Development of the novel cyanobacterial strains producing novel fatty acids and expressing the inducible cell-lysing system

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Microalgae have high potential to produce oils and can be cultivated even on the non-arable lands, thus, they are considered as suitable source of the renewable materials. However, products available form them are limiting based on their metabolism.

Fatty acid methylesters from the lipids in microalgae attract attention as a biodiesel. However, the majority of the fatty acids (FAs) from the microalgae are polyunsaturated. Thus, the biodiesels derived from them are fluid and easy to handle as a liquid fuel, but labile against oxidation by O_2 in the atmosphere. We attempted to produce cyclopropane FAs in the cyanobacterium *Synechocystis* sp. PCC 6803 by heterologous expression of the *cfa* gene for cyclopropane FA synthase from *Escherichia coli* to produce novel FAs that are sufficiently fluid and stable in response to oxidization. We successfully synthesized C19 cyclopropane FA from oleate (18:1Δ9) in the *Synechocystis* cells expressing the *cfa* gene. The *Synechocystis* cells produce di- and tri-unsaturated FAs, linoleate (18:2Δ9,12) and linolenate (18:3Δ6,9,12), at 34˚C. These polyunsaturated FAs were not converted into the cyclopropane FAs. By the co-expression of the *desC2* genes for *sn*-2 specific Δ9 desaturase which converts palmitate (16:0) to palmitolate (16:1Δ9) in the membrane lipids, the cells also produced C17 cyclopropane FA. In order to increase the content of cyclopropane FA, we inactivate the *desA* and *desD* genes for Δ12 and Δ6 desaturases, respectively, to prohibit the synthesis of polyunsaturated FAs. The cells expressed the *cfa* and *desC2* genes and inactivated the *desA* and *desD* genes produced up to 30% of cyclopropane FAs in the total FAs. Because all the known oxygenic-photosynthetic organisms do not produce cyclopropane FAs, it is interested in the effect of production of the cyclopropane FAs on the photosynthetic activity. The production of CFAs in *Synechocytis* did not altered growth rate and photosynthetic activity at high temperature. These results indicate that cyanobacteria might have a potential to produce novel FAs via photosynthesis.

The most of products by microalgae are accumulated in the cells. To obtain them we need to harvest the cells from the large volume of culture media and extract from the cells harvested. Generally, the cell walls of microalgae are rather tough to break, making the cost for cell breakdown expensive. Then, we developed *Synechocystis* cells which have inducible cell-lyse system by the imitation of phosphate. When the cells were cultured in phosphate-deficient (-P) conditions, the cells of the strain were lysed. Whilst the wild-type cells were not lysed. During the -P conditions protein concentrations in the lysed cells were remarkably increased. This system might be applied for saving the extraction cost.

Keywords: cyclopropane fatty acid, desaturase, phosphate-deficiency, *Synechocystis*, two-component system