De La Salle University – Dasmariñas
GRADUATE PROGRAM

SCREENING OF POTENTIAL SOIL BACTERIAL ISOLATES FOR BIODEGRADATION OF LOW-DENSITY POLYETHYLENE (LDPE) STRIPS

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ABSTRACT

Name of Institution: De La Salle University – Dasmariñas

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Title: Screening of Potential Soil Bacterial Isolates for Biodegradation of Low-Density Polyethylene (LDPE) Strips

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STATEMENT OF THE PROBLEM:

This research was intended to isolate and identify bacteria from soil samples obtained from the landfill in Carmona, Cavite. Likewise, it aimed to test their inherent potential to degrade low-density polyethylene plastic polymers through biodegradability assessment tests. Specifically, the study answered the following questions:

1. What were the identities of the potential bacterial isolates from the landfill in Carmona, Cavite?
2. Which among the bacterial isolates were capable of degrading low-density polyethylene strips *in vitro* based on the following tests?
    2.1 Optical Density Test

2.2 Modified Petri Dish Screen Test

3. Was there a difference in the weight of the low-density polyethylene strips before and after incubation?
4. Was there a difference in the infra red signature of low-density polyethylene before and after incubation with the test isolates?

SCOPE AND COVERAGE:

The bacterial isolates from the soil samples of the landfill in Carmona, Cavite were identified to their species level. The assessment of their ability to degrade low-density polyethylene strips employed the following tests: optical density test, modified Petri dish screen test, dry weight method, and Fourier Transform Infra Red (FT-IR) spectrophotometry. The plastic polymer used in this study was made of 100 per cent low-density polyethylene (Glad brand) resins, shredded and/or cut into strips measuring 1 cm^2. No attempt was made to trace the product of degradation. The experiment was conducted at the research laboratory of the College of Science, De La Salle University Dasmarinas from September 2002 to April 2003.

METHODOLOGY:

The study used the experimental design, which investigated the effect of the growth and metabolism of bacterial isolates (independent variable) on low-density polyethylene strips as sole carbon sources in a controlled laboratory conditions. The study utilized standard isolation and

identification procedures, references and tests, instruments and materials to validly identify the bacterial isolates to their species and account for the biodegradability of the strips.

The "Standard Methods of Analysis for Soil, Plant Tissue, Water and Fertilizer" of the PCARR (1980) was used as a general guideline for soil sampling. An enrichment medium with the following chemical specifications (g/L H_2O) [$(NH_4)_2SO_4$, 2.0; K_2HPO_4, 1.2; KH_2PO_4, 0.4; $MgSO_4$, 0.25; $CaCl_2$, 0.02; $FeSO_4$, 0.001; Trace elements (salts of iron, copper, cobalt, manganese molybdenum, zinc and boron all at 10 mg/L), 1.0 ml at pH 7.0] was added with shredded photooxidized plastic of pure polyethylene resin to serve as the only carbon source. These same components were added with 1.5 g of agar per 100 ml of the broth to make enrichment plates and slants (PBSA). Tryptic Soy Agar was chosen as routine isolation media for bacterial organisms found in the environment. Erlenmeyer flasks (125-ml volume) with 50 ml enrichment broth (PBSB), sterilized, aerated and incubated with 5 grams of soil from each station served as containers for the preliminary and secondary cultures. Streak plating was performed on nutrient agar and enrichment plates to allow thorough isolation and to ensure potential for polyethylene degradation. The morphological characteristics of the colonies were taken at the 48-hour period. A series of morphological and physiological characterization and

reference to the Bergey's Manual of Determinative Bacteriology completed the identification of bacteria down to the species level.

Assessment of biodegradability utilized standard procedures for comparison of optical density before and after incubation, Petri dish screen test adopted from Griffin (1994), dry weight (Vaghaye, 1996; Griffin, 1994), and FT-IR spectroscopy.

Four strips of photooxidized polyethylene measuring 1 cm^2 served as the sole carbon sources of the isolates in cotton-sealed test flasks with an aluminum cover. Aeration (with Thermolyne shaker) was applied to enhance microbial activity for 21 to 28 days. Each test flask was inoculated with approximately 510 ug/ml (absorbance of approximately 0.4 at 600 nm) of test bacteria, equalized through the use of spectrophotometer.

Measurements of optical density of the broth before and after 28 days of incubation with the test isolates were compared. The difference in the optical density of the two broth samples indicated the ability of the isolates to metabolize the supplemented carbon source. The polyethylene strips were examined for the amount of growth on their surfaces and ratings were given using the scheme used by ISO 846 for assessing fungal/bacterial resistance of plastics.

Determination of the percent weight reduction of the test polymer including those in the control after incubation completed the dry weight test.

The Fourier Transform Infra Red (FT-IR) was utilized to detect any changes in the chemical composition of the material before and after its incubation with the test isolates.

MAJOR FINDINGS:

The following findings resulted from the study:

1. Soil bacteria from the landfill in Carmona, Cavite were isolated and identified as follows: S1C1, *Pseudomonas alcaligenes*; S1C2 and S1C5, *Bacillus macerans*; S2C3, actinomycete; S3C3, *Pseudomonas putida*; S3C4, *Bacillus subtilis*; S4C1, *Bacillus polymyxa*; S4C4, *Bacillus cereus*; S5C3, *Bacillus. firmus*; S5C4, *Bacillus cereus*; and DC4, *Micrococcus sp.*
2. Measurement of the optical density of the enrichment broth after incubation with the bacterial isolates and test polymer gave the following results: *Pseudomonas alcaligenes* (S1C1), 0.0; *Bacillus macerans* (S1C2), 0.038; *Bacillus macerans* (S1C5), 0.014; actinomycete (S2C3), 0.0; *Pseudomonas putida* (S3C3), 0.044; *Bacillus subtilis* (S3C4), 0.01; *Bacillus polymyxa* (S4C1), 0.012; *Bacillus cereus* (S4C4), 0.014; *Bacillus firmus* (S5C3), 0.012; *Bacillus cereus* (S5C4), 0.0; *Micrococcus sp.* (DC4), 0.0.

   Based on the scheme used by ISO 846, the ratings given to the isolates in the Petri dish screen test for assessing fungal/bacterial resistance of plastics were as follows: *Pseudomonas alcaligenes*

(S1C1), 0; *Bacillus macerans* (S1C2), 0; *Bacillus macerans* (S1C5), 1; actinomycete (S2C3), 1; *Pseudomonas putida* (S3C3), 0; *Bacillus subtilis* (S3C4), 0; *Bacillus polymyxa* (S4C1), 1; *Bacillus. cereus* (S4C4), 0; *Bacillus firmus* (S5C3), 0; *Bacillus cereus* (S5C4), 0; and *Micrococcus sp.* (DC4), 1. A rate of 1 was evaluated as "the material containing nutritive substances."

3. Percent weight reductions of LDPE with bacterial isolates were recorded as follows: *Pseudomonas alcaligenes* (S1C1), 5.13 per cent; *Bacillus macerans* (S1C2), 5.24 per cent; *Bacillus macerans* (S1C5), 3.07 per cent actinomycete (S2C3), 4.88 per cent; *Pseudomonas putida* (S3C3), 3.85 per cent; *Bacillus subtilis* (S3C4), 6.46 per cent; *Bacillus polymyxa* (S4C1), 4.65 per cent; *Bacillus. cereus* (S4C4), 2.47 per cent; *Bacillus firmus* (S5C3), 9.63 per cent; *Bacillus cereus* (S5C4), 9.30 per cent; and *Micrococcus sp.* (DC4), 3.84 per cent.
4. FT-IR measurements of the LDPE strips incubated with the different isolates, on the other hand, showed no significant chemical changes on LDPE.

CONCLUSIONS:

Based on the results of this research, it is concluded that:

1. Eleven bacterial isolates were identified on the soil samples from the landfill in Carmona, Cavite. These were the S1C1, *Pseudomonas alcaligenes*; S1C2 and S1C5, *Bacillus macerans*; S2C3, actinomycete;

S3C3, *Pseudomonas putida*; S3C4, *Bacillus subtilis*; S4C1, *Bacillus polymyxa*; S4C4, *Bacillus cereus*; S5C3, *Bacillus. firmus*; S5C4, *Bacillus cereus*; and DC4, *Micrococcus sp.*

2. Isolate from station 3 identified as *Pseudomonas putida* (S3C3) incurred the highest growth based on the optical density test with LDPE as the sole carbon source. In the Petri Dish Screen Test, the following bacterial isolates were given a rating of 1: *Bacillus macerans* (S1C5), actinomycete (S2C3), *Bacillus polymyxa* (S4C1), and *Micrococcus sp.* (DC4).
3. There was a difference in the weight of low-density polyethylene strips before and after incubation. Weight loss of LDPE was found highest with the isolate from station 5, *Bacillus firmus* at 9.63 per cent.
4. No degradation was recorded with FT-IR Spectroscopy.

RECOMMENDATIONS:

Based on the findings and analysis made by the researcher, the following are recommended:

1. Further investigation and testing must be worked on with *Pseudomonas putida* and *Bacillus firmus* for further characterization.
2. Since sorption is an important factor in biodegradation, further study on factors that would encourage sorption should be done.
3. Furthermore, a consortium of microorganisms must also be considered in biodegradation study.

4. If grown in the laboratory, vigorous aeration must be of primary concern in addition to the continuous culture condition of the system.

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