

Fellowship ID : BR230401

2023年 7月 29日

YYYY/MM/DD

独立行政法人日本学術振興会理事長 殿  
To: President, Japan Society for the Promotion of Science

## 研究活動報告書 Research Report

### 1. 受入研究者/ Host researcher

受入研究機関・部局・職  
Name of Host Institution, Department and  
Title

東京大学・大学院農学生命科学研究科・准教授

受入研究者氏名  
Host Researcher's Name

岡田 茂

### 2. 外国人招へい研究者/ Fellow

所属研究機関・部局・職  
Name of Institution, Department and Title

Texas A&M University, Department of Biochemistry & Biophysics,  
Associate Professor

外国人招へい研究者氏名  
Fellow's Name

Timothy Patric DEVARENNE

### 3. 採用期間/ Fellowship Period

2023年7月6日

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2023年7月25日

### 4. 研究課題/ Research Theme

石油生産微細緑藻ボツリオコッカス ブラウニーのゲノム解析

### 5. 研究活動報告/ Research Report

#### (1) 研究活動の概要・成果/ Summary of Research Results

For this BRIDGE Fellowship there were two main goals: 1. To meet with research collaborators to discuss ongoing and future research projects and 2. To extract high molecular weight genomic DNA from the green microalga *Botryococcus braunii*. I will first describe my interactions and meetings I had with the research collaborators followed by the genomic DNA extraction.

#### Research Collaboration Meetings:

**Dr. Shigeru Okada**, University of Tokyo, Tokyo: Dr. Okada was the host for this BRIDGE fellowship visit and he has been a long time research collaborator with myself (Fig. 1). During the visit Dr. Okada and I discussed several issues related to our ongoing research collaborations:

1. Revisions to a manuscript we are both authors on and is under review at the journal *Algal Research*. The manuscript covers the topic of genomics analysis for *B. braunii*.
2. The content of a manuscript Dr. Okada is currently working on that is related to the classification of the S race of *B. braunii*.
3. Plans for our future research collaborations on *B. braunii* and possible visits to each others laboratories in the future.



Fig. 1. Myself (left) and Dr. Okada (right) meeting in his office.

(注) 採入期間終了後3ヶ月以内に提出

※ (Note) Submit the form within 3 months after the expiration of fellowship.

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4. I gave a presentation about the research being conducted in my laboratory (see section 2 below).
5. I extracted high molecular weight (HMW) genomic DNA (gDNA) from *B. braunii* for use in genome sequencing (see next section below).

**Dr. Koji Kawamura**, Osaka Institute of Technology, Osaka: Dr. Kawamura is a new collaborator on mine, but he has been working on *B. braunii* with Dr. Okada for several years. He is interested in genomics research for *B. braunii* and thus was interested in the current *B. braunii* genomics research being conducted in my laboratory. I traveled from Tokyo to Osaka on July 13 and 14 to meet with Dr. Kawamura and the members of his laboratory. We discussed several issues and conducted some research:

1. On the first day that I arrived in Osaka, I gave a short overview to Dr. Kawamura's laboratory about the research being carried out in my laboratory related to *B. braunii*.
2. We discussed the best approaches for conducting genomics research including DNA extraction and processing, the best sequencing platforms, and approaches for assembling genomes.
3. We discussed Dr. Kawamura's plans to come to my laboratory in January, 2024 to get hands on experience with how researchers in my laboratory conduct genome assembly.
4. On my last day in Osaka, I showed Dr. Kawamura how to extract the high molecular weight (HMW) genomic DNA (gDNA) from *B. braunii* that is needed for the sequencing related to genome assembly.
5. On my final night in Osaka, Dr. Kawamura and his lab members took me out to dinner for tonkotsu ramen (Fig. 2).



Fig. 2. Tonkotsu ramen dinner with Dr. Kawamura. Dr. Kawamura (left), myself (right).

**Dr. Daisuke Umeno**, Waseda University, Tokyo: Dr. Umeno and I have been collaborating for about 4 years. He is a world expert on the research technique called directed evolution. In this process a gene is randomly mutated, expressed to protein in bacteria, and the resulting mutant proteins screened for desirable changes in functionality. My laboratory has been applying directed evolution to a hydrocarbon biosynthesis gene/protein from the L race of *B. braunii* and have been using advice from Dr. Umeno to optimize our experiments. During my meetings with Dr. Umeno we discussed the following:

1. A postdoctoral researcher in my laboratory, Dr. Ivette Cornejo-Corona, has been awarded a JSPS Short Term Postdoctoral Fellowship to visit Dr. Umeno's laboratory at Waseda University to gain further experience with the directed evolution experiments. This visit will be for 3 months beginning in August, 2023.
2. We discussed the plans for the research Dr. Cornejo-Corona will perform in the Umeno laboratory.
3. We discussed the housing arrangements for Dr. Cornejo-Corona.
4. After our discussions Dr. Umeno and I went out for a dinner of pork shabu shabu (Fig. 3).



Fig. 3. Pork shabu shabu dinner with Dr. Umeno. Dr. Umeno (left), myself (right).

**Genomic DNA extraction:**

The goal of this research was to extract high molecular weight (HMW) genomic DNA (gDNA) from the green microalga *Botryococcus braunii*. This alga is divided into three chemical races termed A, B, and L that are defined by the type of hydrocarbon each race produces. In recent years a new chemical race, the S race, has been defined. However, Dr. Okada has evidence that the S race is actually a variant of the L race. One way to definitively determine which race the S race belongs to is to sequence its genome. My laboratory has recently completed the sequencing and assembly of the genomes for the A, B, and L races. An analysis of the putative S race genome in comparison to the A, B, and L race genomes will help us determine the correct classification of the S race. In order to sequence the S race genome HMW gDNA is needed. Thus, my goal was to extract the needed HMW gDNA from the S

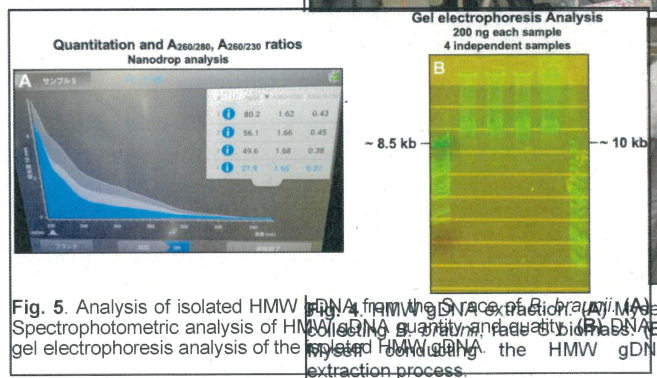
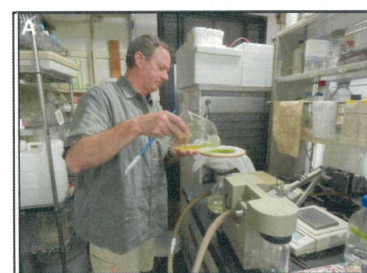


Fig. 5. Analysis of isolated HMW gDNA from the S race of *B. braunii*. A) Spectrophotometric analysis of HMW gDNA quantity and quality (A260/280, A260/230) of the isolated HMW gDNA. B) Gel electrophoresis analysis of the isolated HMW gDNA. The HMW gDNA extraction process.

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race. Dr. Okada grows the S race his laboratory and I do not grow the S race in my own laboratory. The initial step in this process is to collect the biomass of the S race from a culture of this alga. This is done by filtering the algal culture through a nylon mesh to separate the biomass from the liquid of the culture (Fig. 4A). A sample of the isolated biomass is then used to extract the HMW gDNA using a protocol specific for *B. braunii* that was developed in my laboratory (see Fig. 4B showing myself carrying out the HMW gDNA extraction). Once the HMW gDNA is isolated the quality is analyzed spectrophotometrically (Fig. 5A) and by DNA gel electrophoresis (Fig. 5B). This analysis shows that while decent amounts of HMW gDNA were isolated (see ng/ $\mu$ l column in Fig. 5A) it was contaminated with high amounts of polysaccharides (a low A260/A230 ratio in Fig. 5A). The analysis of the HMW gDNA by gel electrophoresis shows the size of the DNA is in a range greater than 10,000 base pairs (10 kb, Fig. 5B). Overall, this analysis indicates that while the size of HMW gDNA is good the quality is not so good due to polysaccharide contamination. Thus, it may not be suitable for genome sequencing. In order to address this issue, I brought some biomass of the S race back to the USA to allow the graduate student in my laboratory to repeat the HMW gDNA extraction to get a better quality sample suitable for genome sequencing.

(2) 主な研究発表 (雑誌論文、学会、集会、知的財産権等) / Main Research Publications

During the fellowship a research publication was not produced. However, I did give an hour long research seminar at the University of Tokyo, mostly to the members of Dr. Okada's laboratory and a few other researchers from his department (Fig. 6A). In this presentation I presented the recent research results from my laboratory focusing on the genomics studies we have carried out on the A, B, and L races of *B. braunii*. After the presentation, Dr. Okada and the members of his laboratory had a small party to allow the students to interact with me and practice their English speaking skills (Fig. 6B).

(3) その他/ Remarks

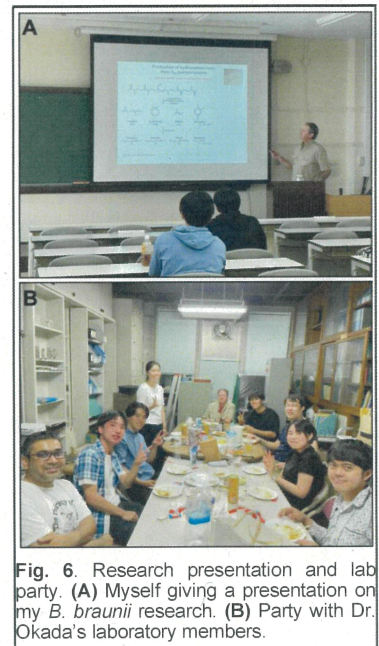


Fig. 6. Research presentation and lab party. (A) Myself giving a presentation on my *B. braunii* research. (B) Party with Dr. Okada's laboratory members.

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