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Screening microalgae native to Quebec for wastewater treatment and biodiesel production

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HIGHLIGHTS

- A novel microalgal screening method using 12 well microplates was used.
- 100 strains from local (Quebec) freshwater lakes and rivers were characterized.
- A number that showed good growth at 10 °C or high (20–45%) lipid content.
- Some showed a high capacity for nutrient removal.

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ABSTRACT

Biodiesel production from microalgae lipids is being considered as a potential source of renewable energy. However, practical production processes will probably require the use of local strains adapted to prevailing climatic conditions. This report describes the isolation of 100 microalgal strains from freshwater lakes and rivers located in the vicinity of Montreal, Quebec, Canada. Strains were identified and surveyed for their growth on secondary effluent from a municipal wastewater treatment plant (La Prairie, QC, Canada) using a simple and high throughput microalgal screening method employing 12 well plates. The biomass and lipid productivity of these strains on wastewater were compared to a synthetic medium under different temperatures (10 ± 2 °C and 22 ± 2 °C) and a number identified that showed good growth at 10 °C, gave a high lipid content (ranging from 20% to 45% of dry weight) or a high capacity for nutrient removal.

1. Introduction

The need for sustainable sources of energy is rapidly increasing due to the increase in the world's population, industrialization and greater demand for transportation. Conventional sources of energy, such as oil, natural gas, and coal, are nonrenewable and their use has caused extensive damage to the environment by increasing the atmospheric load of carbon dioxide and other greenhouse gases (GHGs) that are causing disastrous global climatic changes (Abdelaziz et al., 2013a,b). The highly productive terrestrial bioenergy crops, such as soybean oil and palm, are challenging feedstocks due to their effect on the world food supply. The use of non-edible crops as feedstock, seen by some as desirable, often diverts land from the production of food crops, and neither type can match the potentially high productivity of microalgae (Leite et al., 2013). Biodiesel derived from microalgal lipids has received much attention as it holds the promise to provide low carbon, renewable feedstocks without adversely affecting the food supply or the environment. Although microalgae have many desirable characteristics; faster growth rates, higher photosynthetic efficiencies, greater biomass and lipid productivities, there are however some significant challenges that need to be overcome. Large scale biofuel production will probably require the use of strains that are adapted to and competitive in local environmental conditions, Thus there is a need for the effective and rapid isolation of microalgal strains with potentially high intrinsic lipid content and rapid growth and biomass productivities (Demirbas, 2011; Elliott et al., 2012).

One of the major hurdles in the development of microalgal based biodiesel is that at present the overall cost for microalgal biodiesel production is much higher than that from other bioenergy crops. Thus, selection of an energy and cost effective production strategy will play a very important role in achieving competitive biodiesel prices. Selection of high lipid-producing microalgae, cheap nutrient sources, suitable cultivation locations, rapid cultivation and harvesting methods and efficient oil extraction techniques are criteria that should be considered (Duong et al., 2012). Here, we focus on screening around 100 freshwater strains of

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native microalgae to select the most suitable high lipid-accumulating microalgal strains and the use of wastewater as a production medium, thus potentially greatly reducing microalgal cultivation costs.

Microalgae, in addition to serving as a biofuel feedstock, are potential candidates for wastewater treatment. The discharge of incompletely treated wastewater can lead to eutrophication of surrounding waters and ecosystem damage due to the high amounts of nitrogen and phosphorus (Rawat et al., 2011). The high energy requirements and costs associated with wastewater treatment and nutrient removal with existing chemical and physical based technologies remains a challenge for municipalities, governments and industries (Christenson and Sims, 2011). Using microalgae based wastewater treatment potentially has a number of benefits; wastewater treatment can be coupled to biomass production for biofuel production, offsetting the utilization of unsustainable amounts of freshwater and commercial fertilizers otherwise required for microalgal cultivation. This option promises to reduce microalgal cultivation costs and the energy required for wastewater treatment as well as permitting resource recovery and recycling (Abdelaziz et al., 2013a; Cho et al., 2011; Pittman et al., 2011). Suitable wastewaters, rich in nutrients, in particular nitrogen and phosphorus, are available from slaughterhouse wastes, agricultural/industrial wastes, dairy effluents, compost plant and municipal waste. Growing algae on these waters is an attractive means to decontamination of heavily polluted wastewaters while at the same time providing high yields of biomass for the production of biofuels, organic chemicals, and other commercial products.

Municipal wastewater is one of the main sources of pollution to surface water in Canada, especially since many treatment plants, including those of major cities like Montreal, only carry out rudimentary treatment due to the lack of suitable regulations (Environmental Canada, 2010). An ideal sewage treatment process would consist of three stages; primary treatment to remove heavy solids, secondary treatment, often using microorganisms, to remove BOD (biological oxygen demand), and tertiary treatment to remove the remaining fixed nitrogen and phosphate. Algae can be used either in the secondary treatment process, where they generate the required oxygen through photosynthesis (Oswald et al., 1953), or in tertiary treatment, where they remove the excess nutrients (nitrate and phosphate) (Gutzeit et al., 2005; Munoz and Guieysse, 2006).

Temperature is an important environmental parameter affecting algal growth. Temperatures ranging between 15 and 25 °C are usually considered optimal for algal growth with lower temperatures resulting in decreased growth rates. However, these temperature specific effects most likely vary from one species to another (Goldman and Carpenter, 1974). Although, nutrient uptake and photosynthesis might be expected in general to be lower at lower temperatures, algal strains that are native to cold climates might be capable of achieving treatment goals with high growth rates and good lipid production (Powell et al., 2008). The recent isolation of a novel yellow–green cold tolerant species from snowfields in Colorado, USA, with a lipid content of 55% demonstrates the potential for cold climate algae as strong candidates for biofuel production (Nelson et al., 2013).

Algal samples were collected from five different locations in the vicinity of Montreal, Quebec, Canada. A native culture collection of more than 100 unialgal strains has been established and characterized. As far as we are aware this is the first description of isolation and characterization for biofuels production of any microalgal strains in Quebec. Thus, this work establishes for the first time knowledge about useful properties of microalgae native to Quebec. Here we report on the use of a high throughput 12 well microplate process to survey 100 strains from this collection for growth on municipal wastewater (WW) and synthetic Bold Basal Medium (BBM) at 10 ± 2 °C and 22 ± 2 °C. Additionally, the strains were screened for their capacity for nutrient removal and biofuel production. The results show that the collection microalgae is highly diverse, with genera of various algal classes showing a variety of growth rates under different conditions, different levels of lipid production and differing abilities to carryout nutrient removal.

2. Methods

2.1. Sampling and isolation

Water samples were collected from five different locations; three fresh water lakes (Lac Croche (45°59′24.37″N 74° 0′21.01″W) and Lac Pilon (46°0′14.02″N 74°1′7.09″W), University of Montreal biological station (45°59′17.11″N 74°0′20.55″W)), situated in the Laurentian region north of Montreal, Canada; and two on each side of the Saint Lawrence river, situated approximately 10 km downstream from the confluence with the Ottawa river, where the water of both rivers are not yet totally mixed (45°25′39.12″N 73°49′15.78″W and 45°21′23.36″N 73°48′49.96″W). Sampling at each site was conducted during the spring, summer and fall. Coarse material, potentially including zooplankton, was removed on site by filtration through a 50 μm mesh net and then samples were stored in cool boxes for transportation to the laboratory. Once in the laboratory, the water samples were filtered through a series of membranes of decreasing mesh size (33, 20 and 0.45 μm). The retention products of each membrane was taken using a sterile swab, and directly plated on BBM agar plates (Andersen, 2005) and incubated in a light chamber at 20 ± 2 °C. In all the experiments reported in this study, no special provisions were made for CO_2 supply. Thus, all cultivations were with atmospheric CO_2. Light was provided by warm white fluorescent bulbs at 25 W/m^2 operated on a light/dark cycle of 12/12 h. After growth, different colonies were inoculated in 125 ml Erlenmeyer flasks containing 70 ml of BBM medium and incubated in a light mounted shaker at 20 ± 2 °C, with shaking at 120 RPM and a light intensity of 21.2 W/m^2 using a photoperiod of 12 h light: dark. Isolates were then kept in falcon tubes with the same medium for the further analysis.

2.2. Strain identification

Samples of the different algal cultures were examined morphologically in a light microscope for preliminary identification and confirmation that the cultures were unialgal using a NIKON Eclipse E600 microscope with an attached NIKON digital camera DXM 1200F. Preliminary identification of the algal cultures was made using a field guide (Prescott, 1978).

2.3. Screening for growth

One hundred isolates were assessed for the ability to grow at 10 ± 2 °C or 22 ± 2 °C on the secondary effluent from a municipal wastewater treatment plant (La Praire, QC, Canada) and BBM medium (Andersen, 2005). The nitrate and phosphate content of the wastewater was determined as described below giving an estimated N:P ratio of 37:1 with a phosphate concentration of 3 mg l^{-1} (Table 1). Strains were inoculated (1% v/v of OD_{600} value 1.0) in 12 well flat bottom plates (Falcon tissue culture plates, USA) containing either 4 ml sterile municipal wastewater or BBM medium (both media were sterilized using filtration apparatus using Millipore membrane filter with a 0.45 μm pore size) and incubated for 14 days in a photoincubator at 10 ± 2 °C or 22 ± 2 °C at a light intensity of 40 W m^{-2} and a 12:12 h light/dark cycle. Growth was quantified daily by measuring the optical density (OD_{600})