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Using microalgal communities for high CO_2-tolerant strain selection

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ABSTRACT

Certain species of microalgae are capable of growing under high concentrations of carbon dioxide (CO_2). These microalgae have potential to be used for sequestering CO_2 released as industrial pollutants using a phototrophic carbon fixation system. While some CO_2-tolerant algal species have been identified from existing algal culture collections, this study explored a community-based approach to enrich and isolate CO_2-tolerant microalgae. Meanwhile, we monitored the change of bacterial and microalgal communities during the CO_2 enrichment period based on the 16S and 18S rRNA gene sequences. Four different treatments were set up in the laboratory to test the effect of nutrient and CO_2 on the natural planktonic community. At the end of the enrichment experiment (17 days), green algae (Chlorophyta), especially *Scenedesmus* species, dominated the microalgal community when the water samples were enriched with high CO_2 (10%) and nutrient. The dominance of species in the CO_2-enriched samples was also evident in the clonal isolation of microalgae at the end of the experiment. This study clearly demonstrates that the supplementation of high levels of CO_2 to a natural phytoplankton community is an efficient way to enrich and isolate CO_2-tolerant microalgae. The community-based approach described here poses several advantages over the traditional culture-based screening method for isolating microalgae with specific characteristics.

1. Introduction

Carbon dioxide (CO_2) accounts for nearly 80% of the total greenhouse gas (GHG) emissions worldwide, and most members of the United Nations have committed themselves to significantly reduce their GHG emissions [1]. Exhaust gases from power plants attribute to ca. 40% of the U.S. annual CO_2 emission in 2010, and the concentration of CO_2 in power plant exhausts varies from 10 to 15% depending on the source of fuels [2]. Phototrophic algae fix CO_2 and incorporate to biomass. Phototrophic carbon fixation through microalgae cultivation has been proposed as a biological way to mitigate CO_2 pollution, especially for the sequestration of CO_2 from industrial exhaust gases such as flue gases [3–7]. Therefore, the ideal microalgae candidates for sequestering CO_2 in flue gases should be able to grow under high CO_2 concentration (e.g. ≥10%).

Photosynthesis performed by microorganisms (including cyanobacteria and eukaryotic microalgae) is an ancient process [8,9]. Photosynthetic eukaryotes inhabited coastal waters ca. 1.4–1.9 billion years (Gyr) ago [10–12]. Green algae such as Chlorophytes (for example, *Scenedesmus* and *Chlamydomonas*), are thought to be the dominant phytoplankton in the Mesoproterozoic ocean (0.9–1.6 Gyr ago) and have become much less abundant in the Paleozoic period (0.25–0.54 Gyr ago) [13]. Kasting [14] suggested that the concentration of atmospheric CO_2 decreased from 10% (v/v) at 2.5 Gyr ago to 1% (v/v) at 1 Gyr ago, and continued to decrease. The geological records on algal species and atmospheric CO_2 level suggest that some Chlorophytes species thrived in the high CO_2 environment during the Mesoproterozoic period, and the concentration of atmospheric CO_2 has decreased gradually by CO_2 fixation of Chlorophytes over the geological time. While atmospheric CO_2 decreased, other types of microalgae that can use CO_2 more efficiently emerged [15]. It is possible some current Chlorophytes species still retain the ability or strategy to grow under high CO_2 concentrations. The key mechanisms governing the microalgal tolerance to high CO_2 concentrations could involve the photosynthetic apparatus state transitions, rapid shutdown of CO_2-concentrating mechanisms, or adjustment of fatty acid composition of membranes [16–19].

Some microalgal isolates are able to grow when bubbled with high

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CO_2 concentration [20–24]. One green algal strain, *Chlorella* sp., was able to grow under 100% CO_2 and flue gas, although the maximum growth rate occurring at 10% CO_2 concentration [22]. Another *Chlorella* strain was found to grow faster in 10% CO_2 [25]. Hanagata et al. reported that the green alga *Scenedesmus* sp. could grow under 80% CO_2 conditions, but the maximum cell mass was observed in 10–20% CO_2 concentrations [19]. Another *Scenedesmus* strain was able to grow in a large photobioreactor (500 L) bubbled with flue gas that contains 10–12% CO_2 [23]. *Desmodesmus* spp. could grow under 100% unfiltered flue gas from coal combustion [24]. Other non-green algae can also grow in high CO_2 environment. For example, red algae like some *Cyanidium* species can grow in pure CO_2 [26,27]. Growth of mixotrophic algae like *Euglena gracilis* was enhanced under elevated concentrations of CO_2 (5–45%) [28]. Therefore, it is evident that different microalgae in diverse algal lineages are able to thrive in high CO_2 conditions.

Early studies mainly relied on available algal cultures to test their capability to grow in high CO_2 levels. The approach for screening and selecting CO_2-tolerant strains includes bioprospecting (the search for specific microalgae species from local habitats which are CO_2-tolerant) and acclimation of natural microalgae to high CO_2 concentrations [29]. A high-throughput screening method has been used to discover algal monocultures that can grow in various concentrations of CO_2 [23]. While the culture-based method has been widely used to select desirable algal strains for different purposes, the limitations of this method are multiple: 1) Only a limited number of algal cultures can be tested; 2) maintaining, growing and monitoring of many algal cultures is very time consuming; 3) selected algal strains may not be ideal for the local applications (i.e. use of local water); 4) microalgae grow poorly on the microplates compared to large bioreactors [30]. A recent study shows that a community-based method can be used to enrich and isolate CO_2-tolerant microalgae [24], suggesting that exposing natural phytoplankton communities to the desirable test conditions enables expedited selection of target algal strains from a whole community of microalgae in a particular aquatic ecosystem.

Microalgae are very diverse in the natural environment. It has been estimated that more than one million algal species exist in nature, names for 44,000 of which have probably been published, and 33,248 names have been processed by AlgaeBase (http://www.algaebase.org) [31,32]. Many studies have contributed to better understand the impacts of abiotic or biotic factors to natural environment community shifting. With the use of molecular sequencing technology, the change of prokaryotic and eukaryotic communities can be monitored simultaneously [33–36]. Co-monitoring the variation of different microbial communities in response to a specific change or event (i.e. algal bloom) has become a powerful tool to study the interaction between organisms in the natural environment [37]. It would be interesting to know how microbial communities in natural aquatic bodies respond to high CO_2 exposure, nutrient enrichment, or both. By exposing the natural microalgal communities to high CO_2 condition, we also want to know which kind of microalgal populations will emerge to dominate the community at the end of the experiment.

In this study, we exposed a water sample collected from the Back River, Baltimore to 10% CO_2. Because the concentration of CO_2 in power plant exhausts varies from 10 to 15% depending on the source of fuels. The 10% CO_2 concentration was chosen in this study as we intended to isolate algal strains that are suitable for sequestering CO_2 from the flue gas of power plant. The goal here is to understand how bacterial and microalgal communities change when the natural water is exposed to a high CO_2 concentration. The bacterial and algal community will be analyzed by sequencing the partial 16S and 18S rRNA genes, respectively. Also, we identified and counted the cell density of major microalgae taxa. Ultimately, we isolated CO_2-tolerant algal strains following the CO_2 enrichment experiment.

2. Materials and methods

2.1. Experimental design

The environmental water sample was taken from Back River, Baltimore, Maryland (latitude: 39.300°N, longitude: 76.489°W) on December 12, 2016, which was located close to the Baltimore Back River Wastewater Treatment Plant. Four treatments were set up to expose the environmental sample to 1) 10% CO_2 with high nutrient (BG11 medium); 2) 10% CO_2 without high nutrient; 3) air with high nutrient and 4) air only. All the CO_2 gas was purchased from Airgas USA, LLC (CD USP50), and it was delivered through bubbling with a constant flow. Measurement of the CO_2 concentration was carried out using a GasLab® Sensor Configuration and Data Logging Software (https://www.co2mete r.com/pages/downloads). All treatments were duplicated. A total of 8 bottles (2 L PYREX® Round glass Bottles) were exposed to the same constant light at 21–23 °C under 30–100 μmol photons $m^{-2} s^{-1}$ illumination from 54 watt cool white fluorescent lights (illuminating from the side). The light intensity was adjusted based on the cell density. The light intensity was 30 μmol photons $m^{-2} s^{-1}$ at the beginning of this experiment when the cell density was low. The light intensity was 100 μmol photons $m^{-2} s^{-1}$ at the end of this experiment when the cell density was high. We provided our cultures a sufficient mix by bubbling with CO_2/air to avoid settling and the light limitation.

2.2. Sample collection

Cell density (optical density at 600 nm) and pH were measured daily, and the pH of samples was measured immediately upon sampling. A small subsample (10 mL) was taken from all treatments on day 0, 3, 7, 11, 17. All these samples were fixed with 1% glutaraldehyde (final concentration) for microalgae and bacteria counting, and stored in the dark at 4 °C. Microalgae cells were identified based on morphology according to literature [38]. Microalgae cells were then counted using a Neubauer Hemacytometer which have a center square, subdivided into 25 medium squares, 0.2 × 0.2 mm each in dimension. Counts from the center square are equivalent to counts per 0.1 μL of the sample. The center square was checked at a 100× magnification for even distribution of algae cells after which algae groups (Chlorophyte, diatom, Euglenophyte and dinoflagellate) in all 25 medium squares were counted. Only the top and right edges of each medium square were counted to avoid double counting cells. For day 17 of the treatments, nutrients and high CO_2 or air, samples were diluted to 10% before counting. Identification and counts were done at 100× and 40× magnification respectively using a microscope (Axiplan microscope, Zeiss).

Bacterial samples were stained with SYBR Gold and quantified as described in Chen et al. [39]. Briefly, samples were stained with 2 × SYBR Gold solution (final concentration) for 15 min in the dark after filtered through a 0.2 μm pore-size Anodisc membrane filter (Whatman Inc., Clifton, N.J.). All samples were enumerated under a fluorescent microscope (Axioplan, Zeiss), and visualized with blue-green excitation light (excitation at 480–495 nm).

2.3. Isolation and identification of microalgal strains

Pure culture was isolated from the samples enriched with nutrient and CO_2 by streak plating on petri dishes containing BG11 medium and 1% agar on day 17. After isolation and cultivation of pure cultures, DNA was extracted by DNeasy® PowerWater Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instruction. The purity and concentration of DNA in samples were assessed using NanoDrop ND-1000 (Thermo-Fisher Scientific, Wilmington, DE). Genomic DNA of selected strains was extracted and 18S rRNA gene was amplified using the universal primers for eukaryotes [40]. The PCR product amplified from DNA of each isolate was confirmed as a single band by agarose gel