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RESEARCH ARTICLE

LIPID AND HYDROCARBON CHARACTERIZATION OF NEW GREEN ALGAL ISOLATES FOR BIOFUEL PRODUCTION

Bhuvanesh*, N., Sundaram, S. P. and Balachandar, D.

Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641 003, Tamil Nadu, India.

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ABSTRACT

Three green algal isolates (ALAK 1, ALAK 2 and ALAK 3) from fresh water lakes of Sivagangai district of Tamil Nadu were characterized for various biochemical properties such as chlorophyll, carotenoid, protein, lipid and hydrocarbon. The qualitative analysis of lipids extracted from these algal isolates revealed the feasibility for commercial exploitation of these algal species. The study also revealed ALAK 3 had high potential of biofuel production.

INTRODUCTION

The continued use of fossil fuels is not sustainable, as they are finite resources (Srivastava and Prasad, 2000), and their combustion will lead to increased energy-related emissions of green house gases (GHG). Now there is more concern towards green technology to fight the global warming condition. Microalgae are veritable miniature biochemical factories, and appear more photosynthetically efficient than terrestrial plants (Pirt, 1986). Many algae are exceedingly rich in oil, which can be converted to biodiesel. The oil content of some microalgae exceeds 80% of dry weight of algae biomass (Chisti, 2007; Banerjee *et al.* 2002). In future algae can prove a real source for biofuel. Apart from using for biofuel purpose microalgae can also be used for many industrial purposes. Microalgae are not only sources of food for humans and animals, but are also the sources of a wide range of chemical compounds used in industry, food technology and pharmaceuticals (Spolaore *et al.* 2006). This paper deals the preliminary diversity study of biofuel producing microalgal species that are present in fresh water ponds of Sivagangai district of Tamil Nadu.

MATERIALS AND METHODS

Isolation and purification of algal strains

The samples were collected from different fresh water bodies of Sivagangi, Tamil Nadu, India and cultured in modified Chu 13 medium (Largeau *et al.* 1980) by using enrichment technique. The algae were subjected to purification by serial dilution followed by plating. The individual colonies were isolated and inoculated into liquid medium (modified Chu 13 medium) and incubated at 25 ± 1ºC under 1.2 ± 0.2 Klux light intensity with 16:8 hrs light and dark cycle. The purity of the culture was ensured by repeated plating and by regular observation under microscope.

Morphological and Biochemical properties of microalgal isolates

Morphological properties of isolates were observed under a light microscope (Olympus BH-2, Japan). Biochemical properties used for the identification of isolates included Protein, Chlorophyll, Carotenoid, Lipid and Hydrocarbon content. Protein content in the cell free medium was analyzed by Bradford protein assay (Zor and Selinger, 1996). The chlorophyll and carotenoid content were estimated using Lichtenthaler equations (Lichtenthaler, 1987). Hydrocarbon and lipid content were measured gravimetrically and expressed as dry weight percentage (Dayananda *et al.* 2005; Dayananda *et al.* 2006).

Standardization of media for isolated algal strains

Collected isolates were studied for growth using different autotrophic media such as bold basal medium with vitamins (BBM+V) (Dayananda *et al.* 2007), BG11 medium (Richmond, 1986), modified Chu13 medium (Largeau *et al.* 1980) and AF-6 medium (NIES, 2004) were inoculated uniformly at 0.05% (v/v) inoculums and were incubated at 25 ± 1ºC under 1.2 ± 0.2 Klux light intensity with 16:8 hrs light and dark cycle. All experiments carried out were replicated thrice. Cultures were incubated for a period of 60 days, harvested and analysed for biomass yield.

Qualitative analysis of hydrocarbons extracted from microalgal species by GC-MS

Hydrocarbon was extracted in hexane after homogenizing the dry biomass in a mortar and pestle in the presence of glass powder and the supernatant recovered after centrifugation was evaporated to complete dryness under the stream of nitrogen. Hydrocarbon content was measured gravimetrically and expressed as dry weight percentage (Dayananda *et al.,* 2005;

***Corresponding author:** *bhuva.agri@gmail.com*

Table 1. Evaluation of different media for growth performance of microalgal isolates under in vitro condition

Media	OD at 650 nm		
	ALAK1	ALAK2	ALAK3
MC 13	0.075	0.086	0.099
AF 6	0.062	0.036	0.041
BG 11	0.064	0.067	0.075
BBM+V	0.036	0.059	0.064
Modified Chu 10	0.11	0.18	0.19

Values are mean of three replicates

Table 2. Chlorophyll content (mg/L) of algal strains at pH 7.5

Isolates	Chlorophyll content (mg/L)			
	Incubation period (Days)			
	15	30	45	60
ALAK 1	0.60 ± 0.03	0.68 ± 0.04	0.72 ± 0.04	0.77 ± 0.04
ALAK 2	0.79 ± 0.05	0.84 ± 0.05	0.87 ± 0.05	0.95 ± 0.05
ALAK 3	0.90 ± 0.05	0.92 ± 0.05	0.96 ± 0.06	1.04 ± 0.06

Values are mean ± SE of three replicates

Table 3. Carotenoids content (mg/L) of algal strains at pH 7.5

Isolates	Carotenoids content (mg/L)			
	Incubation period (Days)			
	15	30	45	60
ALAK 1	0.68 ± 0.04	0.71 ± 0.04	0.76 ± 0.04	0.79 ± 0.05
ALAK 2	0.84 ± 0.05	0.81 ± 0.05	0.91 ± 0.05	0.94 ± 0.05
ALAK 3	0.99 ± 0.06	1.10 ± 0.06	1.13 ± 0.07	1.14 ± 0.07

Values are mean ± SE of three replicates

Table 4. Protein content (%) of algal strains at pH 7.5

Isolates	Protein content (%)			
	Incubation period (Days)			
	15	30	45	60
ALAK 1	9.80 ± 0.57	23.20 ± 1.34	35.14 ± 2.03	38.50 ± 2.22
ALAK 2	11.20 ± 0.65	27.40 ± 1.58	43.95 ± 2.54	42.00 ± 2.42
ALAK 3	12.45 ± 0.72	31.54 ± 1.82	50.54 ± 2.92	51.00 ± 2.94

Values are mean ± SE of three replicates

Table 5. Lipid content (%) of algal strains at pH 7.5

Isolates	Lipid content (%)			
	Incubation period (Days)			
	15	30	45	60
ALAK 1	7.95 ± 0.46	12.5 ± 0.72	15.8 ± 0.97	17.3 ± 1.01
ALAK 2	9.12 ± 0.53	13.4 ± 0.77	16.5 ± 1.02	18.2 ± 1.10
ALAK 3	9.95 ± 0.57	14.0 ± 0.81	19.54 ± 1.19	20.7 ± 1.21

Values are mean ± SE of three replicates

Table 6. Hydrocarbon content (%) of algal strains at pH 7.5

Isolates	Hydro carbon (%)			
	Incubation period (Days)			
	15	30	45	60
ALAK 1	1.42 ± 0.08	5.4 ± 0.31	10.02 ± 0.80	12.46 ± 1.07
ALAK 2	1.65 ± 0.10	6.8 ± 0.39	15.25 ± 1.06	16.10 ± 1.31
ALAK 3	1.74 ± 1.10	7.4 ± 0.43	16.19 ± 1.15	17.95 ± 1.35

Values are mean ± SE of three replicates

Table 7. Hydrocarbon profile of microalgal lipid extracts by GC-MS

Isolates	Essential hydrocarbons recorded
ALAK 1	Octadecane, Pentadecane, Docosane, Eicosane and Decane
ALAK 2	Octadecane, Pentadecane and Eicosane
ALAK 3	Octadecane, Pentadecane, Docosane, Eicosane, Decane and Heneicosane

Dayananda *et al.,* 2006).Hydrocarbon extract was purified by column chromatography on silica gel. The hydrocarbon samples were analyzed on SPB-1 column (30 m x 0.32 mm ID x 0.25 µm film thickness) using GC-MS equipped with FID using SPB-1 (poly(dimethysiloxane)) capillary column (30 m x 0.32 mm ID x 0.25 µm film thickness) with a temperature programming 130ºC to 280ºC at a rate of 2ºC/min were identified by comparing their fragmentation pattern with standards (Sigma) and also with NIST library (Dayananda *et al.,* 2005).

RESULTS

On survey made at Sivagangai district of Tamil nadu, three potential isolates of microalgal species were isolated from fresh water ponds. The purity of the cultures were maintained through microscopic observations and the isolates were grown under 30°C with 3000 lux light intensity for 12 h day/night cycle. Further, the growth behaviour of these isolates in different media were performed under in vitro condition and growth as OD at 650 nm revealed that modified Chu-10 medium supported maximum growth of all the isolates than other four media (Table 1). Various biochemical properties which include chlorophyll, carotenoid, protein, lipid and hydrocarbon contents were recorded in modified Chu-10 medium and among the isolates, ALAK 3 was better when compared with the other two strains in all the biochemical properties. Chlorophyll content was recorded in an increasing trend during incubation period of 15- 60 days (Table 2). A highest value of 1.04 ± 0.06 (mg/L) were observed in ALAK 3 60 days after incubation. Carotenoid and protein content were also in an increasing trend (Table 3 and 4). The highest value were observed ALAK 3 (1.14 ± 0.07 (mg/L) and 51.00 ± 2.94 (%) 60 days after incubation). Lipids and hydrocarbon was also recorded higher in ALAK 3. The results are presented in Table 5 and 6. Hydrocarbon profiles of microalgal lipid extracts were identified using GC-MS. The list of identified hydrocarbon is presented in Table 7. ALAK3 isolate had six different hydro carbons such as Octadecane, Pentadecane, Docosane, Eicosane, Decane and Heneicosane while other two isolates having three or five different hydro carbons.

DISCUSSION

Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactive compounds. Studying the diversity of these micro algae in fresh water and marine resources will be always useful to identify novel elite isolates capable of producing hydro carbons and hydrogen which will be useful for commercial ezxplotation. With this objective, three natural isolates of micro algae from Sivagangai district of Tamil Nadu were characterized for their biochemical properties to exploit them as biofuels.

The chlorophyll content of various algal strains of different strains was estimated at pH 7.5. Chlorophyll content decides the photosynthetic rate of a particular strain. According to Waugh and Clark (1986) changes in chlorophyll level are probably controlled as algal density and climatic factors (light and temperature). Photosynthetic efficiency of micro algae is directly related to its growth and hence biofuel production. In our study we were able to identify the species having higher photosynthetic efficiency based on the chlorophyll content in it. The high protein content of various microalgal species is one of the main reasons to consider them as an unconventional source of protein (Soletto *et al*. 2005). In addition, the amino acid pattern of almost all algae compares favorably with that of other food proteins. As the cells are capable of synthesizing all amino acids, they can provide the essential ones to humans and animals (Guerrero *et al.* 2004). This biomass fraction contains valuable proteins for livestock; poultry and fish produce green plastics, green detergents, cleaners, and polymers that are bio-degradable and nontoxic (Will Thurmond, 2009).

Their most important uses of carotenoids are as natural food colorants (e.g., orange juice) and as additive for animal feed (poultry, fish). It also have applications in cosmetics (Del Campo *et al.,* 2000). Hydrocarbons are extracted from the total lipids as the hexane-soluble component and can be converted into useful fuels such as gasoline by catalytic cracking Hillen *et al.* (1982). According to Banerjee *et al.* (2002) the aldehydes generated from fatty acids *via* methylation are converted to saturated hydrocarbons (alkanes). Hydrocarbon is a main constuent of petroleum. Hence it is very important to screen micro algal strains based on its high production of hydro carbon. In present study, we could able to isolate three different microalgal species from the fresh water ponds of Sivagangai district and the biochemical background of the isolates is quite suitable for biofuel production. Among the three different isolates tested, ALAK 3 reported to be better hydrocarbon and lipid contents than other two isolates.

Further, the variability among the strains revealed the diversity and species richness of these algae in various water bodies, which are to be augmented for future prospects. It is well known that some green microalgae can accumulate very large amounts of triacylglycerols in the stationary phase of the growth (Thompson, 1996). The greater differences recognized between the strains would be directly or indirectly related to their discrepancy in hydrocarbon synthesis as reported by Templier *et al*. (1984). Algae producers are especially interested in using algal species with a high triglyceride (TAG) oil content for biodiesel production. Even with algae species with up to 50% oil content, the additional 50% of the biomass remains. The qualitative analysis of present microalgal isolates revealed that at least six different essential hydrocarbons are present in the lipid extract. Hence, it is concluded that diversified microalgal species capable of accumulating hydrocarbons in their cells were isolated from this study and the qualitative analysis of them were done.

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