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HARNESSING THE BIO-DEGRADATIVE POTENTIAL OF *PSEUDOMONAS PUTIDA:* A SUSTAINABLE SOLUTION TO PLASTIC POLLUTION

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Abstract: Plastic pollution is the accumulation of plastic debris in the environment that puts ecosystems, wildlife, and human health at risk because of poor waste management and the durable nature of plastics. Only 9% of the 400 million metric tons of plastic waste produced by plastic pollution is recycled. This urgent issue prompted this investigation into *Pseudomonas putida* capacity to biodegrade two different kinds of plastic: PE and PET. Soil samples were used to isolate *Pseudomonas putida*. On the chosen polymers, the isolated strain biodegradative activity was examined. Within a month, PE was the plastic sample that displayed the most obvious surface deterioration.

Key words: Plastic pollution, *Pseudomonas putida*, Polyethylene (PE), Polyethylene Terephthalate (PET)

INTRODUCTION

Plastic, a term used for a diverse range of polymers, is considered the second name for convenience. The increasing usage of plastics has proportionally intensified an existing environmental crisis: plastic pollution (Kumar, *et al.* 2020). Plastics have transformed everyday life; usage is increasing and annual production is likely to exceed 300 million tonnes by 2010. In this concluding paper to the Theme Issue on Plastics, the Environment and Human Health, we synthesize current understanding of the benefits and concerns surrounding the use of plastics and look to future priorities, challenges and opportunities (Thompson *et al*, 2009). The decomposition of the plastic is the major problem and plastic not decompose easily. A simple plastic bag can take around a hundred years to decompose (Day *et al.*2023). Complex types, such as a single PET bottle, can take 500 years to decompose (Lundell and Thomas, 2020). Plastic waste, apart from contributing to land pollution, affects marine ecosystems adversely too (Thushari and Senevirathna, 2020). In short, plastic pollution is like a ticking environmental bomb that will lead to the degradation of our planet (Shen *et al*. 2020).

Amidst all this, *Pseudomonas putida* has emerged successfully as a natural plastic degrader. The ecologically and industrially important *Pseudomonas putida* belongs to the family Pseudomonadaceae which is known for its versatility (Peix *et al*. 2009). *Pseudomonas putida* can be found in a wide range of ecological habitats, like soil, water bodies, the rhizosphere of plants and certain clinical samples (Silby *et al*. 2011). *Pseudomonas putida* is characterized as gram negative, a catalase producer, and cannot hydrolyse gelatin (Peter *et al*. 2017; Katsuwon and Anderson,1992) Known for its remarkable ability to metabolize and degrade various pollutants and toxic compounds, *Pseudomonas putida* is widely employed for bioremediation purposes (Weimer *et al*. 2020). This species follows a wide range of metabolic pathways and has appeared to be useful for the degradation of synthetic plastic polymers can also grow in the presence of toluene (Wilkes and Aristilde, 2017; Inueo *et al.* 1991). The bacteria use plastic polymers as their carbon source. *Psuedomonas putida* has also displayed degrading activities against plasticizers being used as additives in PVC with the help of enzymatic activities (Lumio, *et al.* 2021).These scientific facts led to this study, where P. putida was harnessed to observe its Bio-degradative abilities on PE and PET.

MATERIALS AND METHODS

Collection and Processing of Samples

Soil samples:

Different soil samples were collected, processed, and suspended in autoclaved nutrient broth (NB) tubes. The tubes were then enriched for 5 days at 32 °C.

Plastic Samples: Polyethylene (PE) samples were collected from landfills, brought to the laboratory for further treatment, and swabbed onto nutrient agar plates.The inoculated plates were incubated at 37 °C for 48 hours.

Isolation of Colonies From Soil Samples

The enriched NB tubes were streaked onto nutrient agar (NA)
plates and incubated at 37 °C for 24 hours to observe growth.

The grown colonies having morphology similar to that of *P. putida* were isolated on Kings A media. The isolation

process was repeated by transferring the isolates to MacConkey agar plates to ensure purity. The selection of colonies on the NA plates was done on a morphological basis. The selected colonies were isolated on MacConkey agar

Biochemical Testing for Identification

Selected strains were subjected to biochemical testing for identification. A series of biochemical tests were performed, and three strains having the same results as P. putida were identified.

Pretreatment of Plastic Films

PE and PET samples were taken, cut into pieces of equal masses, and treated with absolute ethanol to get rid of any existing contamination. The films were subjected to heat treatment (90 ˚C). The films were then disinfected again chemically with absolute ethanol.

Culturing and Harvesting of Films

Pre-treated, weighed, and disinfected films were aseptically added to Luria Bertani (LB) broth. To maintain and ensure asepsis, the films were incubated in a shaking water bath for 24 hours before the inoculation of isolated strains. Two control plastic films were maintained. The remaining films' culture media were inoculated with the isolates, which were then incubated in the shaking water bath at 37 ˚C for one month. After the incubation period, the films were harvested to observe the results.

Table1:*Isolation and identification of bacteria from plastic*

Identification Tests		*P. putida*	Strain 1	Strain 2	Strain 3
Gram Staining		-ve	-ve	-ve	-ve
Lactose Fermentation		-ve	-ve	-ve	-ve
Sucrose Fermentation		-ve	-ve	-ve	-ve
Glucose Fermentation		-ve	-ve	-ve	-ve
TSI Agar		K/NC;-ve H_2S	K/NC; -ve H_2S	K/NC;-ve H_2S	K/NC; -ve H_2S
Nitrate Reduction Test		-ve	-ve	-ve	-ve
Gelatin Hydrolysis		-ve	-ve	-ve	-ve
Catalase Production		+ve	+ve	+ve	+ve
Temperature Sensitivity test	>10˚C	-ve	-ve	-ve	-ve
	>20˚C	-ve	-ve	-ve	-ve

RESULTS AND DISCUSSION

A large variety of polymers and toxic, complex, and aromatic compounds can be tolerated and broken down by *Pseudomonas putida* (Worsey and Williams, 1975; Kai-Chee and Bin. 2008). *Pseudomonas putida* was isolated and used for the breakdown of plastic polymers because of its biodegradative properties ((Wilkes and Aristilde, 2017).

Isolation and Identification of Desired Bacterial Strain

Soil and PE polymer samples were collected from various gardens and landfills sites. Numerous strains were identified. Based on their morphological resemblance to Pseudomonas putida, strains were chosen from this group. Following that, the chosen bacterial strains showed the same outcomes as *Pseudomonas putida.*

Degradation of Plastic Films

Table 2 compares the biodegradation of plastic films caused by the isolated strains. The surface morphology of the films was carefully examined. PE was found to be more degradative as the surface degradation of PE films had already started within the given one-month span. Among these films, the control PE film did not show even a slight change in its texture, and strain#2 had the most wrinkled and very rough surface texture and uneven edges. The rest of the two strains showed degradation on PE films as well. The transition of PE films' surface from smooth to uneven, rough, and wrinkled might be a result of the enzymatic activities of the strains that resulted in the breakdown of complex plastic polymers(Lumio *at al*. 2021; Priyanka and Archana, 2011). Based on the study and the results, *Pseudomonas putida* appeared to be useful for the biodegradation of plastic polymers.

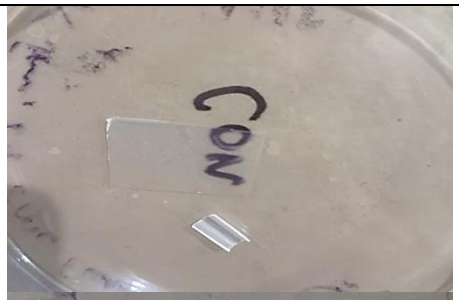
CONCLUSIONS

This study highlights the promising role of Pseudomonas putida in the biodegradation of synthetic plastics, particularly polyethylene (PE). The observed surface alterations in PE films within just one month demonstrate the bacterium's enzymatic potential to initiate polymer breakdown, while the limited effect on PET emphasizes the need for further exploration of strain diversity and optimized conditions. These findings suggest that *Pseudomonas putida* could be harnessed as part of sustainable bioremediation strategies to mitigate the growing challenge of plastic pollution. However, large-scale application will require additional research into enhancing its efficiency, understanding metabolic pathways, and integrating microbial degradation with other waste management practices. Ultimately, the use of *Pseudomonas putida* offers a viable step toward eco-friendly solutions to address the global plastic crisis.

Conflict of interest

Authors declare no conflict of interest.

Table 2: Biodegradation of plastic films by *P. putida*

olony	Figures	Results
ontrol		Both the plastic films, PE and PET, appeared to be new. No textural changes were obtained. The surface felt smooth upon touching.
train 1		The PE film's surface appeared wrinkled. Textural changes were obtained. Surface did not feel smooth. The surface degradation had started. The PET film did not display any visible degradation.
train 2		The PE film's surface appeared wrinkled. More textural changes were obtained as compared to strains#1 and 3. The surface did not feel smooth. Surface degradation had visibly started. The PET film did not display any visible degradation.
train 3		The PE film's surface appeared wrinkled. Great textural changes were obtained as compared to other colony. The surface did not feel smooth. Surface degradation had visibly started. The PET film did not display any visible degradation.

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