論　文　の　要　旨

題目　Isolation of a novel thraustochytrid strain and its application for treatment of food processing wastewater and production of polyunsaturated fatty acids

(新規スラウストキトリッド株の単離とその食品製造排水処理や多価不飽和脂肪酸生産への応用)

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Various polyunsaturated fatty acids (PUFAs) have been known to physiologically support bodily and neural health at cellular level of human and other animals. Deficiencies of PUFAs can be associated with defects in cellular function, which may lead to diseases, as PUFAs are important constituents of cell membranes and cell signaling systems. Among them, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and have been termed as essential due to their core function in human body which is obtained from food as the body could not simply synthesize them.

PUFAs for human consumption have been derived mainly from commercial fish sources in the form of fish oil and the cruder fish meal. Fish oil is extracted from the flesh of fish using physical pressure or enzymatic process, subsequently followed by multiple refining steps to further purify the PUFAs and remove the undesirable odor to make the crude fish oil safe and edible. Fish meal is dried mass made from the flesh of fish that are not used for human consumption. Fish oil and meal are also important feed for fish aquaculture to attain satisfactory growth of fish, among other farm animals in agricultural setting. Because of its beneficial use, fish oil commands such a high price at which highly varies according to its ω-3 purity and profile. The decline of catchment and limited seasonal supply of commercial fish, issue regarding fishery industry sustainability, contamination of mercury in the body of water which in turn gets into the fish, along with issues with the purity of the extracted PUFAs have prompted researched into possible alternative biotechnological sources of PUFAs.

Thraustochytrid, such as *Aurantiochytrium*, *Thraustochytrium*, and *Schizochytrium,* are heterotrophic protists microorganism found in the marine and coastal environment known to produce lipids containing PUFAs. The production of PUFAs by thraustochytrids may provide an easier and less expensive means of producing PUFAs-rich biomass and oil as an alternative to production from fish. Nonetheless, there are still several shortcomings in PUFAs production process using thraustochytrid: preparation of highly-sophisticated artificial growth medium, very specific cultivation condition and method, and subsequent lipid extraction method. Food processing wastewater may be a cost-effective alternative medium to cultivate the heterotrophic thraustocytrids because of its high content of organic matter, absence of toxic compounds within, and generally more economical price. Although sterilization may still be required as a pretreatment to obtain better yield of biomass and PUFAs from the cultivated thraustocytrids after the cultivation process. Also, considering the different qualities of various available food wastewaters and durability and productivity of currently-identified strains of thraustocytrids, adjustment to the chemical properties of the food wastewaters samples might be required to ensure proper growth and high biomass and desired PUFAs productivity (mainly DHA and EPA) of the cultivated strain.

In the present study, we explore the potential of for cultivation in sterilized and unsterilized food processing wastewater. At first, isolation of thraustochytrids was conducted to find the strains durable to wide range of pH, salinity and temperature. Then, food processing wastewater was applied for cultivation of the isolated strain under an unsterile condition to obtain the raw thraustochytrid biomass containing PUFAs. Because confirmation of the digestibility of raw thraustochytrid biomass by fish enzymes would be the first step to exploring the possibility of its use as a fish feed component, the raw biomass of the isolated strain was tested for digestibility by enzymes extracted from fish stomach. Finally, culture conditions of the isolated thraustocytrid to sustain removal of carbon and nitrogen and production of PUFAs in food processing wastewater were explored. This research is divided into following chapter as shown in below.

In Chapter 1, introduced usefulness of PUFAs for human consumption and fish aquaculture, fish oil as a conventional PUFAs and explained the necessity of its alternation. Then, challenges for sustainable production of PUFA using thraustochytrids were addressed.

In Chapter 2, describes isolation and identification of the four isolated four *Aurantiochytrium.* Strains from coastal habitat in Okinawa, Japan were conducted: *Aurantiochytrium* sp. L2R, L2Y, L3W and L4Y based on 18S rRNA gene sequence. Stain L3W showed better specific growth rate and higher biomass and lipid production among the other strains, hence it is chosen for the subsequent cultivation and analysis. Durability of the strain in various growth condition: wide range of pH, salinity, and temperature; were carried out to find the optimum growth condition and versality of the application as biomass yield and PUFAs production by some thraustochytrids can be varied due to changes in physical and chemical conditions of the cultivation and the growth media. Adjustment of pH into the more acidic condition and increase of salinity of the growth media were found to result in suppression of contaminating microorganism without affecting the growth of *Aurantiochytrium* sp. L3W and increase PUFAs productivity.

In Chapter 3, *Aurantiochytrium* sp. L3W was then cultivated in food processing wastewater samples discharged from miso production factory: miso-processing (MP) and bean-boiling (BB) wastewater samples with the specific pH and salinity adjustment on the wastewater sample. The adjustment was done according to the optimum growth condition obtrained from the previous isolation and identification process. In addition, from the perspective of wastewater treatment, removal of dissolved organic carbon (DOC) and dissolved nitrogen (DN) from the wastewater due to consumption by *Aurantiochytrium* sp. L3W culture was evaluated.

As the wastewater might need to be initially diluted to conform with the sewage discharge requirement, effect of the dilution of the wastewater samples was also evaluated. It was found that dilution of the wastewater samples resulted in the reduced biomass and lipid production, but remarkable increase of biomass yield was attained by dilution in terms of the DOC and DN consumptions, i.e. more efficient production. It was found that by diluting more than 5 times, the DN concentration in the BB and MP conforms with the sewage discharge standard, but not the DOC even after use for the cultivation. Longer cultivation duration and multi-step cultivation process may be applied could be applied to address this issue.

In Chapter 4, owing to the durability of the potential of *Aurantiochytrium* sp. L3W in saline and acidic growth condition, cultivation in both sterilized and unsterilized BB and MP was carried out. Cell growth and biomass production in both sterilized and unsterilized wastewater samples were confirmed, albeit unsterilized condition resulted in decreased biomass and PUFAs productivity as expected. The unsterilized condition also resulted in change of extracted PUFAs composition with reduced amount of DHA. The ability to cultivate *Aurantiochytrium* sp. L3W on unsterilized wastewater samples suggest that simultaneous wastewater treatment process and PUFAs production can be readily applied with minimal pretreatment on the food processing wastewater samples.

After successful cultivation of thraustochytrids using food processing wastewater is achieved, then how to most effectively use the harvested biomass is to be considered. Researches on application of thraustochytrids for fish feed supplement or substitute have been carried out to some extent, but the performance of thraustochytrids biomass as fish feed supplement tends to vary among different fish species with varying/limited benefit. Confirmation of the digestibility of raw thraustochytrid biomass by fish enzymes would be the first step to exploring the possibility of its use as a fish feed component. The raw biomass of *Aurantiochytrium* sp. L3W was tested for digestibility by enzymes extracted from rainbow trout stomach. It was found that the *Aurantiochytrium* sp. L3W biomass was fully digestible after 4h with the extracted enzyme assay, hence suggesting proper digestibility of the biomass as fish feed.