Kwansei Gakuin University
Report of Research Outcome

2024/03/15

To President

Department : Science and Technology
Position : Postdoctoral fellow
Name : Matthew Brown

I report the outcome of the research as follows.

Name of the Fund/Program	□Sabbatical leave with grant □Sabbatical leave with no grant □KGU Joint Research □Individual Special Research ☒Postdoctoral fellow ※Please report by designated form as for "International Research Collaboration".
Research Theme	NEDO/AlgalBio – creation of a fast-growing algae strain
Research Site/Venue	Kwansei Gakuin Daigaku, Department of Biosciences
Research period	2023/04/01 ～ 2024/03/31 （12 month）

◆ **Summary of the research outcome** **(approx. 2,500 words)**

Please write down the outcomes in detail regarding the research theme above.

A major focus of our lab currently is the development of a fast-growing strain of the diatom *Phaeodactylum tricornutum* (hereafter *P. tricornutum*) for use within an algal bioreactor. The goal is to utilize this strain as a carbon capture method at a fossil fuel-based power plant, removing CO2 before it is released into the atmosphere. Captured CO2 could then be used either to produce more cells, which subsequently would be used as feedstock for other processes, or used directly by the cells for the production of useful products like biofuel or bioplastics.

To create a fast-growing stain of *P. tricornutum* we have targeted multiple genes for knockout (Table 1). Specifically, multiple genes involved in the production of triglycerides, which are used by the cell as energy storage compounds. The majority of these genes produce diacyl glyceride transferase (DGAT) which catalyzes the final committed step in triglyceride synthesis. The idea is that by reducing carbon flow to these end products we can redirect that carbon into new cells, increasing the growth rate of the culture.

Targeted genes:

AB ID	Gene Name	Protein ID (UniProt)	Genomic Location
17	J9794		2:1107150-1108923
18	J23913	B7GDK2	28:184539-185729
19/20	J31662	J7HZ45	1:645249-646427
21	J41969	KCT1	8:185796-186974
22	J43469	J7I5V7	2:547446-548408
23	J45790	B7FYP1	8:280839-282611
24	J45947	B7FZ92	8:750087-751429
25	J49462	J7I6H6	22:309342-310590
26	J49544	I6QM80	22:574222-575323
27	J49708	B7GBM5	24:16605-19670
28	J49702	B7GBI2	23:497473-489593

Table 1: Genes which have been targeted for knockout. AB refers to AlgalBio, the name of the project, and the ID refers to the particular vector constructed. Gene name is taken from the *P. tricornutum* annotated genome, protein ID is taken from UniProt. The genomic location lists the chromosome number followed but the basepair range encompassing the gene.

Knockouts were created using a CRISPR-Cas9 system developed within our lab. Twelve knockout vectors have been generated (we are targeting two spots for J31662) using the pAC_Cas9_Nickase plasmid (chloramphenicol resistance). Cas9 nickase utilizes two separate gRNA targeting sequences on opposite strands of the DNA, resulting in fewer off-target effects. Vectors were generated using Golden Gate cloning and transformed into 10-beta *E.coli* for amplification. After harvesting the vectors, they were again transformed into S-17 *E.coli* in order to transform *P. tricornutum* via bacterial conjugation. *P.tricornutum* transformants were then plated onto selection media containing chloramphenicol, with only cells which had been successfully transformed able to survive.

Successfully grown cells were then isolated and subject to PCR using primers for the targeted gene. Any cell colony which showed a different-sized band in comparison to wild-type was then resuspended in water and spread onto plates lacking any antibiotic selection. This process was repeated multiple times (between 2-4 for most knockouts) until all the mutant cells tested possessed the same band size, suggesting the presence of a monoclonal mutant isolate. Finally, the DNA sequence of the targeted gene was sequenced and compared to wildtype to confirm the knockout.

Currently, I have successfully isolated monoclonal mutant strains for four of the targeted genes (Table 2). This has been confirmed by sequencing the targeted genes of the strains in question and comparing them to the wild-type strain (i.e. a strain which has not been genetically modified). I am currently screening 4 other strains and will likely have isolated monoclonal mutants within the next month. Finally, I am performing transformation of 3 other strains to make the necessary knockouts.

Gene Name	Transformed	Screened	Monoclonal Isolate
J9794			
J23913	Y	Y	Y
J31662t1			
J31662t2	Y	Y	
J41969			
J43469	Y	Y	Y
J45790	Y	Y	Y
J45947	Y	Y	
J49462	Y	Y	
J49544	Y	Y	
J49708	Y	Y	Y
J49702	Y	Y	

Table 2. Status of knockout creation. Y indicates that a given stage has been completed.

Deadline : Within two months after finishing the research period.

Sabbatical leave with grant: Submit this report to President with confirmation by the dean of

school you belong to.

※Postdoctoral fellow is required to submit this report with confirmation by the dean of graduate school before the end of employment period.

Where to submit : Organization for Research and Development and Outreach (NUC)

◆We put this report on the web of KGU. If there is any problem about it because of difficulties on your research, please let us know.