Standardization of Methods for Characterizing the Physiological Profiles of Aquatic Microbial Communities using EcoPlates

Kim Phan
Augustana College, Rock Island Illinois

Madalyn Murillo
Augustana College, Rock Island Illinois

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Abstract

Microbial physiology is often studied by observing pure cultures of isolated organisms grown in a lab setting. This approach is not realistic when trying to understand how a community of bacteria, which includes a variety of bacterial species, is functioning while coexisting in a specific ecosystem. By using Biolog Ecoplates, the metabolic functioning of a microbial community in response to 31 different carbon sources may be assessed in just one plate. Each well in an EcoPlate contains a single common carbon source and a tetrazolium dye that will turn purple in color if any microbe in the sample is able to metabolize that carbon source. The intensity of the purple color in a well will increase over time in proportion to how much metabolic activity occurred. If water samples taken from the same ecosystem sites at different times are used to inoculate EcoPlates, we can determine if microbial community function has changed over time and use these findings, along with other variables like water chemistry, nitrogen uptake, coliform counts, and temperature and weather patterns, to more completely understand how a freshwater ecosystem is functioning. This pilot study was performed to standardize our sampling, data collection, and data analysis methods by using samples from a local aquatic ecosystem (e.g., the Augustana Slough). Baseline data were also collected for two watersheds in Davenport, IA which will continue to be evaluated this summer for signs of urban stream syndrome in conjunction with an ongoing project through the Augustana Upper Mississippi Center.

Introduction

Analysis of environmental samples using Biolog Ecoplates provides a “fingerprint” of carbon source usage by the microbes present in the sample. This community-level physiological profiling (CLPP) is considered an effective way to detect spatial and temporal changes in microbial communities.

Since environmental samples can be from soil, leaf litter, water or other sources, methods vary somewhat among Ecoplate studies. In this study we assessed various approaches to determine the most reliable methods for detecting changes among microbial communities in samples from two local water sources as preparation for continued research this summer. One sample is used to inoculate an entire Ecoplate, so each plate well may end up containing a slightly different subsample of microbes present in the original community (Fig. 1). The Ecoplate was designed to provide triplicate tests of each carbon source to help compensate for this possibility (Fig. 2). Additionally, we wanted to determine if any of the following variables affected our results for a given sample:

- the volume of inoculum in each well
- incubation temperature
- parafilm wrap during incubation (to prevent evaporation)
- length of incubation
- pattern of well inoculation (e.g., end to end, halfway from alternate ends, etc.)

In order to increase the number of repeat trials, we collected our first samples from the closest natural freshwater source, the Augustana Slough. Our later samples were from two sampling sites along Crow Creek: C3 at 2792 Elk Drive, an urban site near the Mississippi River and C9 on the Crow Valley Golf Club property, near Utica Ridge Road, a more rural site near active farmland. The Crow Creek watershed will continue to be studied this coming summer.

Materials and Methods

Taking care to not disrupt sediments, raw water samples were collected from the following sites using sterile 50 mL conical tubes. Samples from Crow Creek were refrigerated during transport back to campus. Then, the EcoPlates were inoculated using a multichannel pipettor and then incubated using combinations of the following variables:

- Source
- Volume per well
- Incubation temperature
- Incubation length
- Incubation pattern
- Parafilm wrap

For the first day of color change (any slight appearance of purple wells) for each plate, the “metabolic fingerprint” was recorded using Biolog MicroStation, a microplate reader. The plates were read daily until the color of the wells stopped changing, which occurred around 5 days (for Crow Creek samples) and 6 days (for Augustana Slough samples).

Methods for Characterizing the Physiological Profiles of Aquatic Microbial Communities using EcoPlates

Kim L. Phan, Madalyn Murillo
Project Advisor: Dara Wegman-Geedey, Ph.D.
Augustana College, Dept. of Biology, Rock Island, IL 61201

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References


Results

Table 1. Overall measure of community similarity is based on number of carbon sources used. If a carbon source was used even once within the three replicates of a plate, then it was considered positive for that plate. The closer the simple matching coefficient is to 1, the more identity (aka similarity) there is between the “fingerprints” of the organisms in the Ecoplates.

In this study, we determined a standard set of methods for inoculating, incubating, and reading our Ecoplates that gave us reliable data, as measured with simple matching coefficients. We will try a second measurement, average well color development (AWCD), to assess carbon source use over time, which may provide additional insights after a major environmental event (e.g., storm or flooding).

This summer we will continue Ecoplate assessment of microbial communities in the Crow Creek watershed and also a second Davenport, IA watershed, Black Hawk Creek/Walnut Creek. These two water systems have been studied for the past five years by the Upper Mississippi Center (UMC). These microbial data will add to the understanding of ecosystem function in these streams and hopefully contribute to development of management plans for streams and rivers exhibiting urban stream syndrome.4

In this study, we also noted that higher temperature caused faster color development in many wells, likely due to enhanced growth rates of microbial community members, so we will track air and water temperatures in addition to other physical factors.

Conclusion/Discussion

In this pilot study, we determined a standard set of methods for inoculating, incubating, and reading our Ecoplates that gave us reliable data, as measured with simple matching coefficients. We will try a second measurement, average well color development (AWCD), to assess carbon source use over time, which may provide additional insights after a major environmental event (e.g., storm or flooding).

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