MERIT Long Term Overseas Dispatch

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Abstract

I spent my 2 months (from April 22nd to June 21st, 2018) exchange in Weng Laboratory at Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. My host, Prof. Jing-Ke Weng, a member of Whitehead Institute, is an assistant professor in the Department of Biology at Massachusetts Institute of Technology (MIT). In this report I depict the background of my exchange, the research project I was engaged in, and my life at Whitehead Institute and Cambridge.

Background

Whitehead Institute for Biomedical Research is an MIT-related research institute (financially independent from MIT), and more than twenty principal investigators are running cutting-edge research projects in a variety of biomedical fields. This time, I spent my time in Weng Laboratory, where the interests of the members have mainly focused on the elucidation of natural products (especially plant-derived bioactive chemical compounds in Chinese medicine or traditional herbal medicine) or creating recombinant organisms that produce highly valuable, bioactive natural products.

Whitehead Institute

Two years ago I was assigned to analyze an extract sample sent from Weng lab. I, who was an undergraduate student in Fujita lab at the time, had been trying to elucidate molecular structures of natural products, taking advantage of the crystalline sponge (CS) method, a novel X-ray analytical technique invented by Fujita and co-workers. I spent my first year of the Master's course on developing a new workflow that accelerates and facilitates structure elucidation of natural products from this kind of complex, crude extract mixtures that contains no less than six natural products. We were able to publish a paper on this fruitful collaboration through iterative discussions, and that motivated us to move our collaboration forward to the next stage. We expected that we could remove the bottlenecks in synthetic biology by introducing the CS method, that makes us possible to obtain molecular structures of natural products in a short period. Toward this goal I made up my mind to visit and work in the Weng lab in order to learn biological skills and have a chance to find a specific project I should focus on.

Research Project

My project was "Production of kavalactones from *Piper methysticum* in yeast and structure determination of metabolites by the crystalline sponge method." Kava (*Piper methysticum*) is a medicinal plant native to the Polynesian islands and produces bioactive kavalactones that have anxiolytic and analgesic properties. It was widely known that kavalactones are beneficial to human health, though their biosynthetic pathway had remained unknown. In 2018, Weng lab reported potential biosynthetic gene clusters that encodes biosynthetic machineries of kavalactones, and revealed that the biosynthetic origin of kavalactones and the other

intermediates. With kind instructions from my mentor, Dr. Tomáš Pluskal, a postdoctoral researcher who worked on the elucidation of biosynthetic pathways of kavalactones, I attempted the heterologous expressions of these genes in yeast that is one of the widely used host organisms in biology. We adopted an optimized yeast toolkit utilizing Golden Gate assembly to produce plasmids that contain multiple gene clusters. We obtained yeast constructs with biosynthetic gene-containing plasmids through cloning with restriction enzymes and the PCR method, and transformation of the plasmids into either *Escherichia coli* or yeast. After cultivating these recombinant yeasts for several days, metabolite profiles showed the production of kavalactone intermediates. This is the first example of heterologous expression of kava-derived biosynthetic genes in yeast and production of kavalatone intermediates. Meanwhile, I attempted structural analysis of the metabolites by the CS method, and confirmed one kavalactone showed a sign of inclusion into the CS. Upon the completion of this dispatch, I am still working on the structural analysis as follow-up experiments and discussing on the upcoming target of biosynthetic pathway elucidation with Weng Lab members.

Though it was my first experience to perform biological experiments because I am a chemisty student, I was able to obtain a recombinant yeast system within the two-month period of my dispatch. The knowledge and skills of synthetic biology I learned in this dispatch is what I would like to focus on in my doctoral research, thus I really appreciate this opportunity that helps me visualize my future research plan more specific.

Life in Cambridge

Quite a few famous universities and colleges are located in the Greater Boston area, including MIT and Harvard, together with academia-oriented startups and research institutes of leading companies. There are also Japanese researches and students, and I was able to have both academic and social interactions among them by joining the holiday events by Japanese communities such as BJRF and Greater Boston UTokyo Alumni Club.

Lab meetings are held once a week, and most of the members joined in special seminars once or more a week. Sometimes I found it difficult to understand what they meant in their talks on cutting-edge biological research, that were quite unusual topics to chemistry students including me. I also had a chance to give a talk on a chemical topic (the CS method we have developed in Fujita lab) in Weng lab meetings. It was great that in prior to this I was once trained to arrange a representation that is for people who have different research interests through MERIT colloquium I. This talk seemed to give members basic knowledge of the CS method, helped them plan better ideas of future collaborations.

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