



Production of Omega-3 Fatty Acids in *Cymbella* sp

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ABSTRACT

Natural limitations favour a novel approach for the production of omega-3 fatty acids. A series of PUFAs including Eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA) have widespread nutritional and pharmaceutical values. This study investigated the potential production of these two economically important fatty acids from algae. The microorganisms used were Cymbella cistula and Cymbella ventricosa isolated from Koovam River and Porur Lake, Chennai, Tamilnadu and India. The use of Cymbella sp. is preferred since it produced considerable amounts of EPA and DHA. This paper presents the results on the ratios of EPA and DHA produced by these microalgae and recovery aspects.

KEYWORDS: Algae, Diatom, Cymbella, Cistula, Ventricosa, Fatty acid, omega-3 fatty acids.

INTRODUCTION

Omega-3 long chain fatty acids are increasingly of significant commercial interest in that they have been recognized as important dietary compounds for preventing arteriosclerosis and coronary heart disease, and for alleviating inflammatory conditions such as arthritis. These beneficial effects of omega-3 long chain fatty acids result both from the competitive inhibition of compounds produced from omega-6 fatty acids, and from the direct production of beneficial compounds (eicosanoids) from the omega-3 fatty acids. Currently the only commercially available dietary source of omega-3 long chain fatty acids is the oils from pressed fish flesh, which can contain up to 20-30% of these fatty acids. The beneficial effects of these long chain omega-3 fatty acids can be obtained by eating fatty fish several times a week or by consuming fish oil capsules. Reliance on fish oil as the sole source of long chain omega-3 fatty acids is complicated by the significant taste, odor and stability problems associated with fish oil. This complication limits the use of fish oil as a food additive or food supplement. Fish do not synthesize long chain omega-3 fatty acids in significant quantities. Rather they acquire them through their diet by eating zooplankton that has fed on algae. Marine algae, the primary producers in the marine food chain, are also the primary synthesizers of omega-3 fatty acids. Only a very few species of bacteria and fungi produce these highly unsaturated fatty acids. Algal-based technologies for the production of these fatty acids are attractive because they can eliminate many of the taste and odor problems associated with fish. The first attempts at developing these technologies in the early 1980s focused on photosynthetic strains of microalgae for production in outdoor ponds. Heterotrophic production has several potential advantages over photosynthetic production including: (1) a high degree of process control, which can facilitate rapid growth and production, easy maintenance of a monoculture, and consistent/reproducible products; and (2) lower costs for harvesting the biomass because of the higher cell densities normally achieved with this type of production. These advantages may offset the high capital costs for a large-scale fermentation facility if high product productivities can be consistently achieved. Algal oil, which is made from ocean algae, is the one plant source of omega 3 fatty acids that contains pre-formed DHA. Algal oil is usually sold as a dietary supplement. Algae oil is a very strong source of DHA and EPA omega-3 fatty acids. Marine algae, such as zooplankton and phytoplankton are actually the primary source of DHA and EPA. The marine fish eat the algae and then store the omega-3 in their fat, which is why fish oil has such a high amount of both DHA and EPA. DHA derived from algae is now commercially available and has similar health benefits as DHA derived from fish oil. Some algae are also high in EPA, ie. Nannochloropsis sp. and Spirulina, but, EPA-rich microalgae oil is still limited. DHA-

microalgae oil is obviously a better source of DHA, compared to flax seed oil. This is because there are two more enzymatic conversions your body must perform to get to DHA from ALA. Vegetarians with low levels of eicosapentaenoic acid and docosahexaenoic acid, supplemented with 1 gram of algae derived DHA per day, and significantly elevated their levels of both DHA and EPA after eight weeks (Lipids 40 (8): 807-814). This is evidence that DHA derived from microalgae is a much more potent source of DHA and EPA compared to ALA derived from flax oil. So now that you know DHA from algae oil is an exceedingly better vegetarian source of omega-3 fatty acids. Several studies that have shown that eicosapentaenoic acid and docosahexaenoic acid derived from fish oil both have cardiovascular benefits. Similarly, DHA from algae oil also has the same cardiovascular benefits. In one study, vegetarians that supplemented with 1 gram of algae oil a day decreased their triglyceride levels by 23%, after eight weeks. Microalgae oil is such a great source of omega-3 fatty acids for all vegetarians.

MATERIALS AND METHODS

Collection of material

Algal cultures isolated from Koovam River and Porur Lake, Chennai, Tamilnadu and India were used in the present study. Soil samples were collected and serially diluted in sterile F/2 medium. A 100ul of all dilutions were spread over 2% agar plates. The plates were incubated at 21°C for 21 days with 2000 lux. The isolates obtained were subcultures and purified on agar plates and identified as *Cymbella cistula* and *Cymbella ventricosa* by standard protocol.

Extraction

Algal biomass was crushed in a mortar and pestle and 2g of that was subjected to saponification, unsaponified materials were removed using 25ml of methylene chloride using separating funnel. Addition of 50 ml acetic acid liberated the free fatty acids, 50 ml acetone was added. Finally to obtain a concentrated form of only omega -3 fatty acids, a base and alcohol mixture was used.

Gas Liquid chromatography (GLC)

Obtained fatty acid fractions were subjected to GC analysis. The chromatographic column used was a fused silica (Rtx1 Fused Silica) capillary column (30m x 0.25mm ID). Nitrogen was used as the carrier gas at a flow rate of 1.84 ml/min. The column temperature was 180°C and the detector temperature was 250°C. The injection volume was 20µl and performed in split mode 50:0. Fish oil preparations were obtained from capsules available commercially from local pharmacies. Hexane was used for dissolving and diluting the samples before GC analysis.

RESULTS AND DISCUSSION

Fatty acids are abundant in most organisms. Each class or group of organisms is known to have distinctive fatty acids profile which enables us to use them to produce biologically important fatty acids as well as to use them as biomarker of typical class of organisms. It has been reported earlier that several algae are able to produce large quantities of EPA and DHA fatty acids.

In the present study, 15 diatom algal cultures were isolated from Koovam River and initially screened for the production of PUFA and two were shortlisted for further studies. The growth conditions were kept uniform to minimize the variation in fatty acids composition. Both *Cymbella* was grown on F/2 medium initially and later and later transferred to a medium containing Aculeacin A. Aculeacin A is considered as inhibitors of enzymes involved in carbohydrate metabolism in *Cymbella* sp for synthesis of highly unsaturated fatty acids. However, in the present study the concentration of DHA and EPA was increased in Aculeacin A containing medium than that was grown in F/2 medium. Aculeacin A act as inhibitors of carbohydrates metabolism enzymes for producing higher EPA and DHA, the cell growth was not inhibited. Detailed studies are now in process to optimize the dosage levels of Aculeacin A.

Out of two cultures *Cymbella cistula* produced 7.47 mg/g of DHA and 0.29 mg /g of EPA (Figure: 2). Interestingly, both Aculeacin A and F/2 medium inoculated with *Cymbella cistula* revealed higher concentrations of DHA than EPA (Figure:2 &3). Algae belonging to *Cymbella* sp were able to produce up to 27 mg/ g of EPA and many of these are good producers of fatty acids. The major fatty acid produced by most of algae is the Palmitic acid. The low levels of EPA in our study might due to the inactivation of EPA synthesizing enzymes at room temperatures. This study has clearly shown that substantial amounts of DHA as compared to EPA were produced by *Cymbella cistula* and lesser

extent of *Cymbella ventricosa* (Figure:4). Of the two, *Cymbella* sp, *Cymbella cistula* was a better producer of DHA.

Figure.1.GC analysis of EPA & DHA standards from fish oil capsule

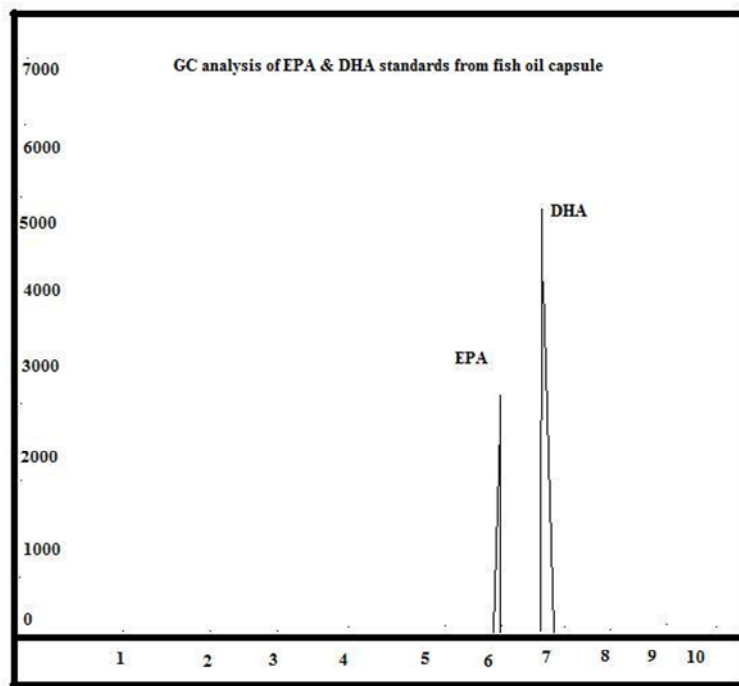


Figure 2. GC analysis of *Cymbella cistula* grown in F/2 medium

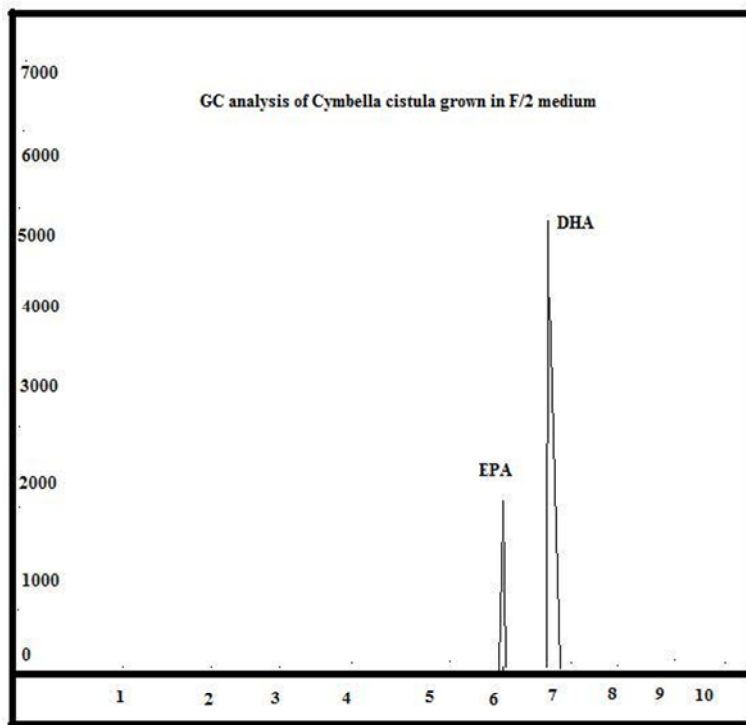


Figure .3. GC analysis of *Cymbella cistula* grown in Aculeacin A medium

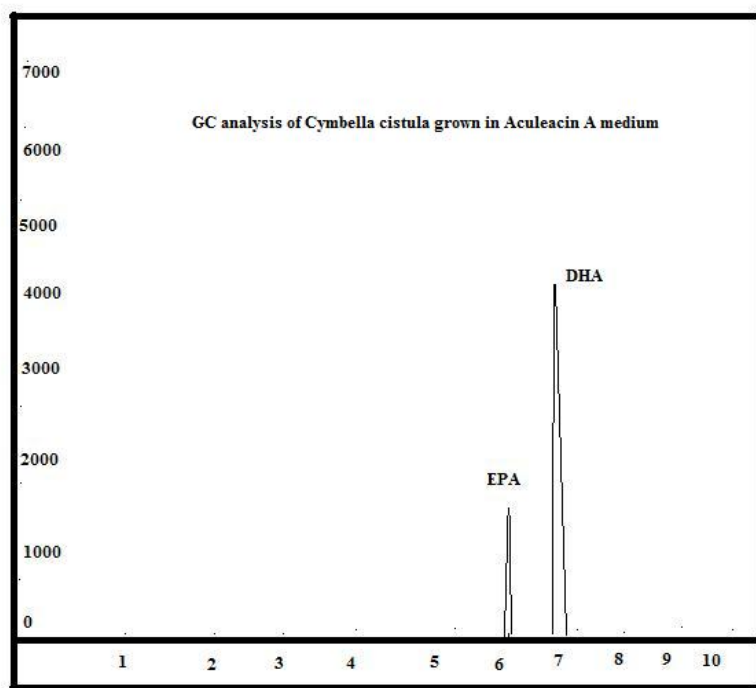
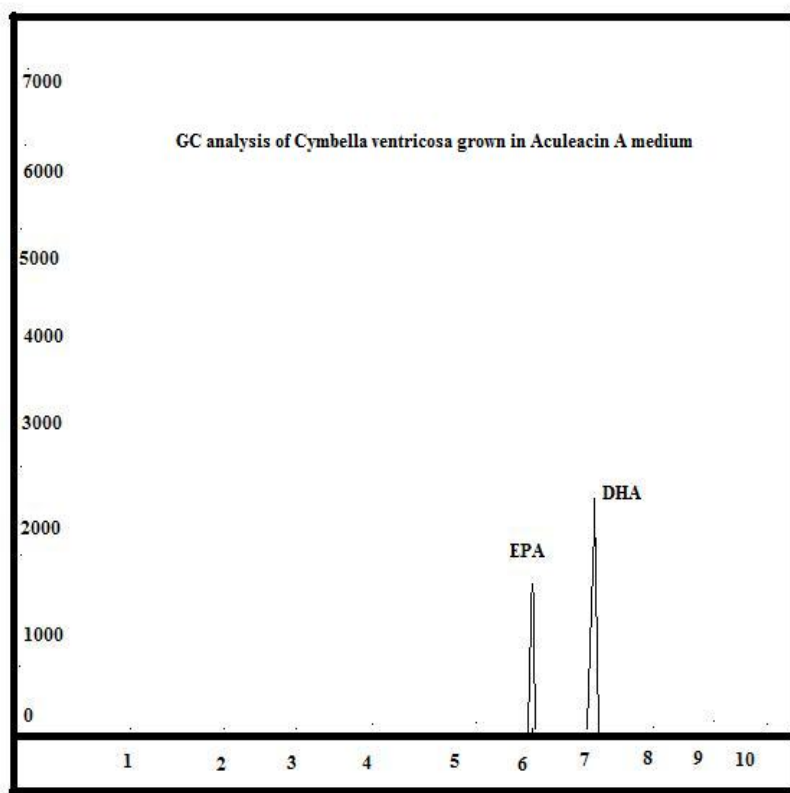


Figure.4. GC analysis of *Cymbella ventricosa* grown in Aculeacin A medium



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