Abstract

Plant physiological studies on oil production in microalgae :
Analysis of lipid synthesizing system and the assessment of the biological invasion risks.

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Pseudochoricystis ellipsoidea is a unicellular green alga, which belongs to the class Trebouxiophyceae. Under nitrogen-starvation conditions, *P. ellipsoidea* accumulates storage lipids consisting primarily of triacylglycerols (TAG) (Satoh *et al*. 2010). Therefore, *P. ellipsoidea* is potentially a viable algal strain for mass biodiesel production. The aim of this study was to investigate the mechanisms of TAG accumulation (chapter 1) and to assess the invasiveness of *P. ellipsoidea* (chapter 2).

Chapter 1 deals with the cellular mechanisms underlying lipid metabolism during oil accumulation. *P. ellipsoidea* was grown in a medium containing nitrate at a concentration of 25 mg/L, under continuous illumination (356 μmol m^{-2} s^{-1}) and aeration (2% CO_2) at 25°C. Because *P. ellipsoidea* consumed nitrogen in the medium for growth, TAG accumulation was triggered by depletion of available nitrogen, presumably depending on cellular C/N ratio.

First, the feature of the TAG accumulating phase will be described. The final step in TAG synthesis in plants is acylated DAG to form TAG. While gene expression of several diacylglycerol acyltransferases (DGATs), which used acyl-CoA as the acyl donor, were upregulated in response to nitrogen starvation, gene expression of a phospholipid:diacylglycerol acyltransferase (PDAT), which used phospholipid as the acyl donor, did not increase during TAG accumulation. DGAT is suggested to be the key enzyme for synthesis of TAG. 5 μM cerulenin, which was a specific inhibitor of the *de novo* fatty acid synthesis, inhibited 96% of the TAG-synthesizing activity, indicating that TAG accumulation was largely attributable to the *de novo* synthesis of fatty acids. Photosynthetic activity decreased during cell growth. Starch was degraded and the gene expression level involved increased, suggesting that carbon reallocation from starch into fatty acids occurred. Comparison of $^{14}CO_2$ released from [1-^{14}C]glucose and [6-^{14}C]glucose revealed that the contribution of pentose phosphate pathway to degrade starch was more than that of the glycolytic pathway. The obtained results for the expression level of genes involved in glycolytic and pentose phosphate pathways were consistent with the C6/C1 experiment. NADPH produced by the pentose phosphate pathway appeared to be in fatty acid biosynthesis.

In the early growth phase, where TAG-synthesizing activity was low and starch-synthesizing activity was high, carbon flux was different from that of the TAG accumulating phase. The feeding experiments with [1-^{14}C]oleic acid suggested that TAG was degraded and likely used for starch synthesis.

TAG-synthesizing activity was less sensitive to inhibition by cerulenin. In the early growth phase, the contribution of *de novo* fatty acid synthesis may be low, or different enzymes may be active in the fatty acid synthesis pathway.

Cellular morphology also changed over the cell growth. Observation with the transmission electron microscopy found autospores inside the cells of the logarithmic phase. In the stationary phase, cell size was smaller and few or no autospores were detected. On the other hand, many small lipid droplets (LDs) were observed in the cell of the logarithmic phase. Later, a few big LDs were present in the cell in the TAG accumulating phase. The membranes of LDs seemed fuse each other. In previous studies, brefeldin A (BFA), a chemical inducer of ER stress, was reported to promote formation of LD in *Chlamydomonas reinhardtii* or *Chlorella vulgaris* (Kim *et al.* 2013, Kato *et al.* 2013). The induction of TAG-synthesizing activity by BFA was detected in this study, as well. Starch granules were observed in chloroplast. Chloroplast was big in the early growth phase, but the degeneration of thylakoid membrane was observed in the TAG accumulating phase.

Chapter 2 assesses the biological invasion risks associated with a massive outdoor cultivation of *P. ellipsoidea.* In this work, a model experiment system was constructed in order to evaluate the survival and growth of *P. ellipsoidea* in various environmental water samples. It was demonstrated that *P. ellipsoidea* survives in aquatic environments. *P. ellipsoidea* grew well in water containing >2 mg/L nitrate, but no growth was observed in water containing 3 mg/L of ammonia if the nitrate concentration was <1 mg/L. The addition of nitrate to the environmental water at the final concentration of 3 mg/L improved the growth, demonstrating that nitrate is the growth-limiting nutrient in these water samples. The mutant of *P. ellipsoidea* did not use nitrate as a nitrogen source, and it could not grow in the water where the wild type grew well. The risk of survival after leakage would greatly diminish by using such mutant of *P. ellipsoidea*.

The spread of *P. ellipsoidea* cells from an outdoor raceway pond into the surrounding area was investigated over one month by detecting this alga in water-containing vessels installed at variable distances from the raceway pond. While examination under a microscope revealed no significant growth of *P. ellipsoidea*, *psbA* gene of *P. ellipsoidea* was detected by PCR even in vessels placed 150 m apart. The ability of *P. ellipsoidea* to spread via wind was demonstrated, but *P. ellipsoidea* was not detected in the direction the wind was not blowing in.

Reference

Satoh A. *et al.* (2010) J. Jpn. Inst. Energy **89**, 909-913.
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