Isolation and Characterization of Diverse Microorganisms Involved in Carbon Recycling

Jasmine Amaya[1 2], Brandon Enalls[3], Mariam Alsaid[3 4], Romy Chakraborty[3],
[1]Diablo Valley College, [2]2022 Transfer-to-Excellence Research Experiences for Undergraduates Program (TTE REU Program),
[3]Department of Ecology, Lawrence Berkeley National Lab;
[4]College of Natural Resources, UC Berkeley

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Abstract

In previous works, we were able to culture a wider variety of microbes using these naturally occurring carbon sources. We expand upon this work by continuing to isolate and characterize microbes that can use microbial necromass as a carbon and energy source. To accomplish this, we are enriching the microbes in solid media using bacterial cell lysates that will simulate necromass found in nature. We can then extract and sequence the DNA from these isolates, allowing us to give them taxonomic assignments. The collection of isolated microbes are likely involved in recycling biological material in their native environments, highlighting their contribution to the carbon cycle.

Background

- Microbes are among the most abundant life forms found on the planet [1].
- 70% remain uncultured, thus, their physiologies and ecological impacts remain largely mysterious [2].
- Culturing microbes in a laboratory setting is vital to understand the their metabolism and how they obtain energy from the environment.
- Chakraborty group developed successful cultivation techniques to culture diverse subsurface microorganisms and complex carbon sources that are effective with encouraging the growth of diverse bacteria [3].
- Building upon prior research, this research project continues isolating and characterizing microbes using microbial necromass as a carbon source from sediment samples.
- Results help us identify the microbes that may be vital to carbon recycling in natural environments.

Methods

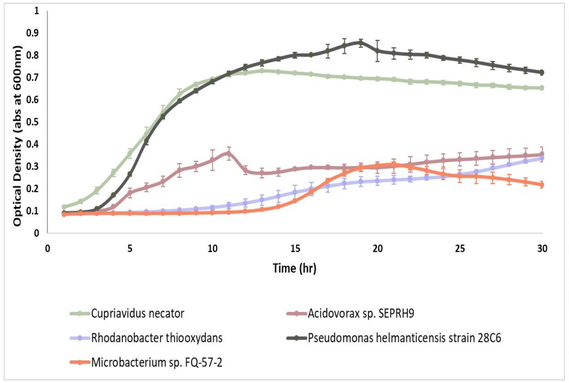

Figure 1. Growth curves for five strains used to make necromass for this project. These strains were chosen in regard to their abundance from our field site and were lysed at mid-exponential growth stage. Error bars represent one standard deviation of experimental triplicates.

Strains	Carbon Concentrations (ppm)
Rhodanobacter thiooxydans	1032.0 ppm
Acidovorax sp. SEPRH9	327.8 ppm
Microbacterium sp. FQ-57-2	448.4 ppm
Cupriavidus necator	385.4 ppm
Pseudomonas helmanticensis strain 28C6.	239.3 ppm

Table 1. Strains chosen to make necromass for this project. Carbon concentrations for the lysates of these strains calculated using a TOC analyzer.

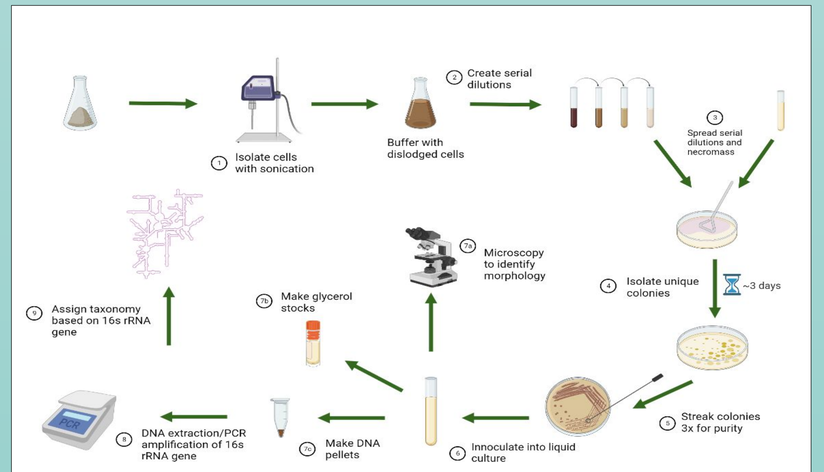

Figure 2. Experimental design in order to isolate and characterize microbes that consume necromass. We used R2A, 1/10 R2A and RCH2 as media throughout the process. Figure was created using BioRender.com.

Results

- On the third transfer streak, the colony's form, consistency, and color were documented. The most commonly seen colony had a circular form, moist consistency, and white or clear color.
- R2A media produces more diversity in microbes that consume necromass compared to 1/10 R2A and RCH2.
- Detected most often were the *Bacillus sp.* strain HY4 on 1/10 R2A and the *Bacillus velezensis* strain ZT-2 on 1/10 R2A and RCH2 media.

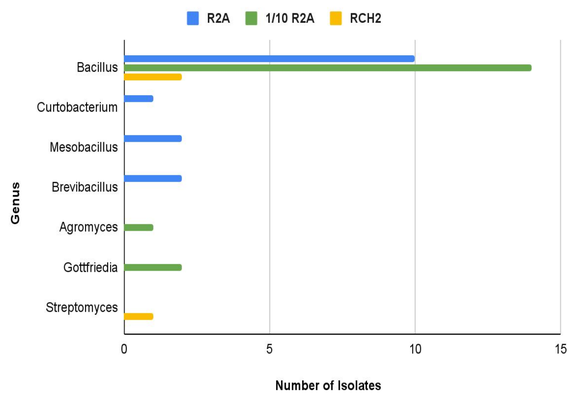

Figure 3. Taxonomic data results separated by type of genera and the number of microbes isolated. The yellow color represents microbes grown on RCH2, the blue color represents microbes grown on R2A and the green represents microbes grown on 1/10 R2A.

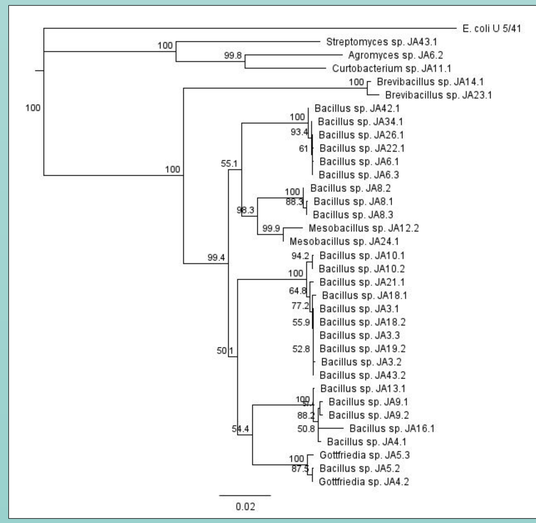

Figure 4. Phylogenetic tree of all the isolates constructed using the 16s rRNA gene. E. Coli strain added as an outlier for comparison.The length of the branches represent how closely related the isolates are to each other. The bootstrap values indicates the reproducibility of each branch.

Conclusions

- Out of thirty-six isolates, seven different genera were found from our one sediment sample.
- Twenty-six of the isolates were members of the genus *Bacillus*.
- We isolated two *Bacillus* strains and a *Streptomyces bungoensis* strain from RCH2 media using only necromass as a carbon source. This indicates that these strains likely contribute to carbon recycling.
- To further our research, we can attempt to grow all of our isolates on RCH2 proving that the microbes isolated are only consuming the necromass instead of nutrients from the more nutrient rich media.

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Contact Information

Jasmine Amaya
jasninaamaya@gmail.com

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