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Gas Chromatography - Mass Spectrometric Determination of Fatty Acid Methyl Esters of Four Marine Cyanobacterial Species.

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ABSTRACT

The present study reports the production of fatty acid methyl esters by four marine Cyanobacterial species. Microalgae are renewable source containing rich lipids in their body and has the potential to refill the partial energy demands in an eco-friendly way. *Cyanobacteria* have received a considerable attention in recent years as excellent organisms for renewable biofuel production. *Cyanobateria* contain significant quantities of lipids and some of them are also rich in essential fatty acids such as linoleic acids and gamma linoleic acids. Besides nutritional value the fatty acids of *Cyanobacteria* generally used to clarify the taxonomical problems. The Gas Chromotography - Mass Spectroscopy analysis for fatty acids revealed that the most of the identified fatty acids were unsaturated fatty acids(92%).The prevalent unsaturated fatty acids were Phthalic acid ($C_6H_{22}O_4$) and Squalene ($C_{30}H_{50}$) followed by saturated fatty acid Silane ($C_{28}H_{60}O_2Si$) (8%).

Keywords: *Cyanobacteria*, biodiesel, GC-MS, saturated fatty acids and fatty acid methyl ester.

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INTRODUCTION

The increased concern for security of the oil supply and the negative impact of fossil fuels on the environment, particularly green house gas emissions, has put pressure on society to find renewable fuel alternatives. Recent soaring oil prices, diminishing world oil reserves and the environmental deterioration associated with fossil fuel consumption have generated renewed interest in using 'Algae' as an alternative and renewable feedstock for fuel production. *Cyanobacteria* are gram – negative photoautotrophic prokaryotes having 'higher plant type' oxygenic photosynthesis [1,2]. Certain *Cyanobacteria* differentiate a small fraction of their cells into heterocysts, the site of aerobic nitrogen fixation. The significant role of these N_2- fixing microorganisms in improving the fertility of wetlands such as rice paddy fields, at the sole expense of photosynthetic energy produced on their own is well documented [3-5]. The availability of powerful techniques allow the biotechnological application of *Cyanobacteria* to produce specific products, to biodegrade organic pollutants in surface waters, to control mosquitoes and for many different other purposes [6]. Some of the filamentous *Cyanobacteria* tend to have large quantities (25 to 60% of total) of poly unsaturated fatty acids [7-9]. A few Cyanobacterial strains, which show facultative anoxygenic CO_2 photo assimilation with sulphite as electron donor, lack poly unsaturated fatty acids in their lipids [10-12]. *Cyanobacteria* possess certain properties which have entitled them to be one of the most promising feed stocks for bioenergy generation [13,14]. *Cyanobacteria* are a diverse group of photosynthetic organisms found in different habitats. In marine waters, they form an important component of primary producers and hence employed in mariculture practice [15,16]. They store reserve food materials which are the source of pigments, lipids, vitamins and proteins [17,18]. *Cyanobacteria* are one of the organisms widely used in food industries and in biotechnological applications. In the sea water habitats they form water blooms exerting secondary metabolites toxic to aquatic fauna [19]. There are some health benefits of polyunsaturated fatty acids for aquatic organisms which has spurred interest in their commercial production [20,21]. Lipds are most effective source of storage energy, function as insulators of delicate internal organs and hormones and play an important role as the structural constituents of most of the cellular membranes [22-23]. The need of energy is increasing continuously because of increase in industrialization and population. Biodiesel are fatty acid methyl esters that are derived from triglycerides by transesterification process. The fatty acids of *Cyanobacteria* are either saturated or unsaturated. They can also tolerate environmental stresses such as heat, cold, dessication, salinity etc. The present study aims at the comparision of fatty acids profiling of four different marine Cyanobacterial species.

MATERIALS AND METHODS

The four different marine Cyanobacterial cultures were obtained from National Facility of Marine *Cyanobacteria* (NFMC), Bharatidasan University, Thiruchirapalli, Tamil Nadu. We maintained pure strains on BG-11 medium at a 24±2°C under a light intensity of 1500 lux and light and dark cycles of 16:8hrs.

Lipid extraction:

The extraction of total lipids was done according to MIDI (Microbial Identification System) protocol [24]. A loopful of Cyanobacterial pure culture was taken in a screw capped glass tube and 1ml of Reagent I (45gm NaOH + 150ml CH_3OH + 150ml distilled water) was added to it. The tubes were vigorously vortexed for 5-10 seconds and returned to the water bath (100°C) to complete the 30 minutes heating (Saponification step). The cooled tubes were uncapped, 2ml of Reagent II was added, the tubes were capped and briefly vortexed. After vortexing, the tubes were heated for 10 minutes at 80°C. (This methylation step is critical with time and temperature).Then 1.25 ml of Reagent III (200ml Hexane + 200ml methyl tetra butyl ether) was added to the cooled tubes was followed by recapping and gently tumbling on a clinical rotator for about 10 minutes. The tubes were uncapped again and lower phase was pipetted out and discarded (Extraction step).About 3ml of Reagent IV (10.8gm NaOH + 900ml distilled water) was added to the organic phase remaining in the tube, then the tubes were recapped and tumbled for 5 minutes. Then the tubes were uncapped about 2/3 of the organic phase was pipette into GC vial which was capped and ready for analysis.

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Gas Chromatography –Mass Spectroscopy condition:

The collected fatty acid methyl esters were processed with GC and MS at VIT, Vellore, India. Gas Chromatography analysis was performed on a Sherlock fatty acid identification system (New York, U.S.A.) fitted with cross linked methyl silicon fused capillary column (30.0m × 250µm), flame ionization detector and sampler. Helium was used as carrier gas. The sample was injected at oven temperature of 60°C for 2 minutes and then raised to 300°C at the rate of 10°C/ min, hold for 6 minutes and then to 240°C.

RESULTS

Table 1 - Distribution of Fatty acids in four marine cyanobacterial species.

S.No.	Retention values	Fatty acids	*Oscillatoria sps*. BDU 142191	*Phormidium tenue* BDU 141753	*Lyngbya sps* BDU 90901	*Synechoccus elongatus* BDHKU 10201
1	22.566	C16:O4	+	+	+	+
2	24.577	C30:O	+	+	+	+
3	29.029	C28:O2	+	+	-	-
4	31.305	C14:O2	+	-	+	-
5	16.729	C16:O4	-	+	-	+
6	17.184	C16:O4	-	+	-	+
7	17.344	C17:O4	-	+	-	+
8	17.990	C18:O4	-	+	-	-
9	31.300	C14:O	-	+	-	+
10	29.204	C14:O3	-	-	+	+
11	17.705	C16:O4	-	-	-	+
12	18.040	C18:O4	-	-	-	+

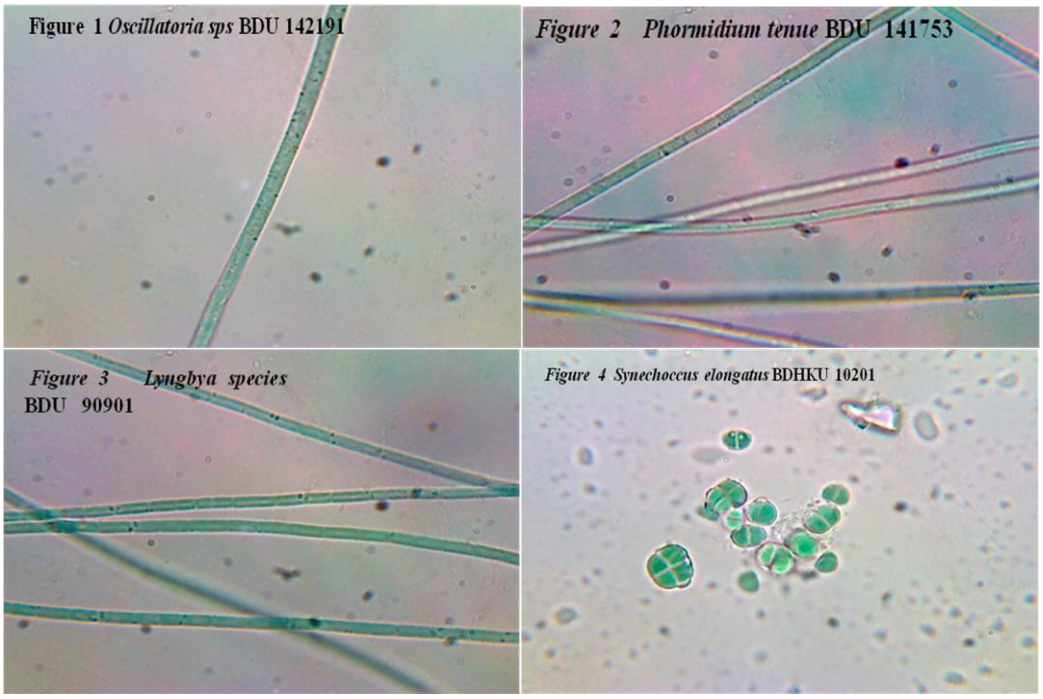

Figure 1 *Oscillatoria sps* BDU 142191
Figure 2 *Phormidium tenue* BDU 141753
Figure 3 *Lyngbya sps* BDU 90901
Figure 4 *Synechoccus elongatus* BDHKU 10201

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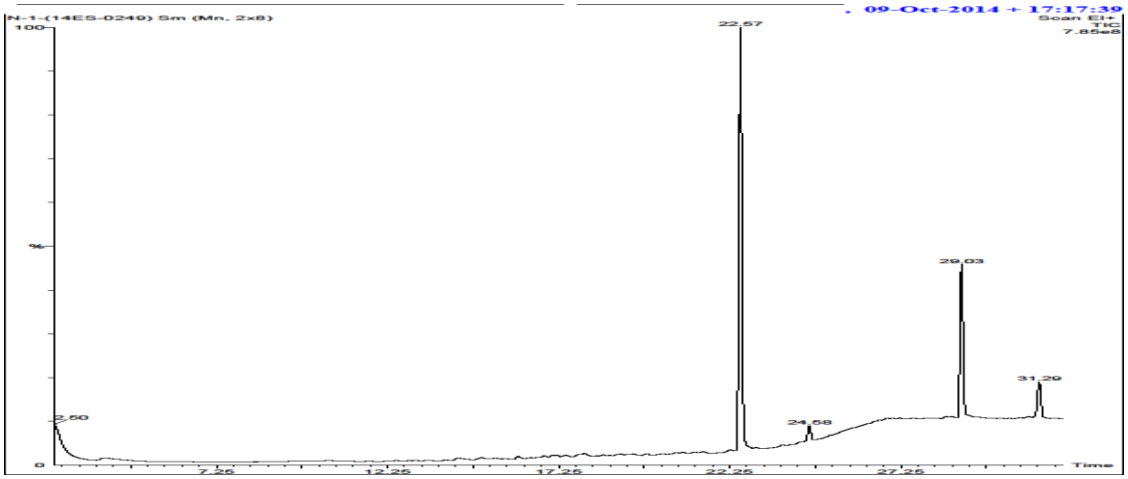

Figure 5 GC-MS spectrum of *Oscillatoria sps*. BDU 142191

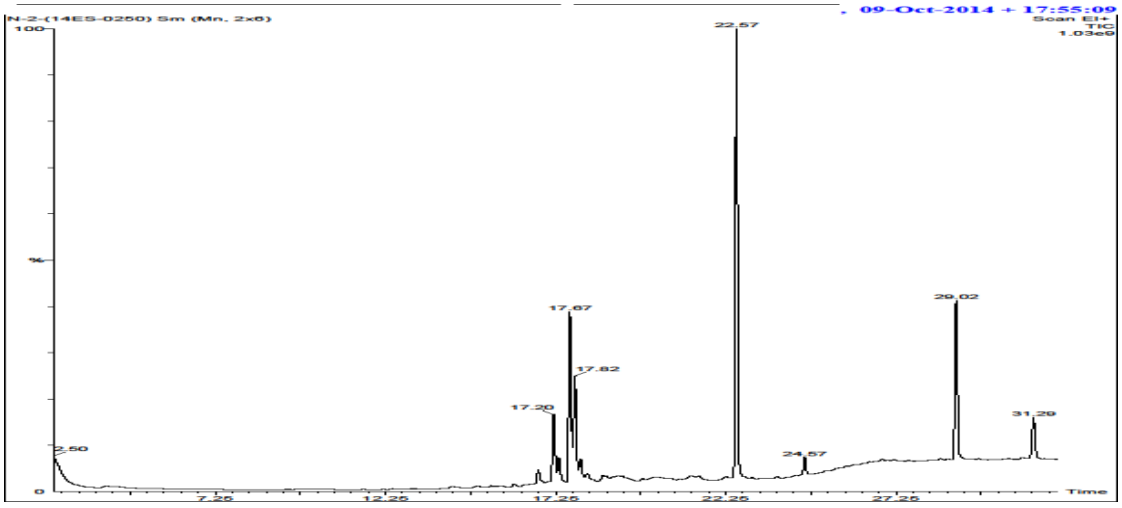

Figure 6 GC-MS spectrum of *Phormidium tenue* BDU 141753

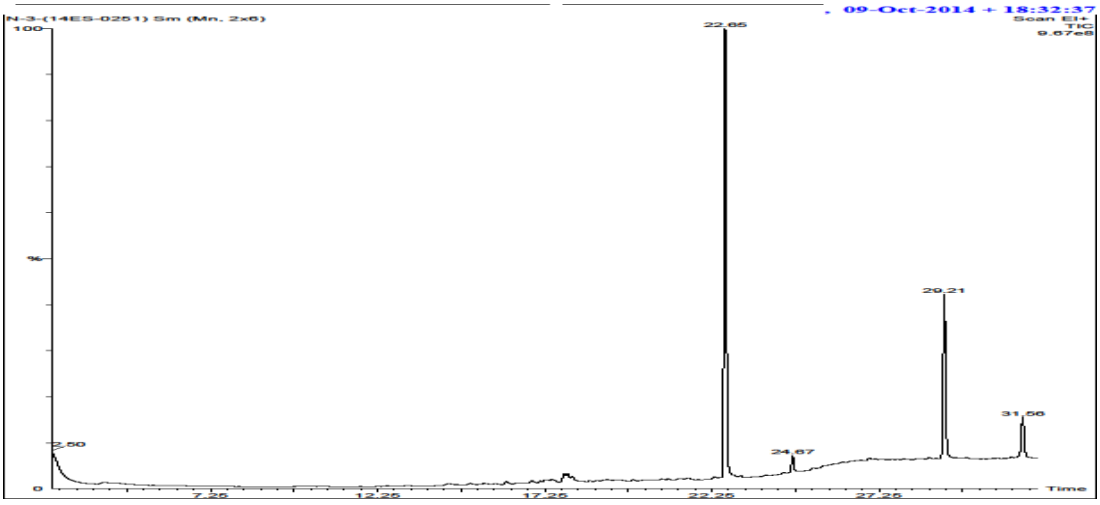

Figure 7 GC-MS spectrum of *Lyngbya sps* BDU 90901

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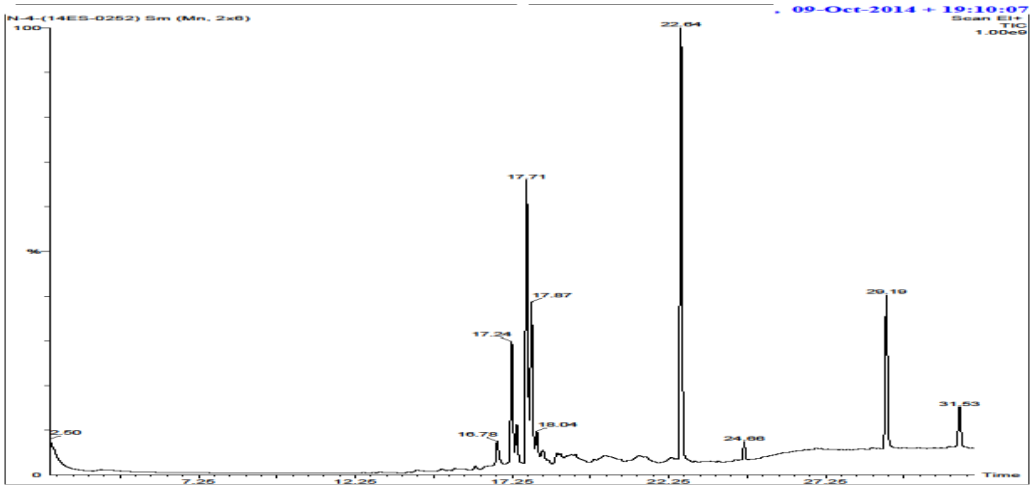

Figure 8 GC-MS spectrum of *Synechoccus elongatus* BDHKU 10201

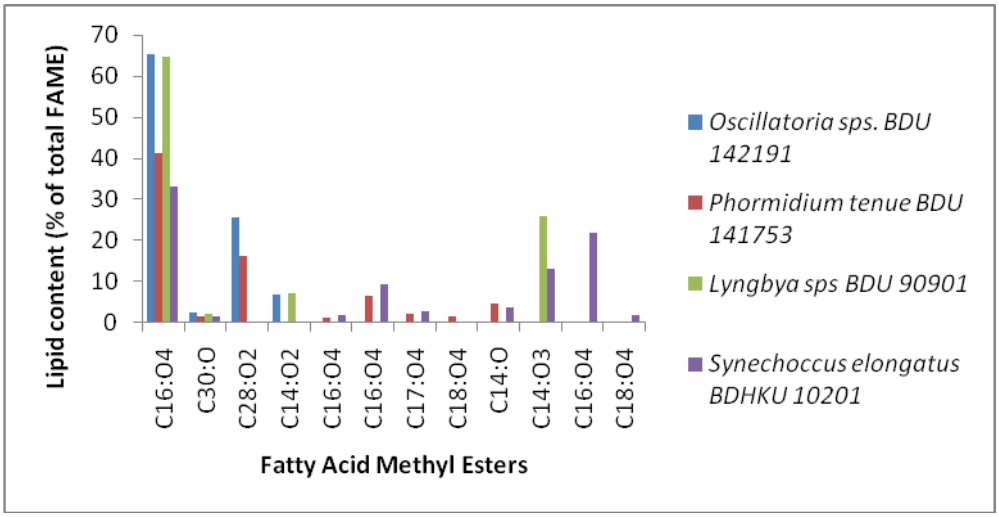

Figure 9 Array of fatty acids in four marine cyanobacterial species

DISCUSSION

Most *Cyanobacteria* are a common source of wide range of fats, oils, hydrocarbons and sterols with potential not only as a renewable source of liquid fuels but also for the production of a range of pharmacologically and industrially important products [25]. The application of *Cyanobacteria* in the production of these latter compounds is only just being explored and their importance has yet to be determined [26]. New developments in the chemical industries, particularly in the area of converting natural products to industrial feedstocks, will further enhance the range of commercially important products synthesized by *Cyanobacteria* [27,28]. From the four Cyanobacterial strains twelve fatty acids were identified using GC-MS analysis. These fatty acids were identified ranging between C14to C30, in which Phthalic acid (C16:O4) was predominant in all four Cyanobacterial strains. In *Oscillatoria sps* BDU 142191, unsaturated fatty acids of Squalene (C30:O), C14:O2 and saturated fatty acid C28:O2 were present. In *Phormidium tenue* BDU 141753 saturated fatty acids Silanes (C28:O2) and unsaturated fatty acids of C30: O, C16:O4, C17:O4, C18:O4 and C14:O were identified. In *Lyngbya sps* BDU 90901 unsaturated fatty acids C30: O, C14:O2 and C14:O3 were recognized. In *Synechoccus elongatus* BDHKU 10201 unsaturated fatty acids C30: O, C16:O4, C17:O4, C14: O, C14:O3 and C18:O4 were occurred. Di (2-ethylhexyl) phthalate is widely used as a plasticizer in flexible vinyl product [29,30]. Plastics may contain from 1to 40% of Di (2-ethylhexyl) phthalate by weight and are used in consumer products such as imitation leather, rainwear, footwear, upholstery, flooring, wire and cable, tablecloths, shower curtains, food packaging materials and children's toys [31,32]. Poly vinyl chloride (PVC) containing Di (2-ethlyhexyl) phthalate is also used for tubing and containers for blood products and transfusions [33].

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CONCLUSION

Most *Cyanobacteria* are a common source of a wide range of fats, oils, hydrocarbons and sterols with potential not only as a renewable source of liquid fuels but also for the production of a range of pharmacological and industrially important products. The present work concluded that GC- MS analysis of four marine cyanobacterial strains *Oscillatoria sps*. BDU 142191, *Phormidium tenue* BDU 141753, *Lyngbya sps* BDU 90901, *Synechoccus elongatus* BDHKU 10201, revealed that the presence of unsaturated fatty acids (92%) C_{16}:O_4 (Phthalic acid), C_{30}:H_{50} (Squalene) .Saturated fatty acids such as Silanes C_{26}:O_2 (8%) were present *in Oscillatoria sps and Phormidium tenue.*

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