In Search of Plastic Degraders using a Winogradsky Column

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Introduction

From the Great Pacific Garbage Patch to micro and nanoplastic particles in our farmland and crops, plastic pollution continues to accumulate. Plastic is nearly impossible to remove from the environment due to its resistance to biodegradation. In recent years, however, some bacteria have demonstrated an uncanny ability to metabolize plastics. To test this, we exposed plastic strips to potential plastic degraders for 15 months using Winogradsky columns. We isolated bacteria from the plastic, sequenced it, and weighed the plastic to verify if plastic degradation had occurred.

Methods

Collection of Samples and Culturing of Winogradsky Columns

Winogradsky columns are tools designed to separate bacterial specimens by their ability to process oxygen. They contain air, water, and collected soil, and over time, the bacteria in the soil samples move to the layer of the column where they can best grow based on the oxygen conditions. Our Winogradsky columns used soil collected from Long Lane Farm in Middletown, CT.

Figure 1: Long Lane Farm

We modified our Winogradsky columns by placing plastic strips (four total) between four different layers: Putative aerobic (maximum oxygen, within the aqueous layer), putative microaerophilic (buried in shallow soil), putative facultative anaerobic (buried in mid-density soil), and putative obligate anaerobic (as deep as possible in the soil). We cultured our Winogradsky columns from March 7, 2020 to June 2021 in the Cohan laboratory space, in a temperature range of 13ºC to 21ºC. Figures 2 and 3 below illustrate our setup.

Initial Culture on Agar Medium

After culturing the Winogradsky columns for over a year, we removed the plastic strips from the columns and vortexed them in water to transfer the bacteria from the plastic to the liquid. Seven test tubes with 1.8 mL sterile water were prepared for each plastic strip to conduct serial dilutions. 0.2mL of the sample liquid was pipet into the first test tube and 0.2mL of each previous dilution was mixed into the following.

Figure 2: Winogradsky column used In experiment

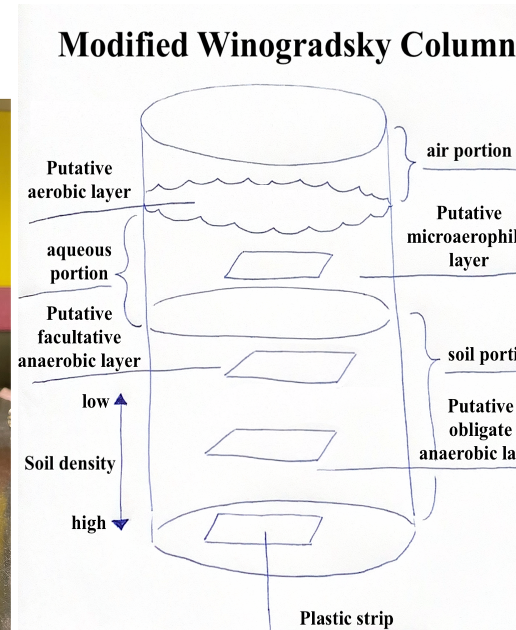

Figure 3: Diagram of modified Winogradsky column

50 µL of dilutions 4 – 7 were plated onto Luria Broth (LB) agar, Bushnell Haas agar, Tryptic Soy Broth (TSB) agar, Nutrient agar, Thioglycollate Broth with Resazurin (TBR) agar, dCGY (difco, Casamino, Glycerol and Yeast extract) agar, and Brewer Anaerobic agar. We plated samples from the putative aerobic layer on regular petri dishes. Samples from the putative microaerophilic layer were plated on petri dishes and placed inside a plastic bag to reduce oxygen exposure. We cultured the solution from the putative facultative anaerobic layer on OxyDish™ petri plates containing the oxyrase enzyme, which can be sealed to prevent oxygen from entering. Finally, we grew the solution from the putative obligate anaerobic layer on OxyDish™ petri plates containing the oxyrase enzyme and placed them inside a GasPak Anaerobic System (shown below in Figures 4 and 5). This system uses a polycarbonate jar, a lid with a gasket to prevent airflow, an indicator strip to demonstrate the presence of oxygen, a disposable gas-generating pouch, and a catalyst to create a completely anaerobic environment.

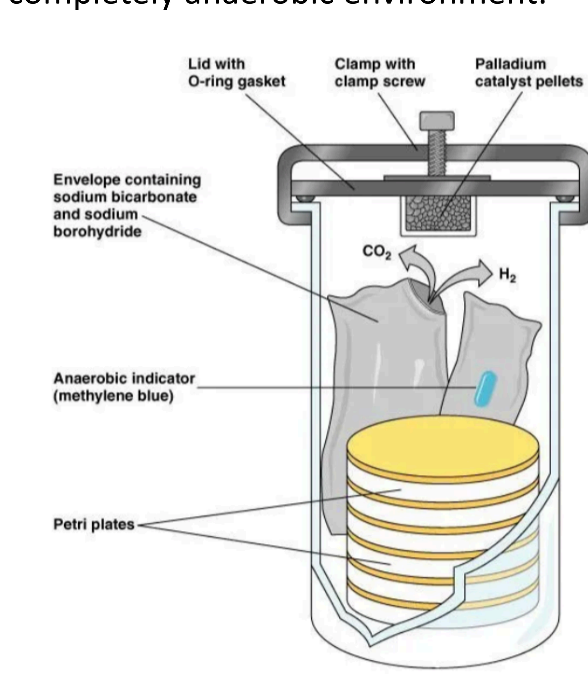

Figure 4 (left): Diagram of GasPak Anaerobic System[1]

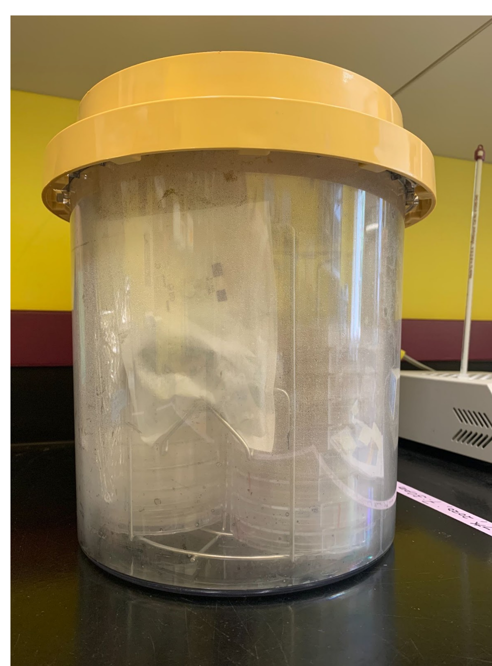

Figure 5 (right): Experimental Setup of GasPak Anaerobic System

We cultured half of our plates at room temperature and half in the incubator at 37ºC, with the exception of the obligate anaerobic bacteria, which were only cultured at room temperature.

Isolation of Samples

We cultured the bacteria on agar medium for an average of 7-14 days. Then we removed individual colonies from petri dishes using a sterile loop and grew them in a liquid culture of LB, TBR, or TSB for 17-18 hours. Figures 6 and 7 illustrate this process.

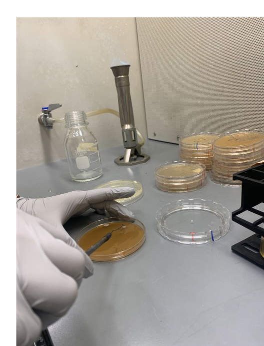

Figure 6 (left)

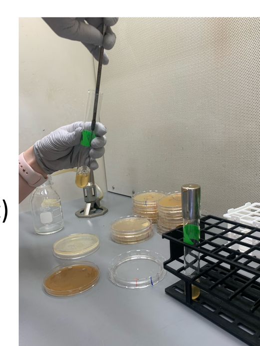

Figure 7 (right)

We transferred the liquid culture into Eppendorf tubes to be sequenced. These pellets were sequenced at the QuintaraBio facility. The 16S rRNA amplification was conducted using the universal bacterial primer 8F and 1492R. Finally, we performed serial dilutions for each sample and streaked the fifth dilution on the appropriate agar plates using glass beads or sterile spreaders.

Once we received the reads of our isolates, we used the Ribosomal Database Project (RDP) site from Michigan State University to classify our isolates at the genus level. We cross-checked against query sequences in the Basic Local Alignment Search Tool (BLAST) site to determine the species. We verified the most recent name for each species using the NCBI Taxonomy Browser.

Findings

We compared the masses of the plastic strips before going into the Winogradsky column to their masses after over a year of exposure to potential plastic degraders.

	S1	S2	S3	S4
Initial Mass (g)	0.0172	0.028	0.028	0.020
Final Mass (g)	0.0120	0.0256	0.0275	0.0142

The mass of the plastic strip in each layer decreased after 16 months, which demonstrates that plastic biodegradation did occur.

Out of our 146 isolates, 126 yielded functional sequences. 41.3% (52 isolates) needed naming. Of those that need naming, 24 (19.0% of the functional sequences) have been discovered, but not classified, and 28 (22.2% of the functional sequences) have not been discovered. 55.6% (70 isolates) have already been classified. 3.17% (4 isolates) had query sequences too short to determine a species. Below are a few interesting strains we have identified.

Strains identified from Putative Aerobic Layer

Domain	Phylum	Class	Order	Family	Genus	Species	Footnotes
Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Geomicrobium	Needs naming	Species already discovered, not classified

This unnamed species was originally discovered in the intestine of a sea cucumber in Nagasaki, Japan[2].

Strains identified from Putative Microaerophilic Layer

Domain	Phylum	Class	Order	Family	Genus	Species	Footnotes
Bacteria	Actino-bacteria	Actino-bacteria	Myco-bacteriales	Nocardiaceae	Aldersonia	Needs naming	Species not yet discovered

The BLAST results for this species were less than 98.7% similar, indicating that this species has not yet been discovered.

Strains identified from Putative Facultative Anaerobic Layer

Domain	Phylum	Class	Order	Family	Genus	Species	Footnotes
Archaea	Crenarchaeota	Thermo-protei	Desulfuro-coccales	Desulfuro-coccaceae	Thermosphaera	Needs naming	Query sequence too short

Although the query sequence was not long enough to determine a species, our identification determined this isolate to be a single-celled organism in the Archaea domain.

Strains identified from Putative Obligate Anaerobic Layer

Domain	Phylum	Class	Order	Family	Genus	Species	Footnotes
Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Needs naming	Species already discovered, not classified

An example of a strain that has already been discovered, but has not been classified.

Conclusions

Many questions remain unanswered about prokaryotic plastic degradation. Over the course of this summer, we successfully isolated and identified bacteria from one of four Winogradsky columns. Next steps in this project will include isolating and identifying strains from the three remaining Winogradsky columns. In addition, more research must be conducted to verify whether or not these bacteria can efficiently metabolize plastic. Thus, future experiments will test the strains we have archived to confirm that the isolates degrade plastics. We will then conduct a chemical analysis of the broths in which this experiment occurs to identify plastic byproducts using Gas Chromatography-Mass Spectrometry (GCMS). Considering that the goal of this project is to pave the way for a product that would remove plastic, micro, and nanoplastics from the environment, a study to determine and optimize the enzymes responsible for plastic degradation in microbes is likely in order. Of the strains we have currently isolated, a significant portion have not yet been classified. We intend to name them, describe them, and add them to the existing bacterial taxonomy. Describing these bacteria will involve full genomic sequencing. A genome sequence can also illustrate subsystems within the organism, as well as accounting for the metabolic capabilities of the bacteria. To study these strains more closely, we must understand their optimal growth conditions. According to Professor Frederick M. Cohan, a fully sequenced and annotated genome would provide enough information about the gene content of a strain to make inferences about its physiology[3]. This suggests that future research about strains that have already been sequenced could be entirely virtual—and needs to be, given the overwhelming number of species that have been discovered in recent years.

Acknowledgements

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