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Exploring the diversity of microalgal physiology for applications in wastewater treatment and biofuel production

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ABSTRACT

A recently established strain collection of freshwater microalgae native to Quebec was examined for physiological diversity. The 100 strains appeared very heterogeneous in terms of growth when they were cultured at 10 ± 2 °C or 22 ± 2 °C on the secondary effluent from a municipal wastewater treatment plant (WW) and defined BBM medium. Scatterplots were used to examine the diversity in physiology that might be present in the collection. These showed a number of interesting results. There was a fair amount of dispersion in growth rates by media type independent of temperature. Surprisingly considering that all the isolates had been initially enriched on BBM, the distribution was quite symmetrical around the iso-growth line, suggesting that enrichment on BBM did not seem to bias the cells for growth on this medium versus WW. As well, considering that all the isolates had been initially enriched at 22 °C, it is quite surprising that the distribution of specific growth rates was quite symmetrical around the iso-growth line with roughly equal numbers of isolates found on either side. Thus enrichment at 22 °C does not seem to bias the cells for growth at this temperature versus 10 °C. The scatterplots obtained when the percentage lipid of cultures grown on BBM were compared with cultures grown on WW at either 10 °C or 22 °C made it apparent that lipid production was favored by growth on WW at either temperature and that lipid production does not seem to be particularly favored by one temperature over the other. When the collection was queried for differences with respect to sampling location, statistical analysis showed that roughly the same degree of physiological diversity was found with samples from the two different aggregate locations.
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1. Introduction

There is a great deal of interest at present at both the research and development levels in microalgal biofuels production systems. A number of very significant challenges remain to be overcome before the dream of sustainable algal biofuels production becomes a reality [1–7]. Among the many challenges, some may be solved by technological advances, e.g. harvesting and effective lipid extraction and conversion to biodiesel, whereas others, including: fast growth rates, high levels of lipid production, competitiveness, tolerance to variation in local conditions, are a function of the biology of the system. These latter goals then can be addressed through strain selection, with native, indigenous strains more likely to be competitive and hardy under local conditions.

Thus, a number of studies involving the isolation of strains in different locales have been undertaken with an eye to biofuels production since the late 1970's over a number of continents and climatic zones [8–17]. Almost invariably these bioprospecting efforts have had a narrow, immediate focus on very specific attributes, such as high lipid productivity, and usually go on to examine in detail the characteristics of only a few strains. Recently, we established a collection of over 100 native freshwater microalgae indigenous to Quebec [15]. We have shown that some of the strains have interesting characteristics in terms of growth and lipid production and have gone on to carry out a RSM (response surface methodology) analysis of lipid production and growth, showing that one of the strains in the collection, PCH90, could grow well at both 22 and 10 °C on secondary wastewater effluent, producing up to 36% lipid [18]. A more in depth look at some of the other strains indicated that there were a significant number that might be of potential interest for biofuels production.

However, such a collection also has the potential of being a valuable resource as a long term genetic resource or in examining local diversity. Algal diversity has long fascinated and in fact was the subject of the first book devoted to scientific photographs [19]. Thus, from the very beginning discussions of algal diversity has been dominated by morphology. In general biodiversity has traditionally been described through taxonomy and taxonomic concepts, and since the phenome used to describe species is usually morphology, taxonomically defined biodiversity of microalgae has been dominated by microscopic examination. Even from this view point microalgal diversity has been regarded as enormous with an estimate of hundreds of thousands of undiscovered species being given more than two decades ago [20].

Traditional taxonomy, arguably the oldest recognized scientific profession (Genesis 2:19) [21], may be at a cross-roads for a number of

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reasons; the number of described species is increasing exponentially, but so are the numbers of taxonomists, with no end in sight [21–23]. Additionally, the naming of species present is not enough to describe the diversity present at a deep level due to: ″hidden″ diversity, the absence of observation of rare species; "cryptic" species, organisms with significant differences below the morphological level [24,25]; and even the recently demonstrated existence of "ecotypes" in marine microalgae [26]. Thus, another descriptive process that looks at diversity on a different level is some type of DNA analysis, with a particularly useful approach called DNA barcoding where using sequence analysis of multiple genetic markers; mitochrondrial cytochrome oxidase (COX), LSU rDNA, and Rubisco (rbcL), can identify new species and uncover cryptic species [27]. This has of course greatly increased the information available about microalgal taxonomic diversity, but at a cost. As the number of sequences accumulates with time, there are fewer and fewer associated with taxonomically recognized names (<20% in 2010), suggesting that taxonomy is on the road to a future without names [28]!

Obviously, each approach has its benefits and should be matched to the specific goal at hand. However, ultimately and on many levels, including ecological and biotechnological (bioprospecting), the most interesting question is, what is the functional diversity that is present. Nevertheless, this is seldom addressed. While it is known that the functional diversity of large culture collections is vast [29], little is known about functional diversity, i.e. physiological and metabolic robustness, within small, region specific collections. Since we had established a collection of one hundred different strains collected from the local waters of Quebec, we were interested in assessing the functional diversity present within this collection. The collection was queried for specific growth rate and lipid productivity on two different media at two different temperatures. The observed diversity was surprisingly large, suggesting that in general desirable microalgal phenotypes can readily be discovered in a restrained geographical search.

2. Materials and methods

2.1. Establishment of the microalgal culture collection

Water samples were collected from five different locations; three freshwater lakes (Lac Croche (45° 59′ 24.37″ N 74° 0′ 21.01″ W), Lac Pilon (46° 0′ 14.02″ N 74° 1′ 7.09″ W) and Lac Triton (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). The detailed sampling protocol and initial isolation were described previously [15]. No enrichment was used other than the use of a filtration step to concentrate the samples [15]. The isolation procedure involved BBM agar plates [15] and incubation in a light chamber at 22 ± 2 °C with atmospheric CO_2 for a period of four to six weeks. Light was provided by warm white fluorescent bulbs at 25 W/m^2 operated on a 12/12 hour light/dark cycle. Individual strains were stored in dim light in 50 ml tubes.

2.2. Growth and lipid production

One hundred isolates were grown at 10 ± 2 °C or 22 ± 2 °C on the secondary effluent from a municipal wastewater treatment plant (La Praire, QC, Canada) and on BBM medium. The nitrate and phosphate content of the wastewater was determined as previously described [15], 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 un-treated 12 well flat bottom plates containing either 4 ml sterile municipal wastewater or BBM medium 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. This method has some

Table 1
Wastewater chemical composition.

Macronutrients		Micronutrients	
Ion[a]	Conc (ppm)	Element	Conc (ppm)
		As	0.034
NO_3^-	110	Be	0.00059
PO_4^+	3.0	Ca	32
		Cd	0.00081
		Co	0.0034
		Cr	0.0048
		Cu	0.085
		Fe	0.0086
		Li	0.055
		Mg	17
		Mn	0.0027
		Mo	0.011
		Ni	0.015
		Pb	0.019
		Se	0.080
		V	0.010
		Zn	0.051

[a] The major species present. There was a very small amount of organic N and N as ammonium, 2.1 ppm total.

variability and an analysis of data obtained in this way indicates that variation between biological replicates done at different times is ±25%. As well, although given the large number of strains it was not possible to carryout replicate samples at the same time, analysis of six duplicates that were included showed that the variation was +25%. Growth was quantified daily by measuring the optical density (OD_{600}) using a microplate reader (Biotek EL800) as previously described [15]. Specific growth rates were calculated using the periodic OD measurements and choosing the exponential growth phase. The cellular content of lipid was determined by Nile Red as described previously [15] and given in Supplementary materials. A Varian Vista MPX ICP-OES spectrophotometer was used to measure the partial elemental composition of the wastewater. Scatterplots were generated using Microsoft Excel and data analysis was made using intrinsic Excel tools or the Regression and Multibase add-ins.

3. Results and discussion

The present article reanalyzes, from a different perspective, an existing data set, one that is too large to completely interpret in a single article. Previously, this collection was used to screen for a few selective strains that either were adept at wastewater treatment or that gave high lipid production under specific conditions. Here we have reexamined this collection (Table S1) in order to determine the extent of functional diversity present. The strains were tentatively identified by microscopic examination (Table S1). The identity of ten strains picked randomly was checked by sequence analysis of the 18 SRNA and in every case agreed with that determined microscopically.

3.1. Culture collection and growth curves

As described previously, we have established a culture collection of microalgae native to freshwaters of Quebec and, using a high throughput 12 well plate procedure, were able to select a few strains showing interesting growth properties, or an apparent capacity for high lipid production [15]. We grew the close to 100 strains at 10 ± 2 °C or 22 ± 2 °C on the secondary effluent from a municipal wastewater treatment plant (La Prairie, QC, Canada) (the chemical composition is given Table 1) and BBM medium. It was apparent from the growth patterns that the collection was very heterogeneous in terms of metabolic properties under these conditions (Figs. S1A, B and S2A, B). A number of interesting questions arose in terms of the diversity in physiology that might be present. Although the strains were originally enriched with synthetic BBM medium, how did their patterns of growth on this

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