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Biofuel Production Concomitant with CO_2 Fixation by Marine Euglenoid, *Eutreptiella* sp.

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Summary

There are great prospects for microalgae in producing biofuel, which have a higher lipid yield per unit area and higher CO_2 fixation capacity than higher plants. Biofuel production using seawater is desirable from the perspective of securing resources because cultivation of microalgae requires large quantities of water. As a freshwater species, *Euglena gracilis* can only survive in salt concentrations of up to 1/3 of seawater, making it unsuitable for cultivation using seawater. In this study, we screened for marine Euglenoid with lipid-producing capacity and found that *Eutreptiella* sp. strain NIES-2325 (hereafter NIES2325) produce WE upon exposure to hypoxia. We tried to elucidate the details of lipid metabolism of NIES2325.

NIES2325 cultures were treated with hypoxia, and as a result, reduction of paramylon content and production of wax esters (carbon length was mainly 26-28) are observed. Comparison of the degraded paramylon and produced WE contents showed that the conversion rate from paramylon to WE was about 7%. This is lower than the conversion rate of 25% in *Euglena gracilis* cultured under autotrophic conditions.

Pyruvate:$NADP^+$ oxidoreductase (PNO) has been considered to be the sole acetyl-CoA source to the WE synthesis system in *Euglena*. In this study, PNO activities in NIES2325 were measured using crude cell extract before and after hypoxic exposure. The PNO specific activity increased approximately 6-fold upon hypoxia exposure. This suggests that PNO has an important function in metabolism under hypoxia than under aerobic conditions in NIES2325.

To analyze the physiological function of isozymes of PNO in NIES2325, this study developed a gene silencing method using RNAi to suppress PNO1 and PNO2 gene expression. Double-stranded RNA was introduced into NIES2325 by electroporation. The mRNA expression levels of cells cultured under aerobic conditions were compared with control cells by semi-quantitative RT-PCR, and the expression of each mRNA was successfully suppressed. In addition, a decrease in WE production under hypoxia was observed in the knockdown cells. These results suggest that the two PNO isozymes of NIES2325 may be involved in the supply of acetyl-CoA in WE production under hypoxia.