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

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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.[1]

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 water 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.

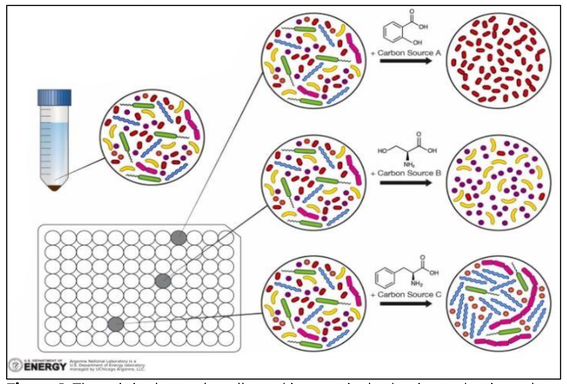

Figure 1. The original sample collected in a conical tube is used to inoculate all wells in a single Ecoplate. This can lead to subsamples of microbes in each well. Image source: April 2018 Webinar by Argonne National Laboratories for BioLOG.

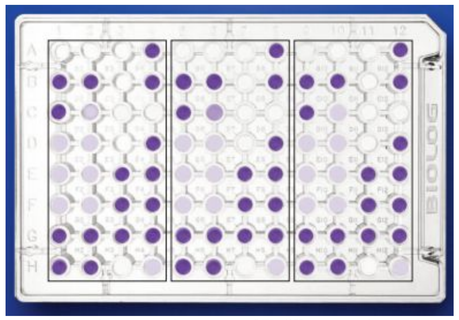

Figure 2. Each Ecoplate tests the usage of 31 different carbon sources in triplicate to help compensate for variation among microbes pipetted into different wells from the same sample. Purple coloration indicates use of the carbon source in that well. Image source: Biolog product site.[2]

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	Inoculation pattern	Parafilm wrap
Augustana Slough (Rock Island, IL)		100 µL	37°C incubator	Shortest: 46 hours	Rows 1-12	Yes
Upper	Lower			Longest: 144 hours		
Crow Creek Watershed (Davenport, IA)		150 µL	Room temperature	Shortest: 72 hours	Rows 1-5 then rows 12-6	No
C3	C9			Longest: 142 hours		

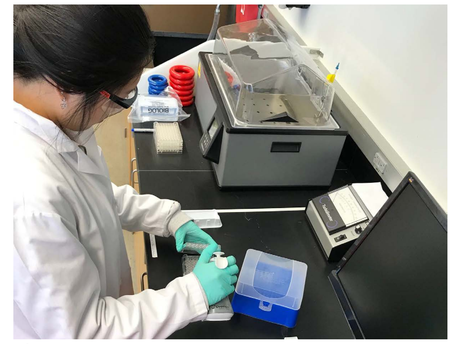

Figure 3. Kim demonstrating her completely on fleek aseptic technique using the Ovation multichannel pipettor to inoculate the Ecoplate

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).

Results

Observations with regard to different variables:

- Sample volume of 100 µL per well is preferable: the 150uL volume caused overflow into neighboring wells. This may have been due to increased fermentation causing bacteria, indicator dye, carbon sources or all three to overflow. (Data not shown)
- Room temperature incubation is preferable: 37℃ incubator caused noticeable evaporation of the Ecoplates by day 3; room temperature is also closer to that of natural water sources; room temperature delayed initial color change by almost 24 hours as compared to 37°C incubator. (Data not shown)
- Absence of parafilm is preferable: using parafilm only slightly reduced evaporation in corner wells; parafilm wrap may have caused increased condensation inside Ecoplate lids leading to well overflow. (Table 1)
- Length of incubation: all plates, regardless of source, volume, temperature, and parafilm, reached maximum values within 5 days of incubation. (Data not shown)
- Pattern of inoculation: carbon use patterns did not differ within replicates in a plate regardless of which inoculation pattern we used. (Data not shown)

Measures of community similarity		
Sample collection location	Simple matching coefficient (S_{SM})	
	First reading	Last reading
C3: parafilm vs no parafilm	0.731	0.914
C9: parafilm vs no parafilm	0.957	0.957
C3 vs C9	0.645	0.924
Upper vs Lower Slough	0.914	0.978
Upper slough: parafilm vs no parafilm	0.978	1.0
Lower slough: parafilm vs no parafilm	0.946	0.946

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.[2] The closer the simple matching coefficient is to 1.0, the more identity (aka similarity) there is between the "fingerprints" of the organisms in the Ecoplates.

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)[3], 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 some bacteria. Summer temperatures may affect growth rates of microbial community members, so we will track air and water temperatures in addition to other physical factors.

References

1. Microbial community analysis with EcoPlates [Internet]. Hayward (CA): Biolog; [cited 2019 Mar 6]. Available from: https://biolog.com/products-portfolio-overview/microbial-community-analysis-with-ecoplates/
2. Mulcahy MP & Edenborn HM. Environmental microbial ecology. 2006. Association for Biology Laboratory Education 28:28-51.
3. Choi K & Dobbs F. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. 1999. J Microbiological Methods (36) 203-213.
4. Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, Morgan RP. 2005. The urban stream syndrome: current knowledge and the search for a cure. J N Am Benthol Soc 24(3):706-23

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