Microbial Community Analysis of Open Ponds for Algal Biodiesel Production

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Abstract. Algae farming in shallow open ponds requires little technology, with low capital and operating expenditures. In relatively uncontrolled ponds there is a high likelihood that microbial contamination will affect algal yield. We are interested in understanding natural contamination as an ecological process to better control the trajectory of microbial community assembly. *Nannochloropsis salina* was grown in small open ponds (100L). Extracted metagenomic DNA was subjected to PCR for amplification of 16S and 18S rRNA genes with the products examined by denaturing gradient gel electrophoresis (DGGE) and excised bands were sequenced. DGGE fingerprints can provide a measure of relatedness between communities that will indicate whether the assembly process is mainly stochastic or deterministic.

Introduction

Recent unexpected variations in oil prices and strong global concerns about climate change have driven scientists to search for alternative energy sources. Terrestrial plants such as rapeseed in Europe and soybean in the US have been used for production of biodiesel [1]. These terrestrial plants require large areas and grow slowly. Algae on the other hand, can produce oil and can be cultured rapidly in smaller areas with minimal effort [2]. Many studies have been done to examine biodiesel production with microalgae at a large industrial scale either in open ponds or in closed photo-bioreactors. Growing algae in an open pond system has its drawbacks, but by keeping the cost of production to a minimum, this may be the best method of algal culture [3]. Uncontrolled open ponds have a high rate of microbial contamination that can affect algal yield [3]. Understanding this natural contamination process gives us better control of microbial community assembly and biodiesel production.

Experiment, Results, Discussion, and Significance

Nannochloropsis salina, a salt-tolerant biodiesel-producing green alga, was grown in cycling batch cultures in small open ponds (100L; 10-cm deep). Time-course samples were monitored through pigment analysis, lipid measurements, and direct microscopic counts. Microbial community analysis was conducted by first extracting metagenomic DNA culture samples and then performing polymerase chain reaction (PCR) of rRNA gene sequences [4]. Touchdown PCR was performed using bacteria-specific forward primer F357 containing a GC clamp and a universal reverse primer R518 [5]. Since our primary inoculum was *Nannochloropsis salina*, an algae-specific primer set (Cyab F 371GC and Cyab R 783mod) were used for specificity [5]. PCR products of similar lengths were separated by melting point characteristics using DGGE, with an acrylamide concentration of 6 or 8% containing a 20% and 70% denaturant (urea and formamide) gradient at 60 ^{O}C for 16 hours. Bands were excised and prepared for sequencing.

Figure 1: Three open-ponds (100L, 10-cm deep) using inflatable kids pool

Open ponds covered with screening were easily established in simplified inexpensive media using bulk ingredients.

Relatively small algal inocula (less than 10%) would grow to carrying capacity in approximately two weeks and could be cycled in batch mode. After three cycles, we found that there was no appreciable colonization by allochthonous algae or cyanobacteria. This could perhaps be due to the brackish growth medium. Microbial contamination was mainly bacterial and showed complex banding patterns in DGGE. Fingerprinting was done in duplicate for verification. Bands were carefully excised, eluted, re-amplified by PCR, and sequenced revealing a diverse consortium of bacteria. Some of the observed taxa included *Belliella, Marinobacter, Pseudomonas,* and *Rhodobacteraceae.*

DNA fingerprints from DGGE can be used to follow microbial populations and generate dendrograms of relatedness between temporal or repetitive samples. Comparing equivalent ponds should allow us to determine whether the microbial community assembly process in open algal ponds is mainly stochastic or mainly deterministic.
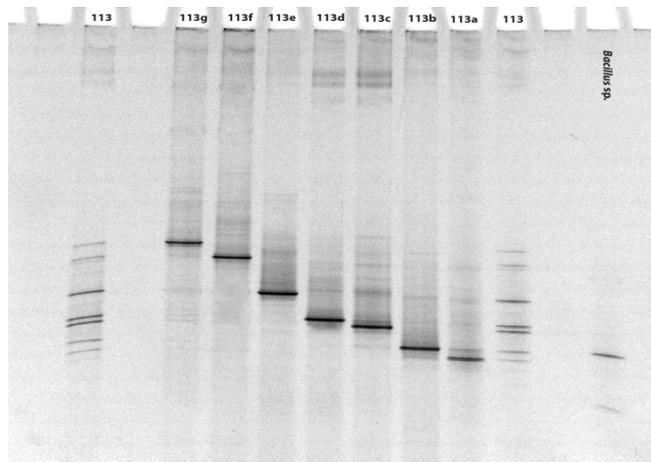

Figure 2: This DGGE gel shows touchdown PCR amplification of amplicons that have been excised and re-run using universal bacterial primer set. The excised bands were sequenced and following organisms have been identified as follows:
113a *Marinobacter* sp
113b *Rhodobacteraceae bacterium*
113d *Alteromonas* sp
113f *Pseudomonas* sp

Conclusions:

This research will provide us with an understanding of variations in community structures of open algal ponds with time. Techniques like DGGE will help us compare the changes in microbial populations in the ponds from the first day of inoculation through a mature culture. Monitoring of wild invasive microbes in the ponds may help to increase yields of the algae producing biodiesel. There may be windows of opportunity where microbial community trajectory can be manipulated to increase yields with minimal inputs.

Acknowledgements:

References:

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