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Characterization and classification of highly productive microalgae strains discovered for biofuel and bioproduct generation

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ABSTRACT

This paper describes the characteristics of microalgal strains that originated out of an isolation and screening project included within the National Alliance for Advanced Biofuels and Bioproducts (NAABB). The project's goal was to identify new potential platform strains with high growth rates and/or lipid productivities. To classify the best performing strains, we conducted a combined microscopic and phylogenetic analysis. Among the best performing strains were many coccoid green algae. Several strains belong to the species *Acutodesmus* (*Scenedesmus*) *obliquus* and to the species *Chlorella sorokiniana*, thus expanding on existing germplasm. Identified at the genus level were some *Desmodesmus* strains and one *Ankistrodesmus* strain. Several strains were classified as belonging to the genus *Coelastrella*, a taxon reported for the first time for North America. Multiple additional strains had ambiguous identities, with some strains possibly representing novel species. Reporting on the above strains, some of which have been tested successfully in outdoor ponds and most of which are deposited at the University of Texas Culture Collection of Algae, is a step forward in expanding the biological resources available for algae biofuel production.

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1. Introduction

Algae are seen as a more appealing biofuel feedstock than land plants because of their faster biomass doubling cycles, more accessible forms of stored carbon, and their ability to thrive on waste or salt water sources [1,2,3]. But the best strains for such production are unlikely to be known or utilized at large scales [4]. Therefore, a need exists for “phyco-prospecting” [4] – the identification of novel algal “platform strains” – which sustain high growth rates and accumulate lipids [5,6,7,8,9]. In addition, strains that produce significant amounts of higher-value products, such as carotenoids, are also desired [1,2,10,11,12,13]. Such novel “platform strains” could, in turn, be developed further via artificial selection, genetic engineering, or other “crop improvement” methods [3,14,15] and used to generate biofuel feedstocks at lower costs.

Commercial scale algae ponds have been operated for more than a decade [3,16,17,18], mainly to harvest pigments and metabolites that are desired as nutritional supplements [10]. The algae cultivated in these ponds include Spirulina (*Arthrospira*), for high protein powder [19,20], *Haematococcus*, for the antioxidant astaxanthin [21,22], and *Dunaliella salina*, for pro-vitamin A production [23,24]. Due to the success demonstrated with these used species [17], the as of yet untapped diversity of microalgae appears to offer possibilities in the field of biofuels [25,26,27], as well as the production of high-value products [1], such as pharmaceuticals [10,13,28].

It is not clear how many microalgae species there are, with estimates running from 70,000 to one million [29,30,31]. Only about 44,000 have been described [29,32]. New species and genera are consistently being discovered; this consistent rate of discovery indicates that a large proportion of undocumented species exists [29].

Based on the known utility of already characterized species and the known absence of knowledge regarding the large amount of undocumented strains, it is hypothesized that previously uncharacterized strains of microalgae exist which exhibit high growth rates and lipid productivities. These novel “strains” may be new species or varieties

Abbreviations: UHPLC, ultra high performance liquid chromatography; PDA, photo diode array; MS, mass spectrometry; APCI, atmospheric pressure chemical ionization; ITS, internal transcribed spacer; SSU, small-subunit; LSU, large-subunit; TAG, triacylglycerols.

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of species with more favorable growth characteristics for biofuel production. Some of these species or strains may also produce high value products. To test this hypothesis, a large-scale effort was conducted to isolate, screen, identify, and characterize microalgae strains that could be used as platform strains for biofuel and/or high-value product generation. For a number of the most promising strains to come out of the screen, a classification was conducted. In deciding on an appropriate barcode, the nuclear rDNA Internal Transcribed Spacer 2 Region (ITS2) was chosen because, unlike the 18S – which often does not vary enough to distinguish algal species [33] – the ITS2 has proved to be a helpful tool for discrimination at the genus [33,34] and species level [35,36,37,38,39,40,41,42]. At the same time, its two dimensional structure is highly conserved throughout the eukaryotes [43,44,45]. Combining the fast evolving sequences with its slowly evolving structure has allowed for it to be used in high level classifications while still discriminating at the low species level, within the same phylogenic tree [37].

The overall goal of our research was to discover new microalgal strains that could enhance the genomic/biological resources available for algae biomass production. This biomass would then be used as feedstock for biofuel and/or for bioproduct generation. The focus of this report is on freshwater algae originating mainly from the NAABB phyco-prospecting project [46]. The objective of this report is to document some of these strains and begin to have a cladistic understanding of where they fall in the algal tree of life. It is hoped that this work may provide some clues for targeted phyco-prospecting approaches in the future.

2. Materials and methods

2.1. Sampling, isolation, and screening

Cells were sampled and screened as described previously in detail [47]. Briefly, environmental samples were collected over the three years of the NAABB project (2010–2012) from a variety of habitats (e.g. freshwater lakes and soils) across the continental U.S. and during all seasons. As described in detail in [47], for strain isolation an approach was taken that combined the traditional plating method [48] with high-throughput flow cytometry using fluorescence aided cell sorting [49,50].

For the first-level screen, in summary, batch cultures of strains were grown in Erlenmeyer flasks in defined minimal media suitable for this selection: C Medium, Bold Basal Medium, and BG11 Medium [51], with the only CO_2 enrichment being the sodium bicarbonate which was added to BG11. Flasks were grown under 50 μE m^{-2} s^{-1} continuous light provided by daylight fluorescent lamps (see the SN-1 for more details on screening methods) at room temperature.

Strains that passed the first-level screen were deemed to be potential producers and were selected for further characterization (Fig. 1). In a second-level screen, the strains were cultivated under identical conditions as in the first screen in multiple different media (C Medium, Bold Basal Medium, BG11) to determine if another growth medium was better suited for use in continued strain characterization (Fig. 1).

In a third-level screen for analysis of actual biomass and lipid production potential, strains were grown at 28 °C in glass columns (3 cm width with 500 mL volume) into which CO_2 enriched air was bubbled through the cultures from the bottom. Twenty-eight degrees Celsius was used at this point in the screen, because it was deemed a good intermediate temperature to find strains that grow well both in the spring and fall as well as in the summer. Cultures were illuminated by daylight fluorescent lamps from one side at an intensity of 200 μmol photons m^{-2} s^{-1}.

Biomass was determined daily by measuring the Ash-Free-Dry-Weight (AFDW, Supplemental Notes 1 (SN-1)). The daily AFDW was graphed using the ggplot package, within the R software environment for statistical computing and graphics (http://cran.r-project.org/), to create weighted polynomial curves, which represented algal growth curves. The doubling time was also calculated in R for the exponential growth phase with the formula log(2)/k, with k being the slope of the growth curve.

When the algal cultures reached their stationary phase, biomass also was taken and analyzed via a gravimetric method to determine the total lipid content [52].

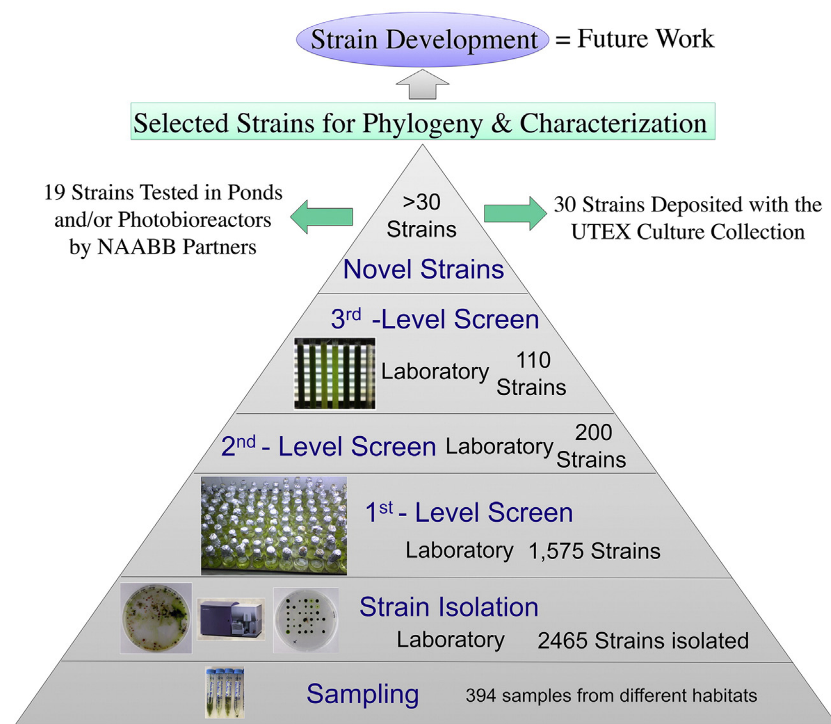

Fig. 1. Schematic summarizing the overall phyco-prospecting process performed in our laboratory as part of the NAABB Consortium to identify the best performing microalgae strains for biofuel production.

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