

Applied studies on the docosahexaenoic acid production using plant biomass by *Aurantiochytrium* species

(*Aurantiochytrium* 属による植物資源を活用した
ドコサヘキサエン酸生産に関する応用研究)

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Abstract

Docosahexaenoic acid (DHA, C22:6 ω 3) is well known as one of the major ω 3-PUFAs, which are abundant in nature and are mainly obtained from fish oil. On the other hand, the demand for fish oil has been increasing in recent years. In order to develop an alternative DHA source, this study focused on the development of a DHA-rich lipid production system by an oleaginous microorganism using plant biomass.

In chapter 1, the author focused on the production of DHA-containing microbial lipids by *Aurantiochytrium* sp. using defatted soybean (DS) as a nitrogen source with a novel isolate, *Aurantiochytrium* sp. 6-2. DS is a plant biomass that could be provided sustainably at a low cost. *Aurantiochytrium* sp. 6-2 could not directly assimilate the DS as a nitrogen source but could grow well in a medium containing DS fermented with rice malt. When cultivated in a fermented DS (FDS) medium, free amino acids from FDS were consumed and *Aurantiochytrium* sp. showed vigorous growth and high DHA productivity. *Aurantiochytrium* sp. 6-2 could grow in the medium with the addition of sufficient sulfate and chloride ions as inorganic nutrients without seawater salt and

showed 15.4 g/L DHA production (in 54.8 g/L total fatty acid production) in 1 L of the FDS medium.

In chapter 2, the author focused on evaluating the production of DHA-containing microbial lipids by *Aurantiochytrium* sp. using starch as a carbon source by co-culture of *Aurantiochytrium* sp. with other microorganisms. The results showed that *Aurantiochytrium* sp. 6-2 could not directly assimilate starch as a carbon source but could grow with the existence of bacteria in a starch-containing medium. Several bacteria were screened for the co-culture with *Aurantiochytrium* sp. by using starch as a carbon source. Three co-culture pairs were selected based on DHA production. *Aurantiochytrium* sp. showed stable growth by using maize starch as a carbon source and produced 3.49 g DHA (in 12.1 g/L total fatty acid production). In the fish-feeding trial, there was no negative effect on the growth of *Scomber japonicus* when microbial DHA was used to replace 50 % of fish oil in the feed.

In chapter 3, the author addressed the development of DHA production by solid-state fermentation of *Aurantiochytrium* sp. Firstly, commercial miso was evaluated as a nitrogen source for the production of DHA-containing microbial lipids by *Aurantiochytrium* sp. The results showed that *Aurantiochytrium* sp. could directly assimilate the commercial miso as a nitrogen source and showed vigorous growth and high DHA productivity, which was similar with the culture by using FDS medium. In particular, *Aurantiochytrium limacinum* SR21 produced 16.2 g/L DHA (in 53.2 g/L total fatty acid production) after 5 days of cultivation. After pre-cultivation using FDS or commercial miso in the liquid culture, solid-state cultivation using defatted soybean as the solid phase was performed, and the results showed that *Aurantiochytrium* species can

further accumulate fatty acid during 3-day cultivation by using DS as substrate in solid-state fermentation and approximately 28.4 % increase of DHA content was recorded. In the fish-feeding trial targeting *S. japonicus*, the partial replacement of fish feed by microbial meal from the solid-state fermentation resulted in no negative effects on the growth of *Scomber japonicus*.

Therefore, microbial DHA produced by *Aurantiochytrium* sp. using FDS or commercial miso as a nitrogen source could provide a stable and sustainable DHA supply. In addition, starch has the potential to replace glucose as a carbon source in order to develop low-cost DHA production. In the feeding trial, microbial DHA was used to replace a part of the fish oil and there was no negative effect on the growth of *S. japonicus*. These results suggest that microbial DHA produced by *Aurantiochytrium* sp using plant biomass could be an alternative source of natural DHA derived from fish oil.