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Enrichment potential of HUFA-rich thraustochytrid *Schizochytrium mangrovei* for the rotifer *Brachionus plicatilis*

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ABSTRACT

An enrichment experiment was performed to evaluate the changes in lipid and essential fatty acid contents of the rotifer *Brachionus plicatilis* fed with freeze-dried cells of tropical thraustochytrid *Schizochytrium mangrovei* (Isolate IAo-1). Rotifers starved for 24 h were fed with *S. mangrovei* cells at 200, 300, 400, 500, 600 and 700 mg L^{-1}. Enrichment was carried out at two periods (Short-term = 5 h; Long-term = 10 h) to determine the optimum time needed for the maximum enrichment of the rotifers. There was an overall significant increase in the total lipid, arachidonic acid (AA) and docosahexaenoic acid (DHA) contents of rotifers after feeding with freeze-dried *S. mangrovei* indicating the successful uptake of these nutrients in the rotifer's biochemical composition. On the other hand, docosapentaenoic acid (DPA) did not change significantly in enriched rotifers. Results of the present study indicate that both factors, feeding concentrations and enrichment periods, significantly affected the lipid, AA and DHA contents of rotifers. Uptakes of lipid, AA and DHA significantly increased with increasing feeding concentrations except for those fed the highest feeding concentration of 700 mg L^{-1} for 10 h. Moreover, lipid and AA contents of enriched rotifers were significantly higher during the short-term enrichment period while DHA contents were significantly higher during the long-term enrichment period. Therefore, it is concluded that the feeding concentration of 700 mg L^{-1} at an enrichment period of 5 h is optimum in the AA and DHA enrichment of rotifers. The strategic scheme of combining the proper amount of enrichment product and the duration of enrichment in boosting the DHA contents of rotifers will effectively ensure a reliable production of nutritionally superior rotifers at a minimal cost. This will ultimately contribute to the success of rearing marine fish larvae in the hatchery.

1. Introduction

Larvae of many marine fish require a certain amount of long-chain highly unsaturated fatty acids (HUFAs) of the n-3 series such as arachidonic acid (AA, 20:4), docosapentaenoic acid (DPA, 22:5), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) for optimum survival and growth. Common sources of these essential fatty acids include: traditional commercial fish oils (by-products of pelagic fisheries); oils rich in DHA/EPA; and, single-celled eukaryotes such as heterotrophic dinoflagellates and phototrophic prymnesiophyceans (Sargent et al., 1997; Leaño and Liao, 2004; Leaño and Liao, 2007). Originally thought to be primitive fungi, the heterotrophic thraustochytrids (e.g. *Schizochytrium* spp.) are now classified under Kingdom Chromista and Phylum Stramenopila, which is closely related to heterokont microalgae (e.g. brown algae and diatoms) (Cavalier-Smith et al., 1994; Parfrey et al., 2006). Commonly known as stramenopiles, thraustochytrids are reported to produce a high amount of fatty acids, particularly DHA (Barclay and Zeller, 1996; Li and Ward, 1994; Yaguchi et al., 1997; Lewis et al., 1999; Fan et al., 2002; Leaño et al., 2003; Leaño and Liao, 2004), which is superior to EPA as feeds for fish larvae.

Fish do not synthesize long-chain HUFAs in significant quantities. They acquire HUFAs through their diet, such as eating zooplankton (e.g. rotifers, crustaceans), which are enriched with these nutrients. Increasing the HUFA content of zooplankton prior to feeding larval fish and shrimp is a common practice in the aquaculture industry (reviewed by Apt and Behrens, 1999). Due to the high cost of producing sufficient and reliable live HUFA-rich microalgae, spray-dried heterotrophic microalgae have been tested as aquaculture feeds. However, the strains tested were selected primarily for their heterotrophic growth potential with only a secondary concern for their nutritional value, particularly on their HUFA content resulting in poor larval feed performance (Laing and Verdugo, 1991). The heterotrophic *Schizochytrium mangrovei* was chosen as the test organism for the enrichment of rotifers in this study because of its high long-chain HUFA contents of the n-3 series, particularly DHA (Leaño et al., 2003). Thraustochytrids offer advantages over other

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sources of HUFA for aquaculture (Leaño and Liao, 2004). Many fish larval aquaculture species require proportionally more DHA than EPA (Furuita et al., 1996; Furuita et al., 1998; Pousao-Ferreira et al., 2001). The HUFA profile of *S. mangrovei* fits this criterion, while most oils from the fish-meal industry contain more EPA than DHA. The cells of *S. mangrovei* are small in size and have excellent suspension characteristics in seawater which facilitate easy ingestion. The physical conditions applied in the drying process can also facilitate easy ingestion by rotifers (Laing and Verdugo, 1991). Freeze-dried *S. mangrovei* may have fragile cell walls as a result of the drying process; this may make the diet more easily digested and physically broken down in the gut of the rotifer, leading to efficient assimilation and utilization. *Schizochytrium* sp. has been shown to enrich and boost the fatty acid and DHA content in rotifers *Brachionus plicatilis* and *Artemia* sp. (Barclay and Zeller, 1996). *Schizochytrium*-based products have also been available in the market for enriching rotifers and *Artemia* sp. in the U.S.A. (www.aquafauna.com). The manufacturer suggests an 8 h enrichment of rotifers prior to feeding to target larval fish. On the other hand, Kraul (2006) reported that a 2 h enrichment of rotifers prior to feeding greatly increases the health and growth rate of marine fish larvae. Unfortunately, the rotifer's fatty acid composition after enrichment for 2 h was not evaluated to support his observation. This study evaluated the potential of locally isolated tropical thaustochytrid *S. mangrovei* (Isolate IAo-1) as live food enrichment for the rotifers *B. plicatilis*. It addressed the need to identify the optimum feeding concentration and period for the enrichment of rotifers *B. plicatilis* using locally isolated tropical *Schizochytrium* species. The changes in the lipid, AA, DPA and DHA contents were analyzed in rotifers enriched with freeze-dried *S. mangrovei* cells at different feeding concentrations during short-term and long-term feeding.

2. Methodology

2.1. Mass production of S. mangrovei

Axenic small-scale heterotrophic mass production of *S. mangrovei* (Isolate IAo-1) in the laboratory was conducted on peptone-yeast-extract high glucose medium (PYG medium) following the procedure of Leaño et al. (2003) with slight modifications. Cultures were grown in a 2 L flask filled with 700 mL culture medium under 150 rpm shaking condition (Orbit Shaker, Labline Instrument Incorporated) at 25 °C for 3–4°d (days). Each culture flask was plugged with sterile cotton to avoid contamination and wrapped with black plastic to eliminate light. These cultures were later used as inoculum for the medium-scale mass production of *S. mangrovei* in a 30 L fermentor carried out at the Biotechnology Laboratory, University of the Philippines, Los Baños, Laguna, Philippines. At late-exponential phase, *S. magrovei* cultures were scaled up by inoculating into sterile 15 L PYG medium in 30 L fermentors as described previously in the small-scale mass production, except that the live cell inoculum was increased from 5% to 10% of the total volume of the culture. Fermentor-cultured *S. mangrovei* was harvested through centrifugation, freeze-dried and stored inside sealed containers at −70 °C until fatty acid analysis or until use in the rotifer enrichment experiments.

2.2. Rotifer source

The rotifers *B. plicatilis* were obtained from a routine culture at the finfish hatchery of the Southeast Asian Fisheries Development Center. They were grown semi-continously on a “green water” system as described by Duray et al. (1996) using live microalgal diet *Nannochloropsis* sp. Prior to the enrichment experiment, rotifers were harvested at the late exponential phase, washed with 0.2 μm-filtered seawater, stocked in 10 L plastic containers at a density of 5000–10,000 individuals mL^{-1}, and provided with adequate aeration. The rotifers were starved for 24 h (hours) to ensure that the gut content was clear of the microalga *Nannochloropsis* sp. with which the rotifers were originally fed.

2.3. Enrichment/feeding experiment

Prior to the experiment, samples from the starved rotifers were taken to determine the initial (time = 0 h) lipid and fatty acid contents. This was designated as time = 0 in the enrichment period.

The remaining starved rotifer suspension was stocked into each of the cone-shaped plastic containers (5 L) filled with 3.5 L filtered seawater. Each rotifer culture was provided with adequate aeration and maintained at a density of 1500 individuals mL^{-1}. Rotifers were then fed with dried *S. mangrovei* freeze-dried cells at different feeding concentrations of 200, 300, 400, 500, 600 and 700 mg L^{-1} of rotifer culture (expressed as mg L^{-1}). Short (time = 5 h) and long (time = 10 h) enrichment periods were tested for all feeding concentrations. Experimental treatments were replicated three times which were randomly assigned. Enrichment experiments were conducted at a temperature (26–27 °C) controlled laboratory.

At the end of each enrichment period, rotifers (120–200 μm in size) were collected from each treatment using a plankton net (mesh size = 37 μm) to separate them from uneaten *S. mangrovei* cells with cell sizes of 8–14 μm (Fan et al., 2002). Rotifer samples were then washed with 0.5 M ammonium formate to remove salts, freeze-dried and stored desiccated at −70 °C until further analysis.

2.4. Lipid and fatty acid methyl ester (FAME) extraction/analysis

Lipid extraction and FAME analysis of starved (initial, time = 0) and enriched rotifers, and sample of freeze-dried *S. mangrovei* cells were done following Lepage and Roy (1984) and Metcalfe and Schmitz (1961) with minor modifications. Lipids were trans-esterified with an acetylchloride/methanol mixture. For quantitative purposes, an internal standard heneicosanoic acid methyl ester (21:0, SIGMA, MO, U.S.A.) was added equivalent to 3.5% of the total weight FAMEs. FAME extracts were evaporated under vacuum, dried under a stream of nitrogen, weighed and resuspended in isooctane (LiChroSolv, Merck, Germany) prior to analysis with a GC-17A gas chromatograph (Shimadzu, Kyoto, Japan). Helium was the carrier gas. Injector and detector temperatures were both at 250 °C. Individual FAME was identified by comparing the relative retention times of the fatty acids with that of the known reference standard and quantified by a Chromatopac C-R7 integrator. FAMEs were quantified using the internal standard method and expressed as percent of the total fatty acids (TFA).

2.5. Statistical analysis

All values on biochemical composition (lipid and fatty acids) are presented on a dry weight basis. Lipid and fatty acid compositions of rotifers after the feeding experiments were analyzed using a two-way ANOVA to compare the effects of different feeding concentrations on the changes in the chemical composition of starved against the enriched rotifers at two enrichment periods. Data showing significant differences at ($P<0.05$) across treatments were further analyzed by Duncan multiple range test (DMRT). All statistical analysis was performed using the statistical program SAS (Statistical Analysis System, Version 8, Windows 2000).

3. Results

3.1. Lipid and fatty acid profiles of S. mangrovei

The lipid and fatty acid profiles of the DHA-rich traustochytrid *S. mangrovei* used as enrichment feed for rotifers *B. plicatilis* are