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ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and Characterization of Polystyrene Degrading Bacteria Isolated from Mealworm *Tenebrio molitor* Gut

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ABSTRACT
The excessive use of durable and degradation-resistant synthetic polymers such as polystyrene as a packaging material has resulted in a rise in environmental pollution. However, it has been confirmed by academic researchers from different countries that yellow mealworms, which are known as the larvae of Tenebrio molitor, can survive when fed with polystyrene (PS) foam as their sole carbon diet. Thus, the current study focuses on the isolation and identification of bacteria produced by the larvae of beetle Tenebrio molitor, which enable them to survive when fed with polystyrene (PS) foam as their sole carbon diet. The gut of the larvae of Tenebrio molitor may contain microorganisms that degrade polystyrene; hence, yellow mealworms are economically among the most important species that could be used for the biodegradation of polystyrene. [Yang Y, Yang J, Wu WM, Zhao J, Song Y, Gao L, Yang R, Jiang L]
Key words: *Polystyrene, Tenebrio molitor, Mealworms, Biodegradation, Bioremediation*

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INTRODUCTION

Plastics are defined as the polymers (solid materials) which on heating become mobile and can be cast into moulds. They are non-metallic moldable compounds and the materials that are made from them can be pushed into any desired shape and size. The most widely used plastics for packaging are polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (Styrofoam) (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), nylons. Plastics commonly used in purpose like packaging, disposal diaper backing, agricultural films and fishing nets. But the major plastics manufacturers questioned the economic viability of recycling at the time, and this is reflected in modern plastic collection.Polyurethane (PU) is a form of petroleum-based plastics that consists of repeating units linked by carbamate bonds. With a global production of 18 million tons in 2016, PU is one of the most extensively used synthetic polymers.The majority of plastic produced is not reused, instead ending up in landfills or polluting the environment as plastic pollution. Plastic pollution can be found in all the world's major water bodies, for example, creating garbage patches in all the world's ocean and contaminating terrestrial ecosystems [1, 2].

MATERIALS AND METHODS [3, 4]

1. Sample Collection: The mealworm was purchased from online website & brought into the laboratory and maintained for further study**.**

2. Isolation of Polystyrene-Degrading Bacteria [5]: The gut cell suspension was prepared from mealworms which were fed with polystyrene as a sole source of carbon in the diet. The mealworms were surface disinfectedby immersing them in 75% ethanolfor 1 min followed by rinsing with sterile 0.85%saline water. The mealworms were then dissected to remove the entire gut from each larva, using surgical blades and forceps.

3. Enrichment and Isolation of Polystyrene Degrading Bacteria: The Mealworm species of were dissected aseptically. Gut was separated to obtain the gut contents for the enrichment of polystyrene degrading bacteria The surfaces of the larvae were sterilized i.e. immersion in 75% ethanol for 3 min and then rinsed 2 times with sterile saline water (SW) Next, the guts of the larvae were drawn out and pooled into a centrifuge tube containing 40 mL of saline and 10mL mealworm gut. After being shaken on a vortex mixer for 5 min, the gut tissues were carefully removedwith a pipette,the remaining suspension wasused as a microbial inoculum andwas transferred into a 150 mL Erlenmeyer flask that contained 1 g of the

small polystyrene pieces and 40 mL of liquid carbon free Basal Medium(LCFBM). This flask was incubated on a rotary shaker (100 rpm) at 30°C.
After one weeks, the residual polystyrene pieces were removed and the cultures were spread across plates with two different media containing complex organic carbon substrates Luria broth (LB) and beef extract peptone agar (BPA). After an incubation period of 24 h, the colonies obtained on above two media were preserved on nutrient agar slant at refrigeration for further study.

4. Characterization & Identification of Isolated Bacteria

Tentative Identification of the bacterial isolates was done by studying their morphological, cultural & biochemical characters & with reference to the Bergy's manual of Systematic Bacteriology of volumes I & II.

5. Estimation of Weight Loss and Molecular Decrease of Polystyrene: 1 g of pre- weight polystyrene block was inoculated in liquid carbon free Basal Medium (LCFBM) with all the obtained isolates. Polystyrene block was weighed at regular interval using the sterile ascetic conditions

6. Determination of Biodegradation of Polystyrene by Bacterial Isolates: Determination of weight loss of degraded polystyrene:

The weight of each block was measured using analytical balance. The weight loss of low-density polystyrene by all the Isolates was calculated by using following formula and the results obtained were compared with untreated polystyrene block as control:

Weight loss (%) = (Initial weight – Final weight) x 100/ Initial weight

RESULTS AND DISCUSSION

Isolation of bacteria from mealworm:

The Polystyrene degrading bacteria were isolated from mealworm gut on Beef Dextrose pentose Agar (BDP) and Luria Broth (LB) agar media. They were purified and preserved for further studies. The isolates were tentatively identified as *Staphylococcus aureus, Moraxellaspp, Bacillus spp, Pseudomonas aeruginosa* and Micrococcus spp. The isolates were designated as PDB1, PDB2, PDB3, PDB4, and PDB5 (Polystyrene degrading bacteria). The results show a study of the physical characteristics of various tentative 5 isolates using Berger's manual volumes I and II and they were very designated as tentatively studied using morphological cultural characteristics, Gram nature of Isolates, biochemical test, and estimation of weight loss gradually at regular intervals.

The bacteria where isolated from dissected gut of mealworm by spreading on respective media. The isolates were identified by morphological, cultural, biochemical and characteristics and designate as PDB1, PBD2, PDB3, PDB4 and PDB5 From the morphological, cultural, biochemical and characteristics obtained isolate obtained tentatively identified *Staphylococcus aureus, Moraxellaspp, Bacillus spp, Pseudomonas aeruginosa and Micrococcusspp.* Amongst *Staphylococcus aureus, Moraxella spp, Bacillus spp, Pseudomonas aeruginosa and Micrococcus spp., Pseudomonas aeruginosa* washaving more potential of degradation of polystyrene than all other isolates. From Mealworm gut, degrading Polystyrene degrading bacteria were isolated on Beef Dextrose pentose Agar (BDP) and Luria Broth (LB) agar. They were purified and preserved for further studies on the slants of above media at refrigeration.

Table-1: Morphological, Cultural Characteristics & Gram Nature of Polystyrene Degrading Isolates:

Colony characteristics:

Sr .No	Tentative Identification of Isolates	Texture	Margin	Colour	Elevation	Isolate D esignations
1	*Staphylococcus aureus*	Smooth	Entire	White	Convex	PDB1
2	*Moraxella spp*	Smooth	Entire	Grayish white	Convex	PDB2
3	*Bacillus Spp.*	Rough	Irregular	White	Flat	PDB3
4	*Pseudomonas aeruginosa*	Smooth	Irregular	Grayish white	Flat	PDB4
5	*Micrococcus spp*	Smooth	Entire	Cream colour	Convex	PDB5

Table -2: Biochemical Test of Isolates

Isolates	Gram nature	Motility	Cell morphology
PDB1	Gram –Positive	Non motile	Cocci in grapes like bunch
PDB2	Gram –Negative	Non motile	Small cocci
PDB3	Gram –positive	Motile	Rod shapes
PDB4	Gram –Negative	Motile	Rod shapes
PDB5	Gram –Positive	Non motile	Cocci shapes tetrad

		PDB1	PDB2	PDB3	PDB4	PDB5
Sugar fermentation	Glucose	+	+	+	+	+
	Lactose	+	+	+	_	+
	Mannitol	+	+	+	+	+
Test	Oxidase	_	+	_	+	+
	Catalase	+	+	+	+	+
	Gas production	_	+	_	+	+
	Urease Hydrolysis	+	_	_	_	_
	Citrate	_	_	+	+	_
	VP/MR	+/+	+/+	+/+	-/-	+/+
	Indole	_	_	_	_	_
	Hemolytic		+ (Alpha)		+ (Beta)	

++ positive test, - + Negative test

1. **Percent weight reductions of polystyrene after 10, 15, 20, 25 and 30 days are shown in Table-3.**

Table-3: The Percent Weight Reduction of Polystyrene by Isolates after 10 Days

Isolate No.	Initial wt. of Polystyrene (in g)	Final wt. Of Polystyrene (in g)	Difference in Polystyrene wt. -g	Residual Weight of polystyrene (in Percentage)
Control	1	1	0	100
PDB 1	1	0.891	0.109	89.1
PDB 2	1	0.974	0.026	97.4
PDB 3	1	0.887	0.113	88.7
PDB 4	1	0.781	0.219	78.1
PDB 5	1	0.791	0.209	79.1

Table-ब्र The Percent Weight Loss of Polystyrene by Isolates After (15 days)

Isolate No.	Initial wt. of Polystyrene (in g.)	Final wt. Of Polystyrene (in g.)	Difference in Polystyrene wt. -g	Residual Weight of polystyrene (in Percentage)
Control	1	1	0	100
PDB 1	1	0.872	0.128	87.2
PDB 2	1	0.954	0.046	95.4
PDB 3	1	0.855	0.145	85.5
PDB 4	1	0.728	0.272	72.8
PDB 5	1	0.769	0.231	76.9

Table-5: Isolates After (20 days) in Terms of Weight Loss

Isolate No.	Initial wt. of Polystyrene (in g.)	Final wt. Of Polystyrene (in g.)	Difference in Polystyrene wt. - g	Residual Weight of polystyrene (in Percentage)
Control	1	1	0	100
PDB 1	1	0.845	0.155	84.5
PDB 2	1	0.937	0.063	93.7
PDB 3	1	0.845	0.155	84.5
PDB 4	1	0.740	0.26	74.0
PDB 5	1	0.745	0.255	74.5

Table-6: Isolates After (25 days) in Terms of Weight Loss

Isolates No.	Initial wt .of polystyrene (in gm)	Final wt .of polystyrene (in gm)	Difference in polystyrene wt.	Residual Weight of polystyrene (in %)
Control	1	1	0	100
PDB 1	1	0.833	0.167	83.3
PDB 2	1	0.829	0.171	82.9
PDB 3	1	0.835	0.165	83.5
PDB 4	1	0.718	0.282	71.8
PDB 5	1	0.732	0.268	73.2

Table -7: Isolates After (30 days) In Terms of Weight Loss

Isolates No.	Initial wt .of polystyrene (in gm)	Final wt .of polystyrene (in gm)	Difference in polystyrene wt.	Residual Weight of polystyrene (in %)
Control	1	1	0	100
PDB 1	1	0.822	0.178	82.2
PDB 2	1	0.818	0.183	81.8
PDB 3	1	0.822	0.178	82.2
PDB 4	1	0.710	0.29	71.0
PDB 5	1	0.720	0.28	72.0

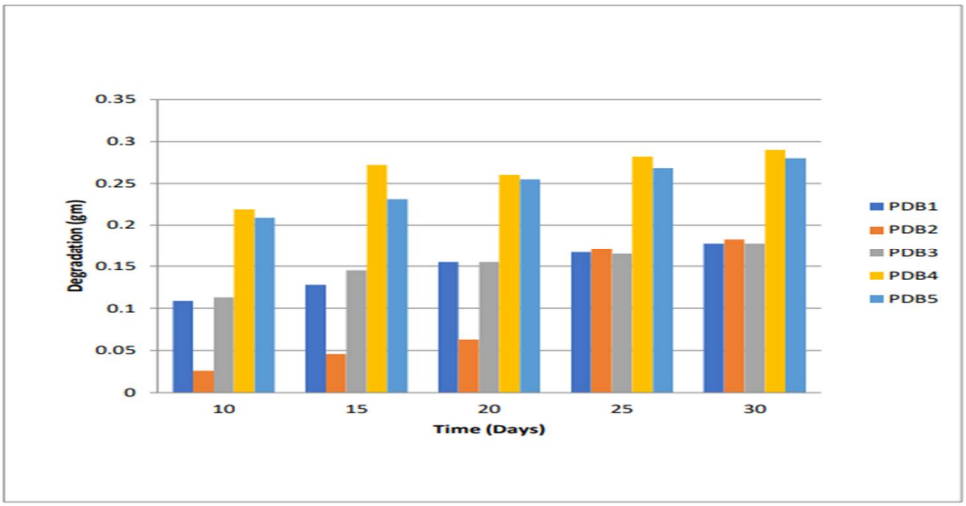
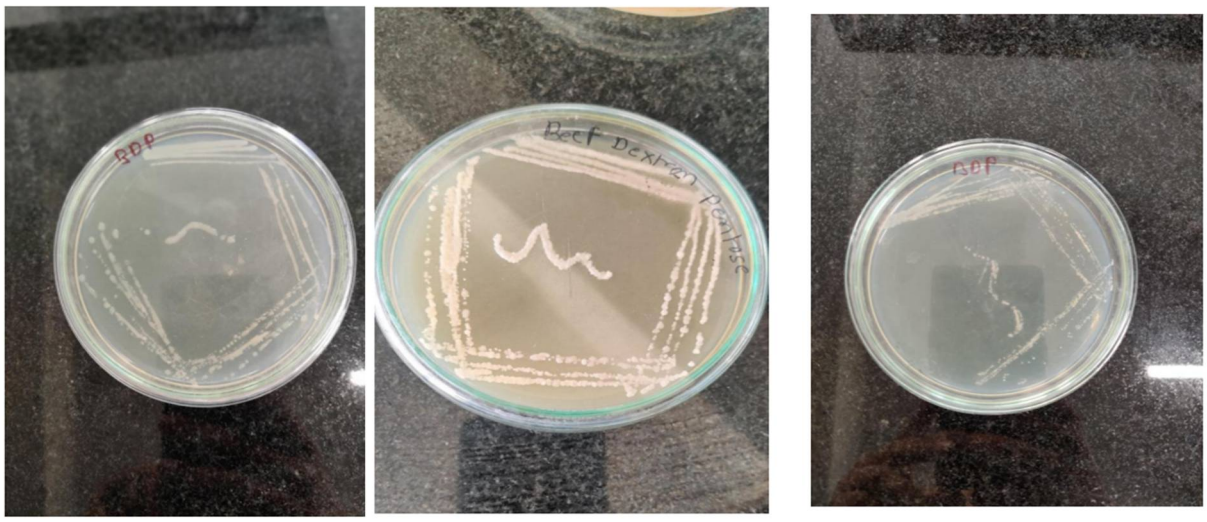


Fig-1: Graphical Representation of Polystyrene Degrading Bacteria throughout Various Time Intervals

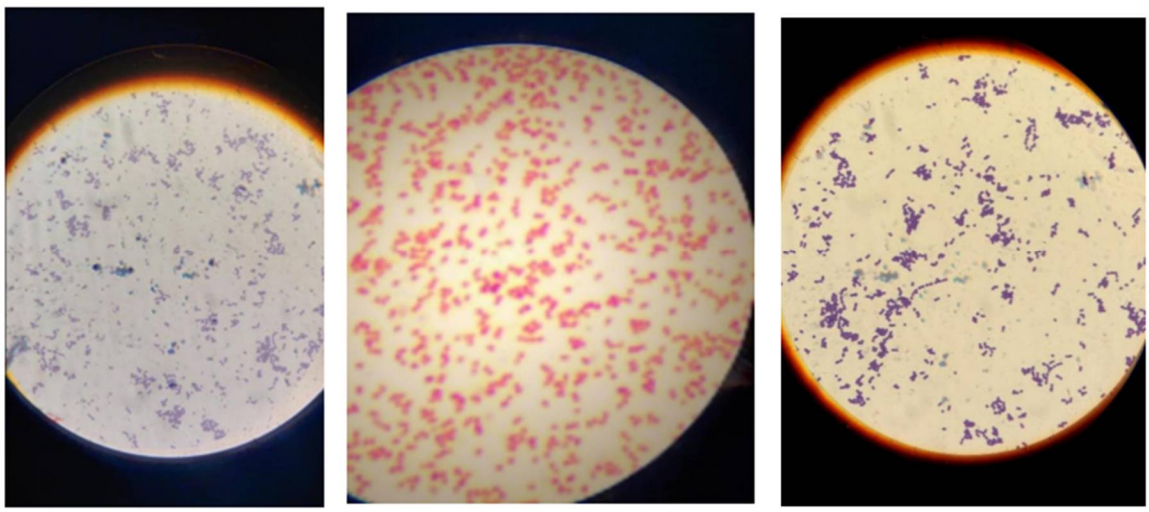
Photoplate-1: Various Bacterial Isolates Obtained After 24 h 30° c Incubation on Beef Dextrose Penrose Agar(BDP):



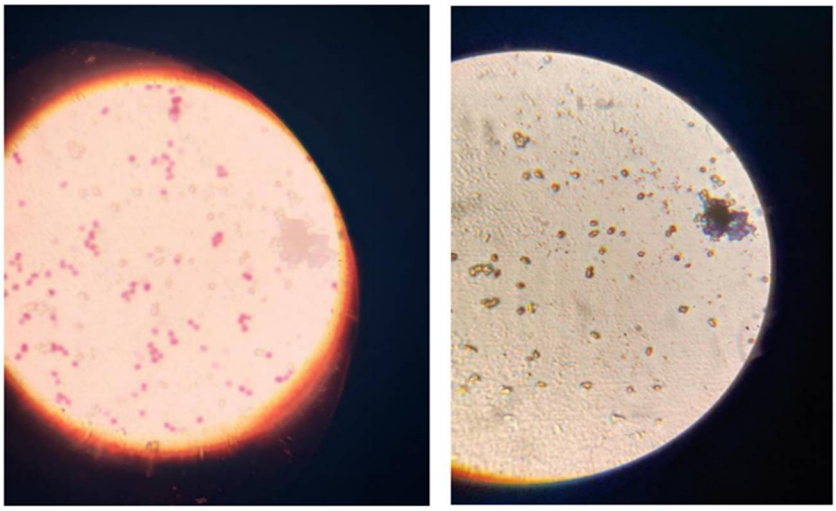
Growth of isolate-PDB1 Growth of isolates –PDB2 Growth of isolates –PDB3

Growth of isolates PDB-4 Growth of isolates PDB-5

Photoplate-2: Microscopic observation of Bacterial isolates



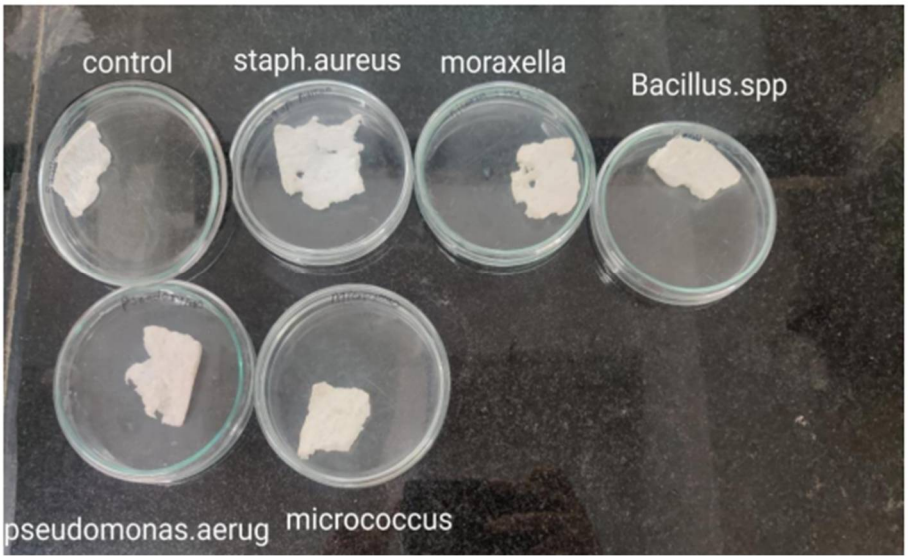
PDB1 PDB-2 PDB-3



PDB -4 PDB-5

Photoplate-3: Polystyrene Degrading By Bacterial Isolates PDB1, PDB2 PDB3, PDB4 and PDB5.

Photoplate-4: Polystyrene Degradation By Five Isolates PDB1,PDB2,PDB3,PDB4 and PDB5



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