stress was induced during gestational days 12-18 in pregnant Long-Evans dams and effects were examined across three generations removed from the stressor (transgenerational) or in each of three generations (multigenerational). With each generation, both stress regimens gradually reduced gestational length, maternal weight gain and behavioural activity, and increased the risk of gestational diabetes. Shorter gestational length was accompanied by delayed offspring development that was recognizable as early as postnatal day 7, with the greatest effect in the F3 offspring of transgenerationally stressed mothers. Stress in F2 mothers altered microRNA expression patterns of the miR-200 family in brain and uterus which regulates pathways related to brain plasticity and parturition, respectively, and also increased placental miR-181a, a human marker of PTB. Furthermore, changes in brain microRNAs related to mental health were partially correlated with changes in placenta, thus providing a mechanism for the identification of predictive epigenetic signatures of disease. The findings indicate that compounding effects of gestational stress propagate across three generations to influence PTB risk and outcomes via microRNA regulation. A family history of stress may program central and peripheral pathways regulating gestational length and health outcomes with potentially lifelong consequences.

NEUROGENIN 2 PRONEURAL ACTIVITY IS REGULATED BY THE POLYCOMB PROTEIN MBT1 IN CORTICAL PROGENITORS Grey Wilkinson[1], Saiqun Li[1], Dawn Zinyk[1], Eko Raharjo[2], Andrey Golubov[3], Igor Kovalchuk[3], Jeff Biernaskie[2], and Carol Schuurmans[1*]

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The neocortex is the region of the brain responsible for high order cognitive functions and sensory processing. The development of the neocortex is under strict temporal regulation, with progenitor cells switching from producing deep-layer neurons first, followed by upper-layer neurons, astrocytes and finally oligodendrocytes. The balance between progenitor proliferation and differentiation is in part regulated by the proneural basic-helix-loop-helix transcription factor Neurogenin 2 (Neurog2), which promotes the differentiation of deep layer neurons in the neocortex. While Neurog2 is expressed in cortical progenitor cells throughout the neurogenic period, loss of this factor leads to defects in cell fate specification only in (early) deep-layer neurons. Similarly, in gain-of-function studies, Neurog2 can promote precocious neurogenesis, but only in early and not later stage cortical progenitors. Thus, the proneural activity of Neurog2 is temporally restricted. We identified the polycomb associated protein, Mbt1 (lethal(3) malignant brain tumor-like protein) as a Neurog2 cofactor that represses Neurog2 transcriptional activity. In gain-of-function experiments in vivo, Mbt1 suppresses neurogenesis in early cortical progenitors, while Mbt1 loss-of-function experiments display the opposite effect, promoting precocious neurogenesis in late stage cortical progenitors. Conversely, $Mbt1^{-/-}$ mutant analysis reveals a dramatic increase in the number of proliferating progenitors suggesting that Mbt1 may also be required for Neurog2 mediated cell cycle exit upon differentiation. Mbt1 may function to suppress cortical neurogenesis by recruiting polycomb repressive complexes to Neurog2 target genes, resulting in the formation of heterochromatin and inhibition of transcription. We found that the reduction in Neurog2 transcriptional efficiency in the late neocortex correlates with a closed chromatin state of target genes, as revealed by FAIRE analyses. Our studies thus support the idea that the temporal regulation of Neurog2 proneural activity is at least in part regulated by epigenetic alterations that are initiated over developmental time.

RFI AND PRE-NATAL NUTRITION OF THE PREGNANT HEIFER: IMPACTS ON BULL CALF SCROTAL CIRCUMFERENCE AND BODY WEIGHT L. L. Wynnyk,[1] F. Paradis,[1,2] J. Kastelic,[3] M. Colazo,[4] L. McKeown,[1] H. Block,[2] C. Li,[1,2] B. Yaremcio,[4] J.A. Basarab,[4] H. Bruce,[1] J. Thundathil,[3] and C. Fitzsimmons[1,2]*

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There is evidence indicating that improvements in feed efficiency may be antagonistic to reproductive traits in beef cattle, and should be carefully considered to avoid negative effects on fertility. Maternal nutrition during gestation can affect offspring growth and reproductive development. Our study investigated effects of maternal diet during gestation, and genetic potential for RFI, on growth and scrotal development in bulls. Pregnant purebred Angus heifers received a ration formulated to gain either 0.5 kg/d (L-diet), or 0.7 kg/d (H-diet), from 30 d until 150 d of gestation. Scrotal circumferences (SC) and weights of bull calves were measured once a month from the ages of 6 to 16 months. SC and weight data were analyzed using SAS 9.2 PROC MIXED for repeated measures, with RFI, maternal diet, time, and their interactions, as fixed effects, and bull age as a covariate. SC was significantly affected by the genetic potential for RFI; high RFI bulls had larger SC. Bull weights were significantly affected by a maternal diet*time interaction where low diet bulls tended to be heavier than high diet bulls as they matured. Therefore, selection for low RFI animals utilizing GrowSafe® tests for RFI, while animals are going through puberty, has the potential to also select for later maturing cattle. Depending upon the source, current breeding values used for RFI may need to be adjusted to remove this bias or a selection index including SC and RFI should be developed. As well, maternal diet during gestation can affect growth traits well into maturity in cattle. This creates a window of opportunity to either ensure or enhance beef production.

PROSPECTS OF DEVELOPING CANOLA WITH VERY LOW LEVELS OF SATURATED FAT M. Tahir

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The healthy oil generally refers to the lower amount of total saturated fats in the oils. Canola oil qualifies as healthy oil based on its low percentage of saturated fats (~7%) and high percentage of mono-unsaturated fats. Canola is also well placed as the healthiest oil when compared to other edible seed oils such as corn and sunflower having 12-13% saturated fats. Reduced sat canola (<3.5%) would meet the demands of the marketplace for foods that lowers saturated fats in human diet and would meet the requirement for allowing L-Sat or Z-Sat fat labels in North America. The Zero-Fat label in North America would provide seed companies additional competitive advantage to DAS Omega-9 oil users and capture intellectual property estate around healthy oils. The commercial viability of L-Sat or Z-Sat canola is strong and is estimated to be up to $420 MM and could open up additional markets for canola oil (i.e., the bottled oil market and additional processed foods applications).

This report will review the current status of low saturated fat canola R&D and IP from the North American canola industry. The various breeding and biotechnology approaches will be presented to reduce the total saturated fat in canola oil. The presentation will also cover the preliminary efforts to produce Brassica germplasm with low saturated fat content using the non-transgenic (Breeding) methods.

DOWN REGULATION OF THE *IND* GENE CAUSES MALE STERILITY IN CANOLA (*BRASSICA NAPUS* L.) Aliaa El-Mezawy[1], Mohammad Al-Forkan[1,2,¶], Limin Wu[1], Randall J Weselake[2], Saleh Shah[1,2]*, and Habibur Rahman[2]*

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A pollination control system, such as male sterility, is useful for economic production of commercial hybrid seed. We developed male sterile canola (*Brassica napus* L.) lines through random T-DNA insertions of the antisense *Arabidopsis thaliana INDEHISCENT* (*IND*) gene. Of the 21 transformants generated through *Agrobacterium* mediated transformation, three (T_0) failed to produce normal anthers and lacked viable pollen grains. Backcrossing the male sterile T_0 plants to the wild type (*wt*) produced normal siliques with viable seeds. Backcrossed progeny (BCF_1) of these three T_0 male sterile plants showed 1:1 segregation for male sterile to male fertile plants. Self-pollinated progeny of the male fertile BCF_1 (i.e. BCF_2 plants) were all fertile. This suggests that male sterility in these transgenic lines is controlled by a single copy of the antisense *IND* gene that functions in a dominant fashion. Molecular analysis confirmed that two of the three transgenic lines carried one copy of the antisense *IND* gene, while the remaining line carried two copies of this gene. The *B. napus IND* gene was down regulated in anthers of the male sterile plants, but not in anthers of the male fertile *wt*. The potential use of this male sterile line in breeding hybrid *Brassica* crops is discussed.

PALLISER'S PROMISE: PRODUCTION OF OILS ENRICHED IN VERY LONG-CHAIN FATTY ACIDS IN *BRASSICA CARINATA* FOR INDUSTRIAL AND NUTRA/PHARMA APPLICATIONS Elizabeth-France Marillia, and David C. Taylor*

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The global demand for vegetable-based oils continues to rise, while the availability of highly productive arable farm land is becoming progressively limited. To meet the requirements of the future, it will be essential to develop new and improved temperate oilseed cultivars adapted to less-than-optimum acreage. An example is the brown soil zone in the semi-arid marginal land area of the south-western Canadian prairies known as Palliser's Triangle, an area encompassing 6.5 M acres, not well-suited to the growth of crops like canola in rotation with wheat. *Brassica carinata* is a species that is well-adapted to growth in semi-arid regions and is highly drought- and heat-tolerant. It is being developed as a new crop platform dedicated to the production of bio-industrial oil feedstocks, most notably oils enriched in very long-chain fatty acids (VLCFAs) like erucic (22:1 c13) and nervonic (24:1 c15). VLCFA-enriched *B. carinata* oils have applications in the manufacture of bio-jet fuels, bio-diesel, enhanced oil recovery surfactants, bio-plastics and many other products. The contributions of such *B. carinata* oil products to bio-based aviation fuels and to the more-efficient extraction of recalcitrant fossil fuel resources for maximum return at drill sites, are both needed to create a more sustainable energy sector. Equally intriguing is that nervonic acid has potential for use in many products in the human health, nutraceutical and pharmaceutical sectors. This presentation will focus on the utilities of VLCFAs, the engineering of high VLCFA *B. carinata* prototypes and the requirements for sustainability and commercialization of this new value-added germplasm.

RECOBINANT PRODUCTION AND CHARACTERIZATION OF TYPE 1 *BRASSICA NAPUS* DIACYLGLYCEROL ACYLTRANSFERASES Michael S. Greer, Xue Pan, Kristian M. Caldo, Guanqun Chen, and Randall J. Weselake*

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Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of *sn*-1, 2 diacylglycerol to produce triacylglycerol (TAG). The DGAT-catalyzed reaction has been a genetic engineering target to increase storage lipid production in both oilseeds and microorganisms. Here, four transcriptionally active *DGAT1* genes were identified and characterized from the oil crop *Brassica napus*. Over-expression of each *BnaDGAT1* in *Saccharomyces cerevisiae* led to an increase TAG biosynthesis. Further studies showed that addition of an N-terminal tag could mask the deleterious influence of the DGATs' native N-terminal amino acid sequences, resulting in increased *in vivo* accumulation of the polypeptides and an increase of up to about 150-fold in *in vitro* microsomal enzyme activity. Phylogenetic analysis of the four *BnaDGAT1* coding sequences revealed that these genes may have diverged into two separate clades relatively early in Brassicaceae history. Although all four forms of BnaDGAT1 could effectively use a range of molecular species of acyl-CoA or *sn*-1,2-diacylglycerol, two of these forms of BnaDGAT1 displayed increased preference for substrates containing linoleic acid (18:2 $\Delta^{9cis,12cis}$). These findings contribute to our understanding of TAG biosynthesis in oilseeds and yeast, and may advance our ability to engineer DGAT1 with desired substrate selectivity properties.

COMMERCIAL CHALLENGES IN THE DEVELOPMENT OF NOVEL SEED OIL PRODUCTS Joseph G. Boothe

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The first crop with genetically engineered seed oil, high-laurate canola, was introduced in 1995. Since that time advances in our basic understanding of plant lipid biosynthesis, coupled with enormous improvements in the tools for creating transgenic plants, have provided researchers with the ability to alter the fatty acid composition of virtually any major oilseed. It is now possible to produce vegetable oils enriched in a variety of fatty acids with enhanced nutritional attributes and desirable chemical feedstock properties. However, despite the early promise, two decades on the commercial potential of the technology has yet to be realized, largely due to market challenges. Development costs and timelines, regulatory considerations, market size and value, and consumer perception can all significantly impact risk and feasibility, dramatically affecting the decision of whether or not to engage in commercial development. This presentation will examine these underlying aspects of commercialization in the context of products currently in the market or under development and identify factors that improve the prospects for success.

ECONOMIC IMPLICATIONS OF CHANGING DEMANDS FOR CANADIAN CANOLA

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Approximately 90% of canola seed, oil, or meal is exported from Canada each year, making Canada the world's largest exporter of rapeseed. However, the 2014 signing of CETA (Comprehensive Economic Trade Agreement), a free trade agreement between Canada and the European Union (EU), has the potential to significantly affect Canadian canola trade. The EU has been notoriously strict when it comes to the regulation of genetically modified (GM) goods, requiring labels on all GM containing foods, and restricting imports of GM commodities. Canada, in which nearly all the canola is GM, exports a relatively small amount of canola and canola-based products to the EU ($23.6 million in 2013). One of the aims of CETA is to increase free trade, with an emphasis on the trade of GM products, such as canola. It is therefore important to understand how CETA might alter Canada's current trade barriers to the EU.

In this study, we consider the possible impact of CETA on the demand for Canadian canola. At the global level, rapeseed (canola) primarily competes with palm and soybean. The latter two oils make up the majority of processed oil production, together contributing to almost 60% of the global market. Rapeseed oil (which includes canola oil produced in Canada) has the third largest share of the global market at 16%. Globally, Canadian canola oil contributes to approximately 12% of the rapeseed oil sector and lags behind the EU and China. Should the free-trade of CETA not extend to Canada's GM canola, or if significant labelling is required, Canadian canola producers may continue to face trade barriers to the EU. Furthermore, substitutes for canola seed and oil have the potential to crowd out the Canadian industry in Europe even further. This could have significant and negative economic effects on the Canadian canola sector. We quantify some of these economic effects under various scenarios.

OPTIMIZING LIPID METABOLISM IN *P. TRICORNUTUM* Kirstin Feussner[1,*], Jennifer Popko[1], Till Ischbeck[1], Richard Haslam[2], Johnathan Napier[2], Inna Khozin-Goldberg[3], Cornelia Herrfurth[1] and Ivo Feussner[1]

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Diatoms are of special interest because they are a major component of phytoplankton communities and believed to be responsible for ¼ of the global productivity. *Phaeodactylum tricornutum* serves as model organism for studying the molecular physiology of diatoms, because its genome has been sequenced and a transformation system is available. Moreover, it produces high amounts of the very long chain-polyunsaturated fatty acid (VLC-PUFA) eicosapentaenoic acid (EPA, $20:5^{\Delta5,8,11,14,17}$). Its fatty acid (FA) profile harbors beside EPA, mainly palmitic acid (16:0) and palmitoleic acid ($16:1^{\Delta9}$) whereas C18 FAs can barely be detected. This unique FA profile makes *Phaeodactylum* very well suited for food and feed applications since it is rich in EPA as well as for the production of feed stocks for the chemical industry out of the mid chain-FA fraction. During stationary phase or nitrogen starvation diatoms accumulate triacylglycerols (TAG) in lipid droplets. However, till now it is neither known which membrane lipids serve as precursors for the TAG formation nor from which cellular membrane these TAG species derive. To contribute to this open question and to increase TAG production, lipidomic and metabolomic analysis of the exponential and stationary growth phase were performed. As expected, the amount of neutral lipids increased during stationary phase and predominantly 16:0 and 16:1 accumulated in the TAG fraction. Additionally, the amounts of different glyco- and phospholipids were analyzed as well as central and specialized metabolites. The obtained data provide now a deeper insight into the lipidome and metabolome of *P. tricornutum* and will help to understand the metabolic pathways of lipid production and storage in diatoms.

ADVANCED BIOPROCESSES FOR INDUSTRIAL PRODUCTION OF HIGH-VALUE COMPOUNDS FROM MICROALGAE Lukas Neutsch*, Matthias Barmettler. Marina Stadler, Iwo Zamora, and Karin Kovar

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Potential uses of microalgae in biotechnology are just beginning to be explored and the list of high value-added compounds obtainable from microalgae is constantly being expanded. It now covers a broad range of substance classes from rare lipids and carbohydrates, to pigments, peptides, recombinant proteins and other molecules with interesting biological activities. However, the use of such compounds in cosmetic, pharmaceutical and other areas of application with high regulatory demands is still limited by the lack of robust and well-defined production systems.

The metabolic plasticity of many species permits cultivation under photoautotrophic, mixotrophic and heterotrophic conditions, with the latter mode allowing for consistent and sustainable production in commercially available closed steel bioreactors. The versatility in feeding regimens (light intensity and spectral composition, carbon source, rate of addition, switch between different trophic modes and nutrient limitation) offers enhanced opportunities for process optimization, but involves a higher degree of complexity in identifying the best configuration.

We aim to improve microalgal bioprocesses by implementing the same level of process control as is applied in other fields of microbial biotechnology (with bacteria or yeasts). Under well-controlled fedbatch heterotrophic conditions, biomass concentrations up to 180 g l^{-1} dry cell weight can be achieved reproducibly and quickly. Specific growth rates can be adjusted to a defined setpoint in order to investigate relationships between product formation kinetics and metabolic burden. In combination with enhanced monitoring tools (e.g. biomass analysis at the single cell level), a deeper understanding of critical process variables can be achieved, paving the way for an expanded use of microalgal-derived substances in the high-value biotechnological industries.

SELECTIVE ANTIBACTERIAL ACTIVITY OF PALMITOLEIC ACID USEFUL FOR POSSIBLE PREVENTION OF ATOPIC DERMATITIS Toshihiro Nagao[1]*, Shigemitsu Tanaka[1], Atsushi Kurata[2], and Noriaki Kishimoto[2]

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Atopic dermatitis (AD) is a chronic inflammatory skin disease occurred by several factors, such as allergen, filaggrin loss mutation, microorganisms, and so on. This study focused on microorganisms, skin microbiome. *Staphylococcus aureus* is observed with high frequency on AD, but is not observed so much on healthy control. Meanwhile, *Staphylococcus epidermidis* is observed with high frequency on healthy control, but is not observed so much on AD. *S. aureus* aggravates AD inflammation through protein A and □-toxin, and *S. epidermidis* represses growth of *S. aureus*. So, selective antibacterial activity, that is, repression of *S. aureus* and no repression of *S. epidermidis*, should be useful for possible prevention of AD.

Palmitoleic acid (9-*cis*-C16:1, PoA) is observed in a few natural oils such as macadamia nut oil. PoA is an isomer of sapienic acid (6-*cis*-C16:1, SA) observed on human skin, and both C16:1 show antibacterial activity against *S. aureus*. Since SA decreases on the AD patient's skin and is rarely observed in natural oils, supplement of PoA to skin should be useful for repression of *S. aureus*. However, PoA shows antibacterial activity against *S. epidermidis*. We thus investigated several factors for selective antibacterial activity of PoA. The results showed that pH was the most important factor. At the neutral and weak alkaline conditions, PoA showed weak and equal activity against two strains. Meanwhile, at the weak acidic conditions, PoA showed selective activity: strong activity against *S. aureus* NBRC13276 and weak activity against *S. epidermidis* NBRC100911.

The most effective supply of PoA is macadamia nut oil including 20% PoA. Unfortunately, the free fatty acid (FFA) from the oil did not show strong activity against *S. aureus*: more than 100 times decreased compared with PoA. This was caused by inhibition of the activity by oleic acid (9-*cis*-C18:1, OA) including in the oil (OA content, 55%).

Sea Buckthorn (*Hippophae rhamnoides*), that is cultivated at Finland, Russia, and Hokkaido in Japan, is a fruits. The fruit contains oils both in pulp and seed. In the case of pulp oil, PoA content was 41%, and OA content was 10%. In this composition, activity against *S. aureus* was not inhibited, and the FFA showed selective antibacterial activity that was suitable for our aim.

DEVELOPMENT OF NOVEL VACCINES AGAINST HUMAN AND AVIAN INFLUENZA VIRUSES

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The outbreaks of flu influenza viruses raised serious concerns to public health and economic loss in poultry industry worldwide. Development of new and effective vaccines to protect humans and animals from influenza virus infection and to control the spread of influenza viruses is an important quest in Taiwan. Both antigen preparation and adjuvant are important components of vaccine. We have recently screened and found a novel TLR2 agonist that induced mouse bone marrow derived dendritic cell maturation and cytokine productions. Here, we explored if this TLR2 agonist can be used as an adjuvant for flu vaccine. Mice vaccinated with influenza hemagglutinin as antigen and the TLR2 agonist as adjuvant induced neutralizing antibodies and Th1 and Th2 responses. The vaccination also protected mice that were challenged with both homologous and heterologous influenza virus strains. We further examined whether or not the TLR2 agonist could be used as an adjuvant for vaccine against bird flu. We used virus like particles (VLP) as antigen. Chicken primed and boosted with H5N2-VLP together with the TLR agonist elicited much higher neutralizing antibody titers than chicken immunized with either H5N2-VLP alone or with alum as adjuvant. Furthermore, chicken immunized with H5N2-VLP together with the TLR2 agonist protected 100% of chicken from H5N2 virus challenge resulting in less virus shedding than chicken immunized with inactivated virus vaccine with alum adjuvant. Our data suggest that these vaccines may be used as novel vaccines against human and bird flu.

TOXICITY OF SQUALENE IN YEAST DEFICIENT IN LIPID STORAGE Martina Garaiova, Martin Valachovic, Roman Holic*, and Ivan Hapala

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Squalene is a lipophilic molecule widely present in the nature. It is a key intermediate in the formation of essential membrane constituents - sterols in eukaryotes and hopanoids in bacteria. As a valuable natural isoprenoid it has many applications in medicine and industry. Limited natural sources of squalene make its production in microorganisms including yeast highly interesting. We recently identified squalene epoxidase encoded by *ERG1* gene as an excellent target for manipulation of squalene levels in the yeast *Saccharomyces cerevisiae*. High accumulation of squalene could be reached in cells treated with terbinafine, a specific inhibitor of squalene epoxidase. However, treatment with higher concentrations of terbinafine was associated with growth defects that can be related to ergosterol deficiency or to the toxicity of accumulated squalene. Accumulated squalene is stored in lipid droplets together with triglycerides and sterol esters. Interestingly, yeast mutants lacking lipid droplets show hypersensitivity to terbinafine. We used this lipid droplet-less mutant to test the potential lipotoxicity of squalene. Analysis of ergosterol and squalene levels in wild-type and lipid droplet-less yeast showed that ergosterol depletion is not involved in terbinafine sensitivity. To prove the toxicity of accumulated squalene we affected squalene levels in lipid droplet-deficient cells by mechanisms independent on squalene epoxidase activity. Reduced squalene production by zaragozic acid (inhibitor of squalene synthase) resulted in growth restoration of terbinafine-treated cells. Our data proved that accumulated squalene is lipotoxic under conditions when it cannot be efficiently stored in lipid droplets. Storage capacity of lipid droplets can thus be a limiting factor of squalene production in yeast.

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SHORT-CUTS THROUGH BIOCATALYSIS FROM VEGETABLE OILS TO VALUE-ADDED INDUSTRIAL COMPOUNDS Hak-Ryul Kim*, Hye-Ran Son, Chakradhar Dasagrandhi, Fei Wang, and In-Hae Choi,

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Structural modification of natural lipids via chemical reaction or microbial bioconversion can change their properties or even create novel functionalities. Enzymatic oxidation of lipids leading to formation of oxylipin is one of those modifications. Hydroxy fatty acids, one of those oxylipins have gained important attentions because of their special properties such as higher viscosity and reactivity compared with other non-hydroxy fatty acids. Recently 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) was produced with high yield from lipid containing oleic acid by bacterial strain *Pseudomonas aeruginosa* PR3, and further study confirmed that DOD contained strong antimicrobial activities against broad range of microorganisms. In this study we tried to modify DOD molecules by enzymatic or physical reaction to create new functionality or to enhance the antimicrobial activity of DOD. After modification of DOD molecules by different ways, we confirmed that the antimicrobial activity of DOD was highly enhanced, suggesting that DOD and its derivatives could be used as an efficient antimicrobial agent for industrial applications.

BRINGING THE CASTOR PLANT BACK AS A CROP IN TEMPERATE REGIONS

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Castor oil serves as a chemical feedstock for production of high value products including lithium grease, surfactants, non-toxic plasticizers, paints, cosmetics, and polymers. These products were in common use, and some still are, but cheap and abundant petroleum coupled with advances in petroleum chemistry provided adequate replacements on a grand scale, even though the qualities of castor oil-based products matched or outperformed those from petroleum.

Under marginal growth conditions, castor can produce oil at levels approaching those of a well-tended soybean field and, with suitable agronomic inputs, the castor plant can achieve oil yields up to 15-20 barrels of oil (>2.5 metric tons of oil) per acre. The presence of toxic proteins has deterred re-introduction of the plant to the farm economy due to potential for toxin exposure during processing and potential for contaminating food crops as an invasive plant. We have developed methods to inactivate or remove toxin during processing and, in collaboration with a castor breeder, have identified low toxin castor varieties. In addition, we have demonstrated an effective method to block invasive propagation of castor in fields, either as volunteers from previous years or from dehiscent castor.

Re-introduction of castor as a crop can help to replace petroleum with a renewable resource. While most petroleum is used for fuel, approximately 30% is used in chemical production. Increased production of castor oil can supplant some of the petrochemical products that replaced castor oil-based products. We hope our research on castor has engendered interest in the re-introduction of castor as a crop in the US. In addition to research fields, there is now a small castor plantation in Florida and additional interest in other states.

NOVEL OILS CONTAINING HYDROXY FATTY ACIDS, POTENTIAL AND CHALLENGES TO COMMERCIAL PRODUCTION Mark A. Smith

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Higher plants synthesize a diverse array of novel fatty acids and unusual seed lipids. Many of these have unrealized potential as industrial feedstocks and in human health/ nutraceutical applications. They also represent a vast genomic resource containing genes encoding enzymes capable of synthesizing and modifying lipids for many different applications. Much of the work characterizing seed oils was conducted over 40 years ago. Developments in analytical technology now allow us to revisit old studies and characterize new oils with increased speed and precision. GC-MS, NMR and Mass Spectrometry represent some of the versatile tools available at NRC for discovery.

Very few plants have the agronomic characteristics necessary for mechanized commercial production. Current approaches in creating novel oils with new fatty acid profiles therefore entail the genetic engineering of established oilseed crops. Although sometimes successful, this is often a complex task requiring the co-expression of multiple genes to reconstitute a biochemical pathway. Fatty acids containing one or more hydroxyl group (HFAs) represent feedstocks with considerable value and potential for a wide range of uses. Engineering their production in commercial oilseed crops is unfortunately highly challenging. The presentation will focus on the discovery and characterization of HFA producing plants, and the engineering of HFA production in oilseeds and microorganisms.

BIOCHEMICAL PROPERTIES OF *LESQUERELLA FENDLERI* LCAT-LIKE PHOSPHOLIPASE A

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Hydroxylated fatty acids (HFA) have valuable applications in the production of industrial materials. Currently, the only commercially available HFA is ricinoleic acid (12-OH 18:1Δ^{cis9}) from castor bean (*Ricinus communis*). Large-scale agricultural production of castor bean, however, is limited by the presence of the highly toxic protein ricin in the seeds. Producing ricinoleic acid in engineered oilseed crops is attractive but largely unsuccessful. One possible bottleneck step is the inefficient release of newly hydroxylated acyl moieties from the *sn*-2 position of phosphatidylcholine (PC) into the acyl-CoA pool for further utilization in triacylglycerol (TAG) synthesis. We recently identified an *Arabidopsis thaliana* LCAT-like PLA (AtLCAT-PLA) with high PLA_2 activity, which can effectively release fatty acids from the *sn*-2 position of PC (Chen et al. *FEBS Lett.* 2012, 586, 373-377). In this study, we characterized the biochemical properties of its homologue from *Lesquerella fendleri*, a plant containing up to 60% hydroxy-fatty acids in its seed oil. Our results indicated that the *L. fendleri LCAT-PLA* (*LfLCAT-PLA*) gene shared about 91% nucleotide homology to *AtLCAT-PLA* and was broadly expressed in all plant tissues including developing seeds. The *LfLCAT-PLA* gene was heterologously expressed in wild type *Saccharomyces cerevisiae.*Tthe recombinant enzyme was purified, and its catalytic properties and substrate specificity were characterized. Co-expression of *Claviceps purpurea Δ-12 hydroxylase, LfLCAT-PLA* and *L. fendleri 3-ketoacyl-CoA synthase* in yeast indicated that LfLCAT-PLA facilitated the channeling of hydroxy-fatty acids from PC to TAG. LfLCAT-PLA's function in hydroxy-fatty acid accumulation in transgenic Arabidopsis was also explored.

METABOLIC ENGINEERING OF HYDROXY FATTY ACID IN HETEROLOGOUS SYSTEMS Dauenpen Meesapyodsuk[1,2], and Xiao Qiu[1,2,*]

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Hydroxy fatty acids are important raw materials with a wide range of industrial uses. In particular, ricinoleic acid (12-hydroxyoctadec-cis-9-enoic acid), a long chain hydroxy fatty acid produced by castor bean (*Ricinus communis*) has many specialized uses in manufacturing of a variety of industrial products such as nylons, lubricants, ink, paints as well as pharmaceuticals and cosmetics. However, due to the presence of a highly potent toxin (ricin), this native plant is not considered as an ideal source for the hydroxy fatty acid production. Therefore, tremendous effort has recently been made in identifying genes from the native plant species involved in the biosynthesis of ricinoleic acid and using these genes to engineer oilseed crops for producing the fatty acid. However, so far a commercially viable level of this fatty acid in transgenic plants has not been achieved. Here we will review our recent work on identification of genes from fungus *Claviceps purpurea* involved in the biosynthesis and assembly of this fatty acid and report new progress towards to metabolic engineering of this fatty acid in microorganisms.

1KP AND THE COMPARATIVE GENOMICS OF BIOLOGICAL PATHWAYS

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1KP is an international multi-disciplinary consortium that has generated transcriptomics data across the *Viridiplantae* (green plants). We have sequenced exemplars of all the major lineages representing about a billion years of evolution, including flowering plants, conifers, ferns, mosses, and streptophytic green algae. These efforts have increased plant gene numbers by a factor of ~50 relative to the public databases.

One of our many goals was to demonstrate that biodiverse sequencing can generate practical benefits. I will discuss how this was accomplished at 3 levels of difficulty. First we discovered novel proteins that enabled the development of improved optogenetics tools for mammalian neuroscience. Second we elucidated, in a relatively short time, the initial stages of a biosynthetic pathway for an experimental anti-cancer drug. Third we used convergent evolution to identify the hundred-plus genes that differentiate c4 vs c3 photosynthesis, *i.e.* are consistently expressed in all c4 dicots across vast taxonomic distances. More generally, and with the continuing decrease in the costs of sequencing, we believe it will be increasingly easy to apply similar methods to learn from *Mother Nature* and billions of years of evolutionary tinkering.

Here are the links to our 1KP publications and related media coverage.
https://pods.iplantcollaborative.org/wiki/display/iptol/OneKP+companion+papers

HIGH-THROUGHPUT OMICS APPROACHES IN GENETICS AND BIOTECHNOLOGY

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The omics approach breaks through the bottleneck of traditional molecular biology and enables a genome-wide perspective to further study functional genomics. In genomics, the next-generation sequencing (NGS) technique provides an efficient method for analyzing high-throughput genetic profiles in model and non-model organisms. This technique is not only used in genome projects, but also applied to other genetic materials, such as in transcriptome, small RNA, and degradome. Whole-transcriptome analysis provides sequence information (gene and non-coding RNA) with expression profiles under various treatments or conditions. Moreover, through its integration with small RNA and degradome profiles, the predicted microRNA and its target can be identified. Furthermore, the transcriptome database provides protein information for protein mass spectra prediction, which can be used for increasing the prediction accuracy of proteomics. Conversely, the proteomics data can be used for confirming the transcriptome profile. Currently, Gateway recombinant system was introduced into virus-induced gene silencing (VIGS) and attenuated viral vector for high-throughput screening the candidate genes in loss-of-function and gain-of-function studies, respectively. These experimental approaches can rapidly generate various genetic materials for further tranecriptome and metabolomics analyses. Base on omics profiles, the network assay and database construction can integrate these omics profiles to identified critical genes that can be applied for metabolomics and genetic engineering.

METABOLOMICS, THE METABOLOMICS INNOVATION CENTRE AND PLANT BIOTECHNOLOGY Rupa Mandal, Jennifer Reid, Danuta Chamot, and David S. Wishart*

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Metabolomics is an emerging field of omics science that is being used in a growing number of applications in food science and plant biotechnology. In this presentation I will give a brief introduction to the field of metabolomics, describe how metabolomics experiments are typically performed and show how it is closely linked to genomics, transcriptomics and proteomics. I will also provide brief background information about The Metabolomics Innovation Centre (TMIC). TMIC, which is located at the University of Alberta, is Canada's national metabolomics laboratory and offers a number of unique facilities, technologies and metabolomic assays that should be of particular interest to both plant and food scientists. To help clarify its potential utility I will provide a short summary of some of the plant metabolomic studies that TMIC has recently undertaken and some of the more interesting findings it has made. I will also describe some of TMICs longer-term or larger-scale plant biotechnology and food science activities. These include characterizing the Alberta Food Metabolome, the construction of the Food Composition Database (FooDB), the PhenolExplorer project and the PhytoMap project.

TOWARDS ELIMINATION OF α-LINOLENIC ACID FROM *BRASSICA* SEED OIL: MUTATIONS IN *FAD3* GENES, MOLECULAR CHARACTERIZATION AND IMPLICATION IN BREEDING

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The traditional *B. napus* canola oil contains about 10% α-linolenic acid (ALA). This fatty acid offers health benefits; however, it oxidizes rapidly resulting in oil which is unstable under frying conditions. Therefore, decreasing the content of ALA in canola seed oil is desirable. ALA content in seed oil is regulated by the *FAD3* gene, which is involved in the conversion of linoleic acid (LA) into ALA. The *B. napus* (AC genome, *n* = 19) carries at least six *FAD3* genes, all of which might be involved in the biosynthesis of ALA. Therefore, mutations in all *FAD3* genes could be required to reduce the content of ALA to near zero in *B. napus*. This would be a difficult task requiring an extremely large mutagenized population. Conversely, *B. oleracea* (C genome, *n* = 9) and *B. rapa* (A genome, *n* = 10), the progenitor species of *B. napus*, carry only half the number of *FAD3* genes; therefore, it would be easier to achieve this in these species. Once the desired mutant gene(s) is identified in the parental species, it could subsequently be utilized in *B. napus* breeding. With this view, *B. oleracea* seeds were treated with 5% and 0.5% ethyl-methane-sulphonate (EMS) and two mutagenized populations were generated. Selection for low ALA content was carried out in these populations up to the M_7 generation and mutant lines with <2.0% ALA was obtained. Molecular analysis of the lines from 5% EMS treatment revealed that the mutation was due to a single nucleotide substitution from G to A in exon 3 of the 'class b' *FAD3* (*BoFAD3-1*) gene. In the case of the low ALA lines from the 0.5% EMS treatment, the phenotype was due to a nonsense mutation within the 'class a' *FAD3* gene (*BoFAD3-2*). Expression of the coding regions of these *FAD3* genes (*BoFAD3-1* and *BoFAD3-2*) from the mutant *B. oleracea* lines in yeast (*Saccharomyces cerevisiae*) cultures resulted in significantly reduced conversion of LA to ALA. Thus, the mutant *B. oleracea* lines developed in this research could potentially be used in breeding for the development of *B. napus* cultivars with further reductions in ALA content, as well as in the breeding of low-ALA *B. carinata* cultivars.

COMPOSITION AND ANTIFUNGAL ACTIVITY OF BALSAM FROM *LIQUIDAMBAR FORMOSANA* HANCE

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Formosan sweet gum (*Liquidambar formosana* Hance) is a tree species endemic in Taiwan. In our study, the composition of balsam from *L. formosana* has been determined by several chromatographic and spectroscopic techniques. Among the 26 compounds identified, three new triterpenoids were detected, namely, 2α,3α-dihydroxyolean-12-en-28-al (**1**), 3α-hydroxyolean-12-en-30-ol (**2**), and 3α-hydroxyolean-2-oxo-12-en-28-al (**3**). The most abundant volatile compounds were β-caryophyllene (22.7 %), α-pinene (23.3 %), and β-pinene (19.6 %), and the most abundant nonvolatile compounds were 3α,25-dihydroxyolean-12-en-28-oic acid (**12**, 19.1 %), oleanonic aldehyde (**9**, 14.0 %), and betulonic acid (**15**, 13.4 %). The compounds 3α,25-dihydroxyolean-12-en-28-oic acid and bornyl cinnamate were found to be inhibitory for white rot (*Lenzites betulina*) and brown rot (*Laetiporus sulphureus*) fungi.

AgING: A NEW EPIGENETIC MODEL FOR STUDYING CELL SENESCENCE Uma Karthika Rajarajacholan and Karl Riabowol*

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Cell senescence is a mechanism that serves as a first line of defence against cancer but also contributes to organismal aging, being responsible for such age-related maladies as immunosenescence, loss of effective wound healing and osteoporosis. Senescence is induced by the cumulative loss of DNA sequences that occurs with each division of normal somatic cells, shortening the telomeres that protect the ends of linear chromosomes. Telomere erosion results in the generation of an ensuing DNA damage signal as cells reach the end of their replicative lifespan in vitro or in vivo. Stresses induced by oncogene or tumor suppressor hyperactivation, oxidative stress, ionizing radiation and other DNA damaging agents result in forms of stress induced premature senescence (SIPS) that show similarities to replicative senescence. Since replicative senescence and SIPS occur over many days and many population doublings in both the body and in mass cultures of primary cells used to study senescence, the sequence of events that occur downstream of damage signaling can be challenging to define. Here we compare a new epigenetics-based model of ING1a-induced senescence with several other forms of senescence. The **IN**hibitor of **G**rowth 1a (ING1a) epigenetic regulator synchronously induces senescence in mass cultures several-fold faster than all other SIPS-inducing agents, taking 24 and 36 hours to activate the Rb/p16, but not the p53 tumor suppressor axis to efficiently induce senescence.

MINIMALLY INVASIVE TECHNIQUES TO VISUALIZE THE RHIZOSPHERE Emil Hallin*

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A combination of several techniques, all well exploited in other disciplines, can be used to enable minimally invasive visualization of intact and in-situ rhizospheres. Neutron based techniques are best suited to dry and sandy soils and work well with aluminum containers (silicon and aluminum are essentially transparent to neutrons). For resolution comparable to small plant cells, high energy synchrotron radiation x-rays can be used for smaller plant containers. Finally, positron emission tomography can also be used to visualize the rhizosphere, and with appropriate detectors can enable nutrient uptake and disposition studies. Current examples of all three techniques are discussed, with a view to their application to provide a minimally invasive 3D imaging of intact rhizospheres with high resolution.

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