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Characterization of microalga *Scenedesmus* sp. ISTGA1 for potential CO_2 sequestration and biodiesel production

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ABSTRACT

The Present study investigates the carbon sequestration potential and biodiesel production ability of a microalga isolated from a marble mining site. The microalgal isolates were grown in BG-11 medium supplemented with sodium bicarbonate ($NaHCO_3$) as a carbon source. The growth behavior of the isolates and rates of $NaH^{14}CO_3$ uptake were considered as screening parameters to determine the most capable microalgal strain. The most efficient microalga was identified as *Scenedesmus* sp. ISTGA1 by 18S rDNA sequencing method. The growth of the isolate was studied under different concentrations of gaseous CO_2 (5–15% v/v) and $NaHCO_3$ (10–200 mM). Results revealed that the isolate attained maximum growth at 100 mM $NaHCO_3$ and 15% CO_2. In the case of 100 mM $NaHCO_3$, chlorophyll content, biomass production and lipid content were 9 μg/L, 1508 mg/L and 301 mg/L respectively. At 15% of CO_2 these characteristics were 12.1 μg/L, 1490 mg/L and 268 mg/L respectively. Lipids were transesterified and FAMEs were analyzed via GC-MS. The FAMEs consisted of saturated (33–35.8%) and unsaturated fatty acids (54–55%) in both the cases of inorganic carbon supplementation, dominated by C16 or C18 fatty acids (>80%), which are appropriate for the production of biodiesel.

1. Introduction

Increase in carbon dioxide concentration in the atmosphere is the prime cause of greenhouse effect leading to the problem of global warming, which in turn have negative impacts on human life [1,2]. Power plants are majorly responsible for CO_2 emissions as the flue gases discharged from them contain very high concentration of CO_2, which usually lies between 15% and 20% v/v [3–6]. Biological fixation of carbon dioxide by photosynthetic microorganisms is a potential alternative as it converts CO_2 into biomass which can be eventually converted into energy in the form of biofuel [7]. Microalgae and cyanobacteria like *Anacystis*, *Botryococcus*, *Chlamydomonas*, *Chlorella*, *Emiliania*, *Monoraphidium*, *Rhodobacter*, *Scenedesmus*, *Spirulina* and *Synechococcus* have been identified and characterized for biological fixation of CO_2 and generating biodiesel [8,9]. Biodiesel has recently gained immense attention as it is renewable, eco-friendly in nature and can be produced by transesterification of oils (triglycerides) obtained from various feedstocks like animal fat, soyabean, canola, palm, corn, jatropha and algae [10–13]. Various microalgal species, when grown in proper optimized environmental conditions, can produce 15–300 times more oil (triacylglycerol), they do not require arable land, grow at much greater densities per unit area and can be grown in fresh water, brown water, saline or oceanic environments and thus are advantageous as compared to other feedstock crop plants like palm and jatropha [11–17]. Various studies have reported that microalgae growing on rocks such as limestone, granite, marble, dolomite, travertine and sandstone produce biofilm composed of extracellular polymeric substances (EPS) containing polysaccharides, lipopolysaccharides, proteins, glycoproteins, lipids, glycolipids, fatty acids and enzymes outside the cells which help the algae to thrive in drastic conditions [18–20]. The EPS produced by these lithic algae can be an added advantage for biodiesel production. Different green microalgal species have been investigated and reported for producing 30–50% lipid of dry cell weight under optimal stress conditions [10,14]. Study of extreme environment like lignite mine in Northern Ontario, Canada has been done to identify stress-resistant and high-lipid producing green microalga *Scenedesmus dimorphus* [21]. Production of large scale of algal biomass is a challenge and there is a need to characterize microalgal strains that can grow robustly in drastic conditions like high concentration of CO_2. About 60 species of microalgae have been well characterized for the purpose to produce large biomass to

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achieve high yields of biofuel [10]. CO_2 fixation by microalgae depends upon several physicochemical parameters like light intensity, temperature, medium composition and pH [22]. Marble/limestone rocks are rich in bicarbonate or carbonate as the major source of inorganic carbon and not only that, the environment is also made CO_2 rich by other inhabiting microorganisms that produce respiratory CO_2 [23,24]. It has been recently reported that microalgae growing on marble rocks are able to grow in an ambience of high concentrations of inorganic carbon and salts and can sustain in adverse conditions of light, temperature, pH, and salinity [23]. Many marine and freshwater microalgal species have been widely reported for CO_2 sequestration and biodiesel production, but new potential strain discovery from unexplored and unusual sites is also important. Thus exploring such untapped realms like marble mining sites, to discover new micro-organisms with enormous potential for efficient CO_2 sequestration and biodiesel production seemed logical and noteworthy [23].

Therefore, in this study, an alga *Scenedesmus* sp. ISTGA1 was isolated from marble mining site, near Alwar, Rajasthan, India and characterized for its CO_2 sequestration potential in terms of growth, lipid content and FAME profile.

2. Materials and methods

2.1. Sampling site

The samples were collected from the Jhiri marble mining site of the palaeoproterozoic metasediment of Aravali subgroup, near Alwar area (27.27° N and 76.18° E) Rajasthan, India. Microalgae growing on the upper portion of the marble rock were scrapped and dissolved in autoclaved distilled water (1:10 w/v) and used as inoculums for isolating microalgae capable of utilizing high Ci concentrations in the form of sodium bicarbonate ($NaHCO_3$) and gaseous CO_2.

2.2. Isolation, purification and cultivation of microalgae

The collected samples were cultivated in BG-11 broth containing (g/L): $NaNO_3$, 1.5; $K_2HPO_4.3H_2O$, 0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; citric acid, 0.006, ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; Na_2CO_3, 0.02 and trace metal solution 1 mL (including H_3BO_3 2.86 g/L, $MnCl_2.4H_2O$ 1.81 g/L, $ZnSO_4.7H_2O$ 0.222 g/L, $Na_2MoO_4.2H_2O$ 0.390 g/L, $CuSO_4.5H_2O$ 0.079 g/L and $Co(NO_3)_2.6H_2O$ 0.494 g/L) at pH 7.4 [25]. The cultivated samples were spread on BG-11 agar plate and incubated at 30 °C. The colonies that appeared were picked and transferred to the same media for purification by sequential culture in BG-11 broth and agar plates. The process was repeated until axenic cultures of different microalgae were obtained. The microalgae were grown in separate 2 L flasks using the BG-11 medium (pH 7.4), incubated at temperature 30 °C and 100 rpm for aeration. Artificial photoperiod of 18 h light and 6 h dark was provided by using four compact fluorescent lamps (50 W each).

2.3. Screening of potential algal strain for efficient CO_2 fixation in terms of growth pattern and metabolic ^{14}C labeling

Microalgal isolates were enriched in BG-11 medium with 100 mM $NaHCO_3$ for 14 days and the growth pattern was observed in terms of optical density (OD) at 680 nm. Microalgal cultures were harvested by centrifugation and washed with milli-Q and resuspended in 1 mL milli-Q water to final densities of $\approx 2.1 \times 10^7$ cells/mL and metabolic labeling was initiated by adding 100 μM $NaH^{14}CO_3$ containing 10 MBq of ^{14}C, shaken for 60 min to determine the CO_2 fixation ability. Microalgal strain showing highest CO_2 fixation rate was shaken separately for 20–80 min for further fixation experiment. The experiment was terminated by adding 2 mL of boiling milli-Q water. Microalgal cells were harvested by centrifugation and were dissolved in boiling milli-Q for 30 min to remove inorganic ^{14}C. The samples were centrifuged and pellets were dried in incubator at 60 °C on a filter paper and added to a final volume of 5 mL cocktail. The samples were assayed for radioactivity using liquid scintillation analyzer (TRI-CARB 2900 TR, Packard Bioscience Company). The rate of $^{14}CO_2$ uptake was expressed in μmol of $NaH^{14}CO_3$ fixed mg/chl/h.

2.4. Identification of the potential microalgal isolate

The microalga was first identified morphologically on the basis of microscopic observation. The identity of the microalgal isolate was further confirmed by 18S rDNA sequence. Genomic DNA was isolated from microalgal strain by manual DNA isolation method [26]. Universal eukaryotic 18S rDNA primers CHLORO F and CHLORO R were used for the amplification of genomic DNA. PCR amplification was performed with a reaction mixture of 0.2 mM of deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, 0.5 mM of each primer, and 1.25 units of *Taq* DNA polymerase. The PCR program followed was: 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min [27]. The analysis of the amplified product obtained after sequencing was compared with the existing database of gene bank, National Center for Biotechnology Information (NCBI) [28,29]. Phylogenetic tree was drawn using MEGA 3.1 software [30].

2.5. Characterization of the isolate by scanning electron microscopy (SEM)

A drop of algal suspension was taken on a coverslip and air dried. It was fixed using 4% (w/v) glutaraldehyde overnight, rinsed in distilled water and dehydrated using 95% ethanol followed by air drying. Air dried sample was coated with 90 Å thick gold–palladium coating in polaron Sc 7640 sputter coater (Carl Zeiss, Germany) for 30 min. Coated sample was viewed at 15 kV by scanning electron microscopy (Leo Electron Microscopy Ltd., Cambridge) [31].

2.6. Growth characterization

Growth was determined by measuring OD_{680} and chlorophyll a content of each culture at regular time intervals during the growth period. Cultures were homogenized and chlorophyll a (chl a) was extracted overnight with 90% acetone from filtered (GF/C, 1.2 μm) samples and measured with a UV–visible spectrophotometer (Cary, 100 Bio, Varian Co., Australia) [32,33]. Other than examining the optimal temperature, all the cultures were incubated at 30 °C, 100 rpm under artificial photoperiod of 18 h light and 6 h dark, provided by four compact fluorescent lamps, 50 W each. After reaching the stationary phase (after 14 days), the microalgal biomass was harvested and oven dried at 100 °C overnight. Biomass productivity (P, mg/L/d) was calculated via following equation:

$$P = \Delta X / \Delta t$$

where ΔX is the increase in biomass concentration (mg/L) over a cultivation time of Δt (d).

2.6.1. Effect of sodium bicarbonate supplementation on cell growth and Chlorophyll a content

Microalgal isolate was cultured in BG-11 medium modified with addition of different concentrations of sodium bicarbonate salt (10,