Characterization of Plastic Degrading Bacteria from Environmental Samples by Genetic and Biochemical Analyses

National Aeronautics and Space Administration

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Abstract

Plastic is the major waste-product during NASA space missions. Recycling this waste-stream to produce other beneficial materials would decrease upmass. There is evidence that suggests some prokaryotes can metabolize synthetic plastics such as polyethylene and polystyrene. Identification, characterization, and engineering of these bacteria, and their eventual incorporation as life support systems would enable space flight beyond lower earth orbit. We will utilize molecular techniques to identify and isolate the most productive bacteria. Environmental samples obtained from locations known to be rich in plastic will be cultured in a laboratory defined-media supplemented with plastic as the sole carbon source. Cultures will be monitored for growth over time. Ribosomal DNA will be amplified from cultures that exhibit growth using Polymerase Chain Reaction and subsequently sequenced to determine the identity of the bacteria. If more specific identification is needed, morphological and physiological profiles of the bacteria will also be conducted by microscopy and biochemical tests. Our goal is to find a bacterial strain that can break down plastics efficiently. The implication for this project would not only benefit space exploration, but it will also make a major impact towards sustainability development on Earth.

Introduction

-Identify the prokaryotes, bacteria or fungi, that can break down polyethylene and polystyrene most efficiently.

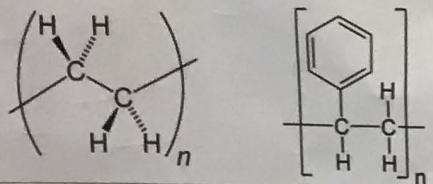
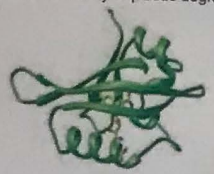


Fig. 1 – Chemical structures of Polyethylene and Polystyrene.

-Transform these bacteria with radial oxygen molecules generating proteins to increase the efficiency of plastic degradation and utilization.



MiniSOG

Fig. 2 – 3D protein structure of the mini-Singlet Oxygen Generator, a light-activated protein.

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Results

-Test tubes filled with environmental samples and plastics were detected to have bacterial proliferation. The ribosomal rDNAs of these strains were isolated and sequenced.

Fig. 3 – Bacteria were retrieved from test tubes, plated on LB agar plates, and incubated at 37°C.

-Investigation of the 16S rRNA genes from unknown bacteria unveiled their close lineages to the genus Ralstonia of the phylum Proteobacteria.

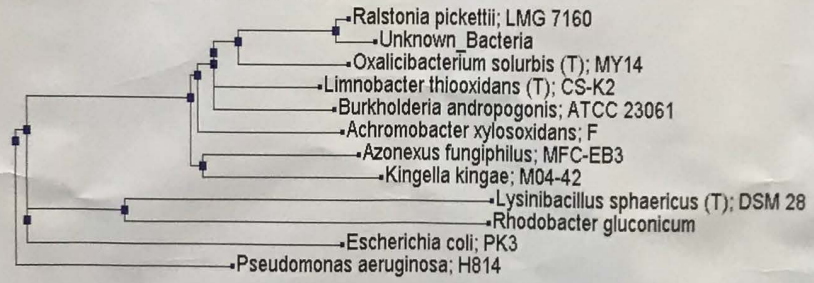


Fig. 4 – Phylogenetic tree of sequenced 16S rDNA from bacteria after examination with computational techniques: Basic Local Alignment Search Tool (BLAST) and Multiple Sequence Alignment.

-Expression of miniSOG proteins was higher in transformed *E. coli* shone with light.
-Excitation at 450 nm and emission at 600 nm.

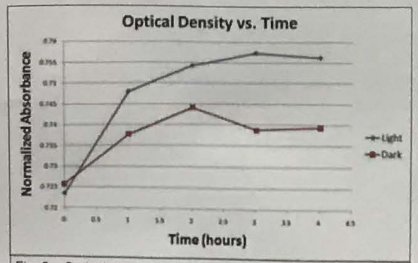


Fig. 5 – Optical density measurements of miniSOG expression with spectrophotometer from samples cultured at 37°C under light or dark conditions.

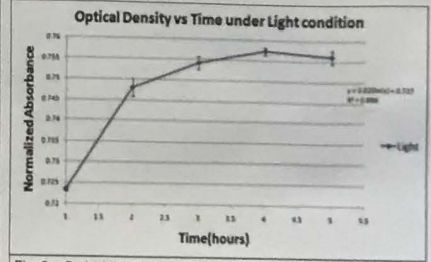
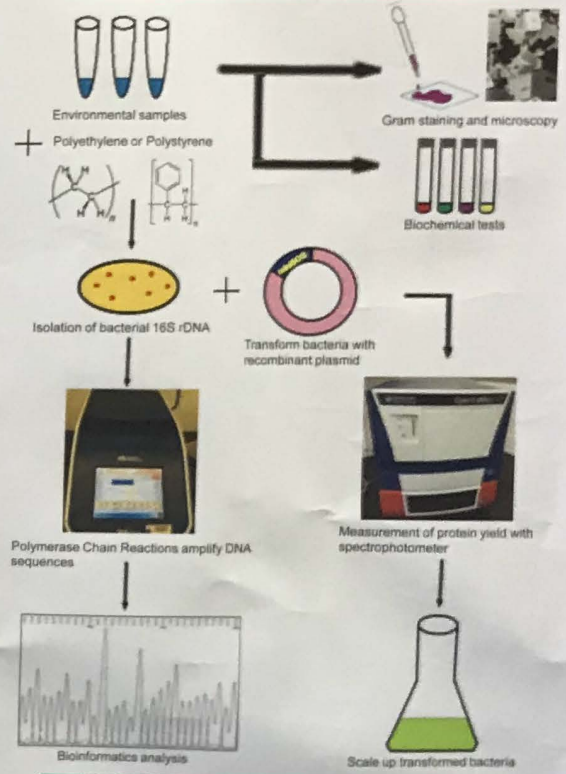


Fig. 6 – Optical density measurements under light condition showed acceptable correlation (R^2=0.886) exposure to light and the increased generation of miniSOG proteins.

Methods



Conclusion

-Bacteria were able to grow in test tubes with polyethylene as the sole carbon source.
-16S rDNA sequencing disclosed the unknown bacteria are closely related to members of the genus Ralstonia.
-Expression of miniSOG proteins was detected in transformed *E. coli*.
-These data confirmed that it is viable to study plastic degrading bacteria in greater details for improving waste management system of spacecrafts.
-The next steps are: characterize these bacteria by Gram staining and biochemical tests, insert miniSOG into their genomes and optimize for plastic degradation efficiency.

Acknowledgement

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