

**BIO 1: Biocatalysis I**

*Chairs: Jun Ogawa, Kyoto University, Japan; and Ching T. Hou, USDA, ARS, NCAUR, USA*

**Identification of Molecular Species of Acylglycerols Containing Hydroxy Fatty Acids of Philippine Wild Edible Mushroom, *Ganoderma lucidum*** Ching T. Hou<sup>\*1</sup>, Jiann-Tsyh Lin<sup>2</sup>, Rich M. Dulay<sup>3</sup>, and Karen Ray<sup>4</sup>, <sup>1</sup>USDA, ARS, NCAUR, USA; <sup>2</sup>WRRC, USDA, USA; <sup>3</sup>Center for R&D, Central Luzon State University, Philippines; <sup>4</sup>NCAUR, USDA, USA

We successfully cultivated four Philippine edible mushrooms in liquid medium: *Ganoderma lucidum*, *Pleurotus cystidiosus*, *Volvariella volvacea* and *Schizophyllum commune*. Among them, *Ganoderma lucidum* grows faster with highest yield. Last year, we reported the identification of molecular species of acylglycerols in the lipid extract of mushroom *G. lucidum* NRRL66208. One hundred and three molecular species of acylglycerols containing all normal fatty acids were identified. The chain lengths of the constituent fatty acids were from 14 to 26 carbon atoms and the numbers of double bonds were from zero to three. Forty-one molecular species of acylglycerols out of 103 contained fatty acids with odd numbered carbon atoms. Now, we studied the polar lipids fraction of this mushroom and identified seventy-two molecular species of triacylglycerols and five molecular species of diacylglycerols containing hydroxy fatty acids (FA). Many odd carbon numbered hydroxy FA constituents of acylglycerols were also identified. The contents of the 77 molecular species of acylglycerols and their constituent hydroxy FA in the lipid extract were: P-19:0-2OH18:2, 0.69%; 2OH18:2-2OH18:2-2OH18:2, 0.60; P-OH19:1-OH19:1, 0.48; S-OH19:1-OH19:1, 0.43; 19:0-19:0-2OH18:2, 0.27

and the total of about 5.8%. The high contents of acylglycerols containing hydroxy FA, odd carbon numbered FA and odd carbon numbered hydroxy FA in edible mushroom might be related to one or more of its health benefits. As far as we are aware of, this mushroom contains the highest amounts of hydroxy FA and odd carbon numbered FA among the human foods. This is the first report on the molecular species of acylglycerols containing hydroxy fatty acids in mushroom.

**Expression of Cyclooxygenase in *Mortierella alpina* 1S-4 for the production of a prostaglandin, PGF2 $\alpha$**  Jun Ogawa<sup>\*1</sup>, Mohd Fazli Farida Asras<sup>1</sup>, Hideaki Nagano<sup>1</sup>, Yoshimi Shimada<sup>1</sup>, Miho Takemura<sup>2</sup>, Shigenobu Kishino<sup>3</sup>, and Akinori Ando<sup>1</sup>, <sup>1</sup>Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan; <sup>2</sup>Res. Ins. Biore. Biotech., Ishikawa Pref. Univ., Japan; <sup>3</sup>Kyoto University, Japan

*Mortierella alpina* 1S-4 is an arachidonic acid (ARA)-producing microorganism. ARA is known to be a biogenetic precursor of prostaglandins and leukotrienes. One of the prostaglandins, PGF2 $\alpha$  is synthesized by cyclooxygenase (COX). PGF2 $\alpha$  is a vital bioactive molecule in pharmaceutical industries especially on the regulation of physiological processes such as blood circulation. However, PGF2 $\alpha$  is limited in nature and has been widely synthesized chemically, yet hardly been produced by any biological process especially by microorganism. In this study, we tested to produce PGF2 $\alpha$  by expressing COX gene from *Gracilaria vermicuphylla* in *M. alpina* 1S-4. We constructed binary vectors which contain GvCOX gene regulated by two constitutively

promoters, His550 with a modest expression level and SSA2 with a higher expression level, and introduced them to *M. alpina* 1S-4 by the *Agrobacterium tumefaciens*-mediated transformation method. A transformant with the promoter His550 was obtained which was capable to produce PGF2 $\alpha$  under an optimal condition. The presence of PGF2 $\alpha$  was detected within the cell with approximately 0.9  $\mu\text{g}/\text{mg}$  of dry cell weight whilst in the cultured medium, approximately 6  $\mu\text{g}/\text{mL}$ . The production of PGF2 $\alpha$  with the His550 promoter was sluggish from 3 to 9 days of the fermentation as compared to that with the SSA2 promoter which consistently increased until the end of the fermentation period. These transformants presented the promising PGF2 $\alpha$  production with approximately 6  $\mu\text{g}/\text{mL}$  culture medium under optimal conditions. These findings provide a solid base for further investigations subsequently enhancing the yield of bioactive products.

#### **Biotechnological Research for the Development of Sustainable Oil Palm Industry**

Ahmad Parveez Ghulam Kadir\*, Rajinder Singh, Meilina Ong-Abdullah Ong-Abdullah, Umi Salamah Ramli, Omar Abdul Rasid, Mohamad Arif Abd Manaf, and Kushairi A, *Malaysian Palm Oil Board, Malaysia*

Biotechnological tools have been proven to effectively improve the output of crops as well as increase their adaptability non-optimal growth conditions. These tools are applied in oil palm R&D efforts to further improve the genetic potential of this most productive oil bearing tree. The successful sequencing of oil palm genome has further boost the potential to improve the productivity of oil palm through biotechnological approaches. The successes have been notable,

especially the isolation of genes linked to important agronomic traits such as the oil fruit form and colour of the exocarp which followed by the released of diagnostic kits known as the SURESAWITTM SHELL and SURESAWITTM VIR. These kits help in the quality control process of the commercial seed production and improving breeding efficiency. Production of industrial oils via genetic engineering of the palm adds an extra dimension to the conventional and traditional exploitation of palm oil. The full complement of genes, promoters, and constructs to achieve all the targeted products are already in place. The successful production of the first transgenic oil palm indicates an important milestone for the utilization of the technology to further diversify the application of palm oil. Additionally, the development of microinjection related techniques for genetic modification of oil palm is also an important strategy to circumvent issues related to genetically modified crops. R&D advancements and availability of molecular diagnostic assays has finally show-cased the true potential of biotechnology in improving oil palm productivity in a sustainable manner.

#### **Screening of Fatty Acids Showing Selective Antibacterial Activity Against Acne-associated Propionibacterium acnes**

Ayaka Uyama<sup>1</sup>, Teizo Sugino<sup>1</sup>, Shimemitsu Tanaka<sup>2</sup>, and Toshihiro Nagao<sup>\*2</sup>, <sup>1</sup>*Momotani juntenkan Ltd., Japan*; <sup>2</sup>*Osaka Research Institute of Industrial Science and Technology, Japan*

*Propionibacterium acnes* is a major commensal bacterium residing on normal human skin, and it plays important roles in maintaining skin health. However, it has also been implicated in the pathogenesis of several diseases such as acne vulgaris. Recent works revealed that P.

acnes clinical isolates can be classified into distinct phylotypes. When *P. acnes* strain type were classified using 16S ribotyping, ribotype (RT) 1-3 subgroup strains were found to be evenly distributed in acne patients and healthy skin. Meanwhile, RT4 and RT5 subgroup strains were found in patients with acne, and a RT6 subgroup strain was found to be associated with healthy skin. These results implied that not all *P. acnes* strain, but the specific *P. acnes* strain, may be concerned in acne. In this study, we aimed screening of antibacterial regents showing selective antibacterial activity against the acne-associated *P. acnes* strains. Many common antibacterial regents such as isopropyl methyl phenol showed almost equal minimum inhibitory concentration (MIC) value against the acne-associated RT4 and RT5 strains and the healthy-associated RT6 strain. Meanwhile, screening of many saturated fatty acids, palmitoleic acid, and oleic acid revealed that a rare fatty acid showed selective antibacterial activity, that is, low MIC values against the acne-associated RT4 and RT5 strains and high MIC value against the healthy-associated RT6 strain. Thus, it was found that the rare fatty acid was useful for an antibacterial reagent selectively suppressing the acne-associated *P. acnes* strains.

**Metabolism of Soy Sugars by Genetically Engineered *Pseudomonas chlororaphis*** Daniel K.Y. Solaiman\*, Richard D. Ashby, and Nicole V. Crocker, *USDA, ARS, ERRC, USA*

Soy molasses contains oligosaccharides (i.e., sucrose, raffinose (Raf), and stachyose (Sta)) that are potential carbon source to support the

growth of microorganism. *Pseudomonas chlororaphis* which can biosynthesize poly(hydroxyalkanoates) (i.e., a biodegradable polymer) and rhamnolipids (i.e., a biosurfactant) however cannot metabolize Raf and Sta. Alpha-galactosidase (a-Gal) enzyme could breakdown Raf and Sta to simpler sugars for use by microorganisms. We previously reported the construction and comparative gene-expression study of two genetically engineered *P. chlororaphis* strains expressing an a-galactosidase gene of *Streptomyces coelicolor* (*a-gal<sub>Sc</sub>*). In this paper, the a-Gal enzyme activity in the two transformant strains was assayed using a chromogenic substrate (i.e., p-nitrophenyl-a-galactopyranoside). The results showed that *P. chlororaphis* [pBS-dAG] strain exhibited 8-time higher a-Gal enzyme activity than the *P. chlororaphis* [chr::AG] did. Cells extracts prepared from [pBS-dAG] strain showed that Raf was hydrolyzed better than Sta, with 32.7% (Raf) and 72.8% (Sta) of the initial substrates remained un-degraded after the assay. EDTA-permeabilized [pBS-dAG] cells were shown to grow (biomass 0.46 g cell-dry-weight/L) and hydrolyze Raf (28% initial substrate remaining), while the wild-type *P. chlororaphis* did not consume Raf and only yielded a biomass of 0.26 g CDW/L. This study paved the way for future research to improve *P. chlororaphis* [pBS-dAG] to use soy molasses and other agricultural-commodity byproducts containing galacto-oligosaccharides (e.g., sugar beet molasses) for cell growth and production of bioproducts.

**Xylose and Levulinic Acid: Two Inexpensive, Renewable Substrates for the Mixed-culture Biosynthesis of Unique poly(hydroxyalkanoate) Polymer Blends with Controllable Properties.**

Richard D. Ashby, Daniel K.Y. Solaiman, and Gary Strahan, *USDA, ARS, ERRC, USA*

Poly(hydroxyalkanoate)s (PHA's) are well-known bacterial polyesters whose compositions are dictated by bacterial strain and culture conditions. Many PHA's exhibit comparable properties to petrochemical polymers (with the added benefit of biodegradability and biocompatibility), but wide-spread use is limited by high production costs. To help circumvent this, inexpensive carbon substrates have been utilized to promote PHA synthesis. Xylose (XYL) and levulinic acid (LA) are inexpensive substrates that have been demonstrated to be effective fermentation precursors for PHA biosynthesis. Previously, we demonstrated that *Burkholderia sacchari* DSM 17165 was capable of synthesizing unique poly-3-hydroxybutyrate-block-3-hydroxyvalerate) (P3HB-block-3HV) with controlled properties from variable ratios of XYL and LA. At the same time, *Azohydromonas lata* DSM 1122 was proven to synthesize random terpolyesters of poly-3HB-co-3HV-co-4-hydroxyvalerate (P3HB-co-3HV-co-4HV) from mixtures of glucose (GLC) and LA. By utilizing mixed substrates containing 1% GLC:1% XYL and various concentrations of LA, mixed culture fermentations of *A. lata* and *B. sacchari* were established as a means of producing polymer mixtures of P3HB-block-3HV and P3HB-co-3HV-co-4HV in varying ratios with controllable mechanical properties. In this presentation we will discuss the details behind these fermentative syntheses and the potential effect of using these renewable feedstocks to create unique PHA

blends and improve the economics of PHA synthesis.

**Biorefinery Process for Valuable Lipid Production by Thraustochytrids** Kenshi

Watanabe, Kim HV Arafiles, Yoshiko Okamura, Takahisa Tajima, Yukihiro Matsumura, Yutaka Nakashimada, and Tsunehiro Aki\*, *Hiroshima University, Japan*

Terrestrial and marine biomass has attracted much attention in the fields of renewable bioenergy and value-added industrial and biological materials production due to the promised sustainability of its use. We have succeeded at using macroalgae to produce methane by marine sediment-derived culture and functional lipids such as polyunsaturated fatty acids, xanthophylls, and terpenoids by using the marine protists, the thraustochytrids. In the biomethane production system, algal polysaccharides were degraded to organic acids that were eventually converted to methane by a marine sediment-derived microbial consortium acclimated to high salinity. Such bacteria that can degrade or convert algal saccharides into suitable substrates for thraustochytrids were explored to serve as intermediate catalysts to establish a composite culture system that ultimately produces the lipids. Fermentation profiles indicated a possibility toward its practical application. Furthermore, assimilation profiles of thraustochytrids has let us to develop a new and ultimate biorefinery system.

**Challenges to Develop Bioprocess for Lignin Paint** Yomi Watanabe\*, *Osaka Research Institute of Industrial Science and Technology, Japan*

Annual report on Forest and Forestry in Japan (2011) estimated that the amount of the unused wooden biomass reached over 20,000 m<sup>2</sup>. Lignin

accounts nearly 40% of that. The realistic way of mass consumption of lignin remains undeveloped as the fractionation of wood chips conventionally used high amount of sulfuric acid, and it was not cost-effective to remove the acid or salt, resulted by the neutralization, from the lignin fraction. The dark color of lignin fraction caused by the sulfuric acid treatment also reduced its quality for reuse. On the other hand, lignin was successfully fractionated by the treatment of cedar tree powder in organic solvent with 1% sulfuric acid (Yamazaki *et al.* JP Application Publication 2013-076067), and was supplied to develop a novel biomass paint. Solubilization of lignin to paint base material is of extreme importance to form the transparent paint film. The solubility of lignin to natural drying oil was improved by the enzymatic treatment of flax seed oil with phenolic alcohol. Unlike the chemical process, the enzymatic treatment of drying oil avoided the color darkening of materials, and would contribute the quality of biomass paint. In the presence of cobalt, the sample paint consists of lignin concentrates, flax seed oil modified by phenolic alcohol, and methyl silicate oligomer, formed a glossy, transparent coating film on a wooden board. The paint surface showed sufficient resistance to food stains; no stains were left after wiping with a damp cloth, and may allow the use of the paint for interiors.

**Enzymatic Characterization of Metabolism of Food Derived Polyunsaturated Fatty acids by Gut Microorganisms Generating Bioactive Fatty Acids.**

Michiki Takeuchi<sup>\*1</sup>, Shigenobu Kishino<sup>1</sup>, Si-Bum Park<sup>1</sup>, Nahoko Kitamura<sup>1</sup>, and Jun Ogawa<sup>2</sup>, <sup>1</sup>Kyoto University, Japan; <sup>2</sup>Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan

Dietary ingredients are metabolized by gut microorganisms, whose metabolites affect host health. Recently, we have revealed polyunsaturated fatty acid saturation metabolism<sup>1)</sup>, which consists of four enzymes, i.e., CLA-HY (hydratase/dehydratase)<sup>2)</sup>, CLA-DH (dehydrogenase)<sup>3)</sup>, CLA-DC (isomerase), and CLA-ER (enone-reductase)<sup>4)</sup>, in *Lactobacillus plantarum* as a model strain of gut microorganisms. This metabolism generates several types of fatty acids such as hydroxy, oxo, and conjugated fatty acids. In those of metabolites, we have revealed some physiological functions. For example, 10-hydroxy-*cis*-12-octadecenoic acid (HYA) has ameliorates intestinal epithelial barrier impairment<sup>5)</sup>. 10-Oxo-*cis*-12-octadecenoic acid (KetoA) activates PPAR $\gamma$ , stimulates adipogenesis and adiponectin secretion<sup>6)</sup>. In this study, we characterized the four enzymes and applied them to produce various kinds of fatty acids such as hydroxy, oxo, conjugated, and non-methylene interrupted fatty acids. 1) S. Kishino *et al.*, *Proc. Natl. Acad. Sci. USA*, 110, 17808–17813 (2013). 2) M. Takeuchi *et al.*, *J. Biosci. Bioeng.*, 119, 636–641 (2015). 3) M. Takeuchi *et al.*, *J. Mol. Catal., B Enzym.*, 117, 7–12 (2015). 4) F. Hou *et al.*, *FEBS Journal*, 282, 1526–1537 (2015). 5) J. Miyamoto *et al.*, *J. Biol. Chem.*, 290, 2902–2918 (2015). 6) T. Goto *et al.*, *Biochem. Biophys. Res. Commun.*, 459, 597–603 (2015).



**BIO 1.1/IOP 1: Biorenewable Polymers**

*Chairs: Richard D. Ashby, USDA, ARS, ERRC, USA; and Baki Hazer, Kapadokya University and Bülent Ecevit University, Turkey*

**Synthesis of Resinic Acid and Lignin Derivative Dimers for Copolymerization with Vegetable Oil-based Monomers** Audrey Llevot\*, *LCPO, France*

The awareness of environmental deterioration and our dependency on depleting fossil feedstocks force research to find innovative solutions in order to design a more sustainable future. With a worldwide plastic production of over 300 million metric tons per year, polymer science represents a very active field in the use of renewable feedstocks. Among the available bioresources, vegetable oils lead to a large platform of aliphatic molecules and to a wide range of thermoplastic and thermoset polymers after modifications. In order to broaden the palette of renewable polymers, other molecules need to be investigated and used to tune the thermomechanical properties of the vegetable oil-based aliphatic polymers. Cycloaliphatic and aromatic compounds are two categories of molecules which enable the synthesis of polymers with high thermal stability and rigidity. In our work, a polycyclic biobased molecule, i.e. resinic acids, and phenolic compounds potentially derived from lignin were studied as comonomers for vegetable oil-based polymers. Both classes of substrates were dimerized in order to get difunctional symmetric synthons. On the one hand, abietic acid dimers synthesized via a cationic mechanism were esterified with undecenol and copolymerized with undecenyl undecenoate by ADMET methodology. On the other hand, we developed a “green” process to dimerize phenolic compounds derived from lignin in large quantity and high yield via enzymatic

catalysis using a laccase. After chemical modifications, the obtained dimers were tested in copolymerization with different fatty acid derivatives. The thermomechanical properties of the polymers will be discussed, as well as the sustainability of their synthesis.

**Dual Cure Alkyds** Mark D. Soucek\*, *University of Akron, USA*

A number of Different approaches have been used to speed the curing/drying process: 1) reactive diluents, allyl ether; 2) change catalyst Fe based; 3) change curing mechanism, moisture, UV or Visible light. Light curable alkyds were synthesized by functionalizing hydroxyl terminated medium and long linseed oil alkyds with methacryloyl chloride or acryloyl chloride. Two glycerol based reactive diluents were prepared by reacting glycerol with methacryloyl chloride or acryloyl chloride. Real time FTIR, photo-DSC and UV-Rheometer were used to study the curing kinetics of UV curable alkyd with 0-30 wt% of reactive diluent. The conversion of methacrylic and acrylic double bonds are above 80% within 10s of radiation at wavelength ranging from 320nm to 500nm. The polymerization rate increases with the addition of reactive diluent, however the final conversion slightly decreases with increasing percentage of reactive diluent due to the formation of crosslinking between UV curable alkyd and reactive diluent limiting the mobility of the reactive double bonds. DMA was utilized to determine the Tg and crosslink density of each system. With the increase of reactive diluent

percentage, initial elastic modulus and crosslink density will also increase.

#### **Reflection of Structural Features of Oils on Properties of Polymeric Materials**

Zoran Petrovic\*, *Pittsburg State University, USA*

Oils are present in all living organisms as an important energy source. They are triglycerides of widely varying composition. Natural oils as a platform for new oleochemicals have several features which make them attractive for a range of new products. They are generally very heterogeneous in structure, length of fatty acids, number of double bonds, with or without functional groups and varying their positions in the fatty acid chains. Generally, oil-based materials are softer than corresponding products from petrochemical sources but structural peculiarities can be beneficial or detrimental depending on application. Effects of specific features of oils on properties will be discussed.

#### **Bio-based Oil Potential in Additive**

**Manufacturing.** Ivan Javni<sup>1</sup>, Olivera Bilic<sup>2</sup>, Jian Hong<sup>2</sup>, Vivek Sharma<sup>1</sup>, Xianmei Wan<sup>1</sup>, and Jamie M. Messman<sup>4</sup>, <sup>1</sup>*Pittsburg State University, USA*; <sup>2</sup>*Kansas Polymer Research Center, Pittsburg State University, USA*; <sup>3</sup>*Dept. of Energy's National Security Campus, managed by Honeywell FMT, LLC, USA*

Additive Manufacturing (AM), or 3D printing is a rising technology that is breaking existing product design and manufacturing methods. This technology is advancing very strongly in capability of making complex elements in low volumes in a rapid and cost-effective way. This technology involves very different processes, such as Fused Deposition Modeling (FDM), Stereolithography (SLA), Selective Laser Melting (SLM), Selective Laser Sintering (SLS), etc. The

progress in production systems and machinery is followed by strong demand for new materials that can meet the specific requirements of new technology. Plastics produced from petrochemicals are common materials in this area. Due to the depleting natural resources and negative effect of carbon dioxide on the environment, there is a strong demand for replacement of petrochemicals with bio-based renewable resources. Bio-based plastics can be made from a variety of natural resources, including oils. Bio-oils are emerging and are promising raw materials for synthesis of a variety of polymers and plastics, including those that can be used in additive manufacturing. Natural oil based non-isocyanate polyurethanes (NIPUs) synthesized from cyclic carbonates and amines are promising new materials for this application. There are some structural specifics of NIPUs which give them excellent adhesive properties. We used soybean oil based NIPUs for improvement of interlayer adhesion of polylactic acid filaments used in AM. The effect of natural oil-based NIPUs on physical and mechanical properties of filaments was evaluated. The experimental results corroborated the presumed NIPUs structure and their effect on filament preparation and 3D printing.

#### **Multifunctional Fatty Acid Macroperoxide Initiators Obtained by the Autoxidization. Synthesis of Block/Graft Copolymers via Free Radical and Ring Opening Polymerization**

Baki Hazer<sup>1,2</sup>, Melike Eren<sup>1</sup>, Elif Ayyıldız<sup>2</sup>, Faruk Bahadır<sup>1</sup>, <sup>1</sup>*Bülent Ecevit University Faculty of Arts and Sciences, Dept. of Chemistry; Faculty of Engineering, Departments of Metallurgical and Materials Engineering, Turkey*; <sup>2</sup> *Bülent Ecevit University Faculty of Arts and Sciences, Nano Technology Engineering, Turkey*

Unsaturated plant oils/fatty acids (UPOFA) can undergo autoxidation under atmospheric conditions to produce macroperoxide initiators. Pure unsaturated fatty acids such as linolenic, linoleic and oleic acid were exposed to air oxygen under daylight at room temperature which is called “ecofriendly autoxidation”. Eco-friendly autoxidation process creates peroxide linkages in order to obtain fatty acid oligomer that is called macroperoxide initiator. Oleic acid macroperoxide initiator was used in the free radical polymerization of styrene in order to evaluate the polymerization kinetics. Because of different functional groups, the macroperoxide initiators were used in the synthesis of block/graft copolymers. Polystyrene-poly oleic acid-polycaprolactone, polystyrene-poly oleic acid-polyethylene glycol, poly N-isopropyl acryl amide-poly oleic acid-polyethylene glycol block/graft copolymers were obtained. Structural and physicochemical characterization of the products was done. Lower critical solution temperature of the thermo responsive double hydrophilic copolymer, poly N-isopropyl acryl amide-poly oleic acid-polyethylene glycol was found to be 36 °C.

**Super Palm Stearin from Enzymatic Directed Interesterification of Palm Oil**

Noor Lida Habi Mat Dian\*<sup>1</sup>, Miskandar Mat Sahri<sup>1</sup>, Tan Chin Ping<sup>2</sup>, and Lai Oi Ming<sup>2</sup>, <sup>1</sup>Malaysian Palm Oil Board, Malaysia; <sup>2</sup>Universiti Putra Malaysia, Malaysia

Enzymatic directed interesterification (EDIE) of palm oil (PO) resulted in an increase in the amount of trisaturated triacylglycerols from about 4.2% to about 28.5%, 6.8 times higher than

Enzymatic directed interesterification (EDIE) of palm oil (PO) resulted in an increase in the amount of trisaturated triacylglycerols from about 4.2% to about 28.5%, 6.8 times higher than the initial amount found in the mother oil. The high melting fraction which concentrated with the trisaturated triacylglycerols is well separated from the low melting fraction (shown by the differential scanning calorimetry melting profile), indicating easy of fractionation. Fractionation of the EDIE PO produced palm stearin (POs) with an iodine value of less than 10. The POs crystallized rapidly and stabilized in mixtures of  $\beta$  and  $\beta'$  crystals. The POs as it is, blended or restructured with vegetable oil via interesterification, and texturized, produced a trans-free hardstock with excellent oil binding capacity, and able to perform effectively as structural fat in the formulation of trans free reduced saturated solid fat products.

**Unexpected Selectivity in the Functionalization of Neat Castor Oil Under Benign Catalyst-free Conditions**

Latchmi Raghunanan\*<sup>1</sup>, and José M. Franco<sup>2</sup>, <sup>1</sup>Trent Centre for Biomaterials Research, Departments of Physics & Astronomy and Chemistry, Trent University, Canada; <sup>2</sup>Pro2TecS-Chemical Product and Process Technology Research Centre. Departamento de Ingeniería Química, Facultad de Ciencias Experimentales, Universidad, Spain

*Abstract Pending*



**BIO 2: Biocatalysis II**

*Chairs: Lu-Kwang Ju, University of Akron, USA; and Masashi Hosokawa, Hokkaido University, Japan*

**Better Understanding of Enzymatic Soy Processing through Modeling Monomeric Sugar Release.** S.M. Mahfuzul Islam and Lu-Kwang Ju,  
<sup>1</sup>*The University of Akron, USA*

Soybean has been extensively used for oil production. After oil extraction, the major components of remaining bean mass (soybean flour) are protein (50%) and carbohydrate (30-35%). Removing carbohydrate gives protein-enriched and digestibility-improved products for feed and food uses, and the carbohydrate converted into monomeric sugars are good fermentation feedstock. We have selected *Aspergillus niger* strains and produced enzyme mixtures of suitably proportioned cellulase, xylanase, pectinase,  $\alpha$ -galactosidase and sucrase for this purpose. Better understanding of how enzyme compositions affect the hydrolysis of different soybean flour carbohydrates (cellulose, hemicellulose, pectin and galactooligosaccharides) is important for further improvement. This has been investigated in this study by modeling the effect of enzyme composition on kinetic release of individual monomeric sugars from different carbohydrate components. The models are valuable for understanding the hydrolysis mechanisms and for progressing toward complete hydrolysis of all types of soybean carbohydrates.

**Biosynthetic Pathways of Functional Carotenoids in Red Seaweed *Pyropia yezoensis***  
Masashi Hosokawa\*, *Hokkaido University, Japan*

*Pyropia yezoensis* is a red seaweed, which is a popular marine food product as “Nori” in eastern Asia. It contains lutein and  $\beta$ -carotene as functional nutrients. However, little is known about carotenoid biosynthetic pathways in red seaweeds including *P. yezoensis*. In this study, we analyzed total carotenoids and discussed their biosynthetic pathways in *P. yezoensis*. Carotenoid fractions were separated from total lipid of *P. yezoensis*. LC-MS and 1H-NMR analysis elucidated biosynthesis of  $\alpha$ -cryptoxanthin and zeinoxanthin as well as  $\alpha$ -,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin and lutein. Furthermore, lutein epoxide was identified as a novel carotenoid in *P. yezoensis*. These results suggest *P. yezoensis* has two biosynthetic pathways from  $\alpha$ -carotene to lutein via  $\alpha$ -cryptoxanthin and zeinoxanthin, and unique metabolic pathway to lutein epoxide from lutein by epoxidase.

**Production of Microbial Lipids using Crude Glycerol** Eiji Sakuradani\*, Naomi Murakawa, and Takaiku Sakamoto, *Tokushima University, Japan*

In recent years, biodiesel fuels are produced using waste vegetable oils as raw materials, but a large amount of crude glycerol is generated as a byproduct at that time. Efficient utilization of crude glycerol is desired. In this study, we tried to isolate microorganisms that efficiently utilize crude glycerol and to evaluate their lipid productivities. We tried to isolate highly

concentrated glycerol-resistant microorganisms from soil and wastewater. As a result, three filamentous fungi were isolated and named N1, N3 and W1 strains. Three strains grew most well when the crude glycerol was 10% in the culture medium, and the amount of total fatty acids reached 4 to 5 mg/ml. The major constituent fatty acids were oleic acid and linoleic acid in all strains.

#### **Alteration of Lipase Selectivity by Protein**

**Engineering** Katja Zorn<sup>1</sup>, Isabel Oroz-Guinea<sup>1</sup>, Henrike Brundiek<sup>2</sup>, and Uwe T. Bornscheuer<sup>\*3</sup>,  
<sup>1</sup>*Institute of Biochemistry, Germany*; <sup>2</sup>*Enzymicals AG, Germany*; <sup>3</sup>*University of Greiswald, Germany*

Abstract pending

#### **Efficient Production of MLCT Oils by Lipase**

**Reactions** Yutaro Kataoka\*, Yoshihiro Ueda, and Hidetaka Uehara, *The Nisshin OilliO Group, Ltd., Japan*

Lipase catalyzed inter-esterification is an excellent method to make a functional structure lipid. It can be conducted at normal temperature and pressure, so deterioration of reactants can be reduced and side reaction can be controlled. Moreover, characteristic structure lipids can be created by lipase's position specificity of esterification. Examples of practical application include cacao butter equivalent for the purpose of improving physical properties and medium and long chain triacylglycerol (MLCT) for nutritional improvement. However, the inter-esterification reaction using lipase has been practically used only in part. One of the major reasons is the cost. Maintaining stability during prolonged reaction is a major subject, as lowering enzyme stability directly leads to increase in cost. However, enzymatic reactions are delicate, so there are

hurdles to fix the proper reaction system and conditions. In these issues, we examined from two points, with a view to manufacturing MLCT. The first plan is to increase the activity of the enzyme itself. We succeeded in enhancing its activity by certain pretreatment to the enzyme. Secondly, by optimizing the reaction system, we were able to suppress the decreasing enzyme stability. We established continuous reaction system by examination of various reaction conditions. We also clarified that the enzyme stability is maintained by pretreating the reaction substrate. From the above studies, we were able to enhance the activity of the enzyme and we achieved the design of the system for stable production. As a result, we have been able to manufacture a high quality MLCT with low cost.

**Preparation of Diethylhexyl Adipate by Lipase-catalyzed Esterification** In-Hwan Kim<sup>\*1</sup>, TaeHoon Kim<sup>2</sup>, Heejin Kim<sup>3</sup>, and Nakyoung Choi<sup>2</sup>, <sup>1</sup>*Korea University, Republic of Korea*; <sup>2</sup>*Korea University, South Korea*; <sup>3</sup>*Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea*

Diethylhexyl adipate (DEHA) has been used for various applications in the chemical industry. Especially, it is used as a plasticizer for polyvinyl chloride and its polymers. In this study, DEHA was synthesized successfully from dimethyl adipate or adipic acid with ethylhexanol via lipase-catalyzed esterification under vacuum condition. An immobilized lipase from *Candida antarctica* (Novozym 435) was used as a biocatalyst in these reactions. Optimum reaction conditions for the synthesis of DEHA from dimethyl adipate and ethylhexanol were a temperature of 50°C, an enzyme loading of 2.5% (based on the total weight of substrate), a

vacuum of 10 torr, and a molar ratio 1:2.5 (dimethyl adipate to ethylhexanol), respectively. Optimum reaction conditions for the synthesis of DEHA from adipic acid and ethylhexanol were a temperature of 50°C, an enzyme loading of 5%, a vacuum of 50 torr, and a molar ratio 1:2.5 (adipic acid to ethylhexanol), respectively. The yield of DEHA was improved significantly and 100% yield of DEHA was obtained, when vacuum was applied.

#### **Enzymatic Preparation of Medium- and Long-chain Diacylglycerols of High Purity in Combination with Solvent Extraction**

Guanghui Li<sup>1</sup>, Jiazi Chen<sup>1</sup>, Zhen Zhang<sup>2</sup>, Ying Li<sup>3</sup>, and Yong Wang<sup>\*4</sup>, <sup>1</sup>Dept. of Food Science and Engineering, Jinan University, Guangzhou, China; <sup>2</sup>South China University of Technology, China; <sup>3</sup>Guangdong Saskatchewan Oilseed Joint Laboratory, Dept. of Food Science and Engineering, Jinan University, China; <sup>4</sup>Jinan University, China

High purity diacylglycerols (DAG) rich in medium chain diacylglycerols (MCD) and medium- and long-chain diacylglycerols (MLCD) were prepared via the enzymatic esterification of monoacylglycerols (MAG) with caprylic acid followed by molecular distillation (MD), solvent fraction and low-temperature centrifugation. The content of DAG in the crude product was 44.8±0.1%, under the selected esterification conditions, which were MAGs/caprylic acid mole ratio of 1:3, reaction temperature of 65°C, reaction time of 30 min and enzyme load of 5 wt.%. Subsequently, the one-step MD and solvent fraction in methanol/ethanol increased the DAG content to 61.3±0.8%. Eventually, the product containing 86.6±0.6% of DAG with 39.3±1.3% of MCD and 47.3±0.6% of MLCD was obtained by the methanol crystallization at 0°C with a water content of 9 wt.% and a 1:3 ratio of

glycerides/methanol (v/v) followed by centrifugation separation.

#### **Stearidonic Acid Soybean Oil – Concentration and Enzymatic Modification** Casimir C. Akoh\*, University of Georgia, USA

High stearidonic acid (SDA, 18:4 n-3) soybean oil (SDASO) is a genetically modified soybean oil intended as a sustainable plant source of n-3 polyunsaturated fatty acid (n-3 PUFA). SDA is a metabolic intermediate in the conversion of  $\alpha$ -linolenic acid to EPA and DHA. SDASO (~23% SDA) was concentrated to yield SDA-enriched triacylglycerol (TAG), diacylglycerol (DAG) and monoacylglycerol (MAG) for possible applications as functional food components. Soybean oil enriched with SDA could be added to the diet to increase n-3 PUFA intake. The SDA content of the original SDASO was increased in several ways: low temperature crystallization/winterization in solvents, lipase-catalyzed hydrolysis, and lipase catalyzed synthesis of structured lipids (SL). SDA yield (82.3%) was attained by performing winterization of FFA in hexane at 10% oil:solvent ratio for 24 h. For TAG, the SDA yield (35.1%) was achieved by using hexane:acetone (10:90, v/v) at 10% oil:solvent ratio and 24 h winterization. For lipase catalyzed reactions, Amano AY lipase was used to hydrolyze previously SDA enriched TAG (48.7% SDA) obtained from low temperature crystallization of SDASO. The best SDA content of TAG (58.6%) was obtained with Amano AY lipase at 2 h incubation (52% hydrolysis). A combination of chemical and enzymatic hydrolysis and acidolysis reaction yielded a TAG with 60% SDA content.

**Enzymatic Preparation of Monogalactosyldiacylglycerols Containing Pinolenic Acid** Byung Hee Kim\*, *Sookmyung Women's University, Korea*

The aim of this study was to enzymatically prepare monogalactosyldiacylglycerols (MGDG) containing pinolenic acid (PLA) with potential appetite suppression effects. PLA-enriched free fatty acid (FFA) mixtures containing ~86 mol% PLA was produced from an FFA fraction obtained from pine nut oil (PLA content, ~13 mol%) by a urea crystallization using methanol and urea-to-FFA weight ratio of 4:1. A commercial MGDG was acidolyzed with PLA-enriched FFA mixtures in acetone using Lipozyme RM IM as the biocatalyst. Optimal reaction conditions were: temperature, 25°C; substrate molar ratio, 1:30 (MGDG:PLA-enriched FFA mixtures); enzyme loading, 20 wt% of total substrates; reaction time, 36 h. An MGDG containing 42.3 mol% PLA was obtained under these conditions.

**ELOVL6 Catalyzes Elongation of n-13:0 and n-15:0 Odd Chain Saturated Fatty Acids in Human Cells.** Zhen Wang<sup>1,2</sup>, Dong Hao Wang<sup>1</sup>, Yuliya Goykhman<sup>1</sup>, Yuanyuan Yan<sup>1</sup>, Peter Lawrence<sup>1</sup>, Kumar S. D. Kothapalli<sup>2</sup>, and J. Thomas Brenna<sup>2</sup>, <sup>1</sup>*Cornell University, USA*; <sup>2</sup>*University of Texas at Austin, USA*

Objective/Hypothesis: Normal odd chain saturated fatty acids (OCSFA), particularly tridecanoic acid (n-13:0), pentadecanoic acid (n-15:0) and heptadecanoic acid (n-17:0), are normal components of dairy, beef and seafood,

and their appearance in plasma is often used as a marker for ruminant fat intake. Human elongases encoded by ELOVL1, ELOVL3, ELOVL6, and ELOVL7 catalyze biosynthesis of the dominant even chain saturated fatty acids (ECSFA), however, there are no reports of elongase function on OCSFA. The objective of this study is to establish specificity of the ELOVL responsible for elongation of OCSFA of quantitative significance in the human diet. Methods Used: To test ELOVL (ELOVL1, 3, 6 and 7) function we constructed expression vectors and transiently transfected them individually into MCF7 cells as a human cell host. ELOVL transfected MCF7 cells were treated with n-13:0, n-15:0, or n-17:0 (80 µM) and products were analyzed by GC-FID and GC-MS. Results: ELOVL6 is the primary ELOVL with activity toward OCSFA, specifically catalyzing n-13:0→n-15:0 and n-15:0→n-17:0. Further, ELOVL7 has moderate activity toward n-15:0→n-17:0 and similarly, ELOVL3 also catalyzes n-13:0→n-15:0. ELOVL1 had no activity toward any OCSFA, and no ELOVL catalyzed n-17:0→n-19:0, consistent with the trace amount of n-19:0 in human tissue. Conclusions: Our data expand ELOVL specificity to OCSFA, providing the first molecular evidence demonstrating ELOVL6 as the major elongase acting on OCSFA (n-13:0 and n-15:0). Studies of food intake relying on OCSFA as biomarker should consider endogenous human metabolism when relying on OCSFA ratios to indicate specific food intake.

**BIO 2.1/H&N 2: Dietary Lipids and the Gut Microbiota**

*Chairs: Barry Tulk, DuPont Nutrition & Health, USA; and Jun Ogawa, Kyoto University, Japan*

**Effect of Diet on the Gut Microbiota** Joanne Slavin\*, *University of Minnesota, USA*

The importance of the gut microbiota for health has long been appreciated. Carbohydrates resistant to digestion and absorption are fermented in the large intestine resulting in the production of short chain fatty acids that are used as gut fuel. Additionally, dietary components beyond carbohydrates are known to alter the gut microbiota and these alterations in the gut microbiota may have health benefits. Dietary fiber, prebiotics, and probiotics are the most studied dietary components that change the gut microbiota. Whether other dietary components, such as proteins and fats alter the gut microbiota is currently hotly debated. New analytical methods have made it possible for consumers to examine the composition of their own gut microbiota, although there are no accepted standards for a “healthy” gut microbiota pattern. Links to disease outcomes, such as obesity, are also driving interest in changing the gut microbiota. Animal studies finding negative changes in the gut microbiota with emulsifiers is an area of concern for oil chemists. Of course, most lifestyle factors, such as exercise, smoking, stress, sleep, BMI, etc also affect the gut microbiota and make the topic controversial and difficult to study.

**Interaction Between Diets and Gut Commensal Bacteria in the Regulation of Immunological Health and Diseases** Jun Kunisawa\*, *NIBIOHN, Japan*

It is well recognized that diets regulate host immune responses. Additionally, accumulating

evidence has revealed a pivotal role of gut commensal bacteria in the regulation of various host biological responses including immunity. In these processes, diets affect the composition and function of gut commensal bacteria and reciprocally gut commensal bacteria is involved in the digestion of diets to consequently produce either useful or harmful metabolites. We have studied the effect of fatty acid composition in dietary oils on the regulation of host immune responses, showing that fatty acid compositions in the dietary oils affect the incidence of allergic and inflammatory diseases. Metabolome and immunological analyses allowed us to identify anti-allergic lipid metabolites and to understand the underlying mechanism. Our recent studies demonstrated that not only host metabolic pathways but also commensal bacteria contribute to both production of anti-allergic and anti-inflammatory lipid metabolites. In this talk, I describe recent findings regarding the immunologic crosstalk between commensal bacteria and dietary oils in the regulation of host immunity and its influence on the development of allergic and inflammatory diseases.

**Role of Bile Acid in Gut Microbiota Alterations in Rats Fed a High-fat Diet** Atsushi Yokota\*, Masamichi Watanabe, Satoshi Ishizuka, and Satoru Fukiya, *Research Faculty of Agriculture, Hokkaido University, Japan*

Mechanisms underlying gut microbiota alterations by high-fat diet (HFD) intake remain unclear. We hypothesized that bile acids (BAs) are involved in this mechanism as BA excretion increases on an HFD and can be a selective



pressure due to their strong antimicrobial activity (=BA hypothesis). We thus tried to verify this hypothesis. We first demonstrated that BA is a host factor that regulates cecal microbiota in rats by feeding experiments. Feeding cholic acid (CA), a common BA in rodents and humans, increased cecal BA concentrations, especially highly bactericidal deoxycholic acid (DCA), by bacterial conversion. Cecal microbiota analysis revealed a significant increase in Firmicutes and a decrease in Bacteroidetes, which were typical alterations reported in HFDs intake. In separate experiments, we found that DCA, common to both humans and rodents, and rodent-specific  $\beta$ -muricholic acid exhibit strong antimicrobial activity. Then, effects of a high-lard-diet feeding on microbiota and BA compositions in rat cecum were investigated. Similar microbiota changes to those observed in the CA-fed rats were detected, which accompanied increases in cecal BA concentrations including both DCA and  $\beta$ -muricholic acid. Examination of DCA sensitivity of the bacterial isolates revealed significantly higher resistance in Firmicutes than in Bacteroidetes. Several operational taxonomic units showed significantly positive (Firmicutes) or negative (Bacteroidetes) correlations to the cecal BA concentrations. Some of them were also significantly increased or decreased accordingly in the CA-fed rats. These results strongly supported BA hypothesis and will contribute to elucidating the relationship between HFD-induced gut microbiota alterations and onsets of metabolic disorders.

### **Correlation Between Dietary Lipid, Gut**

**Microbiota and Health** Jun Ogawa\*, *Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto University, Japan*

Prevalence of metabolic syndrome has stimulated interest in fat metabolism not only by host but also by gut microbiota. Polyunsaturated fatty acids derived from dietary lipids were found to be saturated by gut microbes. We revealed the metabolism in gut lactic acid bacteria, *Lactobacillus plantarum*. The enzyme system was found to consist of four enzymes. The concerned action of these enzymes, i.e., hydratase, dehydrogenase, isomerase, and enone reductase accomplish the saturation and generated hydroxy fatty acids, oxo fatty acids, and conjugated fatty acids as intermediates. We confirmed the existence of these fatty acids in host tissues depending on the existence of gut microbes and evaluated their physiological activity.

1) 10-Hydroxy-cis-12-octadecenoic acid (HYA) ameliorates sulfate sodium-induced colitis in mice by recovering the damage of intestinal epithelial barrier. 2) Oral administration of HYA induced insulin secretion by increasing GLP-1 level. 3) HYA elicited anti-inflammatory effects in vitro in murine enterocytes. 4) 10-Oxo-cis-12-octadecenoic acid (KetoA) induced adipocyte differentiation via the activation of PPARgamma, and increased adiponectin production and insulin-stimulated glucose uptake. 5) Hydroxy fatty acids and oxo fatty acids suppressed fatty acid synthesis by regulating LXR. 6) Enone fatty acids enhanced cellular antioxidative responses preventing multiple diseases induced by oxidative stress. These observations suggest that the dietary fatty acid metabolites by gut microbiota can influence the health of the host.

**Dietary Fatty Acid Metabolism in Gut**

**Microbiota** Shigenobu Kishino\*<sup>1</sup>, Akiko Hirata, Michiki Takeuchi<sup>1</sup>, and Jun Ogawa<sup>2</sup>, <sup>1</sup>Kyoto University, Japan; <sup>2</sup>Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan

Dietary fats are important for human as nutrition. In these days, dietary fats are found to be also important for human health. They are metabolized not only by human but also by gut microorganisms. We revealed two polyunsaturated fatty acid metabolisms in gut microbiota using lactic acid bacteria as model microorganisms. One of them is saturation metabolism in *Lactobacillus plantarum*<sup>1</sup> and the other is hydration metabolism in *Lactobacillus acidophilus*<sup>2</sup>. As to saturation metabolism, the enzyme system was found to consist of four enzymes (hydratase, dehydrogenase, isomerase, enone reductase) and generate hydroxy fatty acids, oxo fatty acids, and conjugated fatty acids as intermediates. The homologous genes encoding these four enzymes were found in genome sequences of many gut microorganisms. Therefore, acting in concert, gut microbiota may mediate the unsaturated fatty acid saturation metabolism in gastrointestinal tract. Furthermore, we confirmed the existence of these fatty acids in host tissues depending on the existence of gut microbes using specific pathogen free (SPF) mouse and germ-free mouse. Successive analysis revealed health promoting activity of these hydroxy and oxo fatty acids, i.e., intestinal epithelial barrier protection, anti-obesity, and anti-diabetic activity, etc. Therefore, we developed novel production system for these fatty acid metabolites using the enzymes from probiotic lactic acid bacteria. These studies could open a new application of gut microbial fatty acid metabolisms and their metabolites for health

promotions. 1) S. Kishino et al., *PNAS*, 110, 17808 (2013). 2) A. Hirata et al., *J. Lipid Res.*, 56, 1340 (2015).

**10-oxo-12(Z)-octadecenoic Acid, a Linoleic Acid Metabolite Produced by Gut Microbiota, Enhances Energy metabolism by Activation of**

**TRPV1** Tsuyoshi Goto\*<sup>1</sup>, Minji Kim<sup>2</sup>, Tomoya Furuzono<sup>2</sup>, Kunitoshi Uchida<sup>3</sup>, Shigenobu Kishino<sup>1</sup>, Haruya Takahashi<sup>2</sup>, Huei-Fen Jheng<sup>4</sup>, Jun Yamazaki<sup>5</sup>, Makoto Tominaga<sup>3</sup>, Jun Ogawa<sup>6</sup>, and Teruo Kawada<sup>2</sup>, <sup>1</sup>Kyoto University, Japan; <sup>2</sup>Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Japan; <sup>3</sup>Okazaki Institute for Integrative Bioscience, National Institute for Physiological Sciences, Japan; <sup>4</sup>Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Japan; <sup>5</sup>Department of Physiological Science and Molecular Biology, Fukuoka Dental College, Japan; <sup>6</sup>Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan

Gut microbiota can regulate the host energy metabolism; however, the underlying mechanisms that could involve gut microbiota-derived compounds remain to be understood. Recently, we found that gut microbiota produces several unique fatty acids from dietary polyunsaturated fatty acids in their saturation metabolism. Interestingly, levels of several these unique fatty acids were much higher in specific pathogen-free mice than in germ-free mice, indicating that these fatty acids are generated through polyunsaturated fatty acids metabolism of gastrointestinal microorganisms. Therefore, in this study, we investigated the effects of KetoA [10-oxo-12(Z)-octadecenoic acid]-a linoleic acid metabolite produced by gut lactic acid bacteria- on whole-body energy metabolism and found that dietary intake of KetoA could enhance

energy expenditure in mice, thereby protecting mice from diet-induced obesity. By using  $\text{Ca}^{2+}$  imaging and whole-cell patch-clamp methods, KetoA was noted to potently activate transient receptor potential vanilloid 1 (TRPV1) and enhance noradrenalin turnover in adipose tissues. In addition, KetoA up-regulated genes that are related to brown adipocyte functions, including uncoupling protein 1 (UCP1) in white adipose tissue (WAT), which was later diminished in the presence of a beta-adrenoreceptor blocker. By using obese and diabetic model KK-A<sup>y</sup> mice, we further show that KetoA intake ameliorated obesity-associated metabolic disorders. In the absence of any observed KetoA-induced antiobesity effect or UCP1 up-regulation in TRPV1-deficient mice, we prove that the antiobesity effect of KetoA was caused by TRPV1 activation-mediated browning in WAT. KetoA produced in the gut could therefore be involved in the regulation of host energy metabolism.

#### **Effects of Fatty Acid Metabolites by a Gut Lactic Acid Bacterium on Lipid Metabolism in NASH**

**Model Mice** Neng Tanty Sofyana<sup>\*1</sup>, Jiawen Zheng<sup>1</sup>, Yuki Manabe<sup>1</sup>, Yuta Yamamoto<sup>2</sup>, Shigenobu Kishino<sup>1</sup>, Jun Ogawa<sup>3</sup>, and Tatsuya Sugawara<sup>4</sup>, <sup>1</sup>Kyoto University, Japan;

<sup>2</sup>Department of Anatomy and Cell Biology, Wakayama Medical University; <sup>3</sup>Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan;

<sup>4</sup>Laboratory of Marine Bioproduct of Technology, Division of Applied Bioscience, Japan

Nonalcoholic steatohepatitis (NASH) is a common liver disease that occurs in people who drink little or no alcohol. The major characteristic of NASH is fat accumulation in the liver, along with inflammation and damage. Factors leading to progressive NASH and inflammation are not well understood, but oxidative stress can be a

possible factor for various liver diseases including nonalcoholic steatohepatitis. Oxo fatty acid, 10-oxo-11 (E)-octadecenoic acid (Keto C), produced by *Lactobacillus Plantarum* from linoleic acid, provided the potent cytoprotective effects against oxidative stress through activation of the Nrf2-ARE pathway (Furumoto et al., 2016. *Toxicology and Applied Pharmacology* 296; 1–9). The aim of the present study was to explore the preventive and therapeutic effects of gut microbial fatty acid metabolites in NASH model mice. Eight-weeks-old male mice, a NASH-cirrhosis-hepatocarcinogenic model, were divided into 3 experimental groups and fed as follows: 1) High-fat diet (HFD) (control); 2) HFD mixed with 0.1% 10-oxo-12 (Z)-octadecenoic acid (Keto A) and 3) HFD mixed with 0.1% Keto C. After 3 weeks, mice were sacrificed. Plasma were used for biochemical analysis and livers were subjected to histological study, mRNA and protein expressions for multiple genes. Keto C increased the HDL cholesterol level in the plasma. There was hardly any difference of fat accumulation in histological study, however, there was no ballooning occurred in Keto C group. Keto C increased the expression level of HDL related genes following suppression of ROS related gene. These results indicated that Keto C has a potent effect in NASH model mice.

#### **Gut Microbiota and Free Fatty Acids Receptors Mediated Host Energy Regulation**

Junki Miyamoto<sup>\*</sup>, and Ikuo Kimura, *Tokyo University of Agriculture and Technology, Japan*

Metabolic disorders, such as obesity and diabetes, arise from disrupted energy homeostasis that depends upon the equilibrium between energy intake and expenditure. Gut microbiota has emerged as a pivotal,

multifactorial mediator in these disorders as it regulates host energy acquisition and metabolism while being modified by diet. Remarkably, the host nutrient-sensing mechanisms of gut microbial metabolites, in particular dietary fatty acids, have been significantly associated with the proneness to obesity and related disorders. Dietary fatty acids are an essential energy source and signaling molecules that regulate various cellular processes and physiological functions. Recently, several orphan G protein-coupled receptors were identified as free fatty acid receptors. FFAR1 and FFAR4 are activated by n-3 or n-6 polyunsaturated fatty acids such as dietary fish and vegetable oil, whereas FFAR2 and FFAR3 are activated by short-chain fatty acids produced by the gut microbial fermentation of dietary fiber. The critical role of gut microbial metabolites as signaling molecules via these FFARs has come to be appreciated, the attention has been focused on the proposed diet-gut microbiota-host homeostasis axis. Functional analyses have revealed that FFARs are critical for metabolic functions, such as peptide hormone secretion and inflammation, and contribute to energy regulation. In this lecture, we summarize the roles of gut microbial metabolites via FFARs in the host energy regulation and present an overview of the current understanding of its physiological functions. We believe that these will provide valuable insights into therapeutic targets for treating metabolic disorder such as obesity and diabetes, and the use of prebiotics to control gut microbiota.

**Effects of the Intake of a Gut Microbial Linoleic Acid Metabolite, 10-hydroxy-cis-12-octadecenoic Acid (HYA), on postprandial Hyperglycemia** Yasunori Yonejima\* and Kohey Kitao, *Nitto Pharmaceutical Industries, LTD.*, Japan

10-Hydroxy-cis-12-octadecenoic acid (HYA) is a gut microbial metabolite derived from linoleic acid, which is a main ingredients of vegetable oil. Several physiological functions of HYA have been reported, e.g., improvement of intestinal epithelial barrier functions (Miyamoto et al., *J. Biol. Chem.*, 2014), reduction of triacylglycerol levels in hepatocytes (Nanthirudjanar et al., *Lipids*, 2015), and protective efficacy against gastric *Helicobacter* infections (Matsui et al., *Helicobacter*, 2017). We now advance application studies of HYA toward industrialization to contribute to human wellbeing and enriched life. It is considered that the sudden elevation of blood glucose levels within several hours after meals damage blood vessels, thereby accelerating arteriosclerosis and increasing the risks of myocardial and cerebral infarctions. For these reasons, it is important even for people with prediabetes to take care of sudden changes in blood glucose levels after meals, and a substance that can be consumed routinely is expected to be effective for preventing diabetes mellitus. Therefore, in this study, the effect of intaking HYA-containing food on postprandial blood glucose level was evaluated via clinical trial. Sixty adults who were susceptible to increase in postprandial blood glucose levels were allowed to consume 50% purity-HYA-containing food and blood glucose levels after

meals were measured. Blood glucose AUC as the primary endpoint was significantly lower after the ingestion of HYA than placebo (Yonejima et al., Prog. Med., 2017). The result suggests that

HYA inhibits the elevation of postprandial blood glucose levels and is expected to have the effect of preventing diabetes mellitus



**BIO 2.2 / PRO 2: Advances in Enzyme Processing Technologies**

*Chairs: Xuebing Xu Wilmar (Shanghai) Biotech R&D Ctr. China; Flavio Galhardo USA*

**Overview and Recent Developments in Degumming, Interesterification and Biodiesel**

Hans Christian Holm\*, *Novozymes A/S, Denmark*

*Abstract not available.*

**Design and Synthesis of New Lipid Molecules by Assembling Nature Segments for Multi-functionalities. An Enzymatic Solution** Zheng Guo\*, *Aarhus University, Denmark*

Thanks to billions of years' evolution, the nature creates the largest biodiversity in both organism species and molecular structures, which afford a sustainable source of structural moieties in connection to activities and functionalities of interests. Over 90% of food ingredients or pharmaceutical excipients are fully or partially from natural molecules; therefore, design and synthesis of new lipid molecules using natural molecular segments as building blocks is an important pathway to acquire novel properties or deliver new functions in food, cosmetic and pharmaceutical industries. In this talk, I will report the concept and strategy in our lab to design and synthesize ultra-long chain fatty acid alcohol esters for cosmetic application; dual-function phenolic-containing DATEM-analogues as excipients for delivery of functionality; and surface-confined molecules as the barriers for prevention of lipid oxidation. We will show enzyme a green technology could optimally serve as a tool contributing to this chemo-enzymatic approach to minimize environmental impact. Not limited to the structural identification of the synthetic structure by MS, FTIT, <sup>1</sup>H/<sup>13</sup>C/<sup>31</sup>P NMR characterization and synthetic skills, an intensive discussion is given to the analysis of the

structure-function relationship by investigation of molecular packing, EPR measurement and AFM imaging etc., to demonstrate how a scientist could use the knowledge in lipid technology and biotechnology solve the technical challenges presented in food and cosmetic industries.

**Value and Potential of Phospholipase C Assisted Enzymatic Degumming in Vegetable Oils.** Ying Zha<sup>1</sup>, Arjen Sein<sup>2</sup>, Steve Gregory<sup>3</sup>, Greg LeFebvre<sup>4</sup>, and Michael Jung<sup>3</sup>, <sup>1</sup>DSM, *The Netherlands*; <sup>2</sup>DSM Biotechnology Center, *The Netherlands*; <sup>3</sup>DSM, *USA*; <sup>4</sup>DSM Food Specialties, *Inc, USA*

The Purifine<sup>®</sup> enzymatic degumming technology of DSM has been showing its value for the past decade. The technology, which uses phospholipase C to convert phospholipids into diglycerides (a form of oil) and a phosphate group, is economically beneficial and sustainable at the same time. It is economically beneficial, because the extra oil yield from diglyceride formation and reduced entrained oil; and it is sustainable, because it reduces the CFP of the entire degumming process. In more recent years, the application of Purifine<sup>®</sup> has been extending from expander soybean oil to canola, as well as flake soybean oil. Dedicated researches at different scales are constantly looking for process and product adaptations to match a specific application. One interesting process can be the sequential combination of PLC and PLA1, which is the enzymatic solution for both higher oil yield and low P value. As a consequence of increased oil yield, the enzymatic degummed gums have much lower volume. This means a less diluted

meal stream containing higher protein content. Besides, as these gums are rich in choline phosphate, they have probably higher nutritional value than the meal, and multiple potential applications.

**Enzymatic Interesterification** Chris Dayton\*,  
*Bunge Limited, USA*

Interesterification is a process that allows the modification of the melting characteristics of a blend of oils without changing the Fatty Acid Composition (FAC) of the original blend. Utilizing interesterification allows the manufacturer to produce a functional product with less saturated fat and with essentially no trans fatty acid. The traditional method for interesterification was to use a strong chemical to rearrange the distribution of the fatty acids in a random fashion with many unwanted side reactions. Commercial enzymes are now available that allow the same rearrangement without the use of harsh chemicals. I will review the Chemical Interesterification (CIE) process and then compare it to the various patented commercial Enzymatic Interesterification (EIE) processes.

**Enzymatic Modification of Menhaden Oil to Incorporate Caprylic and/or Stearic Acid** Sarah A. Willett<sup>\*1</sup>, Casimir C. Akoh<sup>1</sup>, and Silvana Martini<sup>2</sup>,  
<sup>1</sup>*University of Georgia, USA*; <sup>2</sup>*Utah State University, USA*

Structured lipids (SLs) of menhaden oil with caprylic (C8:0) and/or stearic acid (C18:0) were produced enzymatically. The molar ratios of acyl donor substrates were 1:1, 3:1, and 5:1 for C8:0, and 1:1, 2:1, and 3:1 for C18:0. Total mol% incorporation of C8:0 or C18:0 were plotted against molar ratios. Linear interpolation was used to estimate molar ratios that would yield SLs with 20 or 30 mol% incorporation of C8:0 or

C18:0. Enzymatic reactions were also conducted using different blend ratios of caprylic to stearic acid (40:60, 50:50, and 60:40) to produce low saturated fat SLs with melting point range of 25–35°C. Recombinant lipase from *Candida antarctica*, Lipozyme 435, and sn-1,3 specific *Rhizomucor miehei* lipase, Lipozyme RM IM, were compared as biocatalysts in these reactions. Total and sn-2 fatty acid compositions, triacylglycerol (TAG) molecular species, thermal behavior, volatile lipid oxidation products, and oxidative stability were compared. Medium-long-medium (MLM)-type TAGs were produced when C8:0 was the acyl donor, and the 3.03:1 and 4.58:1 ratios incorporated 20 and 30 mol% C8:0, respectively, for 24 h reaction time. A low saturated fat SL was produced when stearic acid was the acyl donor, and the 1.32:1 and 2.41:1 ratios incorporated 20 and 30 mol% C18:0, respectively, for 24 h reaction time. These SLs have the potential for use as nutraceutical due to the conservation of polyunsaturated fatty acids (PUFAs) at the sn-2 position. These SLs also may also be advantageous when formulating food products to reduce consumption of saturated fat and risk of cardiovascular disease.

**Cold Enzymatic Degumming on Sunflower Seed Oil** Ling Hua\*, and Alexey Shevchenko, *Alfa Laval Copenhagen A/S, Denmark*

Physical refining is the most economical refining process in terms of losses and utilities consumption. Degumming is key to overall process performance. Any variation of “special”, “deep”, “uni” degumming are quite demanding in terms of feed oil quality. Uniform feed oil quality with minimum variation is required for a stable process. Such a demand is less critical for an enzymatic degumming process. Even switching

feed from crude to water degummed oil does not required much change in the process. The process is still stable and easy to manage. That is a reason why enzymatic degumming becomes the preferred process for physical refining. For wax containing oils (sunflower, corn) dewaxing is required and in wet dewaxing (high speed separator based process) this is combined with neutralization or washing after neutralization (cold neutralization or cold washing). There are some benefits with this process, but soapstock handling issues are often pushing the industry towards physical refining. Alfa Laval has successfully combined enzymatic degumming with the wet dewaxing process for sunflower oil. Prolonged contact time between enzymes and oil in cold conditions shifts the reaction to the “safe area” when P content after first separator is below 5 ppm and washing does not bring value. Min. 75% waxes are removed simultaneously with the gums. If prolonged cold test is required (36h and longer) a polishing filtration step can be installed after the degumming. Filter aid consumption for this is drastically reduced compared to dry dewaxing process.

#### **How to Overcome the Barrier of Mucilage for Extraction of Omega 3 from Chia Oil?**

Gwendoline Gravé<sup>1</sup>, Sidrine Koumba<sup>1</sup>, Jean-Francois Fabre<sup>2</sup>, Eric Lacroux<sup>3</sup>, Muriel Cerny<sup>4</sup>, Romain Valentin<sup>5</sup>, Othmane Merah<sup>1</sup>, and Zéphirin Mouloungui<sup>4</sup>, <sup>1</sup>INP - ENSIACET, France; <sup>2</sup>LCA UMR1010 INRA-INP/ENSIACET, France; <sup>3</sup>Chimie Agro-Industrielle, France; <sup>4</sup>Laboratoire de Chimie Agro-Industrielle, France; <sup>5</sup>INRA, France

Chia seed is an oleoproteaginous seed and it is also covered by mucilage. This layer of polysaccharide thickens the medium when seeds are immersed in water complicating oleosomes extraction. This work aims to develop a process

to obtain mucilages, oil bodies and fibers from chia seeds. The first step consists of separating polysaccharides of seed's surface by sonication, in order to obtain mucilage<sup>1</sup>, and also to increase the accessibility to oleosomes. Once the polysaccharides layer removed oleosomes can be extracted grounding degumming seeds. Thanks to water and mechanical energy (high-shearing device and high pressure homogenizer) oil bodies as an emulsion<sup>2</sup> are obtained. These emulsions, stabilized by phospholipids and proteins<sup>3</sup>, are mainly composed of triglycerides as omega 3 (63%) and omega 6 (19%). By the addition of a enzymatic lipolysis step, oleosomes become a source of fatty unsaturated acid for production of omega-3 and omega-6 mono-glycerides by glycerolysis or esterification<sup>4</sup> reactions. Besides oil (20%), this process gives access to fibers (30%) and an aqueous phase rich in minerals (9%) and proteins (15%). These oleosomes reveal the potential for the generation of new platform of omega-3 biomolecules. All these compounds have properties for a wide range of applications (alimentation, health, pharmaceutical and cosmetics). 1. Castejón, N. et al. J. Agric. Food Chem. 65, 2572–2579 (2017). 2. Fabre, J.-F., et al. OCL 22, D607 (2015). 3. Deleu, M. et al. Colloids Surf. B Biointerfaces 80, 125–132 (2010). 4. Wallis, C. et al. Waste Manag. 60, 184–190 (2017).

#### **Pilot Enzymatic Production of Medium- and Long-chain Triacylglycerols Using a Solvent-free Packed Bed Reactor Yong Wang\*, Jinan University, China**

*Abstract not available.*

**Lipid Modification by Enzymes and Engineered Microbes** Uwe T. Bornscheuer\*, *University of Greiswald, Germany*

This lecture will provide an overview about recent developments for the use of enzymes as well as microorganisms in the modification of fats and oils [1,2]. Beside the well-established lipases and phospholipases, more recently also CoA-independent acyltransferases as well as isomerases/hydratases and decarboxylases became important for lipid modification. Furthermore, the combination of isolated enzymes in cascade reactions is relevant to make more complex products at higher overall yields. In addition to isolated enzymes, many microorganisms are used to produce biotensides such as sophoro- and rhamnolipids. The recent major developments in metabolic engineering enabled the creation of tailor-designed microorganisms useful for the production of e.g. EPA, wax esters or drop-in biofuels from fats/oil and glucose as renewable resources. [1] Bornscheuer, U.T., Ed. (2018), *Lipid modification by enzymes and engineered microbes*, AOCS press. [2] Bornscheuer, U.T. (2018), *Enzymes in lipid modification*, *Ann. Rev. Food Sci. Technol.* DOI: 10.1146/annurev-food-030117-012336

**Recent Progress of Enzymatic Synthesis of Polymers** Douglas G. Hayes\*, *University of Tennessee, USA*

Hydrolases, particularly lipases, have been proven to be useful enzymes in oleochemistry, including the ability to form polyesters from multifunctional fatty acids and alcohols. The first papers came out in this area in the late 1980s. The research field has continued to expand, to take advantage of newer solvent systems such as ionic liquids and deep eutectic solvents to enhance miscibility, and newer multifunctional molecules such as phenolics (e.g., caffeic acid and cardanol), amino acids, functionalized fatty acids (e.g., via reactions across double bonds and metathesis), polyols, biodiesel co-products (e.g., glycerol carbonate and polyglycerol) and carbohydrates. In addition, the ability to add polymerizable groups to oleochemical synthons, such as the vinyl or acrylate group, via biocatalysis provides a broad platform for chemical synthesis of the modified monomeric units. Recent studies of enzymatic or chemo-enzymatic synthesis of polymers will be reviewed, with applications in lubrication, materials, foods, nanotechnology and other areas.

**BIO 3: PUFA Biotechnology**

*Chairs: Suk-Hoo Yoon, Woosuk University, Republic of Korea; and Tsunehiro Aki, Hiroshima University, Japan*

**Lipase-catalyzed Butanolysis of Echium Oil for the Selective Enrichment in Gamma-linolenic and Stearidonic Acids** Marta C. Corzo-Martinez<sup>1</sup>, Eduardo López<sup>2</sup>, Luis C. Vazquez<sup>1</sup>, Elena Ortego<sup>2</sup>, Erika Olaya<sup>2</sup>, Guillermo Reglero<sup>1</sup>, and Carlos Torres<sup>\*1</sup>, <sup>1</sup>*University Autonoma of Madrid, Spain;* <sup>2</sup>*Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL, CSIC-UAM), Spain*

Hypothesis Enzymatic butanolysis of Echium oil in the presence of *Candida rugosa* and *Rhizopus oryzae* has been studied. Fatty acid selectivity of immobilized *Rhizopus oryzae* lipase towards different fatty acids from Echium oil was investigated. Methodology Powder and immobilized forms of both lipases were compared. Addition of water to the reaction mixture played an important role in the process. Competitive factors for each fatty acid were determined using oleic acid as the reference fatty acid. Reutilization of the biocatalyst in two consecutive trials was carried out to determine the inactivation rate constant (kd) Results Utilization of butanol containing 2.5% w/w of water produced the best results. Complete conversion of triacylglycerols was only achieved with immobilized lipase of *Rhizopus oryzae*. On the contrary, no activity was observed when commercial immobilized lipase from *Candida rugosa* was utilized. Clarification of the oils played a crucial role in the stability of the immobilized lipase from *Rhizopus oryzae* with a kd of 0.0008 h<sup>-1</sup> which indicates that 905 h are required to reach the half life of the lipase (50% of the original activity). Finally, the determined

competitive factors were one order of magnitude lower for stearidonic and gamma-linolenic acid than those calculated for oleic acid, which indicates a very important discrimination of this lipase against these two fatty acids. Conclusions Very good activity, stability, and selectivity of the immobilized lipase from *Rhizopus oryzae* was observed in butanolysis reactions of Echium oil which indicates a plausible industrial application of this bioprocess.

**Efficiency Improvement in the Enzymatic Fractionation of PUFA** Yomi Watanabe<sup>\*1</sup>, Ryosuke Hoshina<sup>2</sup>, Kazumi Katagiri<sup>2</sup>, and Hideaki Kobayashi<sup>2</sup> <sup>1</sup>*Osaka Research Institute of Industrial Science and Technology, Japan;* <sup>2</sup>*Kewpie Corporation, Japan*

The supplementation of n-3 polyunsaturated fatty acids (PUFA) has been widely known to bring various health benefits. Pure 20:5 ethyl ester (EPA-EE) is approved as medicine in Japan and in the US for hyperlipidemia and arteriosclerosis obliterans, while 22:6 (DHA) concentrates are marketed as supplements to prevent cardiovascular diseases, dementia, cognitive disorders, and others. EPA-EE is currently produced industrially by chemical conversion of sardine oil to FAEE, followed by the fractionation and purification by MPLC and/or the rectification. Here, fractionation of EPA and DHA requires careful operation due that the two have similar chemical properties. Thus, the prefractionation step to roughly separate the two in the material oil would improve the efficiency of the final purification steps. Lipase treatment is



good to this end because lipase could discriminate PUFA from other FA and more preferably, EPA from DHA. It was found that the enzymatic treatment at relatively low temperature (5°C) of sardine oil with 3 mol equivalent of ethanol, added stepwise, improved the segregation efficiency of EPA from DHA without significant decrease in the EPA recovery in FAEE fraction, compared to the treatment at 35°C. By the procedure, DHA was recovered mainly in the acyl glycerol fraction. Middle chain (M) FA was enzymatically introduced to the AG fraction to produce MDM type of structured lipid.

#### **Engineering *Yarrowia lipolytica* for the Production of Fatty Alcohols from Sugars and Fats**

Michael Spagnuolo, Murtaza Shabbir Hussain, and Mark Blenner\*, *Clemson University, USA*

Fatty alcohols currently find use in areas such as surfactants, plasticizers, lubricants, fuels, and the cosmetics industry; however, traditional production methods rely on petroleum-derived compounds or the low-yield harvest from plants. Recently, conventional hosts including *E. coli* and *S. cerevisiae* have been used for fatty alcohol production with some success. Oleaginous yeast, such as *Yarrowia lipolytica*, offer significant advantages as a production host for oleochemicals, as the native metabolism already has high flux through its fatty acid biosynthetic pathway; however, efforts to engineer significant fatty alcohol production in this organism have been unsuccessful. Fatty alcohol production in the cytoplasm is hindered by low acyl-CoA availability and competing reactions. As a result, we have targeted fatty alcohol production to the peroxisome, where acyl-CoA flux is directed during beta-oxidation, and where there are

comparatively fewer potential competing reactions. Several acyl-CoA reductases from bacterial and mammalian sources were screened. Gene knockouts and overexpressions were investigated with a recently developed peroxisomal fatty acid sensor for *Y. lipolytica*. Strains with improved peroxisomal fatty alcohol production were found to lack cytoplasmic acyl-CoA synthetase activity. When expressing acyl-CoA reductase, a strain identified through sensor based screening resulted in over 500 mg/L without additional optimization. We will further report on our recent efforts to improve titer and alter the fatty alcohol chain length profile, using both sugar and lipid substrates.

#### **Production of Various PUFAs by Filamentous Fungus *Mortierella alpina***

Eiji Sakuradani<sup>1</sup>, Akinori Ando<sup>2</sup>, Sakayu Shimizu<sup>3</sup>, and Jun Ogawa<sup>2</sup>, <sup>1</sup>*Tokushima University, Japan*; <sup>2</sup>*Div. Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan*; <sup>3</sup>*Kyoto University, Japan*

The filamentous fungus *Mortierella alpina* 1S-4 accumulates a large amount of triacylglycerol containing C20 polyunsaturated fatty acids (PUFAs). Indeed, triacylglycerol production by *M. alpina* 1S-4 can reach 20 g/L of culture broth. The demonstrated health benefits of functional PUFAs have in turn encouraged the search for rich sources of these compounds, including fungal strains showing enhanced production of specific PUFAs. Screening for mutants and targeted gene manipulation of *M. alpina* 1S-4 have elucidated the functions of various enzymes involved in PUFA biosynthesis and established lines with improved PUFA productivity. In some cases, these strains have been used for industrial-scale production of PUFAs, including MA, DGLA, ARA, EPA. In this

study, we described practical PUFAs production through mutant breeding, functional analyses of genes encoding enzymes involved in PUFAs biosynthesis, and recent advances in the production of specific PUFAs through molecular breeding of *M. alpina* 1S-4.

**Practical Eicosapentaenoic Acid (EPA) Production by *Mortierella alpina* Molecular Breeding under Ordinary Temperature** Akinori

Ando<sup>1</sup>, Yuki Takemoto<sup>2</sup>, Ryohei Nakatsuji<sup>3</sup>, Shigeru Hiramoto<sup>4</sup>, Eiji Sakuradani<sup>5</sup>, and Jun Ogawa<sup>1</sup>, <sup>1</sup>Div. Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan; <sup>2</sup>Nisshin Pharma Inc., Japan; <sup>3</sup>Kyoto University, Japan; <sup>4</sup>Nisshin Pharma Inc. Japan; <sup>5</sup>Tokushima University, Japan

*Mortierella alpina* 1S-4 is known to accumulate EPA only when cultivated at a low temperature. However, low temperature causes lower growth. Moreover, undesired other  $\omega$ 3 fatty acids are accumulated because of the broad substrate specificity of the endogenous  $\omega$ 3 desaturase. In this study, we screened for useful heterologous genes which showed rigid substrate specificity and were available even at an ordinary temperature (28°C) for specific EPA production by *Mortierella alpina* molecular breeding. We isolated an EPA-producing microorganism from soil and identified as *Pythium torulosum*. In addition, we selected *Pythium sulcatum* and *Plectospora myriandra*, the related species of *P. torulosum*, based on the high ratio of EPA to ARA. Subsequently, three putative  $\omega$ 3DS genes were isolated from the genomes of these strains (*Ptw3* from *P. torulosum*, *Psw3* from *P. sulcatum* and *PmD17* from *P. myriandra*). The functional analysis of these enzyme genes using yeast at 28°C revealed that *Ptw3* and *Psw3* convert 18- and 20-carbon  $\omega$ 6 fatty acids into  $\omega$ 3 fatty acids,

while *PmD17* only convert 20-carbon  $\omega$ 6 fatty acids. Each enzyme gene introduced into *M. alpina* also showed the same functions. Especially, EPA composition of transformants overexpressed *PmD17* reached at approximately 80% of total fatty acids in test tube scale. Furthermore, this transformant showed 14 g/L fatty acid productivity, composed 52% EPA, in 5L jar fermentor scale. *M. alpina* could be expected as a promising EPA-producer substituted for marine resources.

**Metabolic Engineering for Rare PUFA Production by an Oil-producing Fungus *Mortierella alpina***

Hiroshi Kikukawa<sup>\*1</sup>, Eiji Sakuradani<sup>2</sup>, Akinori Ando<sup>3</sup>, Sakayu Shimizu<sup>4</sup>, and Jun Ogawa<sup>3</sup>, <sup>1</sup>Gifu University, Japan; <sup>2</sup>Tokushima Univ, Japan; <sup>3</sup>Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Japan; <sup>4</sup>Kyoto Univ, Japan

The oil-producing fungus *Mortierella alpina* 1S-4 is known to accumulate a large amount of triacylglycerol containing beneficial C20 polyunsaturated fatty acids (PUFAs), such as arachidonic acid (ARA, 20:4 $\omega$ 6) and eicosapentaenoic acid. To develop an efficient gene targeting system in *M. alpina* 1S-4, we identified and disrupted the *lig4* gene encoding DNA ligase 4 involved in non-homologous end joining on genomic double strand breaks repair. The gene targeting efficiency in the  $\Delta$ *lig4* strain was improved from 3% to 67%. Here, to achieve high production of rare PUFAs by metabolic engineering, dihomog- $\gamma$ -linolenic acid (DGLA, 20:3 $\omega$ 6)-producing strains were constructed with deletion of  $\Delta$ 5-desaturase ( $\Delta$ 5ds) gene encoding a key enzyme of bioconversion of DGLA to ARA using the  $\Delta$ *lig4* strain as a host strain. The ratio of DGLA to total fatty acid in this disruptants reached about 40%, and in contrast, no ARA was

detected. Using the same methodology, Mead acid (MA, 20:3 $\omega$ 9)-producing strains were constructed with deletion of  $\Delta 12ds$  gene encoding a key enzyme involved in bioconversion of  $\omega$ 9-PUFAs to  $\omega$ 6-PUFAs. The disruptants showed no defects in growth and fatty acid production, but exhibited higher MA composition (8.4% of the total fatty acid), without accumulation of  $\omega$ 6- and  $\omega$ 3-PUFAs. From these results, the gene targeting in *M. alpina* 1S-4 is available for molecular breeding, and these disruptants are superior for practical production of rare PUFAs by *M. alpina* 1S-4.

**Lipase-polymer Nanoconjugates for Biosynthesis in Non-aqueous Media: Synthesis and**

**Characterization.** Bianca Perez<sup>1</sup>, Ana Moles<sup>2</sup>, Jannik Pedersen<sup>2</sup>, Steen V. Petersen<sup>2</sup>, Jan Skov S. Pedersen<sup>3</sup>, Adam Perriman<sup>4</sup>, and Zheng Guo<sup>2</sup>,  
<sup>1</sup>Dept. of Engineering, Aarhus University, Denmark; <sup>2</sup>Aarhus University, Denmark;  
<sup>3</sup>Interdisciplinary Nanoscience Center, Aarhus University, Denmark; <sup>4</sup>School of Cellular and Molecular Medicine, Bristol University, UK

Enzyme catalysis has been a prosperous attribute to clean manufacture and green process; however their low thermostability and poor activity in non-aqueous media, has restricts its extensive application. Furthermore, generic engineering for a hyperthermophilic enzyme is highly time/expenses consuming. In this context, solvent free enzyme-polymer surfactant nanoconjugates have challenge the view that enzymes require a water hydration sphere to be active, offering new opportunities for biosynthesis in no-aqueous media. Here in, we present the synthesis and characterization of novel lipases-polymer nanoconjugates containing different enzyme/polymer ratio. To yield the lipase variants, initially, 40 to 60% of the aspartic

and glutamic acid of the *Thermomyces lanuginose* lipase were modified using N,N'-dimethyl-1,3-propanediamine and carboiimide crosslinker chemistry. Later, the resulted lipase was respectively, made react with different ratio of polymer surfactant. The different enzymes were characterized by MALDI-TOF MS, differential scanning calorimetry, Fourier transform infrared spectroscopy, Circular Dichroism Spectroscopy in the FAR-UV, and Small-angle X-ray scattering. Moreover, the activity of the enzymes was assessed towards hydrolysis and alcoholysis. CD spectroscopy showed that once a 20% of surfactant (in relationship to the aspartic and glutamic acid of the enzyme) is added, the enzyme can recover its secondary structure. In addition, activity assays demonstrated that using 33% or 66% of polymer surfactant in relationship to ASP and GLU of the enzyme, yielded the highest activity in aqueous media. Thus, the most promising enzyme/polymer ratio was used to synthesize other lipase-polymer surfactant nanoconjugates. In summary, results demonstrated the potential of lipases-polymer nanoconjugates for biosynthesis of lipids in non-aqueous media.

**Extraction and Refining of Lipids Containing Arachidonic Acid from Single Cell Oil, *Mortierella* sp.** Suk Hoo Yoon\*, Woosuk University, Korea

An oleaginous fungus was isolated from soil and identified as *Mortierella* sp. (M-12) for producing arachidonic acid (AA). Cell disruption methods, extraction methods, and particle sizes of freeze-dried biomass were tested to achieve maximum extraction of total lipids and AA. M-12 grown in glucose yeast media at 25°C for 7 days contained 35.5% total lipid, and 47% of the total

lipid was AA. Maximum lipid extraction was achieved using a mixture of chloroform and methanol (2:1) as an extraction solvent. The smaller the particle size of the biomass, the better the lipid extraction yield was observed. Particle size of biomass was shown to more strongly affect lipid extraction than extraction time. The highest AA content was observed in the class of neutral lipids. The deacidified oil obtained was bleached, after degumming, using activated clay under a 50–100 mmHg vacuum. The bleaching conditions were partially optimized

as follows: activated clay, 1%, bleaching temperature 90°C, and treatment time 20 min. After bleaching, the color of bleached oil as determined by the Lovibond Tintometer, satisfied the specification for edible fats and oils. The bleaching process also decreased the contents of free fatty acids and phosphorus in the deacidified oil. The acid value of the bleached oil also satisfied the specification for edible fats and oils. It was early shown that the normal bleaching process can be used for the bleaching of heavily-colored microbial lipids for human consumption.

**BIO 3.1/PRO 3.1: Biodiesel**

*Chairs: Per Munk Nielsen, Novozymes A/S, Denmark; Casimir C. Akoh, University of Georgia, USA; and Anders Rancke-Madsen, Novozymes, Denmark*

**Improving Pre-treatment Efficiency of Oil Feedstock using Adsorbent Filter Aids** David Gittins, Li-Chih Hu, and Nathan Dias\*, *Imerys Filtration Minerals Inc., USA*

Physically refined vegetable oil and mixed feedstock are often pretreated to remove contaminants which cause issues in downstream production of biodiesel or edible oil. Adsorbents are typically used in conjunction with filter aid in a dry washing filtration step to reduce contaminants. On their own, adsorbents do a great job of removing phospholipids, soaps, metals, and other contaminants but often suffer the effects of short filtration runs or require a lot of adsorbent or filter aid to meet contaminant reduction and throughput needs. The purpose of this talk is to describe how hybrid adsorbent filter aids combine unique filtration kinetics and surface chemistry to reduce contaminants, reduce overall powder use, and increase filtration cycle in oil pre-treatment.

**Online Real-time Quality Control of Biodiesel using Near-Infrared Spectroscopy** Paul Dawson<sup>1</sup>, Yosra Allouche<sup>2</sup>, and Fabiano de Melas<sup>2</sup>, <sup>1</sup>*BUCHI Corporation, USA*; <sup>2</sup>*BUCHI NIR-Online GmbH, Germany*

Quality control of Biodiesel (B100) is of utmost importance and key parameters to be analyzed are commonly defined in ASTM D6751 and EN-14214. However, the chemical analysis of Biodiesel expensive, time-consuming and generates waste products. Here, we introduce a by-pass based online solution of non-invasive near-infrared (NIR) technology for simultaneous,

real-time measurements of e.g. acid value, ester content, methanol, monoglyceride, total glycerol and water content. In general analytical equipment to be utilized directly in a process stream needs to be capable of coping with harsh industrial environments such as vibrations or extreme climate conditions in case of outdoor installations. To circumvent these challenges NIR spectrometers based on robust diode array technology without any moving parts have been developed. Light emitted from a halogen source is transflected from the sample, collected and diffracted on a stationary grating. Spatially separated light is then detected by means of a diode array followed by automated chemometric analysis. Predicted values are displayed in the control room. Operators may therefore correct process deviations in real-time greatly reducing safety margins or out-of-specification batches leading to an increased overall production capacity. Moreover, the described analytical solution is in full compliance with ATEX II 2G Ex px II T4 and may thus be installed in potentially gas explosive environments.

**FFA Reduction and Production Control** Frankie Mathis\* and Bo Munk, *Tactical Fabrication LLC, USA*.

Tactical Fabrication (TF) has for more than a decade been using Resin Technologies to improve the Biodiesel production. In the past 4 years this has included the functionality of Resin Acid Catalyzed Esterification (RACE) of Free Fatty Acid (FFA) after the Novozymes Eversa enzymatic process. This add-on technology can



be used in any production, but become essential when working with ultra-high FFA feedstock like Brown Grease (BG) or Palm Fatty Acid Distillate (PFAD). The water produced in a BG (FFA >85%) reaction compare to UCO (FFA

### **Integrating Conventional and Enzymatic Approaches Towards Industrial Biodiesel**

**Production** Marcelo Cantele\* and Jose Vladimír de Oliveira, *Tranfertech Gestão de Inovações LTDA, Brazil*

Currently, large scale production of biodiesel has been mostly based on homogeneous, alkali catalysis. Nevertheless, biotechnological production of biodiesel with lipases has received growing consideration and compared to conventional alkali-catalyzed production, the enzymatic process is less energy intensive and produces higher-purity product with less downstream operations. Different from the conventional base-catalyst route, the enzymatic process is very tolerant to high acid and water contents hence allowing the use of unrefined, less expensive, high FFA, lower-grade oils and fats, such as soapstock, acid oils and grease strap. The use of the commercial low-cost, soluble free-lipase Eversa Transform 2.0, recently launched by Novozymes, has been tested and shown to be cost-efficient and sustainable at industrial level. However, plant integration of conventional and enzymatic approaches may be much more advantageous than stand-alone production units as relatively low acid charges (~10 wt%) may be pre-treated by enzymatic esterification allowing then direct inlet through alkali-catalyzed reactor and also olein generated in the basic-transesterification route would be welcome in the soluble free lipase-catalyzed reactor. The rationale of infrastructure for both routes should

be then explored to assure the best strategy towards keeping profit margins at the highest levels. This talk illustrates such scenario, taking into account a biodiesel production unit of around from ~ 100 ton/day, discussing a flow chart of an integrated base and enzyme-catalyzed processes. It is shown that the integrated plant approach may constitute a promising alternative to biodiesel manufacture and can contribute to make the enzymatic biodiesel feasible.

### **Enzymatic Esterification to Handle the FFA in Biodiesel Production** Per Munk Nielsen\*, *Novozymes, Denmark*

Enzymatic esterification to handle the FFA in biodiesel production P.M. Nielsen, A. Rancke-Madsen, H.C. Holm Free fatty acids (FFA) is a separate issue in the production of biodiesel. This goes for both the chemically and the enzymatically catalyzed biodiesel. The chemically biodiesel needs to treat the raw material to obtain a feed stock low enough for the Na-methylate process. This can be physical removal or esterification with acid catalyst. Both ways do have some significant drawbacks. For the biodiesel produced using enzymes as catalyst it is normal with 1–3% FFA in the crude biodiesel coming out of the enzyme reactor. There is one process—enzymatic esterification—that can esterify the FFA whether it is coming with crude oil or it in the crude FAME after the transesterification reaction. The way of controlling the process is by managing the chemical equilibrium which mainly must do with the methanol/water content in the reaction mixture. Several methods for making a production setup have been suggested over the years. This presentation will discuss the

alternative process options and emphasize the pros et contra of the processes and concluding which process is the optimum for esterifying the FFA to FAME.

**Liquid Lipases for Enzymatic Refining:  
Technical Advantages Beyond Green Technology**

Zheng Guo\*, Aarhus University, Denmark

Benefited from tremendous in genetic/protein engineering and fermentation technology, production of liquid enzymes could be achieved at high titer and high purity in low price, which promotes enzyme engineers and industries rethink the strategy and choice of free (liquid) enzymes for industrial application; particularly for production of low value but large quantity products. This work examined catalytic specificity and fatty acid selectivity of five liquid lipases C. antarctica lipase A and B (CAL-A/B), and lipase TL (T. lanuginosus), Eversa Transform and NS in ethanolysis of fish /microalgae oils with the aim to concentrate n-3 PUFAs into monoacylglycerols (MAGs) products. Lipase TL, Eversa Transform & NS entail a much faster reaction and produce higher MAGs yield (>30%); whereas CAL-A obtains the highest concentration of n-3 PUFAs/DHA/EPA into MAGs products (88.30%); followed by lipase NS (81.02%). <sup>13</sup>CNMR analysis indicates that CAL-B and lipase TL are sn-1,3 specific; but CAL-A and lipase Eversa Transform are non-regiospecific or weak sn-2 specific; which plausibly explains high enrichment effect of the latter two lipases. All liquid lipases are observed reusable for a certain times (lipase Eversa Transform up to 12 times), demonstrating their competitive advantage over immobilized form for industrial application because of their higher activity and cheaper operation cost. For a complete utilization of fatty acyls, a further

process is carried out after MAG enriched with high content PUFAs is isolated by short path distillation; where the side products are converted into biodiesels at high yield by the same liquid lipase system but different reaction conditions. Thus a complete value chain is created by liquid enzyme system.

**A New Enzymatic Biodiesel Polishing Process  
Based on Esterification of FFA into Methyl Esters**

Anders Rancke-Madsen\*, Novozymes, Denmark

The use of liquid *Thermomyces lanuginosus* lipase in biodiesel production has been a breakthrough as liquid lipase can handle crude feedstocks with any content of free fatty acids at low economical risks. However, equilibrium level around 2-3% FFA requires caustic polishing step and recycling of 7–8% of fatty matter to avoid compromising yields. A next generation FFA polishing process is based on a liquid version of Candida Antarctica lipase B. This lipase can operate at low water activity making it excellent for converting FFA into methyl esters and thus reaching low equilibrium levels of FFA, thus no or limited caustic FFA polishing is needed. The process is based on conventional technology only including reactor design and water removal techniques. Reaction conditions in lab on crude PFAD based methyl esters are 0.75 kg enzyme/ton oil, 10% w/w dry glycerol, 3.5% w/w dry methanol and 13 hours of reaction time at 104°F/40°C and 250 rpm. After reaction, heavy phase is separated off by centrifugation and methyl ester phase is added dilute caustic for 30 minutes followed by separation by centrifugation, 2% w/w water washing step and drying. The biodiesel yield is 99% with FFA < 0.1% in final biodiesel. Glycerol and methanol is recovered from heavy phase and reused. The

presentation will discuss the different process scenarios and key process parameters and document the overall process robustness with data from lab.

**Soapstock Acidulation using Carbon Dioxide**

Rusty Sutterlin\*, *Inventure Renewables, USA*

The Inventure Carbonate Process converts soapstock, refinery byproduct lipid, Fatty Acid Distillate (FAD), Lecithin or any other fatty material into Free Fatty Acids (FFA) using heat and carbonic acid, as opposed to the traditional mineral acid pathway. The FFA produced is then upgraded to the valuable Olein/Stearin products via downstream purification. The waste water

from this process can be concentrated and sold as an animal feed additive. The feedstock undergoes a continuous pre-treatment step prior to the acidulation with carbon dioxide. The material is then pumped to the acidulation batch reactor where carbon dioxide is introduced at medium pressures. After the reaction takes place, it is allowed to settle where any water present is decanted off and sent through a flash drum to recover carbon dioxide. The material is then routed to a combination of distillation/crystallization to product the final olein/stearin products.

**BIO 4: Plant and Algae Lipid Biotechnology and Genomics**

*Chairs: Jay Shockey, USDA, ARS, SRRC, USA; and Timothy P. Durrett, Kansas State University, USA*

**Genome Editing and Plant Agriculture** Daniel Voytas\*, *University of Minnesota, USA*

The ability to precisely modify plant genomes through homologous recombination (HR) promises to advance both basic and applied plant biology. However, even with the use of sequence-specific nucleases, which stimulate HR by creating targeted DNA double-strand breaks, there are only a handful of studies that report precise editing of endogenous plant genes. Our group has been focusing on two efforts to more effectively modify plant genomes through HR. In one, we are developing new vectors to deliver sequence-specific nucleases and DNA repair templates to plant cells. Specifically, we have been using gemini virus replicons, which function in both monocots and dicots, to amplify nuclease-encoding cassettes and DNA repair templates. In a second effort, we are attempting to achieve HR by either genetically manipulating DNA repair pathways or delivering nucleases and repair templates to cells proficient in HR. Progress on our efforts to optimize gene targeting strategies will be reported.

**Improving the World's Nutrition with Next Generation Canola Oils.** Lorin R. Debonte, Xinmin Deng, Richard Fletcher, Kristin P. Monser-Gray\*, Diliara Iassonova, and Willie Loh, *Cargill Inc., USA*

Over the past 30 years a sustainable supply of high oleic canola oil from Cargill has provided the world's leading food ingredient and food service customers with high stability performance and nutrition free of trans fatty acids. Focusing on consumer needs, a targeted innovation pipeline

involving the integration of trait discovery, plant breeding, contract farm production, crushing/refining, product development, and packaging has delivered leading yield and agronomic performance and a production platform for affordable specialty oils in the global market. Today consumers are increasing their focus on ingredients and the consumption of healthy dietary fats, including EPA DHA omega-3s for heart and brain health. To meet this demand we have partnered with BASF to soon launch the next innovation in canola, a sustainable source of EPA DHA omega-3 fatty acids. A land-based source of EPA DHA omega-3s will reduce harvest pressure on wild fish populations and ensure an inexpensive, nutritious, and delicious seafood supply for people everywhere. Because the production of EPA DHA omega-3s in canola partially uses the endogenous fatty acid biosynthesis machinery, a significant opportunity to improve the yield of EPA DHA omega-3s exists through modulation of endogenous genes. Additionally, in crop production systems, grain yield, oil content and environmental variability are important traits that must be optimized, and which interact with the production of EPA DHA omega-3s. Recent data on genomic and environmental interactions will be presented.

**Generation and Characterization of Multiple Mutated Oilseeds via CRISPR Cas9 Genome Editing** Jay Shockey, Catherine Mason, and Tien Thuy Vuong, *SRRC-ARS-USDA, USA*

The targeted gene editing technique known as CRISPR/Cas9 ('clustered regularly interspaced

short palindromic repeats'/CRISPR associated protein 9) has revolutionized plant biology, and many other fields of science in recent years. This technique holds immense promise as a disease-fighting tool among other medical applications, yet the power of this technique has not been comprehensively applied to the study of plant lipid metabolism. Combining CRISPR-based genome editing with existing molecular biology and plant transformation technologies creates the flexibility to achieve simultaneous elimination of one or more endogenous gene targets while simultaneously delivering one or more transgenes for overexpression. Such approaches allow us to complete complex metabolic engineering strategies in much shorter periods of time than was previously possible and to address some of the bottlenecks that have hampered metabolic engineering efforts for many years. We describe here genome editing tools have been incorporated into existing molecular engineering 'toolkits' and used to rapidly generate highly altered oilseed plant lines. These tools should drastically reduce the months or years of transformation, crossing, segregation, and genotyping that is currently necessary to achieve the same genetic pedigrees typically created using more traditional techniques. How such these and other similar plant lines may be used to produce high levels of unusual fatty acids and other novel chemical feedstocks will be discussed.

#### **CRISPR-Cas9 Genome Editing to Alter Oil Production in the Hexaploid Oilseed Crop**

**Camelina sativa** Jose A. Aznar-Moreno\*, and Timothy P. Durrett, *Kansas State University, USA*

*Camelina sativa* is often regarded as a new industrial oilseed crop due a wide range of

suitable agronomic properties. Therefore, camelina has been the recent focus of studies for the production of unusual lipids such as ricinolenic acid and acetyl-TAG. The production of these unusual lipids can be enhanced by the reduction of specific enzyme activities that compete with the introduced biosynthetic enzymes. Suppression of endogenous gene activity has been tackled by RNA interference (RNAi), resulting in the successful increase of acetyl-TAG levels. Nevertheless, the emergence of genome editing techniques such as CRISPR-Cas9 offer the ability to generate genetically stable camelina lines, as well as knocking out the three homeologues of each gene in the camelina allohexaploid genome. We targeted two camelina enzyme activities, diacylglycerol acyltransferase (DGAT) and phospholipid:diacylglycerol acyltransferase (PDAT) which can catalyze the last step for the synthesis of TAG competing with the synthesis of acetyl-TAG. We designed a guide RNA to target sequences conserved for the three homeologous DGAT1 or PDAT genes to determine whether all three copies can be silenced. In most of the lines, we demonstrate that all three DGAT1 or PDAT genes had been modified in the targeted region for the T1 plants. Further, analysis of T2 seed revealed alterations in the fatty acid profile and reductions in seed oil content consistent with mutations in the target genes. We demonstrate CRISPR-Cas9 is a suitable genome editing technique for improving the genome background and the synthesis of unusual oil.



**Advancing Genomic Solutions in Algae Biofuels and Bioproducts** Eric R. Moellering\*, *Synthetic Genomics, Inc, USA*

Algae have long been proposed as cell factories for the conversion of light and carbon dioxide into renewable biofuels and numerous other bioproducts. While algae are advantaged in their potential use of non-arable land and saline water supplies, improvements in strain performance are required for commercialization. In the case of algae derived bio-diesel, substantial improvements are needed in biomass productivity and lipid partitioning in mass outdoor culture. This presentation will focus on progress in the industrially relevant alga *Nannochloropsis* in i) leveraging genomics capabilities to develop state of the art genome editing tools, and ii) discovery and optimization of important traits for improving biomass productivity and carbon partitioning to lipid.

**Molecular Breeding Tools for Rapid Conversion of Cover Crop Pennycress into a Novel Oilseed Crop** Tim Ulmasov\*, *Arvegenix, USA*

Cover crops are widely recognized among the best solutions to agricultural sustainability problems such as soil erosion and waterways contamination. Yet, today, < 5% of farmers are using them to protect their farmland between the seasons. The reason is economic—it requires ~\$40/acre to grow and remove a cover crop from the field before the next planting. Farmers generally are not convinced that the following crop, such as corn or soy, will have increased yield, allowing them to recoup the investment. As a solution to this dilemma, Arvegenix is developing pennycress into an oilseed crop that can be used as a feedstock for food oil, animal feed and biofuel. If successful, it will result in a

first conversion of a cover crop into a cash crop, maintaining environmental benefits of the former, and generating additional income for the farmer from the latter, all without competing for land with other crops. Domestication of pennycress requires significant improvements in its agronomic and compositional characteristics. To accelerate the development of this novel oilseed crop, we are developing molecular tools that allow discovery and advancement of favorable traits faster and more efficiently. Genome-wide association mapping, shorter cycling times and metabolic pathway engineering will have dramatic effect on our ability to generate and introgress key traits, including compositional improvements of oil and meal. Arvegenix work on applying recent advancements in VIF, RNAi and genome editing, as well as leveraging extensive knowledge of biosynthetic/flowering time pathways in *Arabidopsis* to accelerate domestication of pennycress will be presented.

**Employing Synthetic Biology Approaches to Facilitate Value-added Oil Production in the Oilseed Cover Crop Pennycress** John Sedbrook<sup>1</sup>, Michaela McGinn<sup>1</sup>, Malihe Esfahanian<sup>1</sup>, Sunil Bansal<sup>2</sup>, Brice Jarvis<sup>1</sup>, Taylor Suo<sup>1</sup>, Tara Nazarene<sup>3</sup>, M. David Marks<sup>4</sup>, Ed Cahoon<sup>5</sup>, and Timothy P. Durrett<sup>2</sup>, <sup>1</sup>*Illinois State University, USA*; <sup>2</sup>*Kansas State University, USA*; <sup>3</sup>*University of Nebraska, USA*; <sup>4</sup>*University of Minnesota, USA*; <sup>5</sup>*University of Nebraska-Lincoln, USA*

Pennycress (*Thlaspi arvense*; Field pennycress) is an oilseed plant of the Brassicaceae family, closely related to rapeseed canola, camelina, and *Arabidopsis*. Pennycress is being developed as a profitable oilseed-producing winter cover crop to be grown throughout the 80 million-acre U.S. Midwest

Corn Belt. With its extreme cold tolerance, overwintering growth habit, and relatively short life cycle, pennycress can be planted in the fall in standing corn and harvested in the spring in time to plant full-season soybeans. As a cover crop, pennycress provides ecosystem services including reduced soil erosion and nutrients runoff, habitat for animals/beneficial insects, and early-season food for pollinators on otherwise vacant farmland. Elite high-yielding pennycress varieties producing oil for both food and industrial uses along with edible seed meal can provide additional income to farmers and agribusinesses thereby strengthening rural communities. Efforts are underway to rapidly domesticate pennycress by improving stand establishment, harvestable seed yield, and seed oil and meal quality through breeding, traditional mutagenesis, and synthetic biology approaches. This presentation will highlight our efforts employing synthetic biology methodology including CRISPR genome editing to develop pennycress varieties producing seed oils with unique and value-added fatty acid profiles including low and high erucic acid, acetyl-TAGs, and medium chain fatty acids.

#### **Glycolytic Genes Influences Mesocarp Oil**

**Content in Oil Palm** Jaime Y.S. Low<sup>1</sup>, Nurliyana Y.S. Ruzlan<sup>2</sup>, Noor Azizah Musa Musa<sup>3</sup>, Ai-Ling Ong<sup>3</sup>, David R. Appleton<sup>1</sup>, Fook Tim Chew<sup>4</sup>, Hirzun M. Yusof<sup>2</sup>, and Harikrishna Kulaveerasingam<sup>5</sup>, <sup>1</sup>*Biotechnology & Breeding Department, Sime Darby Plantation R&D Centre, Malaysia;* <sup>2</sup>*Sime Darby Renewables, Sime Darby Plantation Sdn Bhd, Malaysia;* <sup>3</sup>*Biotechnology & Breeding Department, Sime Darby Plantation R&D Centre;* <sup>4</sup>*Dept. of Biological Sciences, National University of Singapore, Singapore;* <sup>5</sup>*Sime Darby Plantation R&D Centre, Malaysia*

The glycolysis pathway has been identified as a key controller of oil biosynthesis in oil palm mesocarp tissue. Four glycolytic pathway genes encoding fructose-1,6-bisphosphate aldolase (EgFBA), triose phosphate isomerase (EgTPI), glyceraldehyde-3-phosphate dehydrogenase (EgGAPDH) and glycerol-3-phosphate dehydrogenase (EgG3PDH) were isolated from mesocarp tissues of Jacq. and expressed separately in. Overexpression of oil palm FBA or G3PDH gene in transgenic yeast exhibited significant increments in lipid accumulation of 16% and 21%, respectively. Whereas reduction in activity of TPI or GAPDH resulted in lower lipid content in yeast. Genetic association analysis for mesocarp oil content in oil palm identified four significantly associated of SNPs on the four genes of interest: EgTPI (SD\_SNP\_000035801), EgFBA (SD\_SNP\_000007765), EgGAPDH (SD\_SNP\_000041011) and EgG3PDH (SD\_SNP\_000008411). We successfully identified palms harboring specific genotypes for each of the SNPs that record higher oil yield in field trials. Further enhancement of lipid accumulation was observed by a combination reduced TPI activity and overexpressed FBA in yeast. Combining ability analysis was also performed for significant SNPs in selected oil palm populations, population subsets harboring homozygous GG.GG were found to be significantly associated with high mesocarp oil content. There was an additive effect for oil content when both SNPs were combined, correlating with our finding of 30% increment in the combination study of EgFBA in TPI yeast strain. Our results provide insights into the genetic basis of glycolytic contribution to lipid biosynthesis in oil palm and may facilitate marker-based breeding for oil yield.

**Recapitulation of Triacylglycerol Biosynthesis Pathways to Increase Hydroxy-Fatty Acid****Accumulation** Daniel Lunn, James Wallis, and John Browse, *Washington State University, USA*

Unusual fatty acids are of high value to agronomic industry as they are important in many industrial applications. Unfortunately, the native species in which these oils naturally occur are often unsuited for large scale agronomic production. One such modified fatty acid is hydroxy-fatty acid (HFA), important for cosmetics, plastics, and lubricants. A primary source of HFA is ricinoleate produced in castor (*Ricinus communis*). Although the cultivation of castor is lucrative it is primarily located in the tropics. In addition, castor contains the toxin ricin and several powerful allergens. To increase the value of HFA exploitation it was hypothesized that the biosynthetic machinery responsible for HFA production could be recapitulated in a more amenable crop. Expression of castor hydroxylase

in *Arabidopsis* (*Arabidopsis thaliana*) and *Camelina* (*Camelina sativa*) have been successful in producing HFA. However, the 17% accumulation of the total seed oil in *Arabidopsis* is far below that of the 90% accumulation in castor and the required level for commercial viability. It was hypothesized that castor triacylglycerol biosynthesis enzymes would have evolved a preference towards the incorporation of modified fatty acid. Expression of individual castor triacylglycerol biosynthesis enzymes with an hydroxylase increased HFA accumulation. We hypothesized that to reach levels required for commercial viability it would be necessary to recapitulate key triacylglycerol biosynthesis pathways. In this work we recapitulate several steps in triacylglycerol biosynthesis using castor homologous enzymes and achieve the highest HFA levels yet reported. These provide a further advancement towards the goal of tailored plant oils.

**BIO 4.1/S&D 4: Biosurfactants and Additives**

*Chairs: Daniel K.Y. Solaiman, USDA, ARS, ERRC, USA; and George A. Smith, Sasol North America, USA*

**Next Generation Castor Oil Ethoxylates** Ollie James\*, Dustin Landry, Liam McMillan, and George Smith, *Sasol North America, USA*

Castor oil ethoxylates have been material of commerce for many years. Castor oil is obtained by pressing the seeds of the castor oil plant. The oil is rich in ricinoleic acid, an unsaturated fatty acid containing a hydroxyl group in the 12 position. Castor oil ethoxylates are typically made by reacting castor oil with ethylene oxide using a base catalyst. The product consists of a complex mixture of ethoxylated fatty acids, ethoxylated partial glycerides and PEG. Castor oil ethoxylates are used as emulsifiers and adjuvants for agrochemical formulations. Surface activity and applications performance depend on the degree of ethoxylation and the species distribution. Recently, new ethoxylation catalysts have been developed which give greater control over reaction kinetics and species distribution. Narrow range catalysts allow for insertion of EO into the ester group but typically are not very effective at ethoxylating secondary alcohols. DMC catalysts are effective at ethoxylation secondary alcohols but give very little ester insertion. The species distribution of castor oil ethoxylates prepared by different catalysts was determined using a combination of LCMS and HPLC. Surface and interfacial tension was determined by Wilhelmy plate and pendant drop measurements. The performance of castor oil ethoxylates in different applications was related to species distribution and surface activity.

**Glycolipid Biosurfactants: Characteristic Curvature and Applications in Microemulsions and Emulsions.** Zheng Xue, Dennis Parrish, Eric Theiner, Khalil Yacoub, Andras Nagy, and Terrence Everson, *Evonik Corporation, USA*

Microbial biosurfactants produced by fermentation exhibit favorable properties such as low toxicity, skin mildness, and biodegradability. In particular, glycolipid biosurfactants such as sophorolipids and rhamnolipids have attracted significant commercial interest, owing to the desirable physiochemical properties of these biosurfactants and advantageous economics of large-scale production. In this study, the characteristic curvatures of sophorolipids and rhamnolipids were measured using microemulsion phase behavior study. Then, based on hydrophilic-lipophilic difference (HLD) calculations, a series of surfactant formulation systems comprising biosurfactants were designed. By tuning the HLD, the compositions of the surfactant formulations were optimized for two applications: 1) microemulsions containing terpenes for hard surface cleaning, and 2) efficient emulsification of oily soils for fabric cleaning. The effects of sophorolipids and rhamnolipids on the interfacial rheological properties, interfacial tension reduction, and emulsification in these two applications were investigated. Formulation procedures and comparative results will be discussed.

**Glucamide Surfactants: Structural and Interfacial Aspects** Brajesh Jha\*, *Colgate Palmolive, US*

Surfactants and co-surfactants are widely used in Personal Care and Home Care formulations to provide benefits such as improved foaming, viscosity, and mildness. Innovation continues in the area of surfactant chemistry to meet a growing demand for surfactants which are more versatile and have a greater safety and sustainability profile, and at the same time do not compromise on delivering performance when formulated into consumer products. Glucamides, although not new, are interesting surfactants, since they are based on sugar chemistry. Built from glucose and natural oils, these surfactants usually have a high Renewable Carbon Index (RCI) providing an advantage over the more conventional nonionic surfactants. This presentation will highlight the basic physicochemical phenomena occurring at the air-liquid or liquid-liquid interface in relation to structure-function of a selected group of glucamide surfactants. The select group consists of R-acyl-N-methyl glucamine at 25°C in distilled water, where R = C8/C10, C12/C14 or coco. Particular emphasis will be on the fundamental properties of these surfactants, such as CMC and their ability to reduce interfacial tension and improve foaming properties compared to other similarly structured surfactants. Glucamides' renewable and performance profile makes them an important class of surfactants to study for the fundamental understanding in the formulation development.

**NMR Investigation of the Effect of pH on Micelle Formation by an Amino Acid-based Surfactant.**

Kevin F. Morris<sup>1</sup>, Gabriel Rothbauer<sup>1</sup>, Elisabeth Rutter<sup>1</sup>, Chelsea Reuter-Seng<sup>1</sup>, Simon Vera<sup>2</sup>, Eugene Billiot<sup>2</sup>, Yayin Fang<sup>3</sup>, and Fereshteh Billiot<sup>2</sup>, <sup>1</sup>*Carthage College, USA*; <sup>2</sup>*Texas A&M Corpus Christi, USA*; <sup>3</sup>*Howard University, USA*

Micelle formation by the anionic amino acid-based surfactant undecyl L-phenylalaninate (und-Phe) was investigated in solutions containing either Na<sup>+</sup>, L-arginine, L-lysine, or L-ornithine counterions. Amino acid-based surfactants like und-Phe are biodegradable, biocompatible, and have a low toxicity. For these reasons, they are used in pharmaceutical and food applications and as selectors in chiral chromatography. NMR spectroscopy was used to measure the surfactant's critical micelle concentration as a function of pH in solutions containing each of the above counterions. NMR diffusion experiments were used to monitor changes in micelle radii with pH and to investigate the fraction of surfactant molecules and counterions bound to the micelles. Finally, two-dimensional NMR experiments were used to study the mechanism of L-arginine and L-lysine binding to und-Phe. In each mixture, the surfactant's critical micelle concentration was smallest at low pH and increased as solutions became more basic. NMR diffusion experiments showed that L-arginine, L-lysine, and L-ornithine bound most strongly to the micelles below pH 9 when the counterions were cationic. Above pH 9 the counterions became zwitterionic and dissociated from the micelle surface. Micelle radii measurements suggested that L-arginine attached to the micelles perpendicular to the micelle surface through its guanidinium functional group with the remainder of the molecule extending into



solution. L-lysine and L-ornithine in contrast, were found to bind parallel to the micelle surface with their two amine functional groups interacting with different surfactant monomers. This binding model was found to be consistent with results from two-dimensional NMR experiments.

#### **Effects of Rhamnolipid on Phagotrophic Algae as Sensitive Ecologically Important Model Organism.**

Krutika Invally, Suo Xiao, and Lu-Kwang Ju\*, *University of Akron, USA*

Surfactants can affect biological activities and pose threats to the aquatic ecosystem. Rhamnolipid biosurfactant has promising agricultural, industrial and biomedical applications. It is important to assess the risk posed by rhamnolipid prior to its wide-spread uses. We have evaluated the effects of rhamnolipid on a phagotrophic alga. The model organism used is a versatile mixotroph capable of photosynthetic, osmotrophic and phagotrophic metabolisms. Phagotrophic flagellates consume small microorganisms like bacteria and blue-green algae. They are ecologically important in transferring organic matter between the microbial and the classic food webs. Without a protective cell wall, they are likely more sensitive to surfactants among aquatic microorganisms. Common synthetic surfactants sodium dodecyl sulfate and Tween 80 were used for comparison. Critical concentrations and/or kinetic profiles for motility loss, cell lysis and membrane permeability were determined. Effects of its more unique phagotrophic metabolism on surfactant sensitivity were also observed.

#### **Application of Sophorolipids to Control Food Pathogens**

Daniel K.Y. Solaiman\*, Richard D. Ashby, Xueting Fan, and Modesto Olanya, *USDA, ARS, ERRC, USA*

Sophorolipids (SLs) are glycolipid-class biosurfactants produced by *Starmerella bombicola* and certain other yeast species. Previous studies had shown that SLs possessed varying degrees of antiviral and antimicrobial activity against viruses, bacteria, and fungi. In this paper, we present the results of our studies in which the antimicrobial activity of SLs was specifically tested against foodborne human pathogens (i.e., *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*). SL varieties isolated from fermentation broths of *S. bombicola* grown on palmitic (C16), stearic (C18), and oleic (C18:1) acid as the lipid substrate were screened in the study. Whereas there was no significant difference observed among the SL varieties (i.e., SL-C16, SL-C18, SL-C18:1) against the test bacteria, the Gram-positive *L. monocytogenes* was however found to be more susceptible to the biosurfactants in comparison to the Gram-negative *E. coli* O157:H7 and *S. enterica*. We further examined the effects of temperature and storage time on the efficacy of SL-C16 against the test bacteria, and found that higher temperature (25°C vs. 5°C) and longer storage time (24 h vs. 30 min) were more effective to decrease the pathogen population. Washing of artificially contaminated spinach leaves (i.e., *E. coli* O157:H7 inoculation) with SL solutions (1% w/v) did not significantly reduce the bacterial population in comparison to washing with water. However, the combined application of SL-C16 and a food sanitizer to treat pathogen-inoculated grape tomatoes led to significant reductions of the bacterial population.

**The Stability of Nanoemulsions and Emulsions Containing Cinnamaldehyde and Biosurfactants, and their Antimicrobial Performance against *Escherichia coli* O157:H7 and *Listeria Monocytogenes***

Kangzi Ren\* and Buddhi Lamsal, *Iowa State University, USA*

Two novel biosurfactants—surfactin and fatty acyl glutamic acid (FA-glu)—were compared with commercial emulsifiers—lecithin, and a mixture of Tween 80 and lauric arginate (TLA) for formation and stability of nanoemulsions and emulsions containing cinnamaldehyde (CM). The nanoemulsions'/emulsions' antimicrobial performance against two common foodborne pathogens *E. coli* O157:H7 and *Listeria monocytogenes* was also evaluated. The objectives of this study were to investigate how the processing parameters affect the emulsions' stability and how the emulsion droplet size affected the antibacterial efficacy. Two emulsifier concentration levels (0.5% w/w and 1% w/w) and two homogenizing pressures (9000 PSI and 18000PSI) were studied for their effect on droplet stability during storage for 46 days at 4, 25, and 37°C. Surfactin, FA-glu and TLA mixture formed nanoemulsions at both concentrations, but lecithin did not. Emulsion droplet sizes did not change significantly during 38 days at all temperatures for surfactin- and TLA mixture-stabilized nanoemulsions. However, FA-glu and lecithin stabilized emulsions coalesced after 13th day when stored at 37°C, FA-glu stabilized emulsion also formed viscous structure during elongated storage days at 4°C. The incorporation of CM in nanoemulsions or emulsion did not lower the minimum inhibitory concentration (MIC) in bacterial broths. However, at the concentrations lower than MIC, nanoemulsions and emulsions containing CM formulated with

FA-glu, lecithin, and TLA, showed enhanced effects in inhibiting bacterial growths compared to CM alone, with smaller droplets inhibiting more.

**Unique Characteristics of Sophorolipid, Yeast Glycolipid Biosurfactants, and its Application as Eco-friendly Bio-detergents.**

Yoshihiko Hirata, Glen Lelyn Quan, Michiaki Araki, and Mizuyuki Ryu, *Saraya, Japan*

Biosurfactants (BSs) are natural amphiphiles which are abundantly produced from a variety of renewable resources by microorganisms. They have been receiving great attention because of their unique properties including higher biodegradability, low toxicity, and versatile biological functions, compared to petroleum-based surfactants. So far, the use of BSs has been limited to a few specialized applications because they have been economically uncompetitive. We started the research on sophorolipid (SL), which is a kind of promising glycolipid BS, for practical use as bio-detergents since 1998. We found that SL is a biodegradable low-foaming surfactant with excellent washing ability and commercialized the only automatic-dishwashing detergent containing SL as surfactant in 2001. At present, our SL brand, SOFORO, is originally being fermented using RSPO (Roundtable on Sustainable Palm Oil)-certified “segregated” palm oil. In this study, we will report about the unique “rinsability” of sophorolipid, which is the activity to reduce the amount of surfactants adsorbing on skin surface, and introduce its recent product application, our new detergent brand “Happy Elephant”.

**Applisurf: Functionality Driven Design and Synthesis of New-to-Nature Glycolipid Biosurfactants.**

Sophie L.K.W. Roelants<sup>1</sup>, Sofie Demaeseneire<sup>2</sup>, and Wim Soetaert<sup>3</sup>, <sup>1</sup>*Bio Base Europe Pilot Plant, Belgium*; <sup>2</sup>*Ghent University, Belgium*; <sup>3</sup>*Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Ghent University, Belgium*

Industrial biotechnology holds the opportunity to use and redirect nature's many inventive capabilities to produce a variety of biological amphiphiles with a variety of (new) potential applications like the fermentative glycolipid portfolio recently developed at InBio.be and BBEPP. However, such technologies are mostly driven by market push and finding the best application for each specific new and thus uncharacterized compound is similar to looking for the needle in the haystack, often resulting in a halt of the innovation. This unfortunate

situation could be alleviated by applying an integrated bioprocess design where application development in the form of functional screening is developed in parallel with microbial strain development, driven by synthetic biology. In the AppliSurf project, a molecular glycolipid portfolio generated through a combination of fermentation and green chemistry will be subjected to HTP functional characterisation for surfactant and biological properties. The generated data will be used to build models defining and predicting structure-function relationships. The latter will allow to specifically design and generate promising candidates for dedicated application experiments by industrial end users. Together, this approach is expected to speed up innovation and market uptake of new, innovative and performance biosurfactants.

**BIO 5: General Biotechnology**

*Chairs: Long Zou, Bunge Creative Solutions Center, USA; Lu-Kwang Ju, University of Akron, USA; and Guoqin Liu, South China University of Technology, China*

**Comparison of Three Methods for Analyses of Triacylglycerols in Cocoa Butter Alternatives**

Jun Jin\*, Qingzhe Jin, and Xingguo Wang, Jiangnan University, China

Objective HPLC-ELSD, HPLC-RID, and HT-GC are the predominant tools for analyses of triacylglycerols. However, there are some confusions in selecting the suitable methods for the determination of cocoa butter alternatives (CBAs) that generally contain symmetrical monounsaturated triacylglycerols. Methods Used The triacylglycerol compositions of typical CBAs, including cocoa butter (CB), soft palm mid-fraction (PMF), soft palm stearin, hard PMF, and mango kernel fat and its stearin (MKFS), were analyzed using the three methods. Standards of StOSt, POST and POP were selected to identify the triacylglycerols and the contents were reported in terms of their relative proportions. Results for CB who is composed of several predominant triacylglycerols, similar percentages were found in StOSt using the three methods. However, significant differences between the HPLC-RID/HT-GC and HPLC-ELSD were observed in POST and POP contents, i.e., 39.4%–42.6% and 17.8–18.9% for HPLC-RID or HT-GC, but 55.9–57.2% and 12.8–15.6% for HPLC-ELSD. For samples with one primary triacylglycerol, e.g., PMFs and MKFS, the differences were highly significant. For instance, 68.0–68.6% POP were detected in the hard PMF using the HPLC-RID or HT-GC, but the value was as high as 90.6% for HPLC-ELSD. Similar results were also concluded from the StOSt-rich MKFS. In addition, only 5–7 peaks could be identified using the HPLC-ELSD,

which was half the number the other two methods did. Conclusions HPLC-RID and HT-GC are suggested to analyze the triacylglycerol compositions of CBAs. It is difficult to identify more triacylglycerols and to quantify their percentages using area normalization method when HPLC-ELSD is applied.

**Enzymatic Processing Methods to Reduce Saturated Fat Content of Oils** Matthew A. Robinson\*, Dow AgroSciences, USA

Oil products with less than 3.5 wt% saturated fat, enabling 0 g of sat fat per serving labeling in the US, have been obtained through plant breeding and genetic modification. One limitation of introducing such products is their long development time frames and associated expense, resulting in currently limited commercial availability. Oil processing methods to reduce the saturated fat content offer the potential for shortened development times and application to a broad range of oil types. In exploring processing methods to reduce saturated fat, two methods were identified to biocatalytically reduce saturated fat. By screening a lipase collection, *Candida antarctica* lipase A (CALA) was identified to selectively hydrolyze saturated fatty acids over monounsaturated and polyunsaturated fatty acids. Partial selective enzymatic hydrolysis using CALA was shown to reduce saturated fat in canola oil from 4.93% to 3.09%, representing a 37% reduction with only 8.8% hydrolysis of the oil. The method was successfully extended to sunflower and soybean oil. A second method was developed using the

known selectively of *Geotrichum candidum* lipase (GCL) to preferentially hydrolyze unsaturated fatty acids. Sunflower oil with 3.75% saturated fat was hydrolyzed using GCL to produce a fatty acid solution with

#### **Optimizing Oil Production by *Mucor circinelloides* using Cheese Whey Permeate**

Juliana M. Leite Nobrega de Moura Bell, Lauryn Chan, Josh Cohen, Gulustan Ozturk, Marie Hennebelle, and Ameer Taha, *University of California, Davis, USA*

Oleaginous fungi have recently emerged as an efficient tool to convert agricultural waste streams into valuable components. The filamentous fungus, *Mucor circinelloides*, was cultivated in whey permeate, a byproduct from the cheese production, to produce an oil-rich fungal biomass. Upstream lactose hydrolysis of the whey permeate increased the biomass yield to 7.8 (g dry biomass/L) compared to that of non-hydrolyzed whey permeate (2.4 g/L). Additionally, pH has shown to be a key parameter affecting the fungal growth and its morphology. The use of a low pH (4.5 vs. 6.5) increased biomass production while avoiding the need of sterilization of the medium. The combination of low pH and pasteurization has shown to avoid microbial competition thus favoring biomass yield. A central composite rotatable design was used to evaluate the effects of a lower pH range (3.6–4.65) and temperature (22–33.6°C) on biomass yield and composition, with cultivation performed in shake flasks at 200 rpm for 72 hours. Highest biomass yield (7.63 g/L) and oil content (18.0%) were observed at high temperature (33.6°C), with values not affected by the pH range evaluated. The predictive model was validated at 33.6°C and pH

4.5 for 7 days. Fungal biomass plateaus at ~9 g/L at 84h, coinciding with the depletion of sugars in the medium. However, the oil content continues to increase after sugar depletion, reaching 24% at 168h. At 168h, lipids were composed of 92% triacylglycerol, 0.8% cholesterol esters, 6.6% phospholipids, and 0.2% free fatty acids. Triacylglycerols were composed primarily of oleic (38%), palmitic (22%), linoleic (11%), gamma-linolenic (9%), palmitoleic (22%), and steric acid (4%).

#### **Effect of Interesterification on the Physicochemical Profiles of Rice Bran Wax-based Modified Fats** Zhen Zhang<sup>\*1</sup>, Huihua Huang<sup>2</sup>, and Yong Wang<sup>3</sup>, <sup>1</sup>*South China University of Technology, China*; <sup>2</sup>*School of Food Science and Engineering, South China University of Technology, China*; <sup>3</sup>*Jinan University, China*

Rice bran wax (RBW), a main byproduct of rice bran oil production, is precipitated out after the oil refining, and abounded in tetracosanoic-acid triacontanol ester (TATE, RC30OOCRC23). RBW possessed high slipping melting point (74.4 °C) and its products hold much potential for food and cosmetic applications. To explore the application of RBW in fats interesterification (IE) modification, RBW was interesterified with palm olein (POL) catalyzed by Lipozyme TL IM and the effects of RBW on the fats' solid fat content (SFC), the crystallization rate, and thermodynamic properties were investigated. Compared with fully hydrogenated rapeseed oil (FHRSO), the crystallization rates of RBW-based IE products were expedited significantly compared with the starting mixture. Meanwhile, the SFC of RBW-based IE sample at 10 °C was decreased a lot, profiles of 20–30 °C were obvious increased conversely with steady values at 35–40



°C. Thereby, the SFC profiles became much smoother. Due to the RBW's single long chain ester structure, the RBW-based IE fats sample with a wide compositional range had a broad melting and crystallization range whereas FHRSO-based IE ones with a homogeneous composition had a narrow range which agree with the SFC findings. Moreover, the crystal size distribution strongly influences the completing temperature of melting and hence results in the variation of melting range. Most of the formulated RBW-based IE sample dominantly the  $\beta'$  crystal form, in agreement with the generally accepted fact that  $\beta'$  is more stable in complex mixtures.

**Substrate Preference of Long Chain acyl-CoA Synthetase for Hydroxy-Fatty Acids** Jesse D. Bengtsson and John Browse, *Washington State University, USA*

Novel fatty acids such as hydroxy-fatty acids (HFA) are accumulated in the triacylglycerol of several plant species such as castor (*Ricinus communis*) and *Physaria* (*Physaria fendleri*). HFA are important for many industrial processes, thus the production of these molecules in easily exploitable crops has the potential for high value. In addition, the production of HFA in non-native species offers a unique opportunity to study the underlying biochemistry involved in triacylglycerol biosynthesis. Past work producing HFA in *Arabidopsis* (*Arabidopsis thaliana*) has demonstrated the importance of enzymes from plants that evolved to accumulate HFA. Expression of castor Diacylglycerol Acyl Transferase 2, which catalyses the terminal step in triacylglycerol biosynthesis, showed increased substrate preference to HFA containing diacylglycerol. In addition, a castor Phospholipase A2 $\alpha$  showed increase specificity and selectivity

towards phosphatidylcholine containing HFA at the sn-2 position of triacylglycerol. We endeavored to elucidate the specificity of further enzymes within triacylglycerol biosynthesis. Long Chain Acyl-CoA Synthetases (LACS) catalyze the formation acyl-CoA from its constituent parts. We hypothesized that to provide adequate acyl-CoA pools for triacylglycerol production these enzymes would require strong substrate specificity. Through work in vitro and in planta we investigated the specificity of LACS enzymes during seed development. The evaluation of LACS from *Arabidopsis* and *P. fendleri*, showed no difference in their ability to use HFA as substrate. These results were surprising and add to our understanding of the key players in triacylglycerol biosynthesis.

**Effective Enrichment of Palmitoleic Acid from Seabuckthorn Oil by Combining Different**

**Methods.** Nakyung Choi<sup>1</sup>, Ju Yeon Chung<sup>1</sup>, Heejin Kim<sup>3</sup>, and In-Hwan Kim<sup>1</sup>, <sup>1</sup>*Korea University, Republic of Korea*; <sup>2</sup>*Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea*

Efficient enrichment of palmitoleic acid was investigated by three different techniques, which were urea complexation, enzymatic esterification, or solvent fractionation. Fatty acid (FA) from sea buckthorn oil, which contained ca. 35 mol% of palmitoleic acid, was used as a substrate. The optimum conditions were a weight ratio of 1:2 (FA to urea) for an urea complexation, a temperature of 25 °C and a water content of 2.5% for an enzymatic esterification, and a temperature of –5°C and a solvent ratio of 1:6 (FA to acetone, wt/v) for a solvent fractionation. As a result, palmitic acid was removed efficiently by urea complexation,

but palmitoleic acid was most efficiently enriched in the enzymatic esterification. Consequently, ca. 68 mol% of palmitoleic acid was attained under the optimum condition. To further improve the content of Palmitoleic acid, a combination of urea complexation and enzymatic sesterification was carried out under the optimum conditions of each reaction. Finally, the maximum palmitoleic acid content of ca. 75 mol% was attained with a yield of ca. 30 mol%.

**Synthesis of 2-docosahexaenoylglycerol by Enzymatic Ethanolysis** Yu Zhang\*, Xiaosan Wang, Shuo Zou, Qingzhe Jin, and Xingguo Wang, *Jiangnan University, China*

Synthesis of 2-docosahexaenoylglycerol with high nutritional value was conducted by enzymatic ethanolysis of algal oil. The effect of lipase type, substrate molar ratio of algal oil to ethanol, reaction time and temperature and lipase load on the content of 2-monoacylglycerols (2-MAGs) in the crude product was investigated. Under the optimal conditions of molar ratio of algal oil to ethanol 1:60 at 30°C for 2 h with 8% Lipozyme 435 (w/w, relative to total reactants) as catalyst, 27%–31% 2-MAGs were obtained in the ethanolysis reaction. Lipozyme 435 exhibited a 1,3-specific selectivity and maintained a stable operational stability after 7 successive reuse cycles. The enzymatic ethanolysis catalyzed by Lipozyme 435 could both synthesize 2-MAGs and concentrate DHA. Further purification of 2-MAGs was performed with solvent extraction by 85% ethanol aqueous solution and hexane, obtaining 95% 2-MAGs in a yield of 67%. The content of DHA in the 2-MAGs

product was 74%, which was 26% higher than algal oil. Therefore, this method is efficient and green for synthesis of 2-docosahexaenoylglycerol.

**Preparation of Diisononyl Adipate via Lipase-catalyzed Esterification in a Solvent-free system**

Aree Lee<sup>\*1</sup>, Heejin Kim<sup>2</sup>, and In-Hwan Kim<sup>1</sup>, <sup>1</sup>*Korea University, Republic of Korea*; <sup>2</sup>*Dept. of Public Health Sciences, Graduate School, Korea University, Republic of Korea*

The synthesis of diisononyl adipate was performed with an immobilized lipase from *Candida antarctica* (Lipozyme 435) via direct esterification of adipic acid and isononyl alcohol in a solvent-free system. Diisononyl adipate (DINA) is a plasticizer which has excellent properties of low temperature resistance and permitted in food contact materials. In this study, the effects of four operative parameters, temperature, substrate molar ratio, enzyme loading and vacuum, on the reaction efficiency were investigated. The highest conversion (100%) was successfully achieved in following reaction conditions: a temperature of 50°C, a 1:3 molar ratio of adipic acid and isononyl alcohol, an enzyme load of 2% (relative to weight of total substrate) and a vacuum of 50torr. Furthermore, enzyme immobilization was proceeded to substitute commercial enzyme. Eversa Transform 2.0 which is widely used for biodiesel production was immobilized on Lewatit VP OC 1600, a macroporous hydrophobic resin. As a result, self-immobilized enzyme appears to be active catalyst as commercial enzyme and reached 100% yield of DINA at different optimal operative conditions.

**BIO-P: Biotechnology Poster Session**

*Chairs: Byung Hee Kim, Sookmyung Women's University, Korea; and Shigenobu Kishino, Kyoto University, Japan*

**Hall B**

**1. Isoflavone Phosphate Synthetase from *Bacillus subtilis* BCRC80517.** Chen Hsu<sup>1</sup> and Nan-Wei Su<sup>2</sup>, <sup>1</sup>National Taiwan University, Dept. of Agricultural Chemistry, Taiwan; <sup>2</sup>National Taiwan University, Taiwan

Genistein and daidzein are the major isoflavone aglycones found in soybean and have beneficial effects on human health. However, their oral bioavailability is hampered by their low solubility. Our previous study revealed water-soluble phosphorylated isoflavones generated via microbial biotransformation. However, isoflavone-phosphorylating metabolism remain still uncharacterized. Here, we identified a novel isoflavone phosphate synthetase (IFPS) from *Bacillus subtilis* BCRC80517. IPFS was purified to homogeneity through (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation, DEAE anion-exchange chromatography, Phenyl hydrophobic interaction chromatography and Superdex 75 gel filtration with 183-fold purification efficiency. The protein sequence was identified by LC-MS/MS, contained 839 amino acids, and the molecular mass was 95.4 kDa. IPFS use only ATP as the phosphate donor, and Mg<sup>2+</sup> is a dominant cofactor on the activity rather than Mn<sup>2+</sup>. The optimal conditions of enzyme activity were pH 7.5 and 40°C. IPFS was stable in the pH range of 6.5–8.5 with lower than 40°C. The kinetic parameters of IPFS were determined to be as follows: K<sub>m</sub> for genistein = 0.10 ± 0.01 μM, V<sub>max</sub> = 1.28 ± 0.03 μmol·min<sup>-1</sup>·mg<sup>-1</sup> and K<sub>cat</sub> = 2.02 ± 0.05 s<sup>-1</sup>; K<sub>m</sub> for daidzein = 0.16 ± 0.01 μM, V<sub>max</sub> = 0.50 ± 0.01 μmol·min<sup>-1</sup>·mg<sup>-1</sup> and K<sub>cat</sub> = 0.78 ± 0.03 s<sup>-1</sup>.

Further, we investigated the substrate specificity. IPFS also showed enzymatic activity toward flavone (apigenin, luteolin), flavonol (kaempferol, quercetin) and flavanone (hesperetin, naringenin). Our finding provides new insights into the previously unknown isoflavones phosphorylation enzyme.

**2. Fungal Fermentation of De-Hulled Ground Barley to Increase Protein Levels.** Burgandy R. Roberts<sup>1</sup>, Bishnu Karki<sup>2</sup>, Jacob Zahler<sup>1</sup>, Michael Brown<sup>3</sup>, and William Gibbons<sup>1</sup>, <sup>1</sup>South Dakota State University, USA; <sup>2</sup>Dept. of Biology and Microbiology, South Dakota State University, USA; <sup>3</sup>Dept. of Natural Resource Management, South Dakota State University, USA

In recent years, the cost and usage of barley (*Hordeum vulgare*) had decreased substantially. On the other hand, the cost of fish meal continues to rise due to high demand for aquaculture. To address both issues this study uses de-hulled ground barley as a feed source to concentrate and increase protein concentration for uses in aquaculture feed. *Trichoderma reesei* (NRRL-3653) was used in the solid and submerged state fermentation to increase the protein titer of the barley. Among these two processes, solid state fermentation was found to be more effective as total protein concentration and protein yield were better than that of the submerged process. During the submerged process, there was significant loss in the total mass of the feedstock. Additionally, minimal processing such as washing with a water prior to fermentation process was also evaluated. Using

just a simple wash step prior to autoclaving was able to concentrate the protein significantly. Currently work is in progress to find out if the protein concentration could be further improved by fermenting the washed barley. These results showed that barley could be a possible low-cost substitute for current aquaculture fishmeal.

**3. Optimization of Fungal Stimulation and Processing Parameters to Maximize Glyceollin Production in Soybeans.** Stephanie A. Wootton<sup>1</sup>, Bishnu Karki<sup>2</sup>, Mark Berhow<sup>3</sup>, and William Gibbons<sup>1</sup>, <sup>1</sup>*South Dakota State University, USA*; <sup>2</sup>*Dept. of Biology and Microbiology, South Dakota State University, USA*; <sup>3</sup>*USDA National Center for Agricultural Utilization Research, USA*

As size and number of world populations continue to increase, the ability to feed those populations becomes progressively challenging. Recurrent use of antibiotics disseminates an inevitable increase in antibiotic-resistant microorganisms. This drives a need to explore sources of natural antimicrobial compounds and then to consider how to apply such compounds. One way to possibly apply natural antimicrobials is supplementing them into feeds. Studies have shown fungal infection promotes a response by soybean seeds to accrue glyceollin; however, the glyceollin titers are at low levels. In this study our goal is to evaluate and identify the optimal processing parameters to producing high glyceollin content in soybeans using the fungal metabolic process. In a previous study, our research team at South Dakota State University identified *Trichoderma reesei* NRRL 3653 as the best performing strain, stimulating the highest total glyceollin yield among several other tested strains. Hence, in this study, various processing parameters such as seed soaking time, inoculation method, incubation time, seed

germination effect, seed varieties, etc. have been evaluated. Although the research is at the early stage, the preliminary findings have exhibited there is no major parallel between the seed germination and glyceollins level in soybeans. Likewise, increase in fungal incubation time increased the glyceollins production in soybeans (0 mg/g at 0 hrs Vs. 0.812 mg/g at 120 hrs of incubation). We fully expect that process optimization will allow us to enhance the glyceollin titer in soybeans considerably; thereby, boosting the commercial value of soybean and its market.

**4. An Integrated Multi-omics Study on Lipid Turnover of *Schizochytrium* sp. S31 Cultured on Glycerol.** Ming Chang, Tao Zhang, Ruijie Liu, Qingzhe Jin, and Xingguo Wang, *Jiangnan University, China*

Objective: Lipid turnover in *Schizochytrium* sp. supports a preferential degradation of saturated fatty acids and reservation of polyunsaturated acids. The objective of this study was to investigate the degradation metabolism of triacylglycerol (TG) during lipid turnover. Methods: Lipidomic analyzing, genomic sequencing and RNA-seq were performed at different cultivation stages of *Schizochytrium* sp. S31 to obtain lipid profiles and gene expression levels related to lipid turnover. Results: Lipidomic analysis revealed that lipolysis of TG mainly happened to 16:0/16:0/14:0-TG, 16:0/16:0/16:0-TG, 16:0/14:0/22:5-TG, and 16:0/22:5/22:6-TG, accompanying with an increased levels of 22:6/22:5-PA and 22:6/22:6-PA in phosphatidic acids (PA). RNA-seq results indicated that lipase involved in the TG hydrolysis might be Stgl4p or Stgl9p as confirmed by the high-level transcription (1.31 folds and 1.86 folds,

respectively). Moreover, the diacylglycerol kinase 1 gene (DGK1) was up-regulated for 1.44 folds, while the phosphatidic acid hydrolase gene (PAH) was down-regulated for 1.39 folds. This phenomenon was in favor of an efficient formation of PA from diacylglycerol (DG). Conclusion: DG generated after preferential lipolysis of TG with palmitic acid (16:0) or myristic acid (14:0) was utilized for PA synthesis. This study would enrich the current knowledge regarding lipid turnover and contribute to the enhancement of DHA production by *Schizochytrium*.

#### 5. An Effective Method for Deacidification of High-acid Rice Bran Oil by Enzymatic Amidation.

Xingguo Wang and Xiaosan Wang\*, *Jiangnan University, China*

The current study aimed to reduce free fatty acids (FFA) present in high-acid rice bran oil (HRBO) to an acceptable level. To achieve the goal, an improved method of deacidification of HRBO by an enzymatic amidation reaction between FFA and ethanolamine is described. The reaction conditions were optimized to minimize the FFA content of HRBO. Under optimal reaction conditions (2% of Lipozyme 435, about 1:1 mass ratio of oil to solvent and at 76 °C), acid value of HRBO was reduced from 21.5 to 1.6 mg/g after 4 h reaction. The final oil product was rich in fatty acid ethanolamides (11.9 wt%) which are desirable bioactive lipids. A comparison study showed that compared to esterification deacidification using glycerol or monoacylglycerols (MAG) as acyl acceptor, enzymatic deacidification by amidation can be completed in a much shorter time because amidation reaction is much more favorable than the esterification. This is the first time that

ethanolamine is used as acyl acceptor to enzymatically deacidify a high-FFA oil. Such an enzymatic route is highly effective and environmentally desirable.

#### 6. Dendritic Nanomolecules as Drug Carriers: Solubilization, Sustained Release and Biocompatibility Study. Ravindra V. Movliya and Pravinkumar M. Patel, V. P. & R. P. T. P. Sc. College, India

Formulation of poorly water soluble drugs is one of the challenging tasks in pharmaceutical research and development as it decreases bioavailability and increases side effects. We have developed dendritic generations up to G3 with 128 terminal hydroxyl groups as solubility enhancers used as drug solubility enhancer and delivery of same drug. Drug loaded Dendrimer were characterized by FT-IR spectroscopy and Sustained release study was evaluated and compared with that of free Drug using dialysis bag method. Biocompatibility and toxicity of G3 Dendrimers were evaluated by Hemolysis and MTT assay test of A-549 lung cancer cell lines.

#### 7. Sequential Liquefaction of *Nicotiana tabacum* Stems Biomass by Crude Polyhydric Alcohols for the Production of Polyols and Rigid Polyurethane Foams. Chiragkumar M. Patel, Industrial Chemistry Dept., V. P. & R. P. T. P. Science College, India

In this work, *Nicotiana tabacum* stalks and castor oil-based polyol was synthesized via two-step process. Preliminarily, stalks were liquefied using acid catalyst to procure glycol-glycoside and the optimized conditions for liquefaction of *N. tabacum* stem's biomass was 150°C temperature for 180 min time using PTSA as catalyst. Progressively, the glycol-glycoside obtained from the former step was further



reacted with castor oil in the presence of lithium hydroxide to get dark brown-coloured polyol with hydroxyl value was running in between 200 and 400 IOH. Glycol-glycoside and polyols were characterized by chemical and instrumental methods. Further by employing open-cup method involving the mixing of polyol and isocyanate adducts, the desired poly urethane rigid foam was obtained. The product was tested for their physical, mechanical, thermal, and morphological characteristics, while the thermal conductivity was in the range of 0.013 to 0.017 kcal/mh.AQ1 The performed study may yield high quality rigid or semi-rigid polyurethane.