Laboratory for Microbial Ecology
Department of Earth, Ecological and Environmental Sciences
University of Toledo

Community level physiological profiling (CLPP)

Background information

Community level physiological profiling (CLPP) owes its beginnings to the development of the BIOLOG system in the late 1980s. The system was developed to identify bacteria of clinical importance by assessing each bacterium's usage of any of 95 different carbon sources in one microtiter plate.

BIOLOG Microbial Community Analysis
EcoPlate™

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

Distribution of carbon sources in the BIOLOG EcoPlate.

Originally, the principal users of the BIOLOG system were pharmaceutical, biotech, cosmetics, and medical device companies, as well as labs testing for diseases of humans, animals and plants, labs performing environmental monitoring, and companies or organizations involved in production or testing of food and drink. In the 1990s, environmental researchers realized that useful physiological data concerning "whole" communities could be generated by inoculating mixed microbial assemblages in to the BIOLOG plates and noting the response of the mixed community to the carbon sources.

Since prokaryotic communities can be considered functional units characterized by the sum of the metabolic properties of individual bacteria, CLPP represents a sensitive and rapid method for assessing the potential metabolic diversity of microbial communities. Furthermore, the ecological relevance of certain contaminants such as hydrocarbons, pesticides and metals to soil bacterial

communities can also be assessed. CLPP involves inoculating mixed microbial communities into BIOLOG-GN (for Gram negative bacteria), -GP (Gram positive), or –ECO (environmental) microplates that contain 95 (GN and GP plates) or 31 (ECO plates) single carbon sources in addition to a tetrazolium dye. The utilization of any carbon source by the microbial community results in the respiration-dependent reduction of the dye and purple color formation that can be quantified and monitored over time.

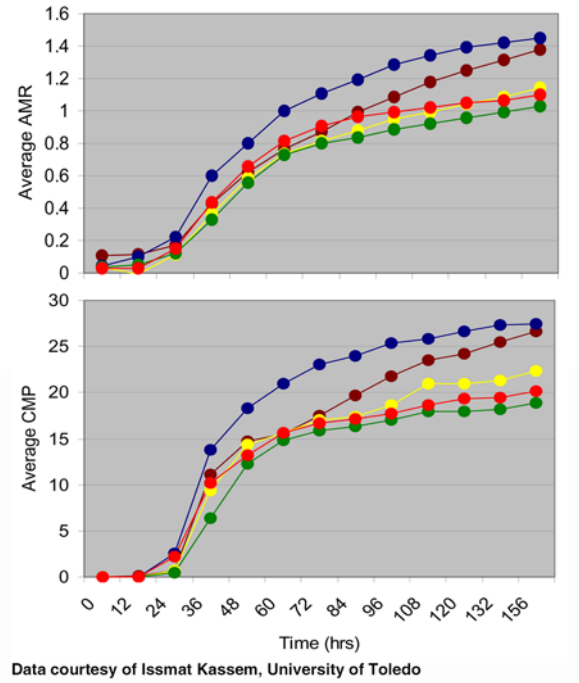
Example of a BIOLOG plate inoculated with a dilution of soil and incubated for 48 hours. The purple wells contain carbon sources that were used by the microbial community. The intensity of the purple coloration indicates the degree of carbon source usage by the community.

Following an empirically determined incubation period, color patterns in the 96-well matrix and intensity (O.D.) of color formation can be used to determine two parameters to describe the microbial community:

(i) The **average metabolic response** (AMR) describes the average respiration of the C-sources by the microbial community and provides a single metric by which communities can be compared. The AMR is calculated as the average of the mean difference between the O.D. of the C-source-containing wells and the control well.

(ii) The **community metabolic diversity** (CMD) is represented by the number of substrates utilized by the microbial community and is analogous to community functional richness. CMD is calculated by summing the number of positive responses (purple-colored wells) observed following incubation.

Each of these parameters can be plotted over incubation time to provide a metabolic kinetic of the culturable microbial community (figure below).



CLPP profiles comparing the AMR and CMD of five classes of lake bottom sediments. Sediment classes: freshly dredged (red line), sampled from open lake-disposal site (green line), surface stored for 20 years (blue line), 5 years (brown line) and less than one year (yellow line).

Materials

10 mM phosphate buffer (sterile, pH 7.0)
200 ml milk dilution bottle
Five - 50 ml Falcon tubes
200 and 1000 µl pipettes with tips
Multichannel pipette to deliver 100 µl with tips
Three BIOLOG GN, GP, or one EcoPlate
Sterile plastic multichannel reservoir

- Wear gloves throughout the entire protocol.
- Do not cross-contaminate your samples OR THE SOLUTIONS. Be aware of your pipette tip.
- Work clean, either on fresh blue bench paper, in the hood, or on a freshly ethanol treated bench top.
- Do not use a vortex at any point in this protocol unless specified.

The protocol in brief

Using a dilution of your sample that will generate approximately 10^5 cfu per 100 ml, you will inoculate a BIOLOG microtiter plate. The plate will be incubated for one week, during which five measurements of color formation in the wells of the plate will be taken. The data generated by the measurements will be used to construct curves describing the AMR and CMD over time.

A. Setting up the dilution series

The dilution series for CLPP is performed exactly the same as for dilution plating. In fact, refer to your results of the dilution plating experiment to determine the proper dilution with which to inoculate your BIOLOG plate.

1. Based on your dilution plate results, determine the dilution of your sample that will result in approximately 10^5 cfu in 100 μl of sample.

    1a. This is an important step, as it is necessary to inoculate all BIOLOG plates with the same number of bacterial per well to facilitate sample-to-sample comparisons.

    Hint: this dilution will likely be three dilutions lower than the one that resulted in a countable plate in your dilution plating experiments. For example, assume the 10^{-6} dilution resulted in 100 cfu on the plate. Since you plated 100 μl of each dilution, this implies that 100 μl of that dilution contains 100 cfu. It follows that to achieve 10^5 cfu, you must pick the 10^{-3} dilution to use for the CLPP experiment.

2. Perform a serial dilution in sterile 15 ml falcon tubes as in the dilution plating protocol until you reach the desired dilution necessary to achieve 10^5 cfu 100 μl. You will need approximately 10 ml of inoculum to fill the BIOLOG wells, so be sure to adjust your dilutions so that you prepare approximately 12 ml of inoculum to ensure enough inoculum is available (dilute 1.2 ml in 10.8 ml at each dilution step).

3. Dispense the contents of the falcon tube that contains the proper dilution into a sterile multichannel pipette reservoir.

4. Remove the cover of a BIOLOG microtitre plate. With a multichannel pipette, transfer 100 μl of the inoculum from the reservoir into each well of the plate.

5. Replace the cover of the plate and label the edge with your name, the date and sample type.

6. Place the plate into a plastic container containing moist paper towels and incubate at 25^{o} C.

B. Measuring the OD of the microtiter plate wells

7. The activity in the plates is measured using a microplate reader set for absorbance at 590 nm. You will be shown how to take the first measurement in class (t = 0) and how the plate reader computer software works.

8. Ideally the formation of purple coloration (indicating microbial metabolism of the 95 carbon sources) in each of the wells is performed every 12-24 hours for one week. In addition to the t=0 reading, we will measure them four times for you (t = 1d, 2d, 3d, and 6d).

9. Once all measurements are taken, the files will be converted to excel spreadsheets and emailed to you. You will be responsible for calculating and plotting two parameters, the **Average Metabolic Response (AMR)** and **Community Metabolic Diversity (CMD)**, described below.

C. Data anlaysis

10. Calculate the **average metabolic response** (AMR) and the **community metabolic diversity** (CMD) of your sample.

    The **AMR** is calculated by averaging the mean difference between the O.D. of the C-source-containing wells and the control well.

$$\text{AMR} = \frac{\sum(O.D.well - O.D.neg)}{95}, \text{ where}$$

    (*O.D. well – O.D. neg*) is the optical density of each carbon source–containing well minus the O.D. of the negative control well.

    The **CMD** is calculated by summing the number of positive responses (purple-colored wells) observed following incubation. A threshold O.D. must be established above which the purple coloration indicates a usage of the carbon source by the microbial community. This threshold is commonly set at O.D. = 0.25.

    These values should be calculated for each measurement date and then graphed as a function of incubation time.

Further reading

Garland, J.L. 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol. Ecol. 24:289-300.

Konopka, A. L. Oliver, and R.F. Turco. 1998. The use of carbon substrate utilization patterns in environmental and ecological microbiology. Microb. Ecol. 35:103-115.

Lehman, M.R. F.S. Colwell, and J.L. Garland. 1997. Physiological profiling of indigenous aquatic microbial communities to determine toxic effects of metals. Environ. Toxicol. Chem. 16:2232-2241.

Preston-Mafham, J., L. Boddy, and P.F. Randerson. 2002. Analysis of microbial community functional diversity using sole-carbon source utilization profiles – a critique. FEMS Microbiol. Ecol. 42:1-14.