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Evaluating Plastic Degradation Capabilities and Plant Growth-Promoting Traits of Isolates Recovered from Plastic Dumping Sites

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Abstract

This study aimed to investigate the potential of microorganisms to degrade plastic waste while also promoting plant growth in plastic landfills. The study isolated soil bacteria, specifically *Pseudomonas sp*. and *Bacillus spp*., from the landfill in the Namakkal area using the clear zone and weight loss methods. Successive enrichment using LDPE as the sole carbon source resulted in 22 bacterial isolates, seven of which displayed a clear zone of inhibition for polyethylene glycol (PEG) degradation, while five showed inhibition zones for plastic powder degradation. The highest degradation rate was observed in IS14 (45.9 ± 1.11%), followed by IS9 (41.66 ± 1.41%). Additionally, IS14 exhibited positive results for phosphate solubilization, N_2 fixation, ammonia production, siderophores production, and hydrogen cyanide (HCN) production. The 16s rRNA sequencing confirmed IS19 as *Pseudomonas stutzeri*. Overall, this study highlights the potential of plant growth-promoting factors derived from plastic-degrading isolates for sustainable agriculture and environmental remediation, though further research is necessary to fully understand the mechanisms behind these activities.

***Key words*:** LDPE, PEG, plastic degradation, PGPF, *Pseudomonas stutzeri*

Globally, plastic waste has emerged as a major environmental concern, causing soil pollution. Being non-biodegradable synthetic materials, plastics persist in the environment for extended periods. Inadequate disposal and mismanagement of plastic waste result in its accumulation in soils, leading to severe repercussions on soil health, ecosystems, and human well-being [1]. Contamination of agricultural soils is widespread, showing spatial and temporal variations. Consequently, there is a growing global focus among scientists and stakeholders on addressing plastic pollution in agricultural soils. Soil plastic pollution originates from diverse sources, like packaging, agricultural films, microplastics from industrial and domestic activities, and improper waste disposal of plastic debris. The presence of plastic in the soil brings about various adverse effects [2].

Over time, plastic fragments accumulate in the soil and break down into microplastics, releasing additives that can harm soil health. The effects of plastics on soil properties and fertility depend on factors such as size, shape, and chemical composition. The presence of conventional plastic mulch films in the soil can hinder water absorption, reduce water retention, disrupt microbial communities and macrofauna, and decrease soil fertility. Consequently, this can lead to negative effects on plant growth and crop yields. These negative impacts are particularly noticeable when high concentrations of plastic (>240 kg/ha) are present, such as when non-biodegradable mulch films are repeatedly left in the soil or incorporated through tilling [3].

Burning plastic waste creates harmful substances and worsens climate change. Dumping it in landfills is also not a long-term solution, as it takes hundreds of years for plastic to break down naturally. However, promising research reveals that microorganisms like bacteria, fungi, insects, and their gut microorganisms can break down different types of plastics such as PE, PS, PP, PVC, PUR, and PET [4]. While ample evidence exists regarding the ability of plastics to degrade in the presence of microbes within the same environment, there is a notable gap in research exploring the potential synergistic role of microbes. Specifically, there is a lack of investigation into whether microbes can not only effectively break down plastics but also play a collaborative role in safeguarding soil fertility and fostering crop growth. By engaging in this synergistic process, microbes could potentially degrade plastics without adverse side effects, simultaneously providing benefits to both crop growth and the overall environmental ecosystem. Building on this concept, the present study was to investigate whether plant growth-promoting bacteria possess the ability to degrade plastic materials.

MATERIALS AND METHODS

Collection of soil and isolating the bacteria

Soil samples were collected from the different dumpsites and landfills in Namakkal, Tamil Nadu, India. After serving as a waste disposal site for over 10 years, 10 samples were randomly collected from various sites using closed, sterile

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containers and transported to the laboratory in an icebox. The samples were homogenized, stored at 4 °C, and later utilized for analysis. LDPE powder was obtained from Merck, India. Low-density polyethylene films for biodegradation studies were acquired from a local market and cut into 1.5 cm × 1.5 cm pieces.

Enrichment of culture and isolation of LDPE-degrading isolates

Culture enrichment for LDPE-utilizing bacteria involved suspending 1 g of soil in sterile saline water and incubating it at 120 rpm for 4 h. A portion of the soil suspension was transferred into an Erlenmeyer flask with sterile mineral salt broth and 0.2% LDPE powder and incubated at 35 °C. After a week, the enriched culture was transferred successively for four cycles. Serially diluted samples were spread on nutrient agar plates, and isolated colonies were transferred to nutrient broth for further study. This method facilitated the isolation of bacteria adapted to LDPE as a carbon source from the soil environment [5].

Screening of LDPE-degrading isolates

The screening for low-density polyethylene (LDPE) degradation involved the clear zone method using a synthetic medium. This medium, comprising 0.2% (w/v) polyethylene glycol and 15% (w/v) agar, was autoclaved and poured into sterile Petri plates. After solidification, isolated colonies from nutrient agar were inoculated and incubated at 30 °C for 2 weeks. Plates were stained with a 0.1% Coomassie Brilliant Blue solution, and a destaining process revealed a clear zone around the colony. Coomassie Brilliant Blue solution, prepared with 0.1% (w/v) Coomassie Blue in 40% (v/v) methanol and 10% (v/v) acetic acid, was used for staining. The destaining solution, consisting of 40% (v/v) methanol in 10% (v/v) acetic acid, was applied to visualize the clear zone produced by bacteria. This method identified bacteria as polyethylene degraders if a clear zone appeared against a blue background [5].

Biodegradation studies

In this study, the biodegradation efficiency of bacterial isolates on untreated LDPE was assessed using 0.2% (w/v) films (1.5 × 1.5 cm). The films were prepared by drying overnight, disinfecting with 70% ethanol, and air-drying in a laminar flow chamber. Aseptically, the films were added to Erlenmeyer flasks with a sterile mineral salt medium supplemented with 0.01% (w/v) yeast extract. Inoculation with 1 ml of a 24-hour-old culture was done for each flask, followed by incubation on a rotary shaker at 35 °C and 120 rpm for 60 days. Sterile controls were maintained. The extent of LDPE film biodegradation after 60 days was determined using the weight-loss method. The weight loss was calculated and compared based on the following formula [6].

$$\text{Weight loss (\%)} = (\text{Initial weight} - \text{Final weight}) / \text{Initial weight} \times 100.$$

Screening of plant growth-promoting factor (PGPF)-producing isolates

From the above-tested parameters, positive isolates were subjected to the determination of PGPF. The determination of PGPF was assessed using a modified qualitative method based on the protocol developed by Selvi and Thangaraj [7]. Bacterial cultures were inoculated into nutrient broth supplemented with tryptophan (1 mg/ml) and incubated at 35 ± 2 °C for 7 days. Following incubation, two drops of orthophosphoric acid and 4 ml of Salkowski's reagent were added to the culture. Salkowski's reagent consisted of 50 ml of 35% perchloric acid and 1 ml of 0.5 mM $FeCl_3$. The occurrence of a pink color development after the addition of the reagents indicated the production of IAA.

The production of siderophores by the isolates was assessed using solid CAS (Chrome Azurol S) agar plates. The isolates were inoculated onto CAS blue agar media and incubated at 35 ± 2 °C for 72 hours. The presence of a yellow-orange halo zone surrounding the colony indicated the production and release of siderophores on the agar plate. To determine ammonia production, the isolates were inoculated into peptone water and incubated for 48 hours at 35 ± 2°C. Following incubation, 0.5 ml of Nessler's reagent was added to each tube. A positive test for ammonia production was indicated by the observation of a color change from brown to yellow.

To assess hydrogen cyanide (HCN) production, bacterial cultures were streaked onto nutrient agar medium supplemented with 4.4 g/l of glycine. Inside the lid of each plate, a Whatman filter paper No. 1 soaked in a solution of 0.5% picric acid in 2% sodium carbonate was placed. The plates were sealed with parafilm and incubated at 35 ± 2 °C for 4 days. The presence of an orange-to-red color development on the filter paper indicated the production of HCN.

Nitrogen-fixing bacteria can be isolated from the soil using yeast extract mannitol selective culture media (YEM) supplemented with bromthymol blue (BTB). In this medium, the presence of yellow halos around the colonies on a blue background indicates a positive result for nitrogen-fixing bacteria.

RESULTS AND DISCUSSION

Regulating plastic pollution has become a pressing need in today's world. In response to the urgent environmental challenge posed by plastic pollution, the current research conducted the isolation of a soil bacterium identified as *Pseudomonas sp* and *Bacillus sp* which exhibits the remarkable ability to degrade two extensively utilized polymers. One viable solution to reduce plastic pollution is through the process of microbial degradation, harnessing the abilities of microorganisms to break down both organic and inorganic molecules. Soil, as a vital natural resource, serves as a rich reservoir of diverse microorganisms capable of contributing to this degradation process. The degradation of various types of synthetic plastics by microorganisms sourced from different ecosystems, including agricultural soils, aquatic environments, and landfills, has been extensive [8]. Built upon this point, the study aimed to identify microorganisms present in a plastic dumping site and assess their capability to degrade plastic materials.

Isolation of the bacteria was carried out on a minimal agar medium, and a total of twenty-two bacterial isolates were obtained. Screening of bacterial isolates for PEG and plastic powder degradation was carried out by the clear zone method. Out of the 22 isolates tested, seven exhibited a distinct, clear zone of inhibition on the plate containing PEG (polyethylene glycol), while five isolates displayed a zone of inhibition on the plate containing plastic powder. The study underscores the potential for bacteria found in specific environmental conditions, such as landfill sites, to exhibit enhanced plastic-degrading capabilities. This is supported by earlier research from Biki *et al*. [9], demonstrating that microbes identified from landfill sites are capable of degrading plastic. In a recent study, Nademo *et al*. [5] also screened the LDPE-degrading isolates through the clear zone method with a Coomassie brilliant blue solution.

In many LDPE biodegradation studies, the weight loss method was used to determine the microbial consumption of polymers [5], [10-12]. In our study, the percentage of LDPE weight loss was calculated, and the highest value was recorded by isolates IS9 and IS14 (41.66 ± 1.41% and 45.9 ± 1.11%, respectively). This indicates a superior degrading ability compared to the findings of Kalia and Dhanya [13], who reported 4.38% (untreated) and 12.09% (xylene-treated) LDPE film weight loss after 30 days of incubation with bacteria.

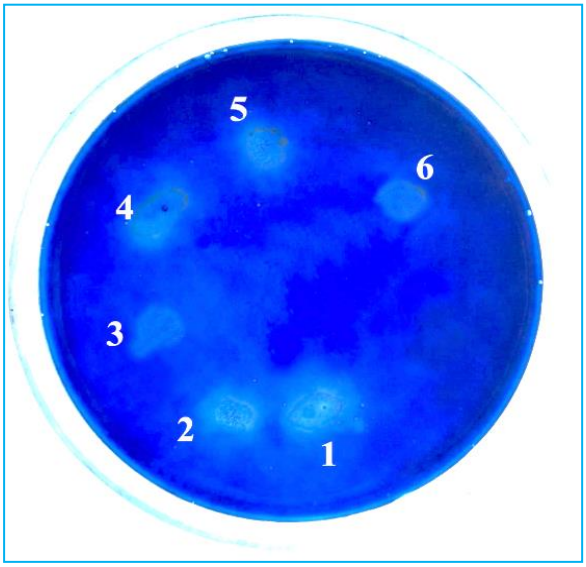

Fig 1 Isolation and screening of LDPE-degrading bacteria the clear zone formed by the isolates after 2 weeks of incubation in mineral salt medium supplemented with 0.2% polyethylene glycol Isolate 1, 2, and 4 as positive and isolates 3 and 6 as negative

The present study demonstrates that soil bacteria sourced from solid waste dump sites exhibit significant efficacy in degrading virgin polyethylene. Unlike previous research that often assessed the biodegradability of LDPE after abiotic pretreatment, such as UV irradiation, chemical oxidation, or thermal treatment [14], our work specifically utilized untreated LDPE film for the biodegradation study, yielding promising results. Our study is unique in that it evaluated LDPE degradation within a shorter time frame than previous research. While previous studies examined LDPE degradation over 90 days, we focused on biodegradability over a compressed 30-day period using plastic strips.

The role of environmental conditions in determining polymer degradation pathways is crucial. Under favorable conditions, polymers can fully decompose into organic acids, CH_4, CO_2, and H_2O. Additionally, the microbes used in plastic degradation are eco-friendly and beneficial to plant growth. This study examined the presence of plant growth hormones and a factor within two potential isolates responsible for plastic degradation to support this hypothesis. According to biochemical results, potential isolates were confirmed as *Bacillus* sp and *Pseudomonas* sp.

Bacillus sp. in the present study exhibited positive results for IAA production, consistent with previous findings in *Pseudomonas* sp. [15]. Furthermore, both *Bacillus* sp. and *Pseudomonas* sp. isolates demonstrated positive traits for ammonia production and phosphate solubilization, mirroring similar characteristics observed in plant growth-promoting isolates from municipal solid waste composts in Delhi, India [16].

In the present study, Pseudomonas sp. revealed positive results for siderophore production, contributing to plant growth enhancement and bioactive effects. Although siderophore-producing isolates are mostly isolated from cultivated soil, our findings are consistent with the 2017 study by Chaudhary *et al.* [17]. It documented the presence of siderophore-producing *Pseudomonas* sp. in various soil samples, including uncultivated sites near petrol pumps and garages.

In this study, *Pseudomonas* sp. demonstrated a positive effect on the production of hydrogen cyanide (HCN), a chemical compound known as a plant growth promoter and biocontrol agent against plant pathogens. Previous research has highlighted the protective role of HCN-producing rhizospheric isolates in protecting plants against root diseases caused by soil-borne fungi. In addition, both isolates exhibited positive results for their ability to fix nitrogen during the assay. Despite anecdotal studies examining the presence of plant growth hormones and factors in agricultural soils, limited research has focused on non-cultivated soil sources. A recent study was observed the production of plant growth-promoting factors isolated from coal mine soil samples in Gujarat [18].

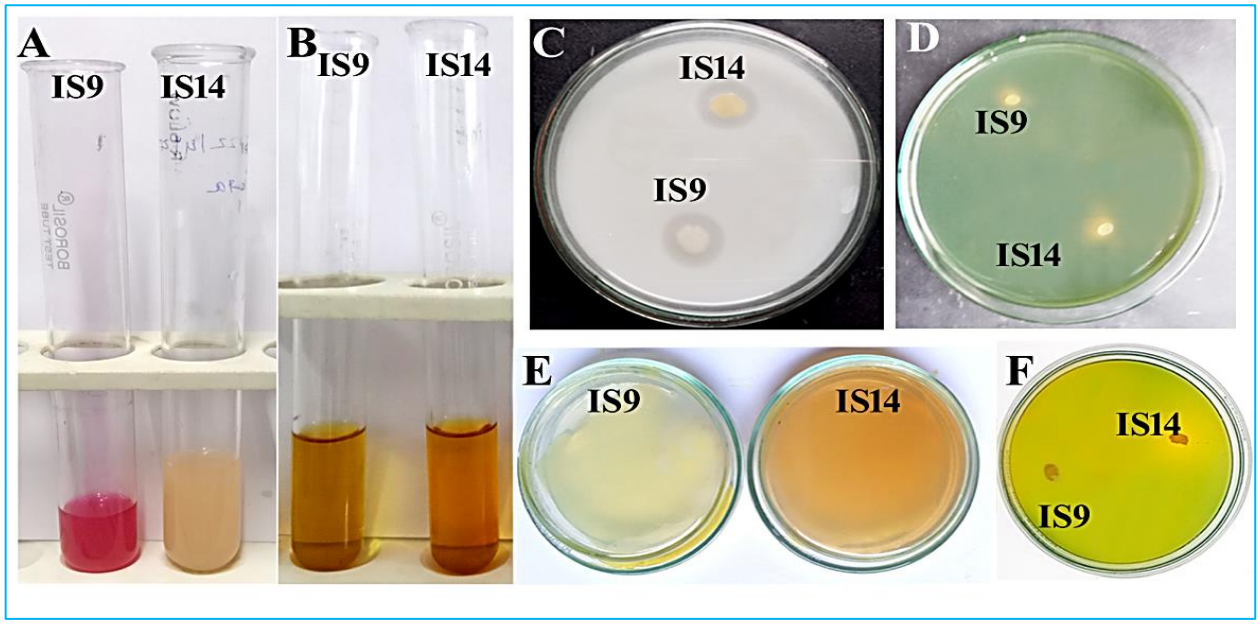

Fig 2 Determination of plant growth-promoting factors in plastic degrading isolates
A. IAA production, B. Ammonia production, C. Phosphate solubilization, D. Nitrogen fixation, E. HCN production, F. Siderophore production

Furthermore, in the present study, potential isolates of *Pseudomona sp* were subjected to 16srRNA sequence analysis for identification of species level. According to the blast result, such isolates as *Pseudomonas stutzeri.* Many strains of *Pseudomonas stutzeri* have been isolated from contaminated soil sites, and it has been identified as a proficient plastic degrader, particularly of polyethylene glycol (PEG). Although several studies have investigated the plastic degradation ability of Pseudomonas sp., no recent study has documented the inability of *Pseudomonas stutzeri* to degrade plastic or to express plant growth-promoting factors. This study presents the discovery of a bacterium, *Pseudomonas stutzeri*, which demonstrates both plastic degradation capabilities and plant growth-promoting properties, marking the first time these dual properties have been identified in a single bacterium.

CONCLUSION

Plastic pollution has become a pressing challenge worldwide, and new solutions are urgently needed to address its negative impact on the environment. The results of this research suggest that these microbes derived from specific natural environments, such as landfill sites, can exhibit enhanced plastic-degrading capabilities. These bacteria not only exhibit plastic-degrading capabilities but also produce growth hormones, siderophores, and hydrogen cyanide. In addition, these bacteria produce growth hormones, siderophores, and hydrogen cyanide. These findings may provide new avenues for bioremediation of plastic pollution and contribute to the sustainable development of agricultural practices. The identification of specific bacteria with dual capabilities further emphasizes the multifaceted benefits that certain microbial isolates may offer for environmental remediation and agricultural development. Furthermore, the identification of potential isolates such as *Pseudomonas stutzeri* with both plastic degradation and plant growth-promoting properties represents a significant advance in this field. Overall, this study provides valuable insights into the potential of natural resources and their microbes to address the current environmental challenges facing our planet.

LITERATURE CITED

1. Godfrey L. 2019. Waste plastic, the challenge facing developing countries—Ban it, change it, collect it? *Recycling* 4(1): 1-6. https://doi.org/10.3390/recycling4010003
2. Kumar R, Verma A, Shome A, Sinha R, Sinha S, Jha PK, Kumar R, Kumar P, Shubham D, Das S, Sharma P, Vara Prasad PV. 2021. Impacts of plastic pollution on ecosystem services, sustainable development goals, and need to focus on circular economy and policy interventions. *Sustainability* 13(17): 9963.
3. Hofmann T, Ghoshal S, Tufenkji N, Adamowski JF, Bayen S, Chen Q, Demokritou P, Flury M, Hüffer T, Ivleva NP, Ji R, Leask RL, Maric M, Mitrano DM, Sander M, Pahl S, Rillig MC, Walker TR, White JC, Wilkinson KJ. 2023. Plastics can be used more sustainably in agriculture. *Communications Earth and Environment* 4(1): 332.
4. Tao, Xuanyu, Ouyang, Huanrong, Zhou A, Wang D, Matlock H, Morgan JS, Ren AT, Mu, Dashuai, Pan, Chongle, Zhu X, Han A, Zhou J. 2023. Polyethylene degradation by a Rhodococcous strain isolated from naturally weathered plastic waste enrichment. *Environmental Science and Technology* 57(37): 13901-13911. https://doi.org/10.1021/acs.est.3c03778
5. Nademo ZM, Shibeshi NT, Gemeda MT. 2023. Isolation and screening of low-density polyethylene (LDPE) bags degrading bacteria from Addis Ababa municipal solid waste disposal site "Koshe". *Annals of Microbiology* 73(1): 6.
6. Montazer Z, Habibi Najafi MB, Levin DB. 2020. Challenges with verifying microbial degradation of polyethylene. *Polymers* 12(1): 123. https://doi.org/10.3390/polym12010123
7. Selvi S, Thangaraj N. 2023. Isolation and biological analysis of plant growth promoting factors from vegetable cultivate soil samples of Namakkal district, Tamil Nadu, India. *Research Journal of Agricultural Sciences* 14(2): 543-547.
8. Ali SS, Elsamahy T, Al-Tohamy R, Zhu D, Mahmoud YA, Koutra E. 2021. Plastic wastes biodegradation: Mechanisms, challenges and future prospects. *Science of the Total Environment* 780: 146-590.
9. Biki SP, Mahmud S, Akhter S, Rahman J, Rix JJ, Al Bachchu A. 2021. Polyethylene degradation by *Ralstonia* sp. Strain SKM2 and *Bacillus* Sp. Strain SM1 isolated from land fill soil site. *Environmental Technology and Innovation* 22: 101-495.
10. Jamil SUU, Zada S, Khan I, Sajjad W, Rafiq M, Shah AA, Hasan F. 2017. Biodegradation of polyethylene by bacterial strains isolated from Kashmir cave, Buner, Pakistan. *Journal of Cave and Karst Studies* 79(1): 73-80. https://doi.org/10.4311/2015MB0133
11. Tareen A, Saeed, Saira, Iqbal A, Batool, Rida. 2021. Biodeterioration of microplastics: A promising step towards plastics waste management. *Polymers* 14(11): 2275. 2022. https://doi.org/10.3390/polym14112275
12. Ali S, Rehman A, Hussain SZ, Bukhari DA. 2023. Characterization of plastic degrading bacteria isolated from sewage wastewater. *Saudi Journal of Biological Sciences* 30(5): 103628. https://doi.org/10.1016/j.sjbs.2023.103628
13. Kalia A, Dhanya MS. 2022. Evaluation of biodegradation efficiency of xylene pretreated polyethylene wastes by isolated *Lysinibacillus fusiformis*. *Nature Environment and Pollution Technology* 21: 3. https://doi.org/10.46488/NEPT.2022.v21i03.045
14. Singh B, Sharma A, Vironika. 2023. Biodegradation of municipal plastic wastes collected from solid waste landfills of Jammu, India. *Research Square*. 2-10. https://doi.org/10.21203/rs.3.rs-3224250/v1
15. Khianngam S, Meetum P, Chiangmai PN, Tanasupawat S. 2023. Identification and optimization of indole-3-acetic acid production of endophytic bacteria and their effects on plant growth. *Tropical Life Sciences Research* 34(1): 219-239. https://doi.org/10.21315/tlsr2023.34.1.12
16. Bhardwaj P, Chauhan A, Ranjan A, Mandzhieva SS, Minkina T, Mina U, Rajput VD, Tripathi A. 2023. Assessing growth-promoting activity of bacteria isolated from municipal waste compost on *Solanum lycopersicum* L. *Horticulturae* 9(2): 214. https://doi.org/10.3390/horticulturae9020214
17. Chaudhary Y, Gosavi P, Durve-Gupta A. 2017. Isolation and application of siderophore producing bacteria. *International Journal of Applied Research* 3(4): 246-250.
18. Patel R, Borada DN, Patel A, Shah NJ. 2023. Isolation and characterization of robust plant growth-promoting rhizobacteria from lignite mines, Gujarat. *Journal of Applied Biology and Biotechnology* 11(2): 226-237.