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Growth kinetic and fuel quality parameters as selective criterion for screening biodiesel producing cyanobacterial strains

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ABSTRACT

The efficiency of cyanobacterial strains as biodiesel feedstock varies with the dwelling habitat. Fourteen indigenous heterocystous cyanobacterial strains from rice field ecosystem were screened based on growth kinetic and fuel parameters. The highest biomass productivity was obtained in *Nostoc punctiforme* MBDU 621 (19.22 mg/L/day) followed by *Calothrix* sp. MBDU 701 (13.43 mg/L/day). While lipid productivity and lipid content was highest in *Nostoc spongiaeforme* MBDU 704 (4.45 mg/L/day and 22.5% dwt) followed by *Calothrix* sp. MBDU 701 (1.54 mg/L/day and 10.75% dwt). Among the tested strains, *Nostoc spongiaeforme* MBDU 704 and *Nostoc punctiforme* MBDU 621 were selected as promising strains for good quality biodiesel production by Preference Ranking Organization Method for Enrichment Evaluation (PROMETHEE) and Graphical Analysis for Interactive Assistance (GAIA) analysis.

1. Introduction

Fast depletion of fossil fuels with exploding population mandates global energy agendas towards the development of renewable sources of fuel. Among different generations of renewable feedstock, photosynthetic microalgae serve as a promising biomass for a diverse number of products such as fine chemicals, nutraceuticals, aquaculture, feed and cosmetics in addition to biofuels (Gerardo et al., 2015). Despite much research over the last two decades, microalgal biofuel has not yet become a commercial reality. For the past two years, the collapse in oil prices impose a large economic pressure on biofuel production (Cate and Ball, 2016). To make microalgal biofuels commercially feasible and practically viable, microalgal biomass must be processed in a biorefinery concept i.e. similar to petroleum refinery for extracting multiple products in addition to biofuel (Maurya et al., 2016). Many up-and downstream processes have been successfully integrated during the conversion of microalgal biomass. For example, integrating the upstream microalgal cultivation with wastewater treatment reduces the overall residual waste component and favours a sustainable economy (Mohan et al., 2016). During downstream processing, high volumes of products such as proteins and carbohydrates, and 'low volume high value products' such as astaxanthin, β-carotene, and polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid have also been co-extracted from microalgal biomass, and have significant market demand (Gerardo et al., 2015). Therefore, a biorefinery should be able to produce a gamut of marketable products and energy in a sustainable fashion (Gravitis, 2008). Utilization of additional products help to subsidize the overall fuel costs (Chuck et al., 2015).

Owing to its simple growth requirements, increased growth rate and ease of genetic engineering with developed molecular tools, cyanobacteria serve as an attractive candidate over eukaryotic microalgae in terms of biomass feedstock utilization. Until recently, many researchers have successfully co-produced various valuable products in metabolically engineered cyanobacteria (Angermayr et al., 2015). However, only limited research has occurred in the exploration of natural cyanobacterial species with the potency of producing different commercially important products. Knowledge of the above properties is important in the selection of most suitable strains which can be exploited successfully at a commercial level. With this in view, the present study is carried out to explore the lipid productivity, lipid content and biodiesel properties of phytohormone producing cyanobacterial strains.

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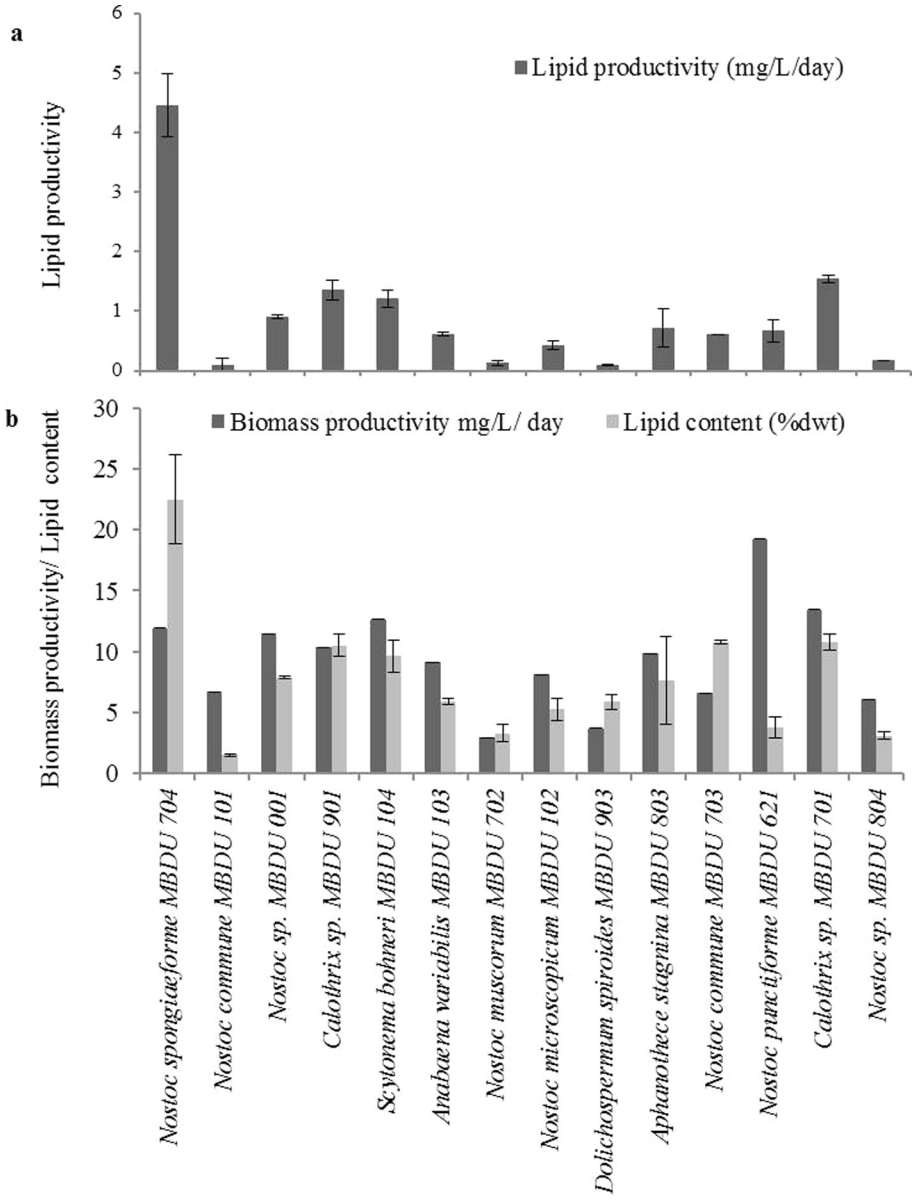

Fig. 1. The performance of (a) Lipid productivity (mg/L/day) (b) biomass productivity (mg/L/day) and lipid content (% dwt) of fourteen cyanobacterial strains. The bar diagram represent the biomass productivity and lipid content and line art represent the lipid productivity. Data values are means (± SE) of three replicates.

2. Methods

2.1. Cultivation of cyanobacterial strains

The fourteen cyanobacterial strains, *Scytonema bohneri* MBDU 104, *Calothrix* sp. MBDU 901, *Nostoc spongiaeforme* MBDU 704, *Nostoc commune* MBDU 101, *Nostoc muscorum* MBDU 702, *Nostoc* sp. MBDU 804, *Anabaena spiroides* MBDU 903, *Nostoc Punctiforme* MBDU 621, *Calothrix* sp. MBDU 701, *Aphanothece stagnina* MBDU 803, *Anabaena variabilis* MBDU 103, *Nostoc sp.* MBDU 001, *Nostoc commune* MBDU 703, and *Nostoc microscopicum* MBDU 102, characterized previously for phytohormone production (Gayathri et al., 2017) were grown in BG-11$_o$ medium in 250 mL Erlenmeyer flask at 28 ± 1 °C under continuous light (50 μE m^{-2} s^{-1}) (Rippka et al., 1979).

2.2. Growth kinetic parameters

The growth kinetic parameters of fourteen strains were determined after harvesting the cells in their stationary growth phase. All the measurements were performed in triplicates. The parameters analyzed included:

- **Biomass productivity (Pb)** indicates the amount of dry biomass produced (mg L^{-1} day^{-1}). For Pb determination, algal suspensions were centrifuged at 3000*g* for 10 min at room temperature and the resulting pellets were washed with deionized water, lyophilized at −40 °C for 48 h and their dry weights were determined gravimetrically.
- **Total lipid content (Lc)**, reported as percentage of the total biomass (% dwt), and determined based on the method by Folch et al., (1957).
- **Volumetric lipid productivity (Lp,** mg L^{-1} day^{-1}), was calculated according to the following equation (Liu et al., 2011).

$$Lp = Pb \times Lc \quad (1)$$

2.3. Total lipid extraction

The total lipid from the tested strains was extracted according to Folch et al., (1957). 40 mg of freeze-dried biomass was extracted with 10 mL of chloroform:methanol (2:1) using pestle and mortar. The extract was filtered through Whatman No. 1 filter paper. To the filtrate three volumes of distilled water was added. The filtrate was then