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Isolation and biochemical characterisation of two thermophilic green algal species- *Asterarcys quadricellulare* and *Chlorella sorokiniana*, which are tolerant to high levels of carbon dioxide and nitric oxide

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ABSTRACT

Atmospheric levels of carbon dioxide (CO_2) and nitric oxide (NO) have been on the rise ever since the beginning of industrialisation. A significant fraction of this increase can be attributed to the emissions from stationary sources such as thermal power plants and steel plants. While there has been an impetus in recent times towards sequestration of these greenhouse gases at source, current technologies are not commercially viable. In this context, microalgae-mediated CO_2 capture and utilization has attracted attention, although several technological challenges remain to be addressed. Importantly, this process will require algal strains that grow fast and are tolerant to high light, temperature and flue gases. The majority of the reported algal strains fail in at least one of these requirements. On account of this, we have isolated two novel green algal strains, which have been identified as *Asterarcys quadricellulare* and *Chlorella sorokiniana*, from water bodies that are located in and around a steel plant in India. These are relatively fast-growing strains with specific growth rates of up to 0.06 h^{-1} and 0.1 h^{-1}, respectively. Furthermore, these strains can tolerate high temperatures of up to 43 °C, high light intensity and high CO_2 and NO levels. When exposed to high CO_2 levels, 55–71% of the dry cell weight comprised of carbohydrates. Additionally, exposure to NO gas along with CO_2 led to an enhanced lipid accumulation of 44%–46% of dry biomass. The high lipid content makes these strains valuable feedstock in biodiesel production, and the high carbohydrate content makes the lipid extracted biomass an attractive source of carbon for biochemical conversion to ethanol. We believe that these strains are promising and ready to be tested with real flue gases under outdoor conditions.

1. Introduction

Carbon dioxide is the biggest contributor of greenhouse gases (GHGs) and accounts for around 76% of the total amount of GHGs responsible for global warming and climate change. The remaining 24% comprises- 16% methane (CH_4), 6.2% nitric oxides (NO_x) and 2.0% fluorinated gases (F-gases) [1]. Globally increasing anthropogenic activities such as industrialisation, urbanisation and transportation are the key drivers of increasing CO_2 levels in the environment. In 2013, direct carbon combustion for energy production generated > 36.1 Gt of CO_2, and by 2100, global CO_2 emissions are expected to reach 51.9 Gt annually, with major implications for the environment and public health [2]. Thus, increasing attention is being paid towards the development of technologies for the capture and sequestration of CO_2 emitted, especially from stationary sources. In light of this, various solutions such as deep ocean injection, surface mineral carbonation and deep well injections have been examined for the sequestration of CO_2 [3]. However, these techniques pose several challenges such as high cost, energy intensiveness, the requirement for large spaces and imposition of a negative impact on the functioning of ecosystems [4–6]. Thus, CO_2 capture with microalgal photosynthesis is being considered as a promising and environmentally sustainable method in the long term [7]. Microalgae represent the base of the food chain in aquatic ecosystems, show many-fold higher aerial photosynthetic rates than terrestrial plants and, thus, have a high CO_2 capture potential [8,9]. Algal biomass is also a feedstock for biofuels such as biodiesel, biogas,

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bioethanol and hydrogen, and has the potential to deliver up to 250 times the amount of oil per acre when compared to soybean [10,11]. Moreover, under stress conditions, some algae accumulate high value food and feed products such as β-carotene, astaxanthin, and α-tocopherol (vitamin E), to name a few [12]. Thus, the photosynthetic capture of CO_2 combines sequestration with its utilization to produce a range of high-value products, is a true biorefinery approach.

An ideal strain for bio-sequestration of CO_2 from industrial flue gases would be one that has (a) high settling capacity (facilitating harvesting), (b) tolerance to the high concentrations of CO_2 and other potentially toxic components that are typically found in flue gases, (c) tolerance to the high light and temperature conditions that are present in outdoor ponds and photobioreactor cultivation systems and (d) high commercial or calorific value. To date, several algal strains that satisfy some of the above requirements have been reported [7,13–15]. However, most of the strains either do not fulfil all the conditions together or they may not have been widely tested/reported for the combined effect of all the stresses. Thus, careful strain selection, testing against the collective effect of these stresses, and detailed physiological and biochemical characterisation under controlled laboratory-scale conditions would be the first steps, before these strains can be tested out in the field.

In the present study, our objective was to isolate, select and characterise algal strains that may be deployed for CO_2 capture from flue gas emissions from steel and power plants. The tropical weather conditions of India, combined with the fact that flue gases are at temperatures that are much higher (up to 120 °C) than ambient, require that thermophilic algal strains be selected. Furthermore, flue gases comprise CO_2 concentrations in the range of 15–25% v/v, and other toxic components such as CO, and NO, which may adversely affect the algal growth. Not many of the reported algal species can survive under such harsh conditions. Hence, we hypothesised that the isolation of algae from the water bodies located in and around steel/power plants in India, would be a useful strategy to screen strains that have the requisite properties. Moreover, the deployment of such thermophilic algal strains will alleviate the need to cool flue gases and, thereby, will reduce the overall cost of the process. Hence, we selected the two fastest growing strains out of several isolated from the effluent ponds located in the vicinity of the selected steel plant and investigated the impact of environmental factors (i.e., temperature and light) on their growth. We also examined the impact of high concentrations of CO_2 and simulated flue gas (CO_2 + NO) on the growth, morphology, maximum biomass production, and biochemical composition of these strains. Lastly, we provide a comparison of our results with the previously reported literature on the other strains that belong to the same genus.

2. Materials and methods

2.1. Isolation and selection of microalgal strains

Water samples were collected from 14 different water bodies that were located in and around the JSW steel plant at Toranagallu in the Bellary-Hospet area (15.07°N 76.55°E) of Karnataka, India. These water bodies store water discharges from different steel-making and power generation units located in the plant. Samples were collected in the month of May, during the day, when the ambient air temperature was between 38 and 46 °C, and temperature of the water was in the range of 30–41 °C. Samples were stored in pre-labelled plastic bottles, brought to our laboratory at IIT Bombay and processed within 24 h of collection. Algal strains were isolated as described earlier [16]. Briefly, the samples were inoculated in Bold's Basal (BB) medium and incubated in a shaker at 125 rpm, under a selection pressure of 37 °C temperature, 250 μmol photons $m^{-2} s^{-1}$ light intensity and 1% CO_2 for five days. These were then sub-cultured in a fresh BB medium, supplemented with antibiotic and antifungal agents and incubated under identical conditions for a week. Serial dilutions of this enriched culture were spread on BB media agar plates, and single colonies were picked up and streaked on new plates. Ten species were isolated in total and, uni-algal cultures were maintained in liquid suspension as well as on agar plates.

Further, the screening of isolates was carried out in a laboratory scale photobioreactor (design and details of the reactor are given in Section 2.3) in batch mode under continuous illumination of 250 μmol photons $m^{-2} s^{-1}$ light intensity, 37 °C temperature, and ambient CO_2 conditions. Based on their specific growth rates the two fastest growing strains ($\mu = 0.045\ h^{-1}$ and $0.048\ h^{-1}$ for algae identified as *Chlorella sorokiniana* and *Asterarcys quadricellulare* [see Results] respectively) were chosen for further studies. The pH of the water bodies from which these two strains were isolated was in the range of 6.9–7.4 (measured over a period of one month).

2.2. Identification of microalgae and phylogenetic analysis

The selected algal strains were sent to GeneOmbio Technologies Pvt. Ltd. Pune, India, for preliminary molecular characterisation through 18 s rRNA gene sequencing. The obtained sequences were subjected to nucleotide BLAST search [17] against the National Center for Biotechnology Information (NCBI) database, to identify species that were closest to these strains. Top 20 hits were aligned using the multiple sequence alignment software MUSCLE [18]. A neighbor-joining method [19] was used to construct a phylogenetic tree for each of the two isolates by using MEGA 7.0 software [20]. Default settings were applied, with an exception that a Bootstrap method (no. of Bootstrap replicates = 500) was used instead of none, as a test of phylogeny in the software. Microscopic characterisation of strains was carried out using a Zeiss Axioskop optical microscope (Zeiss, Gottingen, Germany) with a 10 X ocular and 10 X and 40 X objectives.

2.3. Photobioreactor setup and experimental design

All the growth experiments were conducted in a Photon System Instruments (PSI) Photo Multi-Cultivator MC 1000-OD, which contained eight 100 ml cultivation vessels, all immersed in a temperature-controlled water bath. Each vessel was 20 cm long and 3 cm in diameter and was illuminated by an independent light emitting diode (LED) panel located on its back, emitting highly uniform cool white light. The control unit of the MC 1000-OD was used to set the day/night regime of light, change the light intensity and temperature.

For light- and temperature- tolerance studies, batch cultures were grown in continuous light, and the growth of the strains in the cultivation vessels was quantified by measuring the optical density (OD) at a wavelength of 720 nm. The initial OD for these experiments was set as 0.1, and the study was performed in duplicates. Once optimal light and temperature for growth had been established, for gas-tolerance experiments the reactor was operated at 37 °C, under a 14:10 light-dark cycle that was based on a 14 h sine function with an amplitude of 250 μmol photons $m^{-2} s^{-1}$. The initial cell concentration was 0.6×10^6 cells ml^{-1} and the experiments were performed in triplicates, in a semi-continuous culture mode. During experiments, a working culture volume of 85 ml was maintained in each vessel.

The inoculum for each experiment was prepared by growing the algal strains under the same conditions in which the growth experiment was to be carried out, for at least two weeks with regular sub-culturing at every 3rd or 4th day, when the cells were in their late exponential phase. During experiments, filtered air/gas mixture was bubbled at a fixed aeration rate of 0.45 vvm (gas volumetric flow rate per unit volumetric culture medium) through each vessel. For CO_2 tolerance studies, U.H.P. grade pure CO_2 was premixed with compressed air to obtain three CO_2 concentrations (5 ± 0.5, 10 ± 0.5, 15 ± 0.5% v/v). In simulated flue gas experiments, NO was supplied from a 500 ppm NO cylinder (balanced with N_2) and blended with CO_2 and compressed air to achieve two concentrations, 80 and 160 ppm. All the gas cylinders were obtained from AIR LIQUIDE Australia Limited.