An Analysis of Potential Low Density Polyethylene Degrading Bacterial Species from Several Dump Sites in Jaipur

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Abstract:

This generation is very interested in creating a sustainable environment for the future. Since plastic is one of the main pollutants that affect the environment, it is imperative that plastic be completely eliminated and that the public be made aware of its negative impacts. Because plastics are inexpensive, robust, lightweight, and long-lasting, there is a wide range of uses for them. However, since they produce pollutants and are resistant to biodegradation, they are bad for the environment. Therefore, it is unavoidable that plastic garbage will be removed from the environment. Physical and chemical techniques are often used to reduce plastic trash, however they are expensive and release toxic materials into the environment. Therefore, it is best to use the alternative approach to reduce the pollution caused by plastic. Because of this, the goal of the current project is to employ microorganisms to naturally regulate plastic trash. The efficient microorganisms that break down polyethylene were extracted from the soil of municipal solid waste disposal sites in the Jaipur area. The native microorganisms in the soil samples were isolated using the recommended method, and the physicochemical properties of the soil samples were examined. Ten distinct types of bacteria were isolated and characterised morphologically, phonotypically, and biochemically. Through the use of the clear zone test, the promising polythene-degrading bacterium among the ten isolates was discovered. After undergoing molecular characterisation, the microbe that had shown high performance in the clear zone experiment was identified as Streptomyces lividans. Thus, the results of this research demonstrate that Low Density Polythene may be effectively broken down by Streptomyces lividans that were isolated from the soil of the dumpsite.

Key words: Streptomyces lividans, plastic waste, LDPE, biodegradation, and soil bacteria.

1. INTRODUCTION:

According to Deepranjan Sarkar et al. (2017), pollution is the introduction of any material—solid, liquid, or gas—or energy—heat, sound, or radioactivity—that modifies the environment negatively.

There are several categories of pollution, including noise pollution, solid waste pollution, radioactive pollution, air pollution, water contamination, and land pollution. According to M. Sudhakara et al. (2008), the modern world is also worried about two distinct forms of pollution: noise pollution and solid waste pollution. In developing nations such as India, the creation of solid waste is becoming an increasingly serious environmental and public health issue. One of the main nations that produces

the most solid garbage annually is India. In India, families produce roughly 12 million tonnes of solid trash annually as a result of the country's growing population (Neha Gupta et al., 2015). Household garbage, hotel waste, construction and demolition site debris, and hospital waste are all considered forms of solid waste. Urbanisation and population growth have caused a rapid increase in solid waste production, as well as changes in the garbage's composition. An increasing amount of non-biodegradable garbage is accumulating in the environment due to population growth (G. Gnanavel et al., 2016). The three types of waste materials that make up solid waste are inert waste, recyclable trash, and organic garbage. The biodegradable fraction of solid waste that is classified as organic includes household, garden, and agro waste, among other wastes. The recyclable garbage consists of paper, metals, plastic, and polythene. It is not biodegradable. Sand, gravel, and other materials make up the inert waste (I. Hussein et al., 2018).

Microorganisms immediately break down the organic element of solid trash, while inert garbage also settles on land. However, we have to focus more on the recyclable components of solid waste (Md. Abdur Rakib et al., 2019). Polythene made up the majority of the recyclable garbage. Conventional techniques, such as chemical and physical methods, are used in India to recycle polythene, but they may result in secondary pollution, particularly when recycling plastic through burning, which typically produces some noxious gases like dioxins and furans, which are dangerous greenhouse gases and significantly contribute to the ozone layer's thinning (T. Z. Quazi, 2019). Therefore, it is necessary to use an alternative recycling method for polythene trash in order to avoid secondary contamination. Because of this, the goal of the current research is to provide an environmentally benign alternative to the pollution caused by polythene.

2. MATERIALS AND METHODS:

2.1 Soil sample collection:

At a depth of three to five centimetres, soil samples were taken from the Mathuradaspura, Jaipur, Rajasthan, waste site. A significant part of this landfill, where PE wastes had been deposited for varying lengths of time, is polyethene. As a result, this location was selected for the current work's research area. The Mathuradaspura has five distinct waste sites from which soil samples were randomly taken. Samples A, B, C, D, and E were the names given to the samples that were gathered. The organic farm's agricultural soil served as the control. After being tagged and placed into sterile conical flasks, the soil samples were brought to the lab for examination.

2.2 Soil sample physicochemical analysis:

The gathered soil samples (A, B, C, D, and E) were treated in accordance with standard technique to detect physical, chemical, and microbiological characteristics (O. J. Attoe, 1947). The analysis included key physical characteristics such pH (E.O. McLean, 1982), E.C. (C.S. Piper, 1942), soil texture (American Society for Testing and Materials, 1985), and soil colour (N.P. Kirillovaa et al., 2018). At the M. E. Testing Laboratory in Jaipur, significant chemical factors including soil organic carbon, nitrogen, potassium, calcium, iron, and manganese were examined.

2.3 Microorganism Isolation:

In a 250 ml conical flask with 99 ml of sterile double-distilled water, one gramme of soil sample was added. After thoroughly shaking the soil solution, it was serially diluted from 10-1 to 10-6. In order to separate the microorganisms from various soil samples, the pour plate technique was used. While starch casein agar is utilised for actinomycetes, nutrient agar is employed as a medium to separate the bacteria from the soil. Three duplicates of each dilution were prepared. After that, the plates were cultured for two to seven days at 37°C (S. Deepika and R. Jaya Madhuri, 2015).

2.4 Microorganism identification, characterization, and purification:

The morphological, phenotypical, and biochemical characteristics of the microbial isolates were examined. Studies were conducted on the morphological traits, including configuration, elevation, margin, colour, opacity, size, cell shape, arrangement, and spores. Phenotypic traits were examined, including motility and Gram's response. According to Bergey's Manual of Systematic Bacteriology (2009), the biochemical characterization tests, which included the Methyl Red test, Vogesproskauer test, Catalase test, Oxidase test, Urea hydrolysis, Gelatin hydrolysis, Starch hydrolysis, H2S production, Nitrate reduction, Citrate utilisation, Indole, and Methyl red test, were carried out. After being repeatedly subcultured to produce pure colonies, the selected isolates were stored in slant at 4°C for further research.

2.5 LDPE powder preparation:

The source of low-density polyethylene was J.P. Plastic, located in Chennai, India, 600012. After being chopped into tiny pieces, LDPE films were submerged in xylene and cooked for fifteen minutes. Subsequently, it was allowed to evaporate the xylene before being blended at 3,000 rpm. The resulting LDPE powder was then cleaned again with ethanol to get rid of any remaining xylene, and it was left to dry overnight at 50°C in a hot air oven. For later usage, the finished product was kept at room temperature (Merina Paul Das & Santosh Kumar, 2014).

2.6 Assay for clear zones:

Using the clear zone test, prospective bacteria that degrade polyethylene were screened (R. Usha et al., 2011). At a final concentration of 0.1% (w/v), polyethylene powder was added to the mineral salt medium. The mixture was then sonicated for an hour at 120 rpm in a shaker. The medium was wet sterilised at 120°C and 15 pounds of pressure for 15 minutes after sonication. After the sterilised medium had cooled to 45°C, around 15 ml of the sterilised medium was added to each plate and allowed to cool. The isolated organisms were cultured at 30-35°C for 2-4 weeks after being inoculated on polymer-containing agar plates. We identified the organisms causing the zone of clearing at the molecular level.

2.7 Taxonomy and phylogenetic study of molecules:

Phylogenetic tree building and 16S rRNA sequencing were used to identify specific bacteria molecularly using the technique outlined by C. Elizabeth Rani et al., 2019.

3. RESULTS:

3.1 Soil sample physicochemical analysis:

Table 1 displays the colour of the several soil samples, A, B, C, D, E, and control. The findings of the current investigation showed that the control soil has a sandy loam texture, whereas samples A through E have a sandy clay loam texture (Table 1).

Table 1: Results showing texture and color of the soil samples analyzed.

S. No.	Samples	Color	Sand	Silt	Clay	Class
1	Sample A	Blackish brown	46	15	23	Sandy clay loam
2	Sample B	Blackish brown	48	17	25	Sandy clay loam
3	Sample C	Light brown	50	23	29	Sandy clay loam
4	Sample D	Light brown	41	13	22	Sandy clay loam
5	Sample E	Light Black	54	25	31	Sandy clay loam
6	Control	Reddish brown	44	8	12	Sandy loam

In comparison to other soil samples and the control, the electrical conductivity of soil samples C and D has a higher EC content (Table 2). In this experiment, samples D, C, and E had significantly higher nitrogen contents than the other samples and control. It can be the result of organic pollutants building up at the disposal site. One macronutrient that controls metabolic processes is potassium. In comparison to the other soil samples examined, the exchangeable percentage of potassium in soil sample B is low. A secondary nutrient for plants is calcium. It could control how cells operate. The current study's findings indicate that samples C, D, and E have high calcium levels; this might be because of excessive deposits of products connected to lime stone at the respective disposal sites. In samples A, B, and control, the amount of calcium is minimum to moderate (Table 2).

The current study's findings showed that samples B and C had the highest levels of iron, while the other soil samples had somewhat lower levels. As shown in Table 2, the manganese content of test soil sample C is low and moderate in samples B, D, and E, and somewhat elevated in sample A and control.

Table 2: Results of physico-chemical analysis of different soil samples

Parameter	Control	Sample A	Sample B	Sample C	Sample D	Sample E
pH	7.0 ± 0.03	8.3 ± 0.02	7.6 ± 0.01	8.4 ± 0.04	8.5 ± 0.05	8.3 ± 0.02
EC (mS/cm)	1.21 ± 0.04	1.39 ± 0.06	1.32 ± 0.02	2.24 ± 0.07	3.25 ± 0.09	1.29 ± 0.01
Carbon (ppm)	0.35 ± 0.05	0.39 ± 0.04	0.59 ± 0.02	0.49 ± 0.06	0.57 ± 0.08	0.49 ± 0.09
Nitrogen (ppm)	58.4 ± 0.01	57.6 ± 0.06	66.4 ± 0.03	54.0 ± 0.05	52.4 ± 0.02	51.0 ± 0.08
Potassium (ppm)	21.0 ± 0.07	23.0 ± 0.09	12.5 ± 0.06	26.3 ± 0.08	22.0 ± 0.05	12.5 ± 0.08
Calcium (ppm)	92.0 ± 0.03	95.0 ± 0.03	85 ± 0.02	110 ± 0.04	105 ± 0.05	135 ± 0.02
Fe (ppm)	9.31 ± 0.02	9.43 ± 0.01	10.68 ± 0.03	10.11 ± 0.04	9.16 ± 0.03	9.43 ± 0.04
Mn (ppm)	4.12 ± 0.04	4.17 ± 0.06	3.45 ± 0.05	2.50 ± 0.04	3.08 ± 0.06	3.33 ± 0.08

3.2 Microorganism Isolation:

The findings of the microbial culture showed that soil samples A and B had large colonies of heterotrophic bacteria, whereas the other samples had very few colonies. In sample B, the population of actinomycetes is much higher than in samples A, C, D, and E. In the control soil, no actinomycetes colony was discovered (Table 3).

Table 3: Total heterotrophic bacteria and actinomycetes count of different soil samples

Sample	Bacteria CFU × 10-6 /g	Actinomycetes CFU × 10-5 /g
Sample A	7	2
Sample B	6	8
Sample C	2	3
Sample D	1	2
Sample E	3	1
Control	3	0

CFU - Colony Forming Unit.

All the values are represented as mean ± standard deviation (n = 3)

3.3 Microorganism identification, characterization, and purification:

From the main colonies, ten distinct bacterial species were identified by morphological, phenotypic, and biochemical characterisation investigations. Bacillus amylolyticus, Bacillus firmus, Bacillus subtilis, Bacillus sp., Pseudomonas sp., Achromobacter sp., Staphylococcus sp., and Actinomycetes are the ones that they are. To identify the powerful microorganisms responsible for the breakdown of LDPE, these isolates were put through a low density polythene degradation experiment.

3.4 Assay for clear zones:

The LDPE clear zone test is used to the identified microorganisms. The LDPE powder medium infected with actinomycetes (36 mm) has a sizable clear zone generated in it, according to the findings of the LDPE degradation experiment. Very little zone is seen by the other isolates (Table 3). Thus, our findings unequivocally demonstrated that Streptomyces species function as a powerful LDPE degrader. Studies on molecular characterisation help to further confirm the species of Streptomyces.

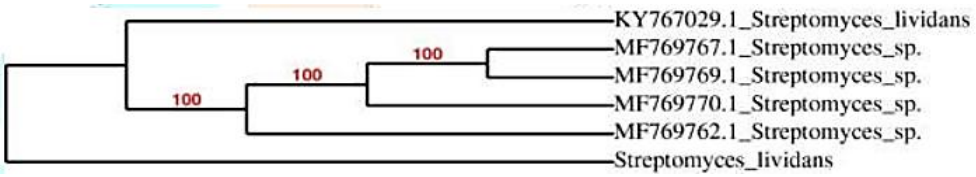
Table 3: Results showing the clear zone formation of the isolates

Isolates	Size of the zone (mm)
Bacillus amylolyticus	15
Bacillus firmus	13
Pseudomonas putida	21
Pseudomonas fluorescence	23
Bacillus subtilis	12
Bacillus sp.	17
Pseudomonas sp.	19
Achromobacter sp.	8
Staphylococcus sp.	11
Actinomycetes sp.	36

3.5 Logarithmic analysis and molecular characterization:

16S rRNA gene sequencing provided further evidence that the putative bacterial strain Streptomyces sp. was capable of digesting LDPE. Thirty-five cycles of PCR were used to amp up the 16S rRNA gene (1404 bp) based on DNA extracts of strain Streptomyces sp. A phylogenetic tree was created using the alignment of the bacterial 16S rRNA gene sequences using the NCBI databases' Blast search (Fig. 1).

Fig. 1: Phylogenetic tree of potential LDPE degrading strain, Streptomyces lividans



4. RESULT AND DISCUSSION:

The capacity of a soil to support plant, animal, and human life is known as soil quality. According to S.S. Kekane et al. (2015), soil quality is the result of the interaction between the soil and various plant, animal, microclimatic, and microbiological elements in a particular region or locale. According to Ku. Smita Tale and Sangita Ingole (2015), environmental and human activity variables dictate the particular equilibrium that the soil maintains among its physical, chemical, and biological components. Therefore, researching the criteria of soil quality aids in understanding its influence on the development and proliferation of living things inside it, particularly microbes. Bearea, M.H. et al., 1997).

One of the most crucial aspects of the soil is its colour. The colour of the soil is affected by microbial activity, minerals, organic content, pollution, and climate. According to Shields et al. (1968), the blackish colour of soil samples A and B indicates fertility and the possibility of a high concentration of organic matter and iron.

Particle size controls the structure and function of the soil, and particle size determines the texture of the soil. Particle size distribution, which is used to determine the texture of the soil, is the number of soil particles distributed according to size in a liquid media. The microbiological activity and water percolation of the soil are regulated by the texture of the soil (Miguel Ángel Martín et al., 2018).

There is a considerable difference in pH between the soil samples A, B, C, D, and E and the other control. According to Pavan M. Kadam (2016), the pH affects the macro and micronutrients in the soil sample and promotes microbial development.

Electrical conductivity is a term used to describe the measurement of salt content in soil (EC). It is a crucial sign of the condition of the soil. An rise in the soil's EC results in a reduction in its microbial activity (Arushi Makkar et al., 2018).

Because soil sample D has a high EC level, it can exhibit lesser microbial activity.

One of the essential macronutrients for the development of microbes and plants is organic carbon.

Sample B had a greater organic carbon content than the control and other samples. The soil's microbial activity could benefit from the increased organic carbon content.

The remaining nitrogen in the soil is present in organic form, with just a little quantity being accessible. According to Chandak Nisha et al. (2017), the microorganisms in the soil have the ability to transform organic nitrogen into a form that is readily accessible. Therefore, the activity of soil microorganisms has a significant impact on the quantity of soluble nitrogen. Because soil sample D has less nitrogen than other soil samples, it may exhibit reduced microbial activity, while soil sample B may exhibit more microbial activity because of its higher nitrogen concentration.

Plant development depends on soil micronutrients like manganese and iron. Because of soil contamination or overuse by plants, these micronutrient levels might be high or low (Deepmala Satpathy, 2014; Arvind K. Shukla and Sanjib K. Behera, 2019). Depending on the kind of pollutants deposited at the site, the macro and micronutrient levels in the soil may grow or decrease. The development and activity of soil microorganisms are greatly impacted by these macro and micronutrient levels. The soil sample B has the ideal pH and nutrient content, which may contribute to its increased microbial activity, according to the findings of the physicochemical study of the soil.

The soil samples B include several colonies of bacteria and actinomycetes. This data may provide us with a clear indication as to why soil sample B has an excess population of actinomycetes, given its ideal pH level and nutrient content. A clear zone experiment was performed on each isolate to identify any prospective microbial species that would be able to degrade LDPE.

According to the results of the clear zone test, a sizable clear zone (36 mm) has emerged on the petri plate that has been inoculated with the actinomycetes species that were separated from soil sample B. Phylogenetic analysis was performed to determine the species name Streptomyces strain. The result showed that the aligned sequence had 97% similarities with Streptomyces lividans. These results allowed us to demonstrate that the microbial strain obtained from sample B is Streptomyces lividans, a member of the actinomycetes family, and that, in laboratory settings, it may be able to digest low density polythene.

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