ISOLATION OF POLYSTYRENE DEGRADING BACTERIA FROM SOIL

Fekadu Haile Abusei, S Dr. K.R. Aswin Sidhaarth

Abstract*: In day to day human activities people are producing goods and services. These processes result in the production of by-products. These by-products are said to be wastes. They are no longer part of the main product. These include solid, liquid and gaseous wastes. Plastics are part of the solid waste. They are dumped continuously into the environment at high rate. Due to this fact plastic waste in the environment imposed a serious challenge in an ecosystem. Most of these synthetic plastic products are not easily degraded in soil in a normal natural process and condition. They may take more than 500 years and even millions of years in some cases to be completely degraded. Polystyrene is one of the plastic types used in many aspects of human life in industries as it has useful characteristics of cheap cost, lightness, versatility, durability, and moisture resistance. However, polystyrene is very stable and extremely hard to degrade in the environment after disposal. On the other hand, Polystyrene can be used as a carbon source for microorganisms similar to many other hydrocarbons. This study is mainly aimed at isolating the most effective polystyrene degrading bacteria from five different soil samples. The soil samples are collected from park, filed farm, garden, waste water area and sludge area soil. In this study, the biodegradation of the plastic is analyzed by carrying out an experiment of in-situ degradation of plastic in soil and laboratory degradation of the same under study on solid culture media for 45 days. The microbial species found associated with the degrading materials were identified as Gram positive and Gram-negative bacteria. The microbial species associated with the polystyrene materials were identified as Bacillus amylolyticus, Bacillus firmus, Pseudomonas putida, Pseudomonas fluroscence, and Bacillus subtilis. Fourier-transform infrared spectroscopy, Gram staining, scanning electron microscope techniques were used in the Isolation and Identification process. The analysis shows that Pseudomonas putida possess greater potential to degrade plastics when compared with other bacteria.

Key words*: A Biodegradation, Plastics, Bacterial Species, Gram Staining

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I. INTRODUCTION

The development of plastics is said to be started with the use of natural materials that had intrinsic plastic properties, such as shellac and chewing gum. The next step in the evolution of plastics involved the chemical modification of natural materials such as rubber, nitrocellulose, collagen and galalite. The first man-made plastic was created by Alexander Parkes who publicly demonstrated it at the Great International Exhibition in London. The material, called Parkesine, was Firsh organic material derived from cellulose that once Frist could be molded The First Plastic retained its shape when Plasyic. Finally, the wide range of completely synthetic materials that we would recognize as modern plastics started to be developed around 100 years ago. The first plastic, based on a synthetic polymer was made from phenol and formaldehyde, with the first viable and cheap synthesis methods invented in 1907, by Leo Hendrik Baekeland, a Belgian-born American living in New York state. Inappropriate use of plastics brought a major challenge and concern to the world.

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Fig. 1. Plastic waste in a city

II. MATERIALS AND METHODOLOGY

A. Soil Samples Collection

Soil samples were collected, for insitu degradation of the plastic in the soil and for the isolation of microorganism from the soil samples, from Vel Tech national hostel and Avadi, Chennai (Tamil Nadu) India. The type of soil samples collected are: - Garden Soil, Sewage Soil, park Soil, Sludge soil and Agricultural Soil.

B. Collection of Plastic Sample

Polystyrene (PS) plastic is a naturally transparent thermoplastic that is available as both a typical solid plastic as well in the form of a rigid foam material. Three cups of PS plastic samples were collected from Vel Tech university canteen.

C. Samples Preparation

The collected soil samples were prepared for the degradation of plastic in the soil samples as it is shown below in Fig. 1

Fig. 2. Soil samples

The weight of the plastic cups was measured using digital beam balance and recorded as an initial data before the polystyrene plastic samples were cut into pieces.

D. Physicochemical Parameters of Soils

The soil characterization was carried out for parameters like pH, moisture, & organic matter (OM) content. Hot air oven, pH meter and electric furnace was used respectively for analyzing these parameters.

1. Soil pH analysis

Soil pH is a measure of the acidity or basicity (alkalinity) of a soil. It is defined as the negative logarithm of the activity of hydronium ions (H+ or H3O+aq) in a solution. In soils, it is measured in a slurry of soil mixed with water. Fig. 3. below shows analysis of soil pH in a laboratory using a pH meter.

Fig. 3. Measuring Soil pH

2. Moisture content analysis

To measure the amount of moisture available in the samples One gram of each soil sample was placed into five different crucible & placed it in a 130 °C hot air oven over night. Then the final weight of each sample was measured.

Fig. 4. Measuring Soil Moisture Content in Hot Air Oven

3. organic matter (OM) content analysis

Soil organic matter is the fraction of the soil that consists of plant or animal tissue resulted by breakdown (decomposition). This data may help to analyze the degrading capacity of microorganisms in that particular soil sample. Fig. 4. below shows analysis of soil organic content in a soil using an Electric Furnace.

a b

Fig. 5. Analysis of Organic Content in A Soil

a. Dried Soil Samples

b. Soil Samples in Electric Furnace

E. Insitu Degradation of Polystyrene in The Soil

Polystyrene plastic cup samples were thoroughly rinsed with tap water and then distilled water, dried the cups under room temperature, took the initial weight of the cups, cut into small strips and then incubated in the dishes containing selected soil.

Fig. f Plastic

F. Bacterial Isolation

It is one of the microbiological techniques used to pick out or selectively isolate microorganisms (specially Bacteria) from a certain given sample (in this case soil) for study or other purposes.

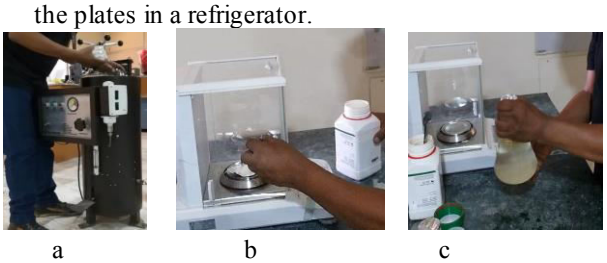
1. Sterilization of equipment

Sterilization is the complete removal of germs from a certain body. All laboratory equipment and materials required for the isolation purpose were serialized using an Autoclave to avoid contamination. The **Autoclave** was made to operate at 121°C (250°F) & at 100 kPa atmospheric **pressure** for 15 minutes. Petri plates, Conical flasks, Inoculation loop, Spatula, Measuring cylinders and Beakers were sterilized.

2. Nutrient agar medium preparation

Nutrient Agar is prepared using the standard procedure.

- 28 g of nutrient agar powder made to be Suspended in 1liter of distilled water,
- the mixture heated while stirring to fully dissolve all components,
- the dissolved mixture was kept in Autoclave at 121 degrees Celsius for 15 minutes,
- allowed it to cool but not solidify, then
- Poured the nutrient agar into each plate and left plates on the sterile surface until the agar has solidified, then
- finally, the lid of each Petri dish Replaced and stored the plates in a refrigerator.



a b c

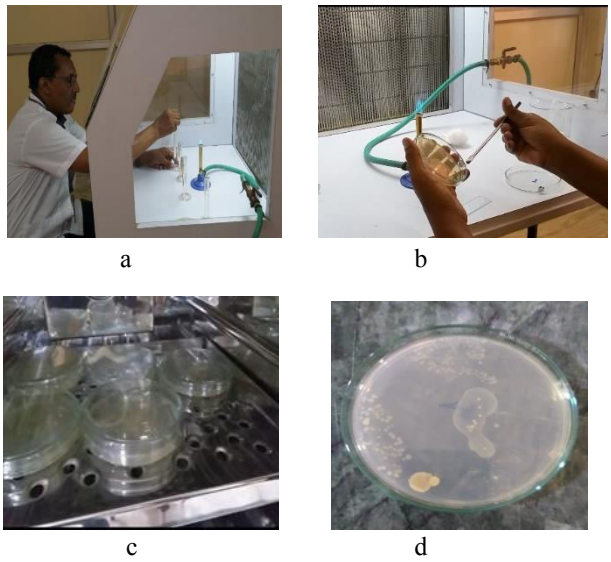
d e f

Fig. 7: Nutrient Agar Media Preparation

a. Sterilization of Equipment in an Autoclave.
b. Weighing the Components of the Media.
c. Mixing the Components within Distilled Water.
d. Heating the Mixture on Hot Plate.
e. Pouring the Nutrient Media onto Petri Plate.
f. Prepared Nutrient Agar Media.

3. Isolation of bacteria

One way to enumerate the number of bacteria present in a soil sample is to utilize dilution and plating methodology. This methodology utilizes agar as a medium for bacterial growth, a process termed, "culturable technology." Because of the vast numbers of bacteria found within soils, a small sample of soil is serially diluted in water, prior to being plated on agar within a Petri plate. Typically, a small amount of soil contained within 0.1 to 1 mL of the diluted soil suspension is "spread" over the surface of the agar plate. Fig. 7. below show the standard procedures followed to isolate bacteria.



a b c d

Fig. 8. a. Serial Dilution
b. Isolation of Bacteria
c. Incubation at 27 °C
d. Bacterial Growth on Nutrient Agar Medium after 2 Days Incubation

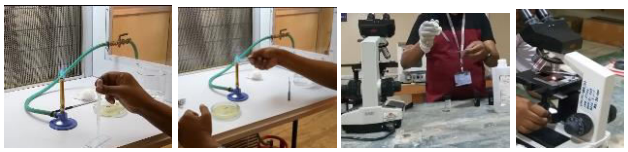
G. Gram Staining

This method is used to distinguish bacterial species in to two large groups. Gram +ve and Gram -ve bacteria.

Procedure:

- Slide with heat fixed smear was placed on staining tray.
- The smear was gently flooded with crystal violet and allowed to stand for 1 minute.
- The slide tilted and gently rinsed with distilled water using a wash bottle.
- Then the smear was gently flooded with Gram's iodine and let stand for 1 minute.
- The slide is then gently rinsed with distilled water.
- Decolorized using 95% ethyl alcohol
- Immediately rinsed with water.
- Flooded with safranin to counter-stain and let to stand for 45 seconds.
- Rinsed with distilled water.
- Dry the slide with bibulous paper and observe the sample under oil immersion Mic.

Fig. 9. Gram Staining Kit



a b c d

Fig. 10: Gram Staining Techniques
a. Preparing Slide Smear
b. Fixation of Bacterial Smear
c. Flooding the smear with chemicals
d. Observing under oil immersion light Microscope

III. RESULT AND DISCUSSION

A. Physicochemical parameters

The soil characterization was carried out for parameters like moisture content (MC), pH, organic matter (OM) percentage & temp. The values obtained are indicated on the following table (Table 1).

Table 1. Physicochemical parameters

Soil Sample	Sample location	pH	% of organic matter	Temp in °C	Moisture content
A	Garden Soil	7.0	2.2	31.0	4.721
B	Sewage Soil	6.8	9.5	29.8	4.872
C	park Soil	6.9	1.7	30.0	4.833
D	Sludge soil	7.1	8.8	31.0	4.830
E	Agri. Soil	7.0	1.8	29.0	4.859

B. Isolated Bacterial Strains

A total of 5 isolates were isolated from the soil samples collected from Vel Tech national hostel and Avadi, Chennai (Tamil Nadu) India. The isolates were purified in order to tilt to the next test and screened for plastic degradation by incubation for 45 days on a nutrient agar media at 37°C temperature conditions. The bacteria which were identified from the above biochemical tests are **Bacillus amylolyticus**, **Bacillus firmus**, **Pseudomonas putida**, **Pseudomonas**

fluroscence, **Bacillus subtilis** by the software PIBWIN (Probabilistic identification of bacteria).

Table 2. Isolated bacterial strains

Sr. No.	Identified Isolates
1	Bacillus amylolyticus
2	Bacillus firmus
3	Pseudomonas putida
4	Pseudomonas fluroscence
5	Bacillus subtilis

C. Identification and Characterization of Isolates

The isolates were identified and characterized on the followings: -

- Colony morphology of the bacterial strain on the basis of serial dilution
- Physiological Test
  - Growth at different temperature
  - Growth at different pH
- Biochemical tests

Table 3: Characteristics of Identified Isolates

a) Colony characteristics and Morphological tests:					
Tests	**Bacillus amylolyticus**	**Bacillus firmus**	**Pseudomonas putida**	**Pseudomonas fluroscence**	**Bacillus subtilis**
Configuration	Circular	Circular	Oval	Oval	Irregul.
Margin	Entire	Rhizoid	Entire	Entire	Irregul.
Surface	Smooth		Smooth	Smoo	Dull
Grams Reaction	-	+	-	-	+
Cell Shape	Rods	Rods	Rods	Rods	Rods
b) Physiological Test					
Growth at temperature					
25°C	+	+	+	+	+
30°C	+	+	+	+	+
37°C	+	+	+	-	+
Growth at Ph					
pH 5.0	-	-	-	-	-
pH 6.0	+	+	+	+	+
pH 7.0	+	+	+	+	+
c) Biochemical tests					
Indole test	-	-	-	-	-
Methyl red test	-	+	+	-	-

D. Degradation of Bacteria Inside the Laboratory

Microbial degradation of plastic on media was analyzed. There is efficiency difference among bacterial species on the process of degrading polyethylene plastic on agar plate media. The degradation inside the laboratory on agar medium is carried out by Pouring the media on Petri dish and allow it to solidify (Fig. 7f). Then inoculate the degraded strips on the medium. Incubate the disc at 37°C for 48 hrs. And the growth is observed on the plate. Now sub culturing is done in every 10 days and observed the % of degradation. (Table 4)

Table 4: Degradation of Bacteria Inside the Laboratory

	DAY 1	**DAY 10**	**DAY 20**	**DAY 30**	**DAY 40**	**DAY 45**
Bacillus amylolyticus	35mg	35mg	35mg	35mg	34mg	34mg
Bacillus firmus	27mg	27mg	27mg	27mg	26mg	26mg
Pseudomonas putida	44mg	44mg	44mg	44mg	43mg	43mg
Pseudomonas fluroscence	45mg	44mg	44mg	43mg	42mg	41mg
Bacillus subtilis	40mg	40mg	39mg	39mg	38mg	37mg

$$\% \text{ Degradation} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100\%$$

Table 5: Result of Degradation of Plastic Sample by Bacteria

Isolates no	Name of bacteria	Initial wt. (mg)	Final wt. (mg)	Difference	Weight Loss/45 days (in %)
1	Bacillus amylolyticus	35mg	34mg	1	97.14
2	Bacillus firmus	27mg	26mg	1	96.29
3	Pseudomonas putida	44mg	43mg	1	97.72
4	Pseudomonas fluroscence	45mg	41mg	4	91.11
5	Bacillus subtilis	40mg	37mg	3	92.5

IV. CONCLUSION AND RECCOMENDATIONS

A. CONCLUSION

A total of five bacterial strains capable of degrading plastic have been isolated from natural soil. Most of the bacterial isolates are Gram positive and belong to genus Bacilli. It can be

concluded that soil contains the potential candidates for bioremediation of plastic wastes.

The isolated microbes were native to the site from where they are collected and might show some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media. This gives some suggestion that these microbes can be used in both natural and artificial conditions for the purpose of degradation of polymers. Our knowledge, microbes cause greatest degradation of polystyrene and plastics. Among the bacteria, viz Pseudomonas putida followed by Bacillus subtilis, Bacillus amyloliticus Bacillus firmus, Pseudomonas fluroscence, having greater degradation ability. It is concluded that isolated strains are solely dependent on plastic for its carbon source. FTIR spectra also confirm the biodegradation of polymer as some chemical changes are seen in surface of polymer. Hence, the further attention is required from microbiologists for commercial degradation and eco-friendly polyethylene.

B. RECOMMENDATION

In the natural environment different microorganisms play an important role in various steps involved in the degradation of natural and synthetic polymers. Studying the synergism between those microorganisms will give insight for future efforts towards the biodegradation of these materials. In addition to screening soil microorganisms, isolating microorganisms from marine, petroleum waste and polymer dump site could lead to new unexplored strains, with superior performance. If one can characterize the genes responsible for the production of degrading enzymes and its regulation by using current genetic engineering tools, one can genetically modify the microorganisms and use them as a superbug for degrading the recalcitrant polyolefins.

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