Comparative transcriptomics to elucidate the water surface-floating mechanism of HIKARIMO

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Research objective

Microalgae fix CO_2 and convert it into several useful compounds owing to their high photosynthetic activity. Especially in the evolving decarbonized society, expectations from the field of applied microbiology, including the fermentation industry, are high in terms of fish and shellfish aquaculture for the production of omega-3 fatty acids and biofuels. However, making the microalgal industry economically feasible remains difficult. One of the most problematic processes in terms of cost, CO_2 balance, and energy balance is the harvesting of microalgal cells from culture. In general, microalgal cell concentrations in culture tend to be lower than those in bacterial or yeast cultures. Therefore, cell harvesting using conventional methods, including centrifugation, incurs significant cost and energy consumption. In this study, we focused on the golden microalga *Chromophyton* sp. (called HIKARIMO in Japanese) (Figure 1), which has the unique property of floating on the water surface and aimed to elucidate its floating mechanism at the molecular level. If cells spontaneously concentrate on the water surface, they can be easily harvested, which is expected to markedly decrease the cost of cell collection, which is a bottleneck in the development of the microalgae bioindustry.

Fig. 1 *Chromophyton* sp. (HIKARIMO) found at Hitachi-city, Ibaraki, Japan

Methods

Genome sequencing of *Chromophyton* sp.

Three isolates (strains SH01, SH02, and SH03) of *Chromophyton* sp. were cultured in AF-6 medium, which is widely used to culture freshwater microalgae. DNA was extracted from the cells of each strain using the cetyltrimethylammonium bromide method. The extracted DNA was subjected to long-read sequencing using PacBio Revio and the obtained read sequences were assembled. Gene prediction was performed using Funannotate, based on the transcriptome information of the SH02 strain obtained as described in the next section. In addition, gene prediction for the SH01 and SH03 strains was performed using BRAKER based

on the predicted gene information of the SH02 strain.

Transcriptome analysis of *Chromophyton* sp.

TRIzol reagent was added to the cultured cells (swimming cells) of strain SH02 and agitated. Chloroform was then added, and the supernatant obtained by centrifugation was purified using the NucleoSpin® RNA kit. In the field, *Chromophyton* sp. was collected with permission from the Hitachi City Office. Floating cells were collected from the Higashinamekawa Hikarimo Park in Hitachi City, Ibaraki Prefecture, where the SH01 strain was isolated. After removing large debris using a cell strainer, the cells were immersed in RNAlater™ Stabilization Solution and transferred to the University of Tsukuba. RNA was extracted in the same manner as described above.

Results

Acquisition of highly accurate genome information of *Chromophyton* sp.

Genome information for the three strains was obtained, and gene predictions were performed. The results showed that the genomic information of the three strains were highly similar in terms of genome size, number of genes, and sequence similarity. This result is consistent with that of previous phylogenetic studies. BUSCO analysis was performed to assess the completeness of the gene predictions. The obtained BUSCO values were sufficiently high compared with those from previous studies that analyzed the genomes of other golden microalgal species.[1)] In addition, metabolic pathway maps were reconstructed from the predicted genes, and the reconstructed metabolic pathways were consistent with the composition of photosynthetic pigments and fatty acids detected in *Chromophyton* sp. These results led us to conclude that we have almost completely identified the genes in the *Chromophyton* sp. genome. The gene sequences predicted in this study were used for the subsequent transcriptome analysis.

Comparative transcriptomes of swimming and floating cells of *Chromophyton* sp.

We successfully optimized the culture conditions, resulting in a significant increase in cell concentration. However, most cells in the culture medium were swimming cells, and only a small fraction were floating cells. To elucidate the mechanism of the water surface-floating phenotype, it was necessary to extract a sufficient amount of RNA from floating cells for transcriptome analysis.

Therefore, we extracted RNA from cells floating in a natural environment. We observed cells floating on the water surface throughout the year at Higashinamekawa Hikarimo Park in Hitachi City, Ibaraki Prefecture, Japan (Figure 1). Floating cells were collected on glass slides and subjected to RNA extraction. As a result, 14.7 μg of RNA was obtained. RNA was also

extracted from the cultured swimming cells, and 13.0 μg of RNA was obtained. When sufficient RNA samples (Table 1) were obtained, sequence data were obtained using a next-generation sequencer and mapped to genomic information.

Table 1 RNA extraction from the floating and swimming cells

	Concentration (ng/μl)	Amount (μg)	A260/280	A260/230
Floating cells	488.5	14.4	2.22	2.28
Swimming cells	434.4	13.0	2.28	2.53

A comparison of transcriptome data obtained from floating and swimming cells indicated that a group of genes encoding proteins with functional domains related to cell division and differentiation were highly expressed in floating cells. Previous studies have reported that the cellular morphology of *Chromophyton* spp. changes when it floats on the water surface.[2,3] This suggests that the group of genes identified in this study may be involved in cell morphological changes.

To further test this hypothesis, we will establish a genetic engineering technique for *Chromophyton* sp. to achieve the knockdown or knockout of the genes found in this study.

Conclusion

In this study, we obtained genomic information on the water-surface planktonic microalga (golden microalga) *Chromophyton* sp. (also known as HIKARIMO) and predicted the genes for transcriptome analysis. RNA was extracted from cultured swimming and floating cells collected from the natural environment. Subsequently, transcriptome analysis was performed. We found that a group of genes encoding proteins potentially involved in cell division and differentiation was highly expressed in floating cells. This suggests that these genes may be related to morphological changes during the transition from swimming to floating cells. The establishment of a genetic engineering technique for *Chromophyton* spp. is planned to further elucidate the mechanism of the flotation phenotype.

References

1) Majda, S., Beisser, D., Boenigk, J. (2021) Nutrient-driven genome evolution revealed by comparative genomics of chrysomonad flagellates. *Commun. Biol.* **4:** 328
2) Karin Petry, W. (1968) Entwicklungsgeschichtliche Untersuchungen an *Chromophyton rosanoffii* und einigen Chlorophyceen *Osterr. Bot. Z.* **115:** 447–481
3) Nomizu, M. (2007), Study on light reflection of water-surface floating golden microalga, *Chromophyton rosanoffii* (HIKARIMO). *Master's Thesis, University of Tsukuba*