Final Scientific Report

Award #: DE-SC0012411

Recipient: Presidents and Fellows of Harvard College

Title: “Unravel lipid accumulation mechanism in oleaginous yeast through single cell systems biology study”

Principal Investigator: Professor Xiaoliang Xie

Collaborators: Shiyou Ding – Michigan State University

3. Executive Summary

Searching for alternative and clean energy is one of the most important tasks today. Our research aimed at finding the best living condition for certain types of oleaginous yeasts for efficient lipid production. We found that R. glutinis yeast cells has great variability in lipid production among cells while Y. lipolytica cells has similar oil production ability. We found some individual cells shows much higher level of oil production. In order to further study these cases, we employed a label-free chemical sensitive microscopy method call stimulated Raman scattering (SRS). With SRS, we could measure the lipid content in each cell. We combined SRS microscopy with microfluidic device so that we can isolate cells with high fat content. We also developed SRS imaging technique that has higher imaging speed, which is highly desirable for high throughput cell screening and sorting. Since these cells has similar genome, it must be the transcriptome caused their difference in oil production. We developed a single cell transcriptome sequencing method to study which genes are responsible for elevated oil production. These methods that are developed for this project can easily be applied for many other areas of research. For example, the single transcriptome can be used to study the transcriptomes of other cell types. The high-speed SRS microscopy techniques can be used to speed up chemical imaging for lable-free histology or imaging distribution of chemicals in tissues of live mice or in humans. The developed microfluidic platform can be used to sort other type of cells, e.g., white blood cells for diagnosis of cancer or other blood diseases.

4. Provide a comparison of the actual accomplishments with the goals and objectives of the project.

The goals and objectives of this project were to combine advanced imaging, single-cell systems biology, and metabolic engineering approaches to understand the lipid accumulation mechanism of oleaginous yeast, and to decouple nitrogen regulation and sugar utilization on lipid production for lignocellulosic advanced biofuel production.

1) We have developed hyperspectral Stimulated Raman Scattering (hsSRS) microscope in our MSU lab that are specifically tailored for fast chemical imaging in complex biological systems. It features high numerical aperture of 1.4 for better spatial resolution and reduced cross-phase modulation. The femtosecond infrared laser was selected for deeper penetration and less photodamage for longer *in-situ* imaging time. Hyperspectral Raman scanning was achieved through spectral focusing where two femtosecond laser beams were linearly chirped to create overlapping pulses in time domain. We are able to achieve transformed limited spectral resolution of ~15cm which provides superior clarity in identifying different types of lipids. A set of sophisticated algorithms based on Multivariate Curve Resolution (MCR) were developed to analyze yeast images and track droplets.
2) Studies of lipid production have been carried out on selected oleaginous yeast strains. Lipid accumulation has been studied with R. glutinis and Y. lipolytica grown in both regular and nitrogen starvation media. hsSRS imaging of the lipid droplets showed considerable variation among individual Y. lipolytica cells in terms of volume and composition, while the R. glutins lipid droplet showed similar Raman response representing more homogenous lipid production. Hyperspectral SRS analysis revealed the spatial distribution of two major lipids-triacylglyceride (TAG) and sterol ester (SE)- where TAG formed cores were surrounded by SE shells. Significantly elevated level of SE/TAG was observed at cellular and sub-cellular levels which could be attributed to epigenetic variation in cell development.
3) Lipid components and quality have been studied with SRS imaging with fast chemical identification. Yeast growth conditions were studied based on their lipid droplets formation. Optimal condition was found in two-stage grown media where the yeast was grown in regular YEPD and transferred to nitrogen starvation media after maturation. The volume and composition of lipid droplets vary significantly depending on the availability of nitrogen source. The difference stems come from the environmental stress as well as the specific cell growth stage. Large number of cells have been fast screened and analyzed by custom-designed microfluidic systems for statistical significance.
4) We developed a novel SRS microscopy technique to improve the imaging speed for high through put cell screening. We developed a frequency modulation spectral focusing SRS technique, which can subtract the on and off resonance Raman band in real time. Furthermore, we developed a simultaneous two-color SRS. We used fiber laser to generate a second Stokes laser at a shifted wavelength. We then combined the two Stokes laser and pump laser in space and time, making the two Stokes lasers to provide a 90 degree modulation phase shift. The signals generated by two Stokes lasers can be read out by two orthogonal channels in the lock-in amplifier and therefore achieve simultaneous two-color SRS. We have demonstrated its ability to perform high speed imaging. They will be helpful for screening cells in high throughput microfluidic cell sorters.
5) For transcriptome profiling, we have successfully developed a single-cell whole-transcriptome amplification assay that to our knowledge offers the highest sensitivity. Because the mRNA content of a single yeast is no more than 5% of that of a human cell, the importance of high sensitivity cannot be over-emphasized for sequencing single yeast transcriptome. Finally, to correlate the lipidomic profile to transcriptomic data for the same yeast cells, we have designed

a microfluidic platform for isolating single yeast cells, imaging each cell with hsSRS microscopy for lipidomic quantification, and collecting each cell for whole-transcriptome amplification and sequencing. These recently developed methods are to probe lipogenesis in relation to transcriptome changes in oleaginous yeasts for sustainable biofuel production.

5. Summarize project activities for the entire period of funding, including original hypotheses, approaches used, problems encountered and departure from planned methodology, and an assessment of their impact on the project results. Include, if applicable, facts, figures, analyses, and assumptions used during the life of the project to support the conclusions.

Hydrocarbon fuel is of great interest in the advanced biofuel research, as they have high energy density and are compatible with current fuel infrastructure. Oleaginous yeasts are a valuable model for these studies because they can accumulate high levels of lipid in the form of triacylglycerols (TAGs) when encountering stress conditions or imbalanced growth. The lipid accumulation in oleaginous yeast is tightly controlled by the amount of nitrogen in the media. It is also known that the lipid production varies dramatically when different sugar, e.g. glucose, xylose is used as carbon source. The efficient utilization of all monomeric sugars of hexoses and pentoses from various lignocellulosic biomass processing approaches is the key for economic lignocellulosic biofuel production. In this project, we have explored lipid production in oleaginous yeast under different nitrogen and sugar conditions at the single-cell level. We've performed these studies under critical conditions in which each yeast cell experience dramatic changes in lipid accumulation. Stimulated Raman Scattering imaging has been optimized and used to image the different types of lipids accumulated in the yeast cell. The newly developed frequency modulation and the simultaneous two-color SRS combined with microfluidic devices will allow us to analyze the morphology and chemical content of lipids of the cells *in vivo* in high throughput. The single-cell transcriptome sequencing will give us more insight into the mechanism of high lipid production. We believe these quantitative microscopy and genomic tools will greatly advance the biofuel research.

6. Identify products developed under the award and technology transfer activities

a. Publications/Presentations:

- "Technologies for Characterizing Molecular and Cellular Systems Related to Bioenergy and Environment", Workshop Report, September 21-23, 2016, convened by U.S. Department of Energy, Office of Science, Office of Biology and Environmental Research. Ding S-Y led the session discussion: Bioenergy and Bioproducts Production, and wrote the report Chapter 2: Cell Wall Composition and Degradation.

- Song B, Li B, Wang X, Shen W, Park S, Collings C, Feng A, Smith SJ, Walton JD, Ding S-Y. Real-Time Imaging Reveals Lytic Polysaccharide Monooxygenase Promotes Cellulase Activity by Increasing Cellulose Accessibility. *Biotechnol. Biofuels*. Accepted.

- Zeng Y, Himmel ME, Ding S-Y. Visualizing Chemical Functionality in Energy Plant Cell Walls. Submitted to *Biotechnol Biofuels*. Accepted.

- Dan F, Yang W, Xie XS. Label-free Imaging of Neurotransmitter Acetylcholine at Neuromuscular Junctions with Stimulated Raman Scattering. *J Am Chem Soc* **139**(2), 583, 2017.

- Yang W, Li A, Suo Y, Lu F, Xie XS Simultaneous two-color stimulated Raman scattering microscopy by adding a fiber amplifier to a 2 ps OPO-based SRS microscope. *Opt Lett* **42**(3), 523, 2017.

- Abolibdeh B, Collings C, Li M, Ding S-Y. A Comparative Study on *Rhodotorula Glutinis* Yeast lipid Production and Growth in Various Types of Corn Biomass Hydrolysates (Poster presentation). University Undergraduate Research and Arts Forum (UURAF), April 9th, 2016.

- Shen W, Chang C-H, Li A, Ding S-Y, and Xie XS. Lipid Production in Single Oleaginous Yeast Cells Using *In Vivo* Label-Free Imaging (Poster presentation). DOE Genome Science Program annual meeting, March 6-9, 2016.

- Ding S-Y and Xie XS. Lipid Production in Single Oleaginous Yeast Cells Using *In Vivo* Label-Free Imaging (Oral presentation). DOE Genome Science Program annual meeting, March 6-9, 2016.

- Lee D, Lu J, Chang S, Loparo JJ and Xie XS. Mapping DNA polymerase errors by single-molecule sequencing. Nucleic Acids Res. 2016 May 16. [Epub ahead of print]

- Ding S-Y. "Real-time imaging of cellulose microfibril and biosynthesis", 253rd ACS National meeting, April 2-16, 2017, San Francisco, CA.

- Ding S-Y. "Real-time imaging of cellulose microfibril and biosynthesis", Tight Interactions, April 6, 2017, Weizmann Institute of Science, Rehovot, Israel

- Ding S-Y. "Plant cell wall composition and degradation" Invited participant and session chair for the workshop, "Technologies for Characterizing Molecular and Cellular Systems Relevant to Bioenergy and Environment" sponsored by the US Department of Energy, Office of Science, The Biological Systems Science Division (BSSD), September 21-23, 2016, in Rockville, Maryland.

- Ding S-Y. "Bioenergy Research at US Department of Energy", August 11, 2016, Biotechnology for Bioeconomy (B4B) Conference, As, Norway.

- Ding S-Y. "In vivo chemical imaging of single cells", July 18, 2016, Guangxi University, Nanning, China.

- Ding S-Y. "Real-time imaging of plant cell wall nanoscale architecture and biodegradation", July 7, 2016, University of British Columbia, Vancouver, Canada.

- Ding S-Y. "Super-resolution real-time imaging of biosystems for biofuels", Sino-US/Canada joint Symposium on Biotechnology and Bioenergy, June 3 – 8, 2016, Wuhan, China.

- Ding S-Y. "Advanced Imaging techniques for biosystems for biofuels", June 7, 2016, Hubei University, Wuhan, China.

- Ding S-Y. "Advanced microscopy techniques for biosystems for biofuels", June 7, 2016, Huazhong University of Science & Technology, Wuhan, China.

- Ding S-Y. "Lipid Production in Single Oleaginous Yeast Cells Using In Vivo Label-Free Imaging", March 8, 2016, 2016 Genomic Sciences Program Annual PI Meeting, Tysons, Virginia.

- Ding S-Y. “Real-time imaging to identify plant cell wall features that affect processing”, January 7, 2016, DOE BESC workshop, Riverside CA.

- Ding S-Y. "Studying lipid accumulation mechanism in oleaginous yeast using hyperspectral SRS microscopy and RNA-Seq in single cells", Genomic Science Contractors-Grantees Meeting XIII/USDA-DOE Plant Feedstock Genomics for Bioenergy Meeting. February 22-25, 2015, Sheraton Tysons Hotel, Virginia.

- Ding S-Y. “Advanced imaging methods in biology”, July 15, 2015, Guangxi University, China.

- Ding S-Y. “Nanoscale visualization of plant cell wall architecture and disassembly”, MIE Bioforum 2014. November 18-21, 2014, Nemuno Sato, Ise-Shima, Japan.

c. Networks or collaborations fostered

- Ding S-Y has severed as **Research Management Committee**: BioFuelsNet (2011-2017), Canada

- Ding S-Y is the **Consortium Partner**: Bioboost: Development of a plant biotechnology platform for low cost production of industrial enzymes to boost biorefinery of lignocellulose biomass, Bioforsk – Norwegian Institute for Agricultural & Environmental Research, Norway

- The hyperspectral Stimulated Raman Scattering (hsSRS) microscopy imaging technology developed through this project has been integrated into the newly awarded Great Lakes Bioenergy Research Center (GLBRC), that is one of the Bioenergy Research Centers (BRCs) sponsored by U.S. Department of Energy, Office of Science, Office of Biology and Environmental Research.