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Screening of plastic degrading bacteria from dumped soil area: A study

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Abstract
Background: Environmental threats from the accumulation of plastic trash are getting worse. Because of the expanding usage of plastics in packaging, biodegradable polymers can be viewed as environmentally beneficial and have a wide range of possible applications.
Aim and Objective: The current study is to determine the screening of plastic degrading bacteria from dumped soil area.
Problem of study:
Plastic has become one of the most pressing environmental issues that we are facing today. India is generating about 3.5 million tons of plastic waste annually and the per capita plastic waste generation has almost doubled over the last five years.
Methodology: This study used the liquid culture technique to examine the biodegradation of polythene bags after a month of incubation. Total heterotrophic bacterial counts in the degrading materials reached up to 0.0275 109 per gram.
Duration of study: Started of work September 2020 up to September 2021.
Results: The microbial species found associated with the degrading materials were identified as two Gram positive and five Gram negative bacteria. The microbial species associated with the polythene materials were identified as *Bacillus subtilis, Bacillus amylolyticus, Arthobacter defluvii*. The efficacy of microbes in the degradation of plastics were analyzed in liquid (shaker) culture method, among the bacteria *Bacillus amylolyticus* degrades plastic more in 1 month (50% weight loss/month) period compared to others and lowest degradation rate was observed in case of *Bacillus subtilis* (20% weight loss/month).
Conclusion: This work reveals that *Bacillus amylolyticus* posses greater potential to degrade plastics when compared with other bacteria.
Therefore, there remains an urgent need to conduct further studies in order to isolate and study plastic-degrading microorganisms. Additionally, the impact of plastic pollution on both environmental and human health should encourage the general public to transition from plastic products to more eco-friendly options.

Keywords: Biodegradation, plastics, degradation, bacillus

1. Introduction
Polymers (strong materials) that become malleable and can be cast into molds when heated are referred to as plastics. They are non-metallic moldable compounds, allowing for the creation of objects in any desired shapes and sizes [1]. Plastics are frequently used for a variety of things, including wrapping, disposable diaper backing, rural movies, and angling nets. Plastics and their use are now prevalent in all economic sectors [25]. Agribusiness, media transmission, construction and development, consumer goods, packaging, health care, and rehabilitation are only a few examples of the high-growth sectors that ensure plastics demand [22].
Plastic is used to package goods including food, medications, cosmetics, cleaners, and chemicals [2].
The most widely used polymers for bundling include polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephtha late (PBT), and nylons. Around 30% of all plastics are used for bundling applications worldwide [3].
One of the main contributors to environmental contamination is low-density polyethylene [3]. A polymer called polyethylene is created from ethylene long chain monomers. The worldwide utility of polyethylene is expanding at a rate of 12% annum and approximately 140 million

tons of synthetic polymers are produced worldwide each year [26]. With such huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue [20]. It takes thousand years for their efficient degradation. Biodegradable polymers are designed to degrade upon disposal by the action of living organisms. Biodegradable polymers generally decompose in varius medium in our environment [9].

1.1 Plastics in the Environment

Plastic is used extensively around the world because to its low production costs, strength, light weight, corrosion resistance, and electrical insulating qualities [11]. In fact, according to current estimates, the amount of polyethylene (PE) produced annually to satisfy this worldwide need ranges from 500 billion to one trillion tons [27].

Plastic takes more than a thousand years to degrade while having beneficial properties for industrial use [10]. Burning plastic emits dioxides and carbon dioxide (CO_2) into the atmosphere in addition to the lengthy period it takes material to degrade [21].

When combined, plastic waste and consumption significantly increase pollution and global warming. Microplastics are produced after big plastic materials are photodegraded by sunlight, which is a serious environmental hazard related to plastics [23]. Due to the potential for contamination of sand and water resources, these hazardous microplastics may end up being accidentally consumed by both terrestrial and aquatic animals as well as people through the food chain. According to recent estimates, at least 276 species, including 44 percent of seabirds and 86 percent of sea turtles, are impacted by plastic pollution in the aquatic environment [24].

1.2 Environmental Impacts of Plastic Pollution

Multiple dangerous and environmentally harmful impacts are brought on by plastic contamination in the marine environment. Wildlife is directly endangered by plastic waste, which has been shown to negatively affect a wide variety of animals [4]. For the majority of species, entanglement in and consumption of plastic materials pose the greatest threats. Particularly young animals frequently become tangled in plastic trash, which can cause major harm as they mature, as well as mobility restrictions that hinder animals from adequately eating and, in the case of mammals, breathing [5].

Marine birds, sea turtles cetaceans, fur seals, sharks, and filter feeders are just a few of the known species that have been significantly impacted by plastic trash. Particularly vulnerable to ingesting plastic items that they mistake for food are marine birds [8]. Plastic consumed by these animals stays in their digestive systems and can impair reproduction by decreasing eating stimulus, gastrointestinal obstruction, stomach enzyme output, and steroid hormone levels [17].

Organic contaminants at high concentrations have been shown to be present in plastic ocean debris. Oceanic plastic debris has frequently been found to contain toxic chemicals, including polychlorinated biphenyls (PCBs), nonylphenol (NP), organic pesticides, such as dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and bisphenol A (BPA) In addition, many of these chemicals can undergo considerable biomagnification and may possibly pose a direct risk to human health [29], which enhances the hazards associated with wildlife ingesting plastic waste [18].

Numerous health issues, such as developmental impairment (neurological impairment, growth abnormalities, and hormonal imbalances), cancer, endocrine disruption, neurobehavioral changes, arthritis, breast cancer, diabetes, and DNA hypomethylation have been linked to and are associated with these toxic substances [16].

Additionally, the movement of potentially invasive and damaging animals into new settings is made easier by the presence of plastic trash [13]. Marine species frequently colonize and then disperse, and there have been several instances of barnacles, bryozoans, polychaetes, dinoflagellates, algae, and molluscs adhering to environmental plastic trash [7].

Animals that live on land have also been seen to travel on maritime trash [14]. Ants have reportedly traveled several kilometers on debris from the Brazilian peninsula to San Sebastian Island, and there have even been reports of iguanas and other big creatures traveling to new Caribbean islands on flotsam [12].

1.3 Effects of plastic accumulation

Plastic is a polymeric material that is, a material whose molecules are very large, often resembling long chains made up of a seemingly endless series of interconnected links. Natural polymers such as rubber and silk exist in abundance, but nature's "plastics" have not been implicated in environmental pollution, because they do not persist in the environment. Today, however, the average consumer comes into daily contact with all kinds of plastic materials that have been developed specifically to defeat natural decay processes materials derived mainly from petroleum that can be molded, cast, spun, or applied as a coating. Since synthetic plastics are largely nonbiodegradable, they tend to persist in natural environments. Moreover, many lightweight single-use plastic products and packaging materials, which account for approximately 50 percent of all plastics produced, are not deposited in containers for subsequent removal to landfills, recycling centres, or incinerators. Instead, they are improperly disposed of at or near the location where they end their usefulness to the consumer. Dropped on the ground, thrown out of a car window, heaped onto an already full trash bin, or inadvertently carried off by a gust of wind, they immediately begin to pollute the environment. Indeed, landscapes littered by plastic packaging have become common in many parts of the world [6]. (Illegal dumping of plastic and overflowing of containment structures also play a role.) Studies from around the world have not shown any particular country or demographic group to be most responsible, though population centres generate the most litter. The causes and effects of plastic pollution are truly worldwide [19].

1.4 Biodegradation

Any physical or chemical change in a polymer brought on by environmental factors such light, heat, moisture, chemical conditions, and biological activity is refer to as plastic degradation. Biodegradable polymers are design to disintegrate when abandoned by living beings. Microorganisms break down plastics by enzymatic actions that cause the polymer to be chain-cleaved into monomers. Polymers partially deteriorate because microorganisms consume polythene film as their only supply of carbon [29].

The metabolic variety of bacteria makes them a valuable resource for cleaning up environmental contamination. Heavy metals including arsenic, mercury, cadmium, and lead as well as oil spills, PCBs, and other contaminants have all been clean up using bacteria. There are enough examples to imply that most chemicals can be used by microorganisms for metabolic processes, if not entirely. In comparison to existing methods of garbage disposal, biodegradation is an appealing alternative since it is often less expensive, may be more effective, and does not generate secondary pollutants as incineration and landfill do [15]. In rare circumstances, the bacterial metabolism of pollutants may even allow for the production of valuable products with economic benefits, such as ethanol for usage in biofuels [17].

The purpose of this study was to isolate microorganism from dumped soil area and screening of the potential polyethylene degrading microorganisms and indentifying the high potential microorganism that degrade the plastics [28].

2. Problem of study

Plastic has become one of the most pressing environmental issues that we are facing today. India is generating about 3.5 million tons of plastic waste annually and the per capita plastic waste generation has almost doubled over the last five years.

3. Aims and Objectives

1. Isolation of microbes from soil-dumped plastics.
2. Identification of tiny organisms that damage plastics that have been removed from plastics.
3. To take into account these microorganisms' ability for biodegradation.

4. Materials and Methods

4.1. Description of the Study Area

Guntur district, Andhra Pradesh consists of 57 revenue mandals under 3 revenue divisions *viz*., Guntur, Tenali and Narasaraopet. It has a geographical area of 11,328 sq. kms, lying between North latitudes $15^{0}18'$ and $16^{0}50'$ and East longitudes $79^{0}10'00''$ and $8^{0}55'00''$ (Fig.1). The annual average rainfall of the district is 889.1 mm, contributed by two monsoons, the South-west and North-east monsoon, contributing 59% and 26%, respectively. Krishna and Naguleru are the main rivers of the district. The district has been gifted with the vast surface and ground water resources. About 3.01 lakh ha area is irrigated by canals and it has vast ground water resources of 1.72 lakh ha. Out of the total geographical area of 11,328 sq. km, 10.27% of the area is covered by forests. The barren and uncultivable land is 3.04% and when combined with wasteland and the current fallows, it accounts for 4.8% of the total land area. The net area sown is 56.81%.

Fig 1: Guntur City Map

4.2. Duration of study

Started of work September 2020 up to September 2021.

1) Sample collection

Plastic sample was collected from the dumped soil of hostel garden, ANU Campus.

2) Isolation

a. Serial dilution

Following the collecting of the plastic sample, 1g of the sample was divided into pieces and added to 9 ml of sterile water to create a 1:10 dilution; 1ml of the 1:10 dilution was then added to the 9 ml of sterile water to create a 1:100 dilution, and so on.

b. Total heterotrophic count

C.F.U. /g= Number of colonies/ inoculums size (ml) X dilution factor

3) Identification

Identification of the isolates were performed according to their morphological, cultural and biochemical characteristics by following Bergey's Mannual of Systematic. All the isolates were subjected to Gram staining and specific biochemical tests.

4.3 Gram staining method

Colony morphology

This was done to determine the morphology of selected strains on the basis of shape, size and color.

4.4 Biochemical tests

Utilizing a biochemical differentiating proof unit (Hibacillus recognizable proof unit, HIMEDIA) and a few manual biochemical techniques, the detached strains' biochemical differentiation was verified.

A standard colorimetric differentiating proof system using common biochemical tests and carbohydrate consumption tests may be called a "Biochemical Recognizable evidence test unit."

4.5 Microbial Degradation of Plastics in Laboratory Condition: Determination of Weight Loss

Pre-weighed, 1-cm-wide circles made of polythene bags were aseptically transferred to a cone-shaped carafe that held 60 ml of culture broth medium that had been infused with a specific bacterial species.

Plastic circles were used to maintain control inside the microbe-free media. For each treatment, certain jars were stored up and cleaned out in a shaker. The plastic circles were collect after a month of shaking, thoroughly cleaned with distilled water, dried in the shade, and then weighed to determine their final weight.

The weight misfortune of the polymers was determined using the data gathered.

5. Results

Table 1: The weight misfortune of the polymers was determined using the data gathered

Dilution no.	Colony morphology	Source	Code	Sl. No.
10^{-1}	Large round white Small	Dumped plastic material from hostel garden.	PLRW	1
	round yellow Small round	Dumped plastic material from hostel garden.	PSRY	2
	white Large irregular	Dumped plastic material from hostel garden.	PSRW	3
	white	Dumped plastic material from hostel garden.	PLIW	4
10^{-2}	Large round pale yellow Small	Dumped plastic material from hostel garden.	PLRP	1
	round yellow Small round	Dumped plastic material from hostel garden.	PSRY	2
	transparent Large irregular	Dumped plastic material from hostel garden.	PSRT	3
	white	Dumped plastic material from hostel garden.	PLIW	4
10^{-3}	Large round white Small	Dumped plastic material from hostel garden.	PLRW	1
	irregular yellow	Dumped plastic material from hostel garden.	PSIW	2
10^{-4}	Large irregular white	Dumped plastic material from hostel garden.	PLIW	1

Table 2: Gram Staining: The bacterial strains are identified from the seven selected strains

Bacterial Strain no	Strain	Shape of the organism	Characteristic	Color
1	PLRW	Rods in chain	Gram -ve, bacillus	Purple
2	PSRY	Coccus in chain	Gram +ve, coccus Gram	Pink
3	PSRW	Coccus in chain	–ve, coccus Gram +ve,	Pink
4	PLIW	Rods in chain Rods	bacillus Gram +ve,	Purple
5	PLRP	in chain Rods in	bacillus Gram –ve,	Pink
6	PSRT	chain Rods in	bacillus Gram –ve,	Pink
7	PSIY	chain	bacillus	Pink

The table contains the bacterial strains which are gram +ve & Gram –ve. Bacterial strains.

5.1 Bacterial count

Table 3: Total heterotrophic bacterial count

Dilution	Number of colonies	Inoculums size (in mL)	CFU/g
10^{-3}	275	0.1	0.0275×10^{9}

Table 4: Result of biochemical test

Sl No:	Catalase test	Manito test	Motility test	Maleate utilization test	Gas production from glucose	Nitrate reduction test	Oxidase test	Citrate utilization test
1	+ve	+ve	Non-motile	-ve	-ve	-ve	+ve	+ve
2	+ve	+ve	Non-motile	+ve	+ve	-ve	+ve	-ve
3	+ve	+ve	Non-motile	-ve	+ve	-ve	+ve	+ve
4	+ve	+ve	Non-motile	-ve	+ve	+ve	+ve	-ve
5	+ve	+ve	Non-motile	-ve	-ve	-ve	+ve	-ve
6	+ve	-ve	Non-motile	+ve	-ve	+ve	+ve	-ve
7	+ve	+ve	Non-motile	-ve	-ve	-ve	+ve	-ve

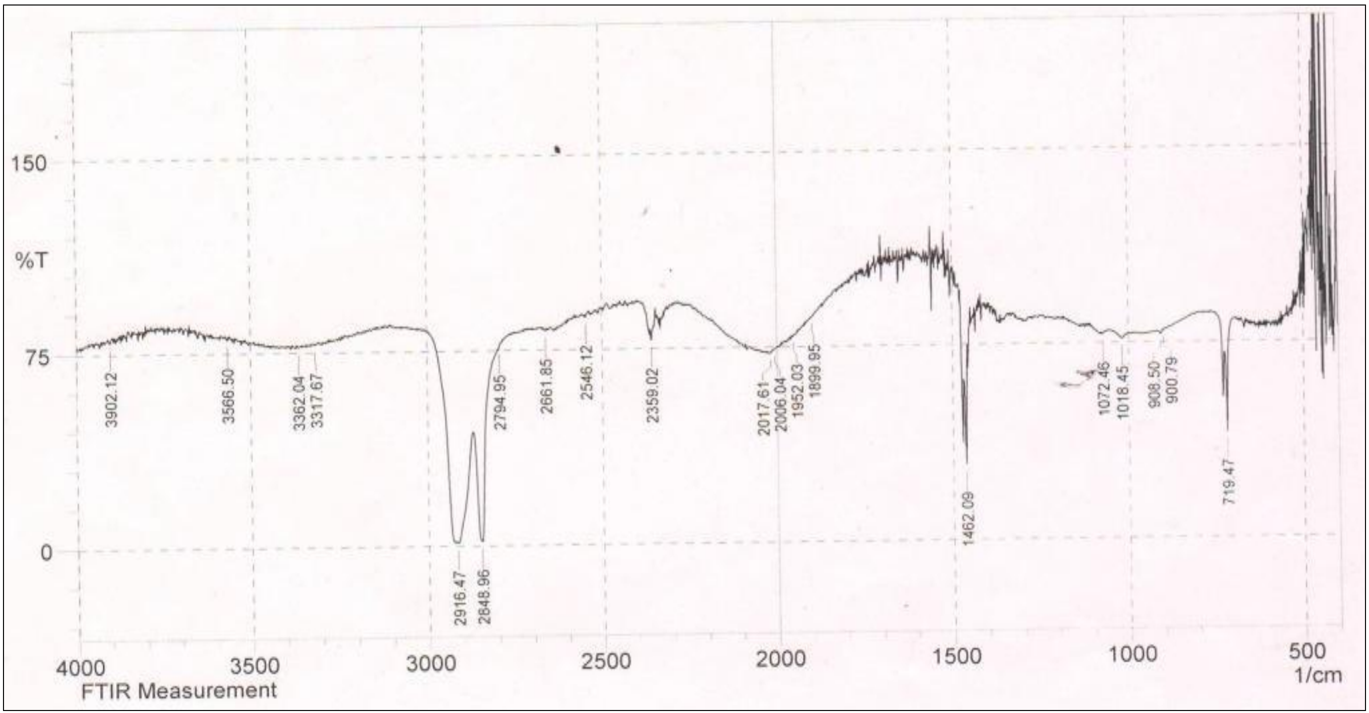

Fig 2: FTIR spectra of plastic before degradation

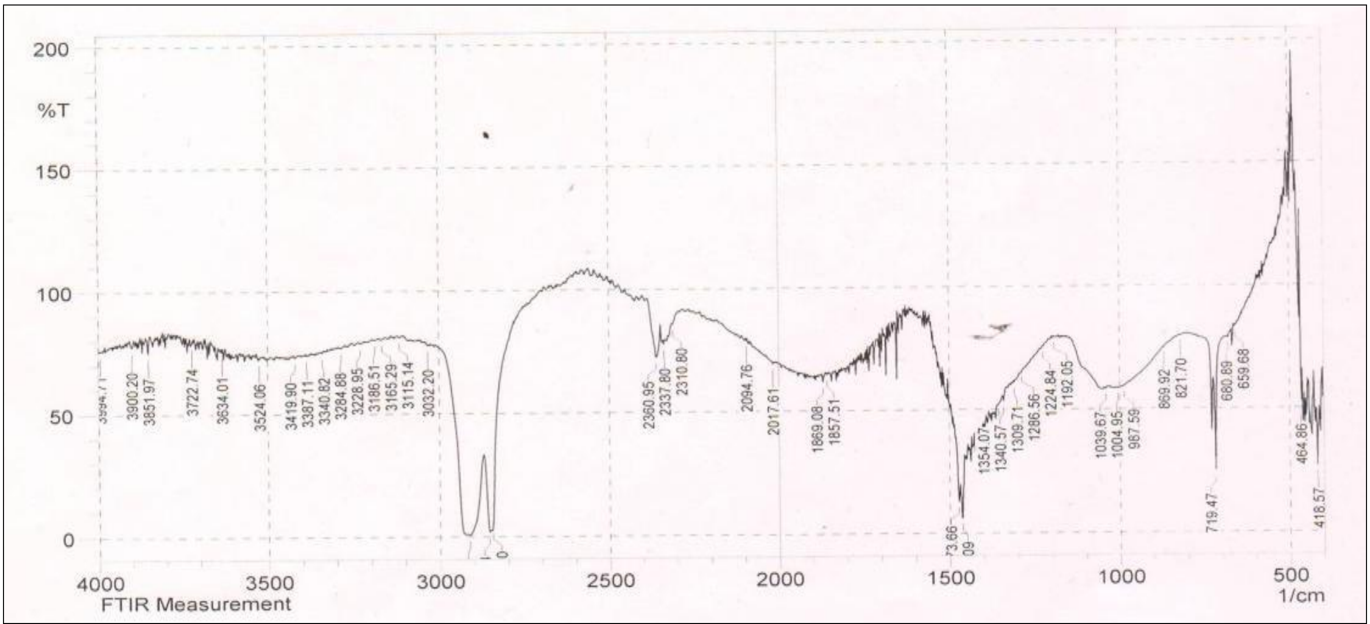

Fig 3: FTIR spectra of plastic after degradation by Pseudomonas putida

- Biochemical tests shows, catalase and oxidase test result of all the strains were found to be positive.
- Mannitol test of the strains were also found positive excluding strain no.6, Motility test shows all the strains are non-motile.
- Citrate test of strain no 1 and 4 were found positive and rest of them showed a negative result.
- Nitrate reduction test of strain no.4 was found positive and rest of them showed a negative result.
- Malonate test shows only strains 4 and 6 gave a positive result.
- The test named gas production from glucose shows strains 2, 3 and 4 showed a positive result.

Table 5: Result of degradation of plastic sample by bacteria after 1 month

Strain No.	Initial wt (mg)	Weight loss/month (in %)	Difference	Final wt (mg)
1	60	20	10	50
2	60	27.65	13	47
3	60	33.33	15	45
4	60	39.53	17	43
5	60	42.85	18	42
6	60	42.85	18	42
7	60	50	20	40

6. Discussion

This study has examined the main issues related to the kinds, applications, and degradability of natural and manufactured polymers as well as disposal techniques and criteria for measuring polymer degradation. The biodegradation of plastics via the liquid culture approach has been another subject of study.

It is clear that under the right circumstances and concentrations, the majority of refractory polymers may be partly broken down. The current study examines soil-based bacteria that can break down plastic and their separation, identification, and derivative abilities. During morphological and biochemical investigation, the microorganism produces a variety of modifications. In this investigation, synthetic plastic samples were gathered from hostel garden soil that had been discarded. This plastic was used to explore how microorganisms that were isolated from them biodegraded them.

Microbial counts in the degrading materials were recorded up to 0.0285×10^9 per gram for total heterotrophic bacteria. The microbial species found associated with the degrading materials were identified as two Gram positive and five Gram negative bacteria.

In the present study pieces of plastics were inoculated in the liquid culture medium containing bacterial isolates and kept for 1 month to observe the percentage of weight loss by bacteria. The result shows the degradative ability of the microorganisms after one month of incubation. The percentage of weight loss due to degradation was found more by *Bacillus amyloliticus.* This shows it has the greater potential of degradation compared to other bacteria.

The bacteria which are identified from the above biochemical tests are *Bacillus Subtilis* (strain- 1), *Bacillus Amylolyticus* (strain-2) *and Arthobacter defluvii* (strain-3) by the software PIBWIN (Probabilistic identification of bacteria). These three bacterial species were also found on the basis of common morphological characteristics.

7. Conclusion

The purpose of the current study was to use microorganisms obtained from soil samples containing garbage to breakdown the plastic strips. The soil samples yielded a wide range of bacterial and fungal isolates. However, a screening process only selected three dominant bacterial and fungal colonies, and these colonies were then recognized based on their morphological and biochemical traits.

The isolated bacteria were local residents of the disposal site for polyethylene and had some natural degradability; however, they had also demonstrated signs of biodegradation in lab settings on synthetic medium, The microscopic organisms were distinguished to be *Bacillus Subtilis, Bacillus Amylolyticus and Arthobacter defluvii., Bacillus amylolyticus* degrades plastic more than that of other bacteria, *Bacillus subtilis* has less capacity to degrade plastic as compared to other bacteria.

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