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Bacterial community changes in an industrial algae production system

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SUMMARY

While microalgae are a promising feedstock for production of fuels and other chemicals, a challenge for the algal bioproducts industry is obtaining consistent, robust algae growth. Algal cultures include complex bacterial communities and can be difficult to manage because specific bacteria can promote or reduce algae growth. To overcome bacterial contamination, algae growers may use closed photobioreactors designed to reduce the number of contaminant organisms. Even with closed systems, bacteria are known to enter and cohabitate, but little is known about these communities. Therefore, the richness, structure, and composition of bacterial communities were characterized in closed photobioreactor cultivations of *Nannochloropsis salina* in F/2 medium at different scales, across nine months spanning late summer – early spring, and during a sequence of serially inoculated cultivations. Using *16S rRNA* sequence data from 275 samples, bacterial communities in small, medium, and large cultures were shown to be significantly different. Larger systems contained richer bacterial communities compared to smaller systems. Relationships between bacterial communities and algae growth were complex. On one hand, blooms of a specific bacterial type were observed in three abnormal, poorly performing replicate cultivations, while on the other, notable changes in the bacterial community structures were observed in a series of serial large-scale batch cultivations that had similar growth rates. Bacteria common to the majority of samples were identified, including a single OTU within the class *Saprospirae* that was found in all samples. This study contributes important information for crop protection in algae systems, and demonstrates the complex ecosystems that need to be understood for consistent, successful industrial algae cultivation. This is the first study to profile bacterial communities during the scale-up process of industrial algae systems.

1. Introduction

Microalgae (herein, “algae”) are photosynthetic unicellular eukaryotes that grow in aquatic or marine environments. For reasons including rapid growth and high lipid content, certain varieties of algae are considered promising biofuels feedstocks [5,31]. Algae may be cultivated on otherwise non-arable land in growth systems that use salt water or wastewater, so production of algae biomass does not necessarily divert land and fresh water from production of traditional agricultural crops [37]. Generally, large-scale industrial growth systems circulate algae, nutrients, and water around open ponds or within closed photobioreactors. Open ponds use a paddle wheel to circulate algae around a constantly exposed raceway. In closed systems, algae cultures are confined in bags or tubes that reduce exposure to the environment. Closed systems have higher capital costs but allow greater control over parameters such as CO_2 and nutrient concentrations while limiting the potential for invasion by unwanted organisms [19,39].

Growers typically desire to cultivate monocultures of algae selected or engineered for traits such as robust growth and accumulation of desired biochemical products (e.g., lipids or other high-value compounds) [37]. Following conventions used with traditional agricultural crops, these high performance algae varieties may be referred to as

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"elite". For production of lipids, several commonly used elite strains are members of *Nannochloropsis*, a genus of marine algae with doubling times on the order of 30 h and lipid contents ranging from 30 to 60% [18,44]. Algae growth parameters are often studied and optimized using laboratory conditions including small-volume cultures, aseptic conditions, and precisely controlled light, temperature and nutrient regimes. Since elite algae have not historically been grown at the large volumes required by the biofuels industry [11], a challenge is translating the productivity of elite strains optimized under highly controlled lab environments to consistent outdoor culture productivity at large scales.

Much like terrestrial crops, algae productivity may be modulated by biotic factors such as weeds, predators, and other microbes. For example, algae with low lipid content that contaminate elite cultures – and compete for resources such as light and nutrients – are considered weeds [13]. Zooplankton grazers prey on small algae [40] such as *Nannochloropsis*. Fungi and bacteria also affect algae productivity [25,40]; however, there is little understanding of the interactions among elite algae and co-resident microbes. The majority of algae pathogens and pests have not been identified, and industry pest management standards are at an early stage of development [14,27].

Bacteria are abundant and dynamic in algae cultures, and bacterial counts commonly reach 1×10^9 cells/mL, outnumbering algae cells 10- to 100-fold [47]. Although bacteria are often considered contaminants that can inhibit algae productivity or terminate algae populations, bacteria-algae interactions have a range of potential outcomes [25,26,32,38]. Algae support bacterial growth by releasing 25% of the total organic carbon fixed by photosynthesis [25,45]. Reciprocally, of hundreds of algae varieties surveyed, over half do not endogenously produce vitamin B12 and therefore require bacteria-produced vitamin B12 for growth [7]. Additionally, specific bacteria may stimulate algae growth through activities including regulation of the amount of available nutrients such as iron, nitrogen, and phosphates [1,12,42], or by releasing phytohormones such as indole-3-acetic acid into the growth environment [8]. In some instances, bacteria reduce algae productivity by competing for these same nutrients [6,21]. In addition to nutrient competition, non-lethal bacterial pathogens may inhibit algae productivity by diverting algal cellular resources from growth to defense. Finally, some bacteria can directly kill algae, causing cultures to collapse [28,46]. Much of this knowledge of algae-bacteria interactions derives from ecological studies of harmful algal blooms in natural environments, with the general aims of identifying bacteria or specific bacterial functions that promote or inhibit such blooms. Of immediate need for the algae bioproducts industry is an understanding of the relationships among elite algae and co-resident bacteria in engineered cultivation systems containing high concentrations of cells and nutrients.

In this study, bacterial communities were monitored during industrial algae production at Solix Biosystems (Fort Collins, CO). At this facility, production involves scale-up from 5-mL algae cultures grown under aseptic conditions to 200-L cultures grown in closed, but not aseptic, photobioreactors. Smaller cultures are used to inoculate larger ones until the 200-L scale is reached. Small cultures of 4 L or less are kept under aseptic laboratory conditions, including sterilized glassware and media, with all handling of open containers occurring in a laminar flow hood. These small cultures are grown under artificial light sources in shaking incubators or on shaking platforms. Medium cultures (20 to 60 L) are grown in flat-panel bioreactors under ambient light in a greenhouse, whereas large cultures (200 L) are grown in closed photobioreactors in an outdoor water basin under natural light. Though medium and large cultivations are grown in closed systems, handling of these cultures involves system components that are not sterile. In addition to opportunities for microbe entry during culture handling, the medium and large closed growth systems are technically more difficult to isolate from microbes in their environment.

It was hypothesized that bacterial communities would differ across growth system scales, across seasonal changes in environmental conditions, and in algae cultivations exhibiting different algae growth rates. To monitor bacterial communities in these *N. salina* cultivation systems, 275 samples were collected from small, medium, and large cultivations over 244 days. From these samples, a region of bacterial *16S rRNA* was amplified and sequenced, and the composition, structure, and richness of bacterial communities associated with *N. salina* were determined. Although different growth systems contained distinct bacterial communities, 16 bacterial OTU were identified in 90% of *N. salina* cultivations, including a single OTU found in all samples. Differences in community composition were observed across *N. salina* growth systems, across the duration of the experiment, and among replicate large-scale cultivations supporting different algae growth rates. Relationships between bacterial community structure and algae growth rates were evaluated.

2. Materials and methods

2.1. Algae growth systems

All samples were collected from cultivations of *Nannochloropsis salina* at a single growth facility operated by Solix Biosystems (Fort Collins, CO). *N. salina* was originally obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota (formerly, Center for Culture of Marine Phytoplankton, CCMP) (Bigelow Laboratory for Ocean Sciences, East Boothbay, ME). All algae cultures were grown in F/2 medium [36]. To scale up the culture volume (Fig. 1A), a single *N. salina* colony was isolated from an F/2 agar plate and grown to high density in 5 mL liquid culture. Cultures were primarily grown in a serial batch mode with a portion of each harvest used to inoculate the subsequent cultivations in the same-volume system, or used to start a new cultivation in larger systems. For this study, culture volumes of 5 mL, 1 L, 2 L, and 4 L are all designated as "small". Sterile technique was used with all small cultures, including growth in sterilized containers and F/2 medium, as well as use of a laminar flow hood during culture handling. Small cultures were maintained on a shaker table rotating at 200 rpm and supplemented under 24-hour artificial light at 50 μE. Cultivations designated as "medium" were grown in variable volume (20–60 L) flat-panel bioreactors aerated with 2% CO_2 at 2.5 vvm (volume gas per volume liquid per minute) in a greenhouse under ambient light. Cultivations designated as "large" were approximately 200 L and grown in enclosed photobioreactors located outdoors in a water basin in which the temperature was maintained between 19 and 26 °C, and pH was maintained at approximately 7.3. System specifics are provided elsewhere [13]. Flow cytometry was used to evaluate the purity of the algal population, and specifically the presence of a *Tetraselmis* sp. that had previously been observed at this site. This analysis revealed that the cultivations contained only low levels of this weedy species: 89.9% of the samples had less than 1% of *Tetraselmis*, 95.3% contained less than 2% of *Tetraselmis*, and 98.9% (3 samples) contained less than 5% of *Tetraselmis* (data not shown).

2.2. Algae cultivation sampling and growth monitoring

A total of 17, 81, and 177 samples were obtained from small, medium and large cultures, respectively. The frequency of sampling varied and not all systems were sampled on every sampling date, but the overall system was sampled at least once per calendar month from July 2011 to March 2012. Whenever a particular system scale was sampled, between 2 and 16 samples were isolated from cultivations within that growth scale. For samples from small cultures, an adjustable pipette was used to transfer 1 mL culture to a microcentrifuge tube in a laminar flow hood. Samples from medium and large systems were drawn using a sterile 10-mL needleless syringe through a non-sterile plastic sample line connected to sample ports at one end of the photobioreactor. To ensure that sample lines and ports were clear of waste