Isolation and Characterization of Plastic Degrading Bacteria Collected From Landfill Sites

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Abstract
The growing demand of plastic leads to global pollution generated from plastic waste. The conventional methods fail to degrade plastic completely so alternative methods are used for degradation. In the present study the collected waste sample screened and three strains having potential for polythene degradation were isolated. Among 3 strains the strain P1 degrade PVC efficiently both in broth and agar media. Biochemical testing and ribotyping characterize the strain P1 as *Bacillus gibsonii* strain pm11.
Keywords: Plastic; Biodegradation; Pollution.
Significance statement: The present study intended to isolate plastic degrading bacteria from dumped site and characterize the bacteria.

1. Introduction

Owing to their durability, stability, waterresistance, versatile natureand minimal cost make plastic as a replacement to other available materials (Priya et al. 2021). It becomesa essential part of our life and on high demand with passing time. The existence of long chain polymeric molecules makes its decomposition process slower (Bakht et al. 2020). Degradation of plastic generates microplastics (with particle size of < 5 mm) responsible forecotoxicological effects (Chen et al., 2020a; 2020b). Landfill, incineration and recycling method is also not suitable due to high cost as well as release of green house gases (Hou et al. 2022; Gao&Sun 2021).Biodegradation i.e, degradation using micro-organisms is an environmental friendly where plastic degraded to CO_2 & H_2O orsubstances with shorter chain, through various biological processes: biodeterioration, biofragmentation and assimilation without liberation of any by-product (Zeenat et al. 2021; Kim et al.2017).The polymer chain break down to oligomers and monomers by groups of living organisms in the biodegradation (Atanasovaet al.2021).Micro-organism's are preferred as they destroy the inert nature of plastic which makes it resistant for degradation (Mohanan et al., 2020; Khalil et al., 2018). Micro-organisms exchangethe carbon in the polymerchains either into micro biomolecules or carbon dioxide and water which contributes to soil fertility, reduction in both plastic accumulation and cost of waste management. Additionally, biodegradable plastics could be helpful for the manufacture of helpful metabolites (monomers and oligomers) (Mir et al.2017;Tokiwa et al. 2009).Currently more priority is given to isolate potent plastic-degrading micro-organisms from plastic-contaminated soil (Muhonja et al. 2018; Bombelli et al. 2017). The present study intended to isolate plastic degrading bacteria from dumped site and characterize the bacteria.

2. Materials & Methods

2.1. Sample collection

Plastic sample was collected from the contaminated soil. Soil samples were collected from dumpsites and landillsusing sterilized spatula and were preserved in polythene bags.

2.2. Isolation

a. Serial dilution

1g of the collected soil sample was cut into pieces followed by addition of 9 ml of sterile water to make 1:10 dilution, then 1ml of the previously diluted sample added to 9ml of sterile water to make 1:100 dilution and so on for further dilutions.

1 mL of diluted sample plated in a media NA (Nutrient Agar) containing 2% of PEG (Polyethylene Glycol) with the pour plate method and incubated at 37°C for 48 hr.

b. Purification

Isolated bacteria grown in a NA media by the streak plate method and this process repeated until pure isolates were maintained. The pure isolates further cultured on MSM (Mineral Salt Medium) supplemented with 2% of PEG and incubated at 37°C for 48 hours.

2.3. Identification

The isolates were identified on the basis of their morphological, cultural and biochemical characteristics by following Bergey'sMannual of Systematic Bacteriology (Kandler and Weiss, 1986).

2.3.1. Morphological

a. Gram staining

A smear of the bacterial culture was prepared on a clean grease free slide with a pre-sterilzed loop. The air dried and heat fixed smear was flooded with crystal violet for 1min after washing with distill water. Then washed with alcohol after flooded with Gram's iodine for 1 min. Finally using saffraninfor 30 secondsslide was counterstained followed by washing with distill water.

b. Colony morphology

The stained slide was then observed under microscope and the morphology of selected strain determined according to their shape, size and colour.

2.3.2. Biochemical

The biochemical identification of the isolated strains was done by using following biochemical methods.

a. Mannitol Test

The bacteria able to ferment mannitol sugar, raises pH of the mediaby producing acids. The change of color from red to yellow shows positive result.

b. Citrate Test

In this test citrate is the onlyavailable carbon source and converted to oxaloacetate by the bacteria. Bacteria turns media into bright blue color gives +ve result.

c. Catalase

The presence of catalase enzyme was tested by inoculating a loopful of culture into tubes containing 3% of hydrogen peroxide solution. The formation of effervescence resulting from breakdown of hydrogen peroxide to O_2 and H_2O indicates positive result.

d. Motility

The isolated microorganism inoculated with approximately 5ml of the nutrient broth and incubated overnight. After that a drop of the NB media was taken on a grooved slide (hanging drop method) and was observed under microscope.

2.4. Microbial degradation of plastic in laboratory condition

Determination of weight loss

Pre-weighed discs of plastic having 1-cm diameter were transferred aseptically to the conical flask containing 50 ml of culture broth medium with bacterial species and incubated in shaker incubator at 150 rpm for 14 days. Liquid broth having plastic broth without microbes was treated as a control. The plastic discs were collected after one month and weighed for final weight after washing thoroughly with distilled water followed by shade-dry. The weight loss of the plastics was determined as follows.

Percentage the lost of dry weight = $\frac{Wi-Wf}{Wi}X100$ Where

W_i = Dry weight before degradation (g) W_f = Dry weight after degradation (g)

2.5. Identification of most potent micro-organism

Genomic DNA of the active plastic-degrading isolates was extracted using phenol, chloroform and iso amyl alcohol. The forward primer (27F) sequence 5′-AGAGTT TGATCCTGGCTCAG-3′ and the reverse primer (1492R) sequence 5′-GGATGAGCCCGCGGCCTA-3′ were used to amplify the 16S rRNA gene. The amplification was performed in a reaction mixture of Polymerase Chain Reaction (PCR) with 10x Taq buffer, 3 U Taq DNA Polymerase, 2.5 mM dNTP mixture, 3.2 mM MgCl2, 133 ng DNA, and double-distilled water, mixed in a final volume of 50 µl. The program for PCR was set as follows: one cycle of 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, with one extension cycle at 72 °C for 7 min. The PCR product was purified, analyzed, and sequenced in a ABI 3130 Genetic Analyzer. The sequence data was aligned and analyzed to identify the Bacteria and its closest neighbors.

3. Results and Discussion

The colony morphology of various isolated strains were studied and presented in the table-1. The identification was done morphologically using grams staining (Table-2) and biochemically with the help of biochemical assays (Table 3). The degrading capacity of the isolated strains was analyzed and represented in the table 4. The weight of plastic sample was taken before and after incubation and difference in weight was calculated.Phylogenetic study of the potent PVC degrading strains based on the 16S rDNA sequence and gel image was shown in Fig. 1.

In the present study isolation, identification and degradative ability of plastic degrading microorganisms from soil was investigated. The isolated microbial species associated with the degrading materials were identified as two Gram positive and one Gram negative bacteria. Micro-organisms degrade polymer at a particular concentrationin the appropriate environment. Strain P1 has high degradation activity both on liquid and agar media.

The isolate can be used as a potent biodegrading agent for waste material containing plastic. The large scale application may benefit researcher for maintaining healthyenvironment. The strain P1 was identified to be *Bacillus gibsonii* strain pm11.

4. Conclusion

The microbial strains were successfully isolated from the soil sample havingbroad prospective to degrade the synthetic polymers like polyvinyl chloride. The *Bacillus gibsonii* strain pm11 was found to possess highest

plastic degrading potential as compared to other strains. These findings will help in bioremediation of plastic waste more efficiently in combination with conventional approach.

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Table no. 1: Colony morphology of the strains on the basis of serial dilution.

S.N.	Dilution Factor	No. of colonies	Colony Characteristics
1	10^{-1}	75 (3 types)	Large round white
			Small irregular yellow
			Small round white
2	10^{-2}	42 (2 types)	Small round transparent
			Large irregular white
3	10^{-3}	10 (1 type)	White branched irregular

Table no. 2: Result of GRAM STAINING

Strain	Shape	Color	Characteristics
P1	Bacilli	Violet	Gram+ve
P2	Bacilli	Red	Gram -ve
P3	Bacilli	Violet	Gram+ve

Table 3: Result of Biochemical test

Biochemical test	Strain P1	Strain P2	Strain P3
Catalase	+ve	+ve	+ve
Mannitol	+ve	-ve	+ve
Citrate	-ve	+ve	-ve
Motility	Non-motile	Non-motile	Non-motile

Table no. 4:Difference in the weight of the plastic samples with different microbial strains after an incubation period of 1 month

Strain	**Agar Media**			**Broth**		
	Initial wt. of PVC (in g)	Final weight of PVC (in g)	Weight loss/month	Initial wt. of PVC (in g)	Final weight of PVC (in g)	Weight loss/month
P1	0.14	0.07	50%	0.12	0.04	66.66%
P2	0.06	0.06	-	0.12	0.1	16.66%
P3	0.06	0.04	33.3%	0.11	0.10	10%