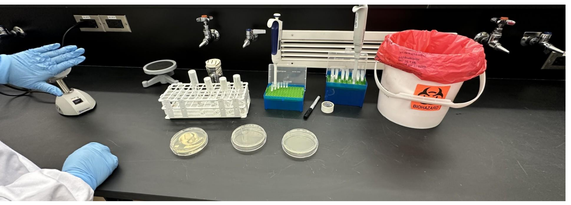
Plastics From The Environment Are Colonized by Bacteria With Possible Plastic Degrading Abilities

Chelsea Day, Suzannah Erickson, Elzena Jenkins
Dr. Barcin Acar
Microbiology 260 Winter 2024

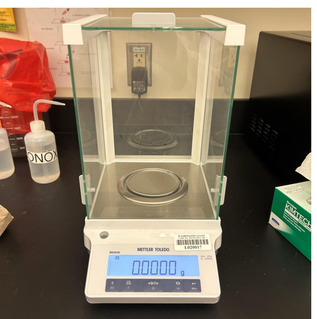
ABSTRACT

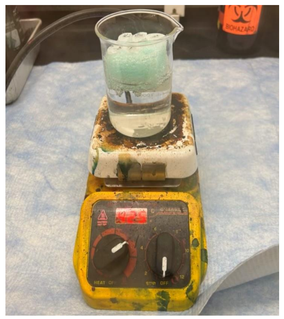
We as a microbiology class in Winter 2024 participated in a CURE (Course with Undergraduate Research Experience). In this class, we wanted to identify the bacteria that are capable of living in no carbon environment, and the only carbon source that is provided would be the plastics. For our experiment, we used biodegradable plastics, PET (Polyethylene Terephthalate), HDPE (High-Density Polyethylene), and LDPE (Low-Density Polyethylene) plastics to collect data. We conducted several bioinformatic and biochemical tests to identify our unknown bacterial characteristics. At the end of this experiment, we identified several unknown bacteria that can survive the non-carbon media with the addition of the plastics. We concluded that Pseudomonas sp. is ubiquitous in these environments and may have plastic degrading properties. More research is needed to confirm our findings.

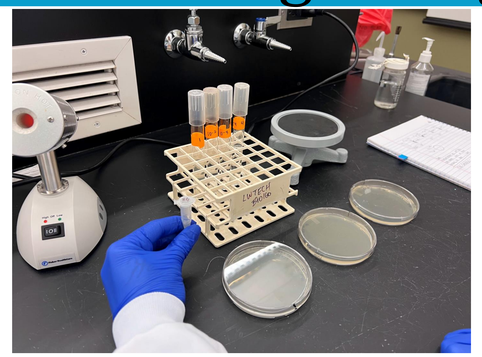


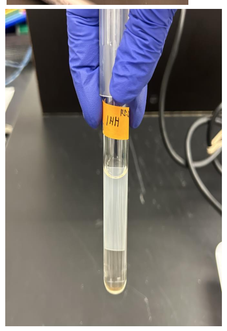
OBJECTIVES

Bacteria was inoculated in carbon-free media, utilizing either single colony or multiple colony cultures alongside various plastics. High Density Polyethylene (#2), Low Density Polyethylene (#4), and Compostable plastic were the three types of plastics examined. Toothpicks served as the control condition, providing carbon. For each condition, duplicate tubes were prepared. The single colony bacteria were then identified using standard microbiology lab techniques.









IDENTIFIED ORGANISMS

Pseudomonas idahonensis
Enterococcus hirae
Pseudomonas palmensis
Pseudomonas protogens
Pseudomonas shahriarae
Pseudomonas qingdaonesis

IDENTIFICATION TESTING

T-Streak – Isolating Single Colony

Gram Stain

Endospore Staining

KOH Testing

Biochemical Testing

Antibiotic Testing

Indole Testing

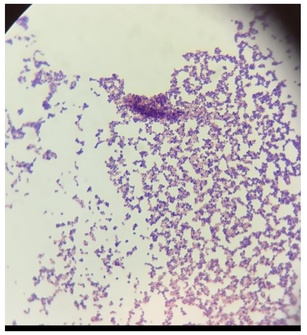
Oxidase Testing

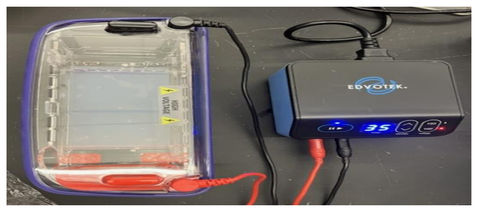
Catalase Testing

Selective/Differential Testing

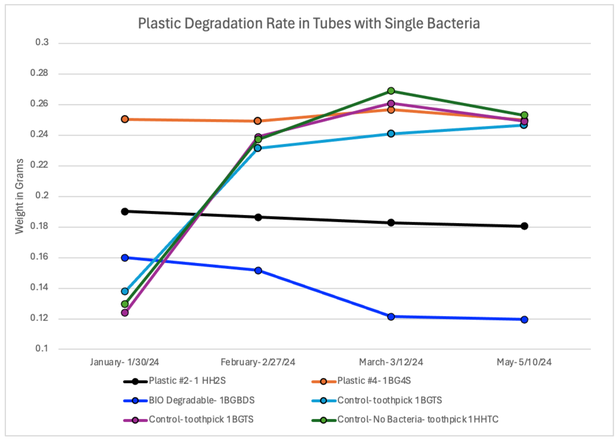
PCR

Electrophoresis

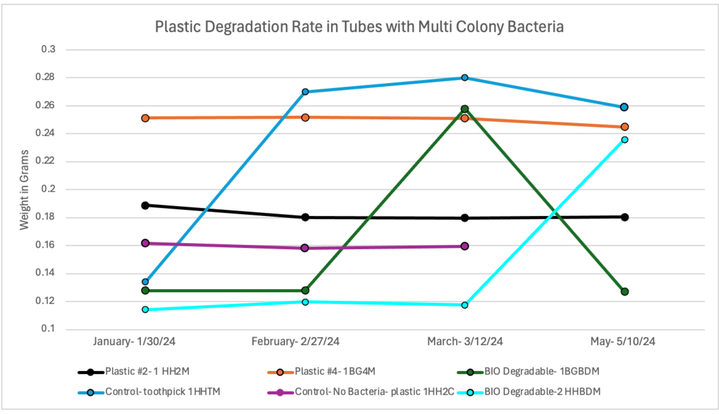




WEIGHING



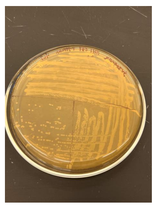
RESULTS

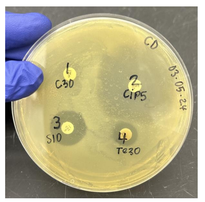


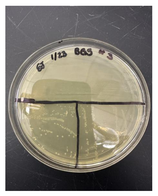
CONCLUSION

- We had fun and enjoyed the process of the Plastic and Environmental Samples being set up to test the hypothesis that if there is success in isolating a plastic-degrading bacteria from an unknown soil sample, then replication of the bacteria and its properties is possible.
- There is a chance that contamination happened during the weighing/washing process. It wasn't easy to pinpoint when or where the contamination was coming from. To avoid this, toothpicks and plastics could be soaked in alcohol for 24 hours before first weight to maximize decontamination throughout. The paper towels used for drying could have been contained. Instead of using them, we could let samples airdry in a sterile environment. The sterile technique surrounding this part of the experiment could have been better. There could be cross-contamination from the weigh scales, gloves, and the liquids used to rinse toothpicks.
- Soil sample HH1 was identified to be *Pseudomonas qingdaonesis. Pseudomonas* was able to start breaking down the HHTM2 toothpick but did not seem to break down the other HHTM, HHTCS, and HHTC toothpicks.
- *Pseudomonas qingdaonesis* is a gram-negative bacterium that cannot ferment xylose, lactose, or produce hydrogen sulfide. It is motile, can ferment glucose, and produce hydrogen sulfide as well as catalase. It is unable to break down tryptophan and urea and cannot produce cytochrome C oxidase. *Pseudomonas* is unable to use citrate as a carbon source, and ammonium dihydrogen phosphate as a nitrogen source. *Pseudomonas* is resistant to Chloramphenicol, sensitive to Streptomycin and Ciprofloxacin, and intermediate to Tetracycline.
- *Pseudomonas protegens* CHA0 is aerobic gram-negative rods, found in various environments including soil, water, and plants. They are non-spore forming, oxidative, and motile. They are mesophilic and can utilize citrate as a source of energy. *P. protegens have* extremely versatile biocontrol properties and are utilized in commercial agriculture as a biological control agent to protect crops from diseases. *P. protegens* was sensitive to Streptomycin and Ciprofloxacin and resistant to Chloramphenicol and Tetracycline. Though with Chloramphenicol and Ciprofloxacin, the zone of inhibition doesn't appear clear compared to Streptomycin and Tetracycline. It displayed some growth making it appear cloudy. This could be the result of the development of antibiotic resistance or from an error related to not correctly plating and spreading the bacteria uniformly.
- The effects of *Pseudomonas protegens* on HDPE plastic display varying data. Test tube one can be seen consistently going down in weight. In contrast, test tube two shows the weight going down for the first two weights and rising for the final weight. This increase could indicate that the bacteria was starting to produce a biofilm. This discrepancy could be due to errors made in weighing or errors within the scale since the control was seen consistently rising. The final control could not be weighed due to mold contamination.

Further testing is needed to determine the full effects of the now-known organisms against HDPE and LDPE plastics, compostable plastics, and carbon-based sterile toothpicks. Science is well, you know; messy!







REFERENCES

(Ruthi et al,. 2023)

(Iglewski,1996)

(Bourafa et al,.2015)

(Aryal, 2022)

(Aryal, 2023)

ACKNOWLEDGMENTS: Winter 24 Microbiology Students, Sara Erickson, Val Koklic

Lake Washington Institute of Technology™
Practical. Purposeful. Promising.