



Characterization of *Priestia megaterium S1*, a polymer degrading gut microbe isolated from the gut of *Tenebrio molitor* larvae fed on Styrofoam

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Abstract

This study reveals that *Tenebrio molitor* larvae are fed with two different feeds i.e., barley bran along with Styrofoam, and barley bran without Styrofoam, the survival percentage of mealworms shows 86 and 89%, respectively. Five isolates namely S1, S2, S3, S4, and S5 were isolated from the gut of Styrofoam-feeding *Tenebrio molitor* larvae and tested for Hydrophobicity percentage, clear zone assay and turbidity measurement. S1 isolate showed best (turbidity percentage of 19.65%, 13.54% hydrophobicity percentage, and 37% zone of clearance) when compared to other isolates, respectively. 16S rRNA characterization of S1 isolate revealed that the isolate belongs to *Priestia megaterium S1*(ON024787). Biodegradation of PE and PS beads by *Priestia megaterium S1* makes physical and structural changes over 180 days, after microbial adhesion to the beads. Growth parameters have shown that the *Priestia megaterium S1* thrives more effectively in the pH (6.5), temperature (28 °C) and at 1.5% LDPE/HDPE/PS concentration there is maximum utilization of carbon and a high percentage survival rate. Significant colonization of the isolate after 30 days over beads of LDPE (52.47%), HDPE (49.26%), and PS (48.11%), respectively. Experimental data revealed that *Priestia megaterium S1* have PE and PS beads degradation capacity, proven by weight loss studies, at 6th-month percentage weight loss of LDPE (36.1%), HDPE (31.9%), and PS (28.6%), the percentage loss of carbon and hydrogen shows higher when compared to control. One month Biological Oxygen Demand (BOD) showed that LDPE (7.4 mg/l), HDPE (7.2 mg/l), PS (6.7 mg/l), and simultaneous studies on CO₂ evolution over LDPE treatment is 5.05 g/l, HDPE (4.26 g/l), and PS (3.91 g/l), respectively. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM) prove the occurrence of biodegradation on the surface of beads. This work highlights that *Priestia megaterium S1* plays a vital role in effectively degrading PE and PS beads.

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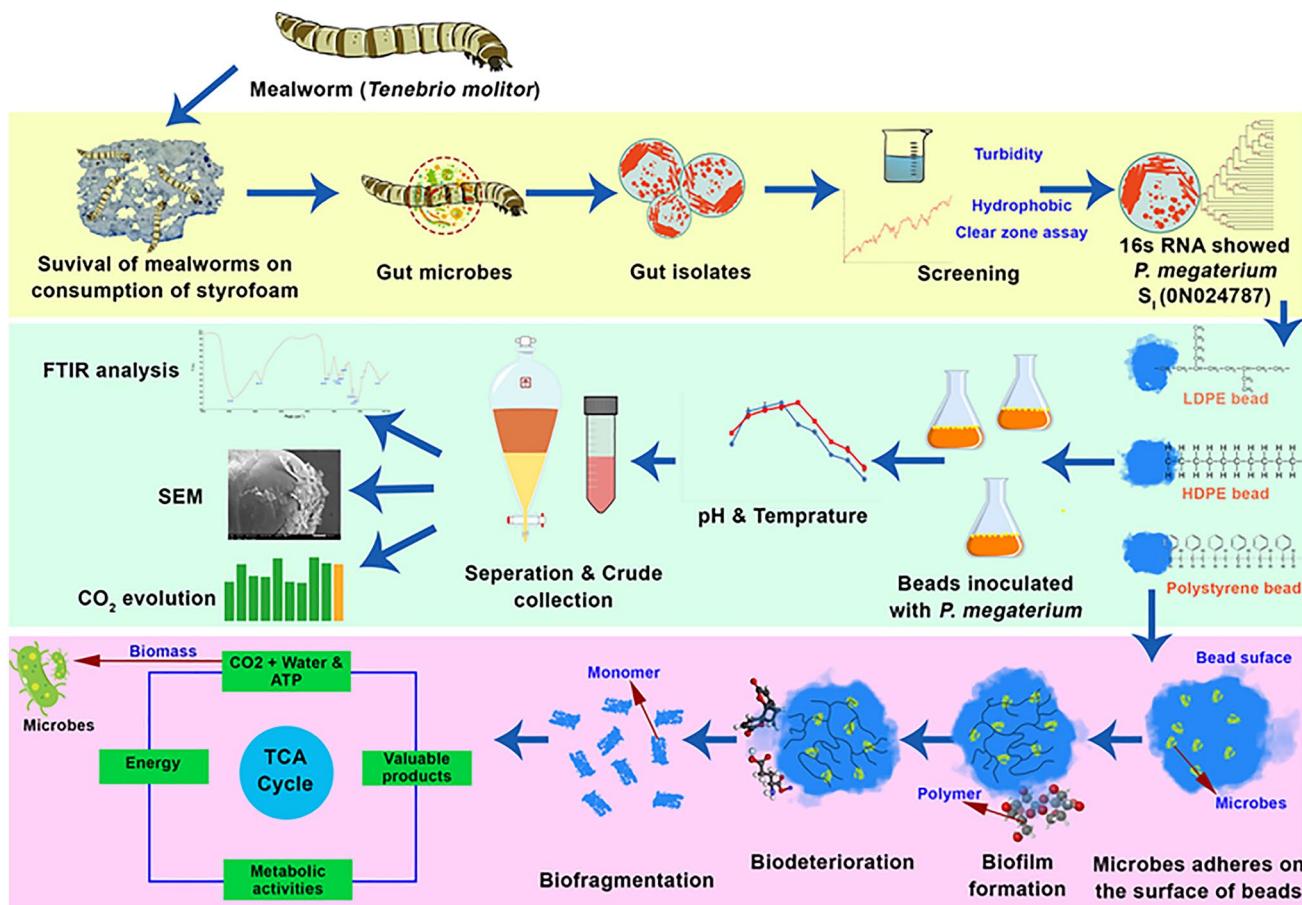
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Graphical abstract



Keywords Biodegradation · Mealworm · Styrofoam · Polyethylene · Polystyrene

Abbreviations

BOD	Biological oxygen demand
FTIR	Fourier Transform Infrared Spectroscopy
HDPE	High-density polyethylene
LDPE	Low density polyethylene
PE	Polyethylene
PM	<i>Priestia megaterium</i>
PS	Polystyrene
PVC	Polyvinyl chloride
SEM	Scanning Electron Microscope
SF	Styrofoam
TM	<i>Tenebrio Molitor</i>

Introduction

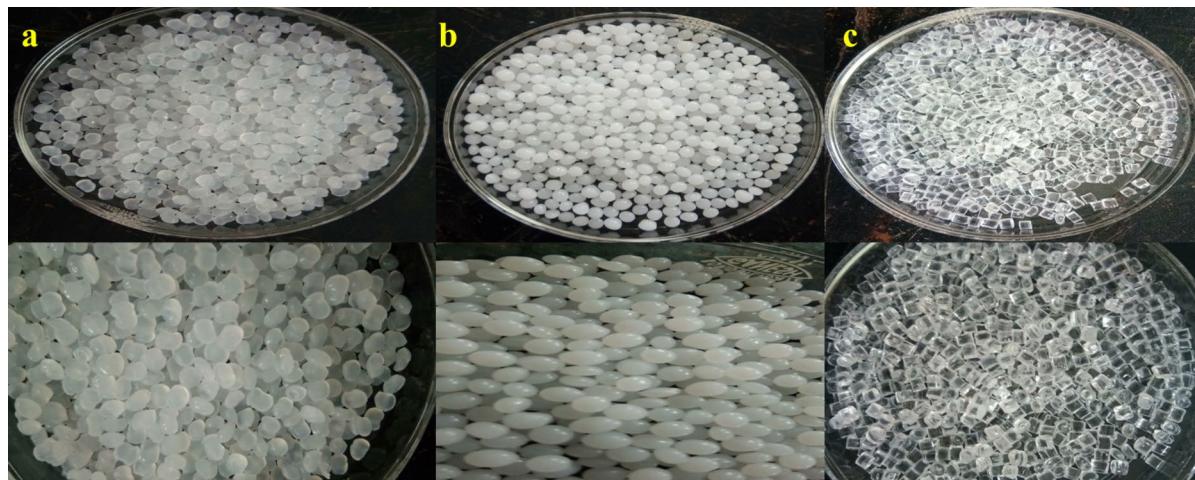
Since 1950, plastics have been manufactured and utilized extensively. By 2020, the worldwide output of plastics had already reached 400 million tons (Mt), by 2035 and 2050, it

may have increased to 800 Mt and 1600 Mt, respectively (Ali et al. 2022; Mastropetros et al. 2022). Plastic is inexpensive, very adaptable, and has several properties that make it suitable for many uses. But these characteristics have also made it an environmental problem. Due to their low cost, hardness, durability, and lightweight, plastic materials have become a necessary component of daily life. Petroleum-based plastics, such as Polystyrene (PS) and Polyethylene (PE), are widely utilized in the construction, automotive, medicinal, culinary, and agricultural sectors (Sánchez 2020). Plastics are widely used because they are affordable, strong, and lightweight materials. Only 18% of plastic garbage gets recycled globally, with an estimated 58% of it being landfilled or directly discharged into the environment (Chamas et al. 2020).

PS and PE are exceedingly tenacious and biodegrade at a very slow pace in natural environments. As a prerequisite for the plastics to be effectively biodegraded, pre-treatments such as photolysis or thermolysis are thought to be necessary (Geyer et al. 2017). Low density polyethylene (LDPE) (used

Table 1 Specification of LDPE, HDPE, and PS beads

S. no	Name of the beads	Molecular formula	Appearance	Identity (IR)	Melt flow index (g/10 min)	Melting point °C
	LDPE	- $(\text{CH}_2-\text{CH}_2)_n-$	White beads or pellets	Conforms	20–30	116
	HDPE	- $(\text{CH}_2-\text{CH}_2)_n-$	White beads or pellets	Conforms	12	125–140
	PS (PS)	- $[\text{CH}_2-\text{CH}(\text{C}_6\text{H}_5)]_n-$	Colorless or translucent beads	Conforms	2.5–3.5	210

**Fig. 1** Shows the **a** LDPE beads, **b** HDPE beads, and **c** PS beads

as wrapping material, food packaging, etc.,) is a flexible PE with special flow characteristics that make it suitable for shaping into films. Due to its high ductility and low tensile strength, it may be stretched to a great extent before breaking. High-density polyethylene (HDPE) (used as trash cans, shampoo bottles, jugs, etc.,) is very thick, dense, crystalline, highly durable, resilient, and impact resistant. PS (used as furniture, water cups, utensils, etc.,) is a thermoplastic that solidifies but melts at around 210 °C. Once melted, PS can be molded into new shapes and then cooled until solid, making it an excellent material for recycling. PS is currently regarded as harming human health and as a suspected carcinogen due to its toxicity.

One of the most popular synthetic polymers is PE, for many years, PE waste contamination has been a problem for both the environment and human health. The most effective and environmentally conscious method of handling plastic trash is biodegradation (Elsamahy et al. 2023). However, given their extremely poor environmental degradation, the problem of plastic pollution is becoming an ongoing concern for everyone (Verschoor et al. 2022). Recent years indicate a rise in studies and an appetite for the biodegradation of plastic-based products, which is facilitated by the action of bacteria and fungus. According to research, these organisms can break down plastics, when combined with insects, and may break down natural polymers into smaller pieces

that help enzymatic processes (Oliveira et al. 2019; Brandon et al. 2020). Although some of them still exhibit poor biodegradation capacity, numerous microorganisms, such as bacteria, fungi, and algae, are effective in degrading plastic.

In recent years, organisms that consume plastic have been discovered to include yellow mealworms *Tenebrio molitor* (TM) larvae, super worms (*Zophobas atratus* larvae), wax moths (*Galleria mellonella* larvae), honeybees, and snails (*Achatina fulica*) (Liu et al. 2020a; Song et al. 2020; Yang et al. 2021; Wang et al. 2022). The scientific field is paying increasing attention to the edible insect TM from the family Tenebrionidae and Order Coleoptera, which is an important tool for the bioconversion of organic waste and plastic, because of its highly diverse gut microbiota (Sangiorgio et al. 2021). According to (Wu and Criddle 2021), TM is one of the few insect species capable of breaking down the lignocellulose matrix of plastic waste composed of Polyvinyl chloride (PVC), (PS), and refractory cellulose waste like cardboard. TM biomass can be altered into a range of materials with marketable utilization, such as feed and food, biomaterials, chitin, chitosan, fertilizers, and biofuels. This can help ensure the viability of the proutilization induction process and, as a result, the transition to a model of circular economy (Azagoh et al. 2015; Ojha et al. 2020). It is yet unknown how polymers are metabolized by insects that consume plastic. Recently, yellow mealworms that have consumed PS were shown to include its

oligomers (such as trimers) and monomers (such as styrene) as well as its derivatives (such as acetophenone, cumyl alcohol, and -methyl styrene) (Tsochatzis et al. 2021).

This study aims to assess the biodegradation of PE and PS by exposing them to gut bacterial strain *Priestia megaterium* (PM) S1 of Styrofoam (SF) fed *TM* larvae. Physical changes of PE and PS beads are characterized by weight loss studies, carbon and hydrogen analysis, Biological oxygen demand (BOD), and the Sturm test. Morphological and functional changes are characterized by Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR).

Materials and methods

The experiment was conducted in the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

Model organism

Tenebrio molitor (Indian meal worm) also known as the darkling beetle, comes under the Order: Coleoptera, Tenebrionidae family.

Test materials

Mealworm

Mealworms were purchased from PISCES-The farm Aquarium shop, Kolkata, West Bengal. The average weight of the mealworm is between (65 to 75 mg/worm) and the larvae typically measure about with a long slender structure. Generally, the mealworm feeds on some dead insects, vegetables, fruits, grains, etc., Collected mealworms are fed with, natural barley bran (protein and lipid-rich) purchased from the cooperative store of Chidambaram, Cuddalore district which has a moisture percentage above 60%. The Mealworm larvae are morphologically identified as *TM Linnaeus*.

LDPE, HDPE, and PS beads

Beads were purchased from SISCO CHEM laboratory chemicals and reagents, Thane, Mumbai, and the specifications are shown in (Table 1; Fig. 1).

Survival of mealworm on consumption of SF (feed test)

The laboratory setup and procedures utilized to grow the mealworms in polypropylene (PP) storage containers were the same as those previously described by (Yang et al. 2018). Mealworms were divided into two groups. The first group

was the control (fed only barley bran) and the second group (fed 1.5 g of SF with barley bran). Polypropylene containers are seeded with 100 mealworms maintained at a controlled temperature of 25 ± 1 °C and humidity of 55–60%. Each group's mealworm pupae and corpses of larvae were collected daily, and the bug quantities were kept. Larval survival rates (SRs) were tracked for 30 days through an interval of 5-day. At each measurement point, dead larvae and molts were taken away from the containers to prevent the surviving larvae from swallowing the dead worms (Peng et al. 2022). To eliminate insect waste and uneaten feed from the environment, the feed was replaced every week (Liu et al. 2020b). Loss of PS biomass and the survival of larvae were monitored and calculated.

Isolation of gut bacterial strains of mealworm

Ten larvae were collected in group 2 (fed with SF and barley bran after 30 days). Each larva was rolled on a thin coating of EtOH (2 ml of EtOH in a typical Petri dish) for 6–7 s to eradicate the microorganisms that survived on the outermost layer of the larvae. The bottom of the larva was shattered using two tweezers to pull out the intestine, and the larva was then allowed to squeeze the internal liquids to flow out. Transfer 1 ml of aliquot suspension in 250-ml Erlenmeyer flask with Nutrient broth 100 ml containing (Beef extract 0.3 g; Peptone 0.5 g; NaCl 0.5 g at pH 7.0) were purchased from SISCO CHEM laboratory chemicals and reagents, Thane, Mumbai, India, supplemented with 10 g of LDPE, HDPE, and PS beads respectively, which was shaken on a rotary shaker (120 rpm) at room temperature. The remaining beads were removed from the broth after 60 days, and the enrichment culture was distributed on Nutrient agar plates. According to the standard method of bacterial isolation, colonies were selected and then distributed on sterile Nutrient agar plates after being cultured for 24 h at room temperature (Yang et al. 2015).

Screening of bacterial strains with LDPE, HDPE, and PS beads

Turbidity measurement

Hundred ml of nutrient broth was taken in a sterile 250 ml conical flask and inoculated with five isolates from the mealworm gut namely S1, S2, S3, S4, and S5. And the flasks were incubated at 28 ± 2 °C for 48 h. The turbidity rate was determined on the 1st, 10th, and 20th day, respectively, using a spectrophotometer at 600 nm.

MATH test

It is a basic and widely utilized method to assess how hydrophobic the bacteria or fungus cell surfaces are towards hydrocarbons. NA medium is prepared according to its composition except for its carbon source. LDPE, HDPE, and PS powder are added to the media with the replacement of carbon source, which is necessary for the growth of bacterial gut isolates, and this was performed using the appropriate procedure (Rosenberg et al. 1980). Using a spectrophotometer at a wavelength of 600 nm, the absorbance of the aqueous phase was measured simultaneously before adding toluene and after adding the toluene sample (control). The findings of MATH tests are known as “Cell Surface Hydrophobicity” (CSH), and they are often shown as the proportion of cells that are partitioned into the hydrocarbon phase. The following formula was used to figure out the percentage of hydrophobicity.

$$\text{Hydrophobicity (\%)} = \frac{(\text{OD of initial bacterial suspension} - \text{OD of final bacterial suspension})}{\text{OD of initial bacterial suspension}} \times 100$$

Clear zone assay (Augusta et al. 1993)

Nutrient agar medium had additions with LDPE, HDPE, and PS bead powders, each at a concentration of 0.1% and sonicating the mixture for 1 h. The medium was sterilized for 20 min at 121 °C and 15 lbs/inch² of pressure. The Petri plates were filled with the medium, which was then given time to set up. The cooled medium was inoculated with mealworm gut bacteria and cultured for two to three days at 28 °C. The Coomassie blue solution was applied to the plates and let to stand for 30 min and then add 10% acetone for decolorization. A zone of clearing surrounding the colonies was detected on the plates. The zone of clearance proved the mealworms' gut bacteria's potential for biodegradation.

Sequencing of bacterial strains

Microbial identification was analyzed and sequenced in IND SEQ GENOMICS, Chennai. The genomic DNA was extracted from the bacterial culture using a standard phenol–chloroform extraction procedure. Amplification of the 16S rRNA gene was conducted using universal primers 27F (5'-GAGAGTTGATCCTGGCTCAG-3') and 1495R (5'-CTACGGCTACCTGTTACGA-3') (Weisburg et al. 1991). The reaction mixture (50 µl) had 0.5 µl of each primer, 400 µM of dNTPs, 2.5 U of Taq polymerase, 1.5 mM of MgCl₂, 50 ng of DNA template and 5 µl of 10×PCR buffer. The PCR was conducted at an initial denaturation temperature of 94 °C, 8 min; followed by 28 cycles of 94 °C, 1 min; 58 °C, 1 min; 72 °C, 2 min; and a final extension at 72 °C for 10 min. The similarity search was conducted in silico using the BLAST database of NCBI. The phylogenetic and molecular evolutionary analyses were conducted using MEGA software version 10 (Tamura et al. 2011).

Optimization of the growth parameters for mealworm gut bacterial strains

Isolated mealworm gut bacteria growth conditions are found by the ideal pH, temperature, and carbon supply required for bacteria to thrive in suitable conditions studied. Hundred ml of nutrient broth was taken in a sterile 250 conical flask with 10 g of LDPE, HDPE, and PS beads, and five different pH conditions were provided 6.0, 6.5, 7.0, 7.5, and 8.0 and they were inoculated with mealworm gut bacteria. The same procedure is followed to optimize the growth at different temperatures (26 °C, 28 °C, 30 °C, 32 °C, and 34 °C), respectively. To optimize the carbon sources different concentrations (0.5%, 1.0%, and 1.5%) of each LDPE, HDPE, and PS were taken to determine the optimum carbon source for growth, the flasks were incubated at 28 °C for 24 h and can be determined using a spectrophotometer at 600 nm.

Fig. 2 Larvae feed SF



Fig. 3 Survival percentage of *TM* larvae

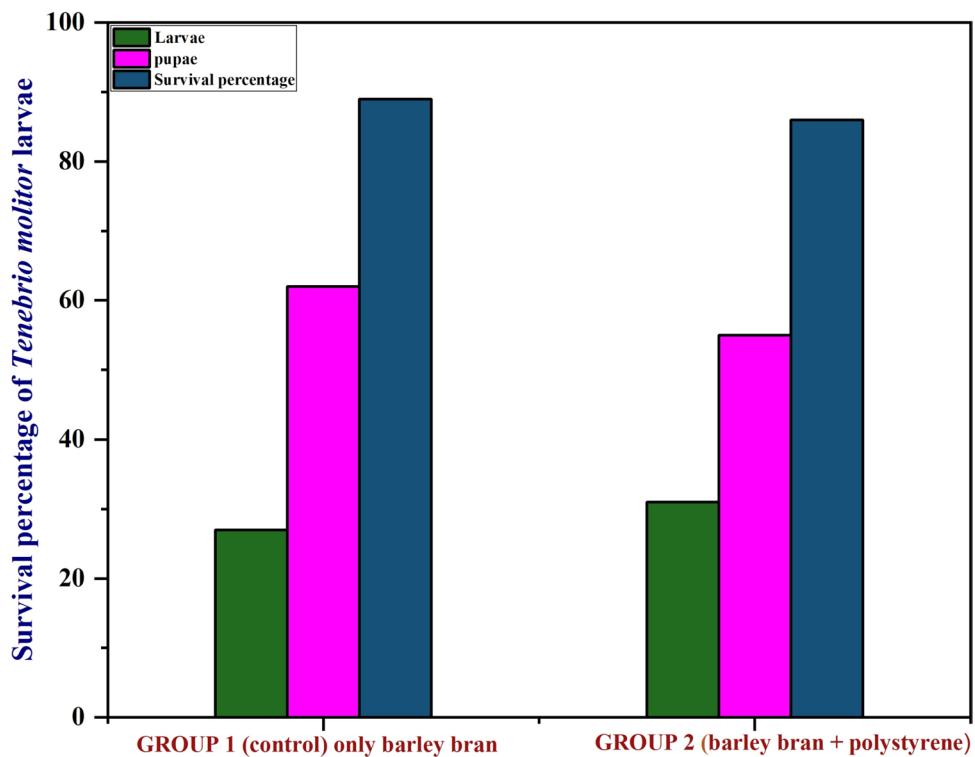


Fig. 4 Turbidity measurement of gut mealworm isolates

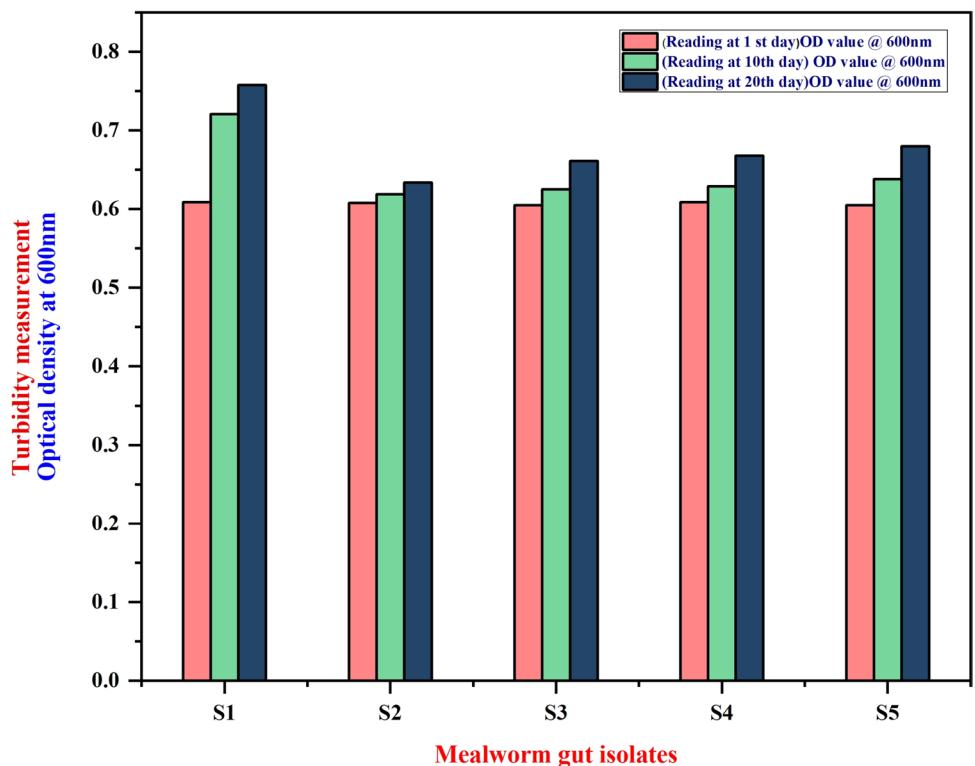
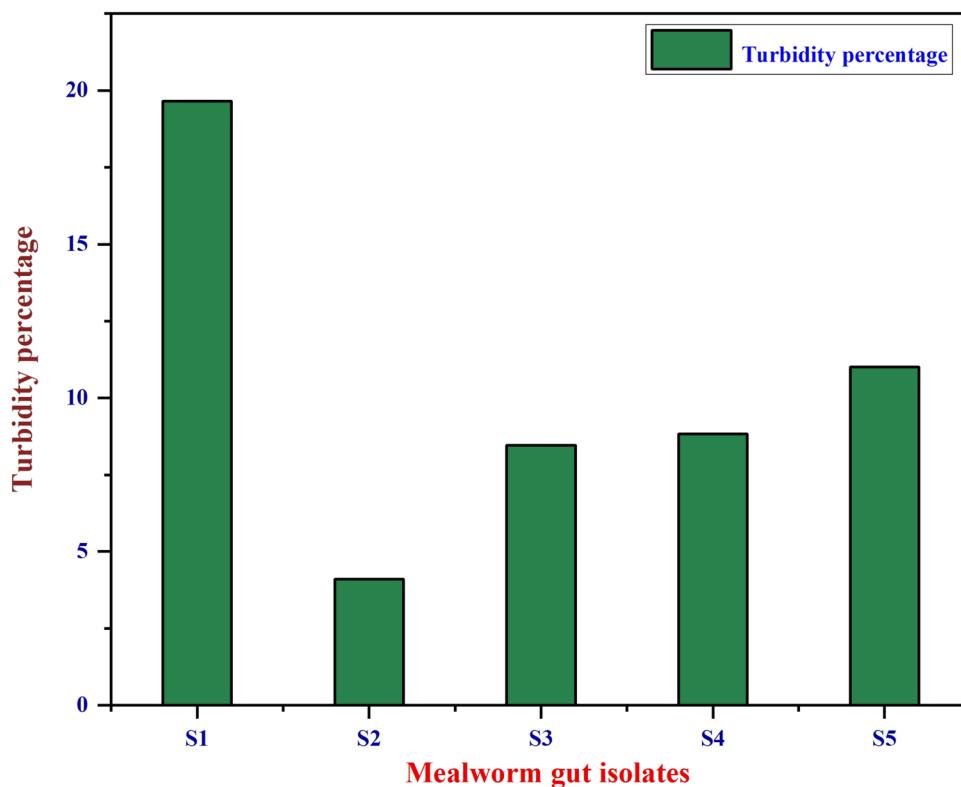


Fig. 5 Turbidity percentage of gut mealworm isolates



Assessing biofilm formation and ability to degrade isolated bacteria

The nutrient broth was used to assess the degradation ability of isolated bacteria and was dispensed into seven test tubes for each bead. After capping, all the test tubes were sterilized at 121 °C for 15 min and allowed to cool. On cooling, 1 g of LDPE, HDPE, and PS beads were added to each test tube and inoculated with drops of isolated bacterial suspension. For each bead control (without inoculation of culture) is maintained. All the tubes were incubated at room temperature 28 °C to 30 °C for 14 days after which each tube was scored for biofilm formation and turbidity indicating utilization of polythene and PS as sole carbon and energy source.

Colonization studies

Colonization studies were done using beads of unique weight and size. 10 g of LDPE, HDPE, and PS beads were weighed and disinfected with 70% ethanol for 30 min and were transferred into 250 ml conical flask containing 150 ml of sterile nutrient broth and inoculated with mealworm gut bacterial isolate and incubated at 28 °C ± 2. Microbial colonies formed clusters, which increased breaching over the surface of beads which exhibits a color change in beads (white to pale yellow), Nutrient broth, and increased the weight of

beads. The colonization of the isolate was observed after 15 and 30 days.

Weight loss studies

The gut mealworm bacterial isolate were inoculated in Nutrient broth containing 10 g LDPE, HDPE, and PS in a 250 ml conical flask. The broth cultures were incubated at 28 °C for 180 days. The degradation was periodically monitored by measuring the weight of the beads. The beads in nutrient broth with mealworm gut bacterial isolate were used as test samples, and the conical flask with beads exposed to media without any bacteria was used as a control. These beads were treated with 2% (w/v) aqueous Sodium Dodecyl Sulphate solution for 4 h while being shaken to remove the bacterial cells and other cell debris (Gilan et al. 2004). Beads were then cleaned using distilled water and 70% ethanol and dried at 70 °C to calculate the percentage of loss of weight using the formula below (Kyaw et al. 2012).

Percentage weight loss

$$= [\text{Initial weight} - \text{Final weight}/\text{Initial weight}] \times 100$$

BOD (Welsh and Smith. 1960)

BOD (Iodimetric method) was done in Department of Chemistry, Annamalai University, India. Alkaline potassium

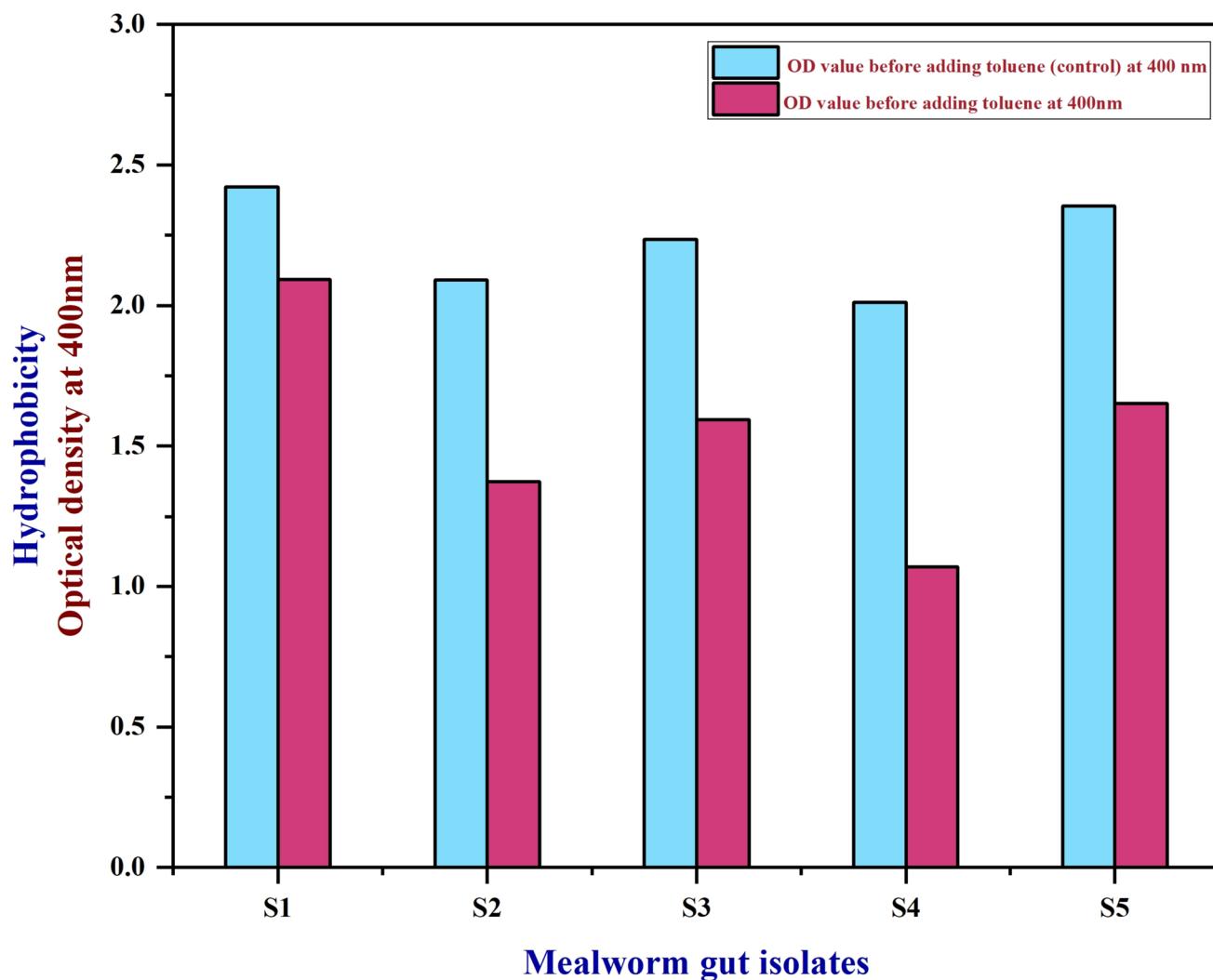


Fig. 6 Measurement of hydrophobicity of Gut mealworm isolates through spectrophotometer

iodide and manganous sulfate were added to the sample. Manganous hydroxide was formed which was oxidized by the dissolved oxygen of the sample to the basic manganic oxide. The liberated oxide, iodine were titrated with a standard solution of sodium thiosulphate using starch as an indicator. 300 ml of sample was taken in a BOD bottle and 2 ml manganese sulphite solution and 2 ml alkali iodine oxide solution were added and allowed to precipitate. The clear supernatant liquid thus obtained was carefully removed and the precipitate was allowed to settle. 2 ml of concentrated sulfuric acid was added to the supernatant and this setup was kept tightly closed for complete mixing. The contents were then titrated with 0.025 N sodium thiosulphate using starch as an indicator.

Carbon and hydrogen in LDPE, HDPE and PS beads after biodegradation by elemental analysis

The micro analyzer was used for the determination of element in LDPE, HDPE, and PS beads after degradation. Carbon, hydrogen, and Sulfur are detected simultaneously using Independent IR detectors. A Thermal Conductivity (TC) detection system is used to monitor nitrogen. The autoloader of the micro-oxygen add-on module was used to load samples for oxygen analysis before they were placed into a high-temperature heating chamber. The oxygen that was released during the heating of the sample was allowed to react with a carbon-rich environment in the furnace to form CO. The CO is swept from the furnace and converted to CO₂ before measurement via an IR detector.

Fig. 7 Hydrophobicity percentage of Gut mealworm isolates

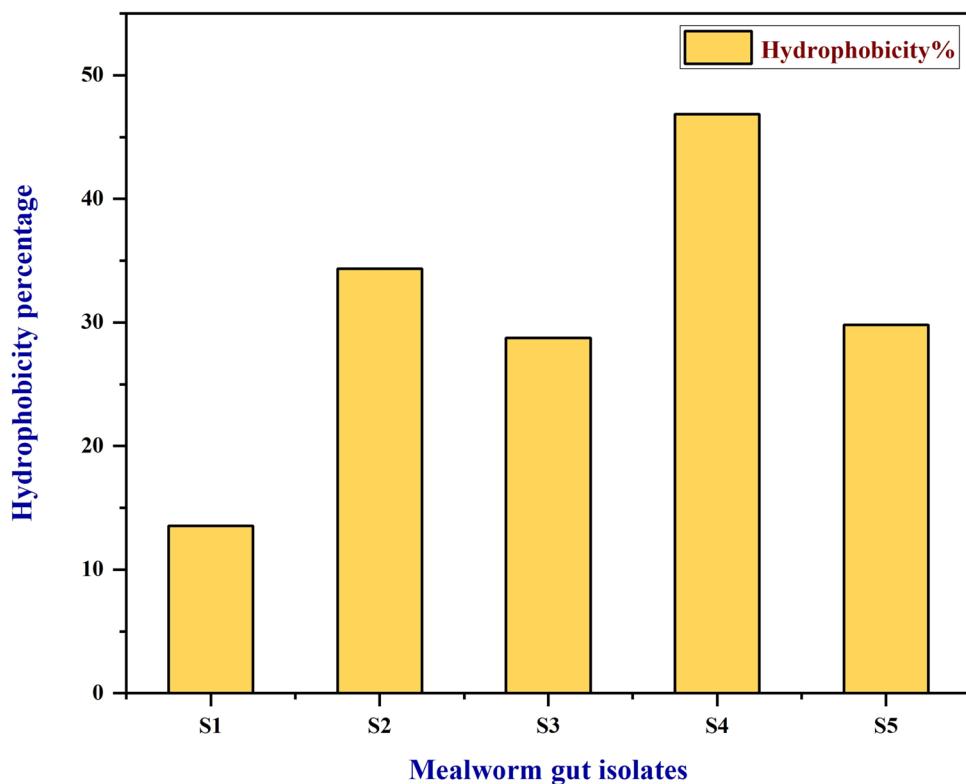


Fig. 8 Dendrogram based on 16 s rRNA gene sequencing showed on *Prestia megaterium* S1 (ON024787), related genera on *Bacillus*, *Metabacillus*, *Cytobacillus* within family Bacillaceae

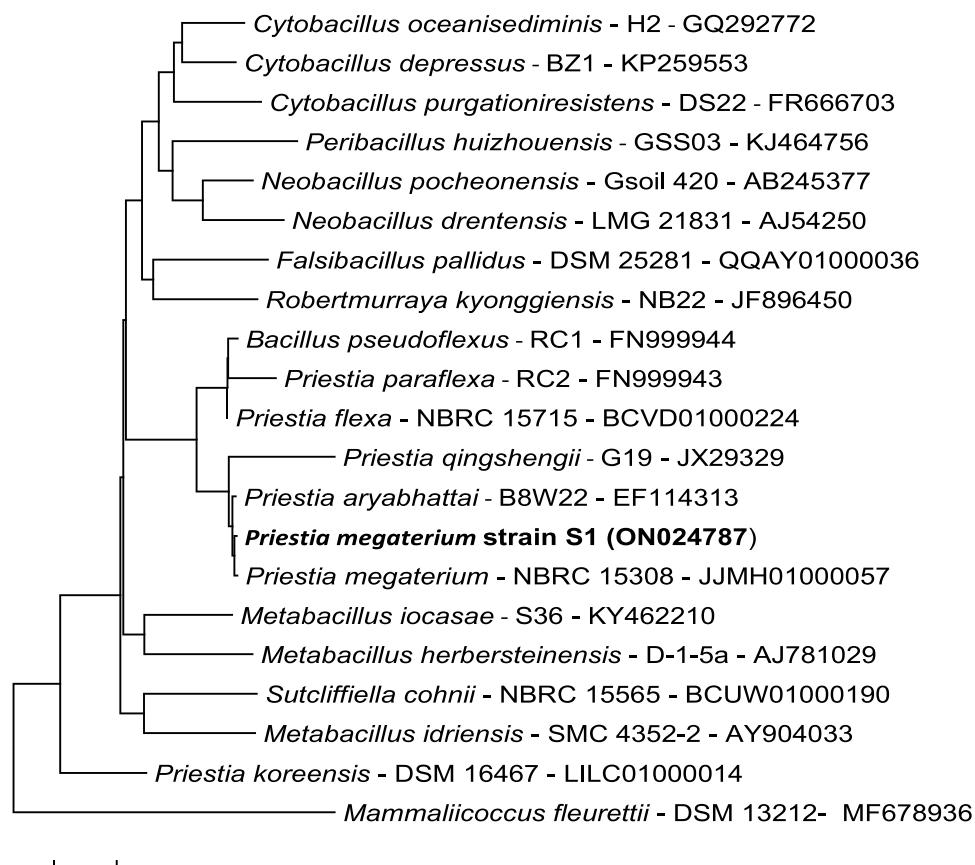


Fig. 9 Growth measurement of *PM S1* at different pH

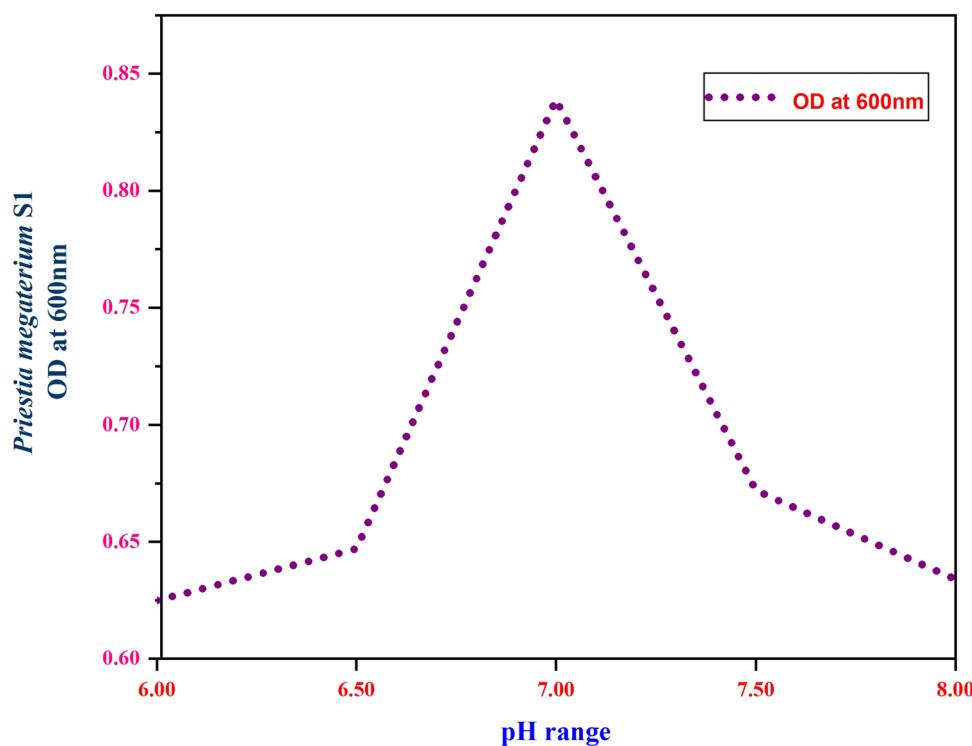
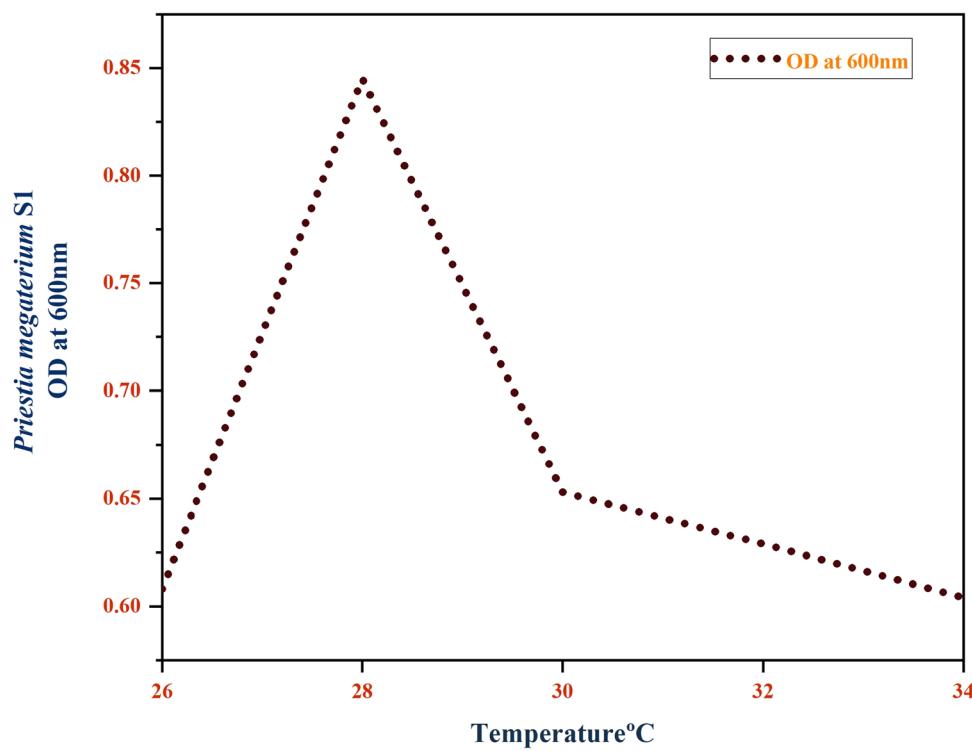


Fig. 10 Growth measurement of *PM S1* at different temperature

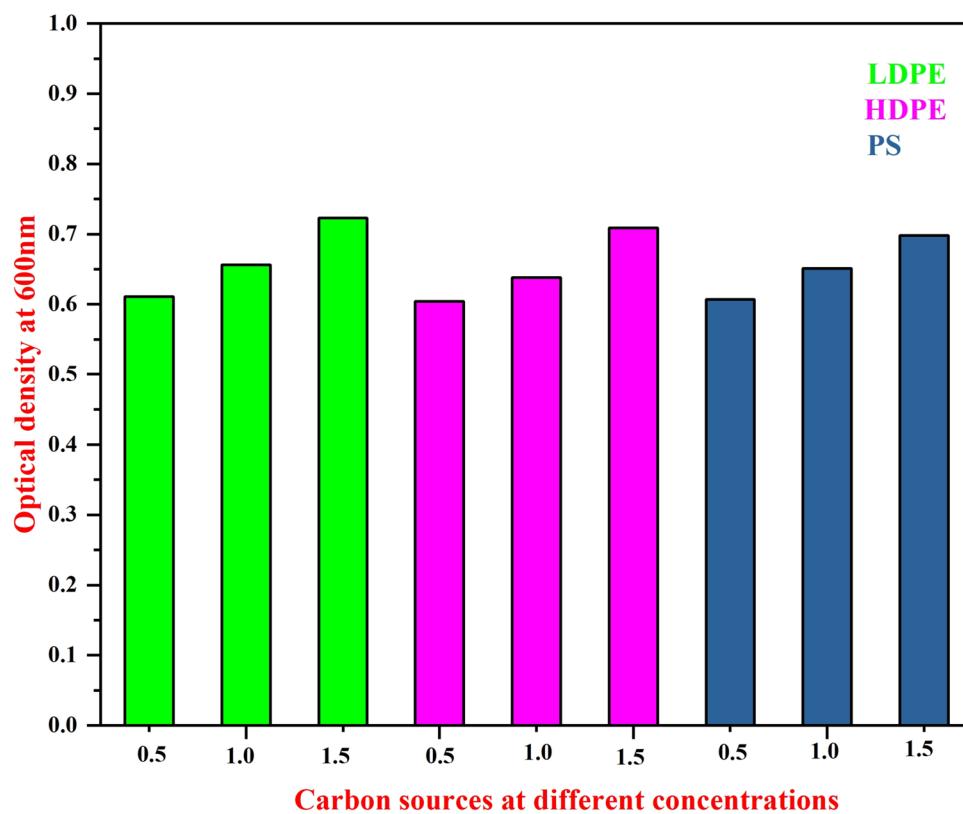


FTIR analysis

FTIR spectroscopy analysis is done in the Department of Chemistry, Annamalai University, Annamalai Nagar, Chidambaram. performed for LDPE, HDPE, and PS beads

before and after the treatment. Agilent technologies at the resolution 8 Range from 4000 to 650 nm were performed to identify the spectrum.

Fig. 11 Survival of *PM* over different concentrations of carbon sources



SEM analysis

SEM analyses were done in the Centralised Instrumentation and Service Laboratory (CISL), Department of Physics, Annamalai University, Annamalai Nagar, Chidambaram. Changes in surface structure and the pattern of texture of LDPE, HDPE, and PS beads after microbial degradation were analyzed by SEM. Distortion in bead surfaces like biofilm formation, adherence of microorganisms, and erosion of beads were examined.

Sturm test

Sturm test was used to determine the CO₂ evolution because of plastic biodegradation (Mueller et al. 1992). 5% inoculum of the isolates was used for the degradation analysis. The culture inoculums for the bacterial isolates were produced in Nutrient and MSM broth, respectively. The test flask, which contained 100 mL of enrichment medium meant for the isolates and without any additional carbon source added pieces of polymer films. As a control, a medium without polymer films was used. For four weeks, the test was carried out at room temperature. The amount of carbon dioxide generated in the test and control flasks was determined gravimetrically after 4 weeks of incubation. The CO₂ that was released because of the breakdown

of the polymeric chain was contained in an absorption flask containing 1 M KOH. Barium chloride solution (0.1 M) was added to the flask consequently, barium chloride precipitates (using CO₂ generated from the breakdown of polymer) were produced. By measuring the amount of CO₂ precipitates produced by the addition of BaCl₂, the amount of CO₂ generated was gravimetrically determined. There were modifications in the test flask result.

Result and discussion

Characterization of SF fed with TM (feed test)

Mealworms may choose to balance their diet ratio and consumption by their nutritional demands (Rho and Lee 2014) and are able to receive bran, which has all the essential nutrients but not in adequate amounts, therefore supplementing the diet is helpful at distinct phases of development (Morales-Ramos et al. 2010). In the mealworm enterprise and laboratory rearing facilities, a diet made up of bran, a water supply (such as fresh vegetables like carrot, apple, potato, or cabbage), and/or a protein source (such as beer yeast, casein, or soy protein) is the most typical diet (Ribeiro et al. 2018). In this study, mealworm was fed with barley bran along with PS visual examination of the PS feedstock and frass resulting in an assessment of the biodegradation

Fig. 12 Colonization of *PM SI* over LDPE, HDPE, and PS bead

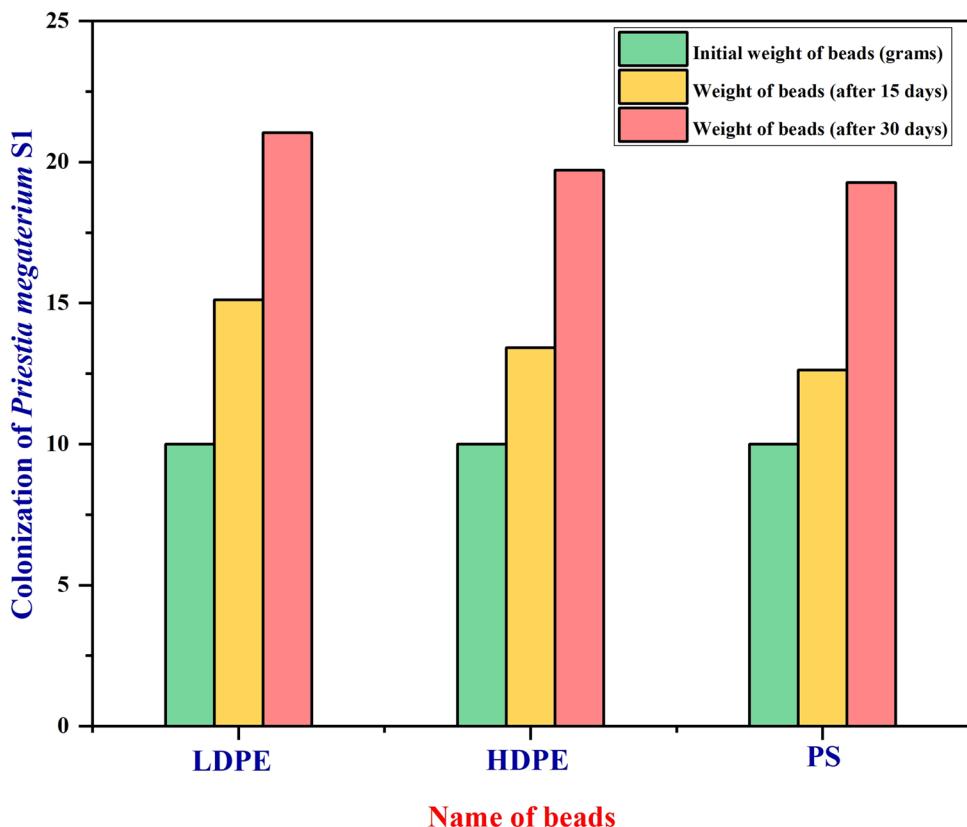


Fig. 13 Colonization percentage of *PM SI*

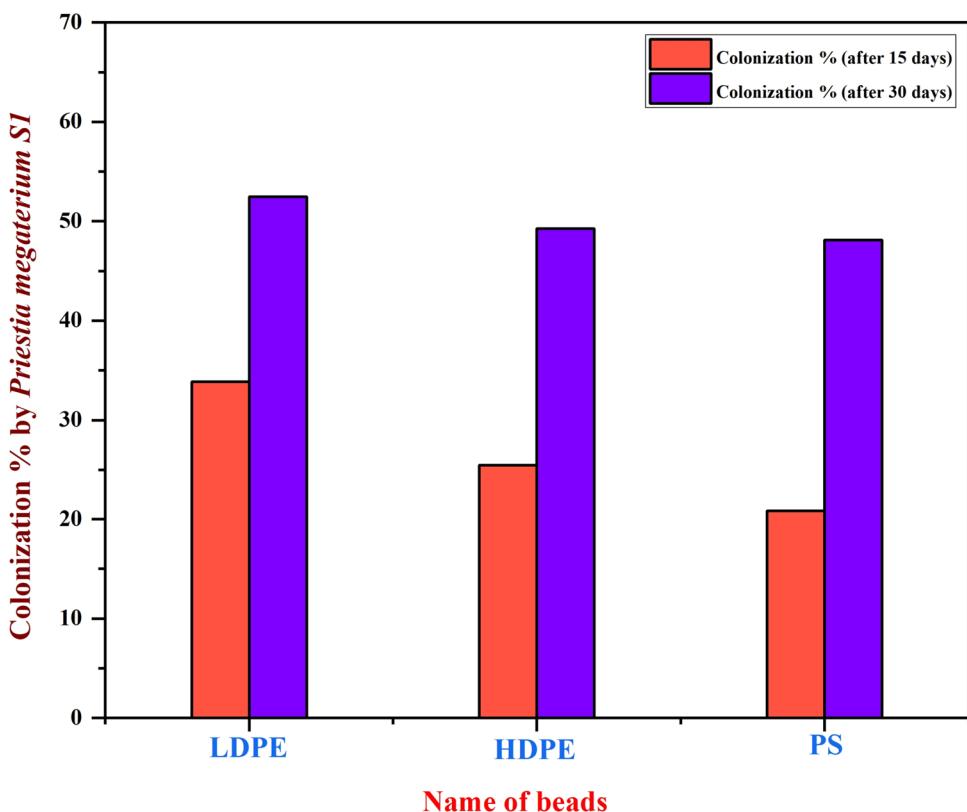


Table 2 Weight loss studies (2nd, 4th, and 6th month)

	2nd month			4th month			6th month						
	Initial weight (g)	Final weight (g) mean value	Weight loss (g) loss	% of weight loss	Initial weight (g)	Final weight (g) mean value	Weight loss (g) loss	% of weight loss	Initial weight (g)	Final weight (g) mean value	Weight loss (g) loss	% of weight loss	
LDPE	Treated with PM	10.00	8.42	1.58±0.1	15.8	10.00	7.75	2.25±0.5	22.5	10.00	6.39	3.61±0.5	36.1
HDPE	Treated with PM	10.00	8.61	1.39±0.5	13.9	10.00	7.93	2.07±0.2	20.7	10.00	6.81	3.19±0.2	31.9
PS	Treated with PM	10.00	8.77	1.23±0.1	12.3	10.00	8.16	1.84±0.1	18.4	10.00	7.14	2.86±0.1	28.6
LDPE, HDPE, PS	Control	10.00	10.00	0.00	0	10.00	10.00	0.00	0	10.00	10.00	0.00	0

and depolymerization of ingested PS. The hollows found on the SF in group 2 indicate that the mealworms were consuming SF for food, which indicates their ability to live when given SF as their only source of carbon. The visual change indicates the rupture on the SF surface, holes, irregular shapes, cracks, and roughening of the surface as shown in (Fig. 2). SF was quickly consumed by mealworms from all sources after being fed. Mealworms that were 2.0–2.5 cm long were examined to be actively feeding, leaving crevices in the SF blocks (Yang et al. 2015). There is a gradual decrease in the mass of PS in group 2 and the number of larvae surviving in each group was recorded after 20 days and shown in (Fig. 3).

The results showed that out of 100 mealworms seeded in each group, only 11 died in group 1 and 14 died in group 2 and slowly developed pupae, the percentage of survival rate of group 2 (86%) was slightly decreased when compared to control. This study implies there is a minimal death rate while feeding SF, hence these mealworms' gut microbes can be used for the biodegradation of plastics in safer environments.

Isolation of gut bacterial strains of TM

Bacterial isolates were isolated from the gut of the SF-eaten mealworm and the colonies were observed in the Nutrient agar plates. *Serratia sp.*, *Staphylococcus sp.*, and *Rhodococcus sp.* were identified and associated with both PS and CS diets by (Mamtimin et al. 2023). The bacterial colonies were picked up and purified by streaking on solidified Nutrient agar plates and in slants. Among them, five isolates were selected based on cell morphology which included the shape, colour and size for further degradation studies.

Screening of bacterial strains

All the isolated mealworm gut bacteria cannot degrade PE and PS, so they were screened to analyze their ability to degrade polythene using a Nutrient agar medium. Screening of these isolates was done by comparing their growth ability. The isolated mealworm gut bacteria were screened for the ability to degrade PE and PS beads by the following assays.

Turbidity measurement

To identify the turbidity rate, S1, S2, S3, S4, and S5 isolates were tested in Nutrient broth, based on the spectrophotometer reading at 600 nm on the 1st, 10th, and 20th day, isolate S1 grew more vigorously and formed biofilm than the other isolates. Turbidity percentages show that S1

Fig. 14 Comparison of percent of weight loss of beads after gut bacterial degradation for 6 months

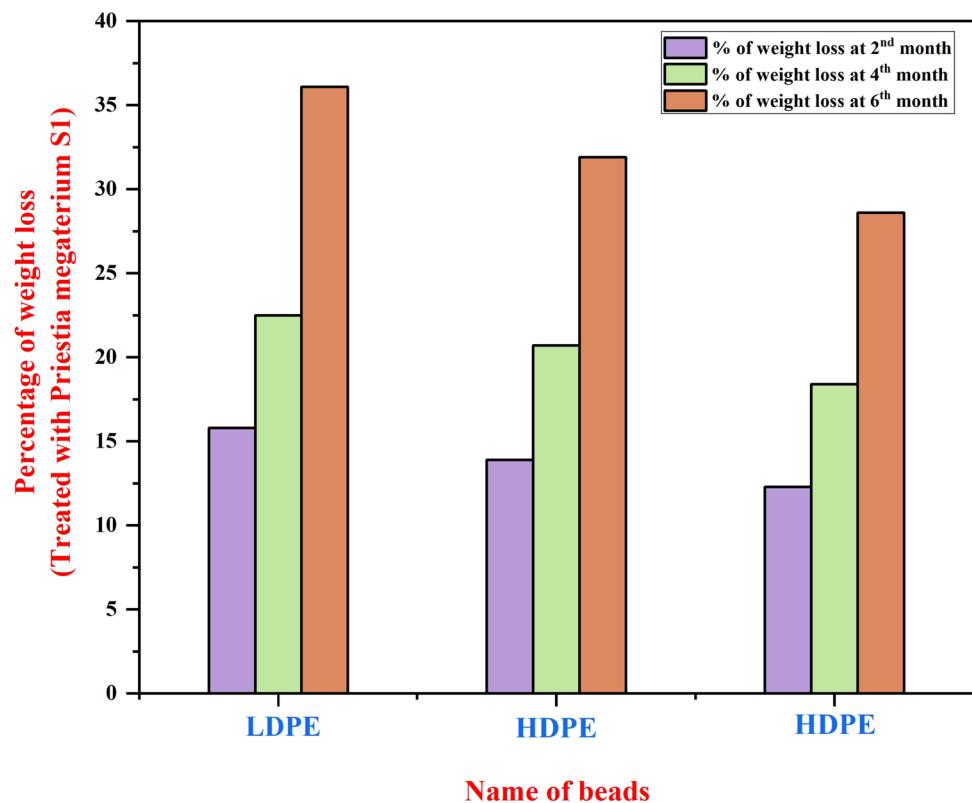


Fig. 15 Effect of Carbon of PM over LDPE, HDPE, and PS beads

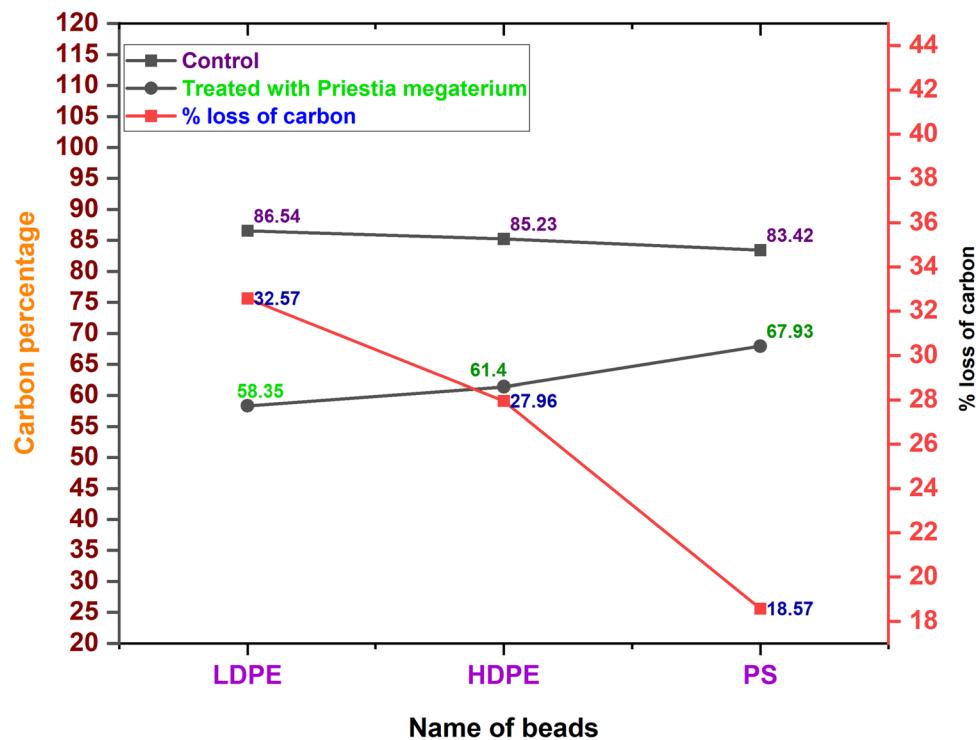
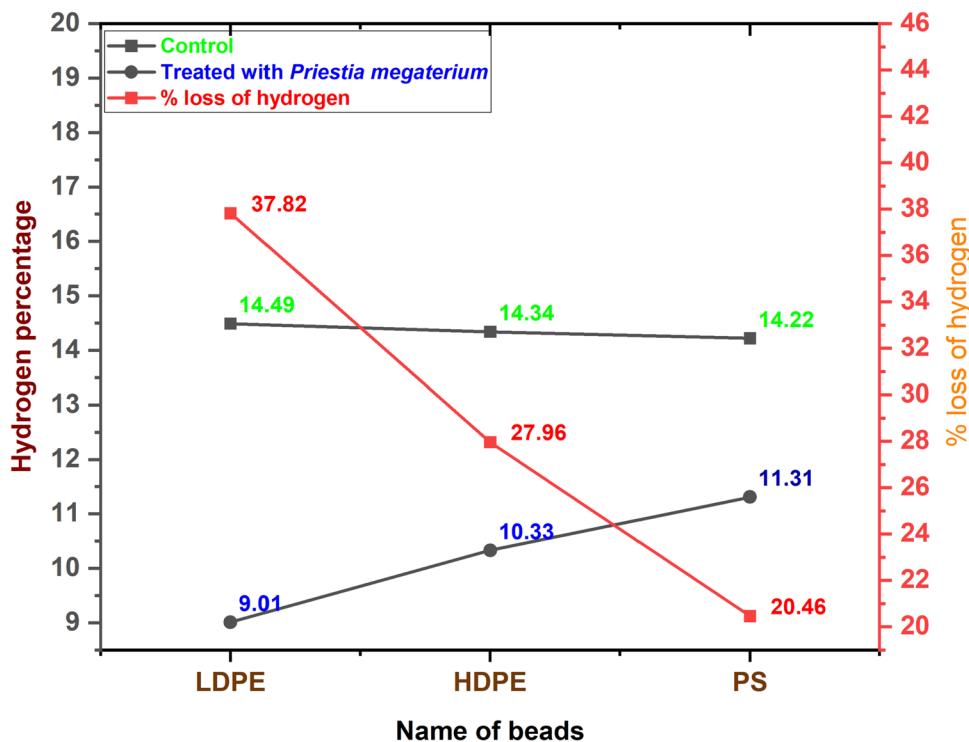


Fig. 16 Effect of Hydrogen of *PM S1* over LDPE, HDPE, and PS beads



isolate form more turbid than the other isolates were shown in (Figs. 4 and 5). High turbidity indicates the high growth rate of bacteria and its effect on adhering over bead surface will be high.

MATH test

According to (Tribedi and Sil 2013) the hydrophobicity of a cell surface impacts bacterial adherence and controls the rates at which non-polar polymers degrade. In carbon-starved conditions, the bacterial surface becomes more hydrophobic and stickier than in non-starved conditions. (Kavitha and Bhuvaneswari 2021). Effective biofilm production often follows a surface's significant bacterial colonization (Gupta et al. 2022). Isolate which was able to degrade PE and PS beads by utilizing their carbon sources as nutrients showed growth on NA medium plate. The bacterial isolates that showed the highest growth on the Nutrient agar medium Petri plate were selected for biodegradation selected beads. Isolated bacteria were designated as S1 to S5. Out of five isolates, S1 showed effective growth on a nutrient agar medium. Five isolates of bacterial strain were designated among them S1 showed effective growth, more adhesion and hydrophobicity and thus showed the proportion of cells partitioned into the hydrocarbon phase. During this experiment, the high hydrophobicity of isolates caused a substantial quantity of biofilm to grow on the bead surface. When strain CGK5 hydrophobic cells were transferred from the aqueous phase to the hexadecane, the turbidity was reduced

by 22.05% at a concentration of hexadecane of 0.2 ml (Gupta et al. 2023). Hydrophobicity values describe the efficiency of isolated mealworm gut bacteria in attaching to hydrophobic surfaces like LDPE, HDPE, and PS. The results of the hydrophobicity of isolated bacteria are represented in (Figs. 6 and 7).

Clear zone assay

The development of the clear zone denotes the production of exo-enzymes, which always results in the solubilization of the polymer, the clear zone test is a relatively straightforward and practical approach for screening bacteria that break down PE (Augusta et al. 1993). Gut bacterial strains of mealworm were screened by clear zone assay to determine their potential in the biodegradation of PE and PS beads. The mealworm gut bacteria were inoculated onto Nutrient Agar medium supplemented with 0.1% LDPE, HDPE, and PS as the only carbon source. Out of five bacterial isolates S1 (37%) was effectively found to biodegrade and utilize it as a source of carbon forming a zone of clearance. S1 isolate produces extracellular enzymes to break down the LDPE, HDPE, and PS thus forming a zone of clearance. This is the preliminary test to determine the biodegradation of PE by the gut microbiome of mealworms.

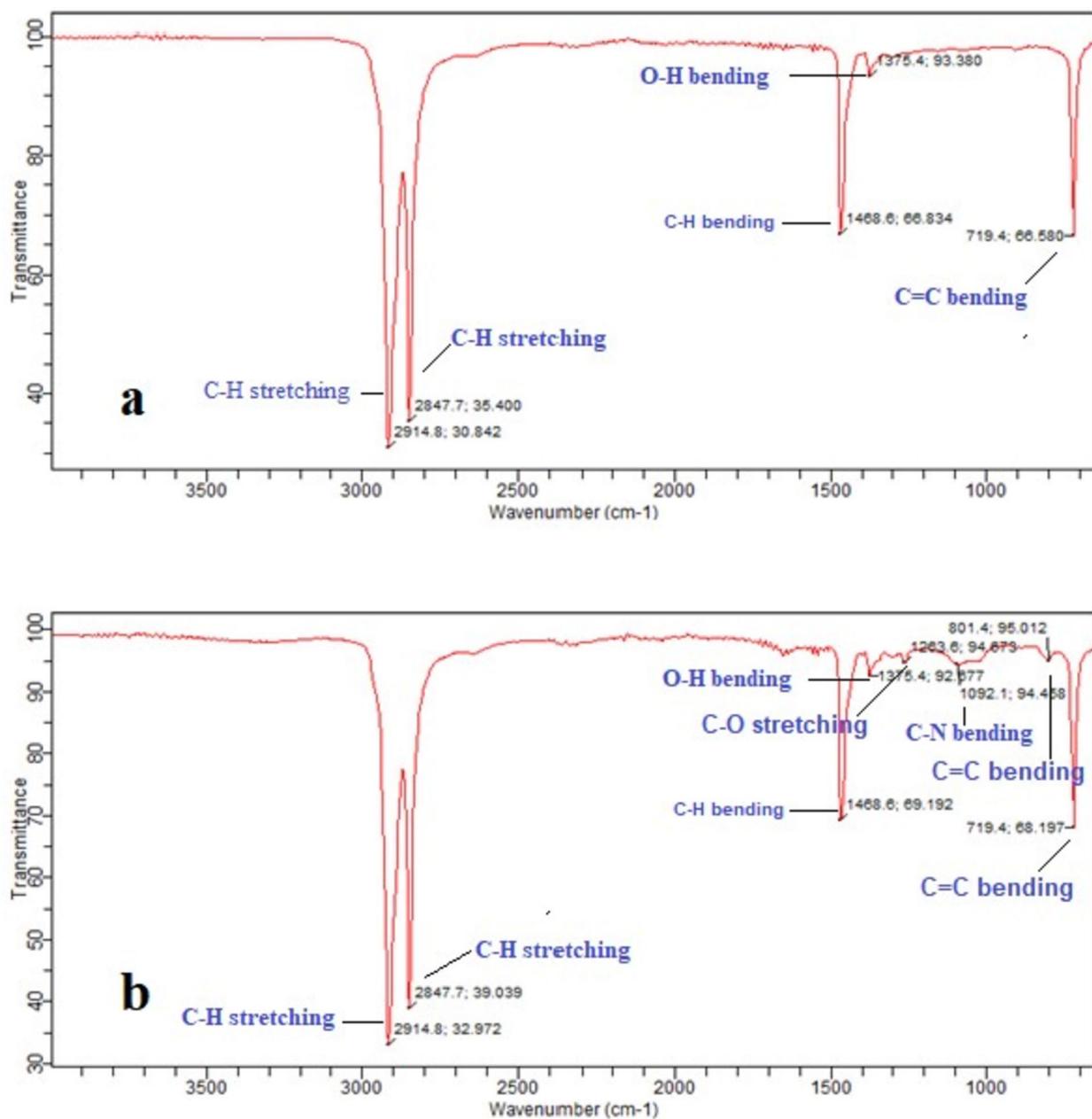


Fig. 17 FTIR spectroscopy of LDPE beads (**a**-control; **b** -Treated with *PM S1*)

16S rRNA sequencing

Five mealworm gut bacterial isolates (S1, S2, S3, S4, & S5) were screened for the potential degradation of PE and PS. S1 isolate were determined positive and utilized PE and PS as the sole source of carbon. Isolate were identified based on their 16S rRNA gene sequence homology. The isolate S1 showed 99% homology with *PM* (ON024787), related genera on *Bacillus*, *Metabacillus*, *Cytobacillus* within family *Bacillaceae*. The analysis confirmed their similarity to the respective species Phylogenetically shown in (Fig. 8).

Growth parameters (pH, temperature, carbon source)

To identify the ideal pH required to facilitate their development, *PM S1* was subjected to five distinct pH settings of 6.0, 6.5, 7.0, 7.5, and 8.0. At pH 6.0 and 8.0, the *PM S1* grew very slightly and grew substantially greater at pH 7.0 were shown in (Fig. 9). To determine the ideal temperature required for the *PM S1*, they were exposed to five different temperatures of 26 °C, 28 °C, 30 °C, 32 °C and 34 °C. Bacteria were found to thrive much more rapidly at

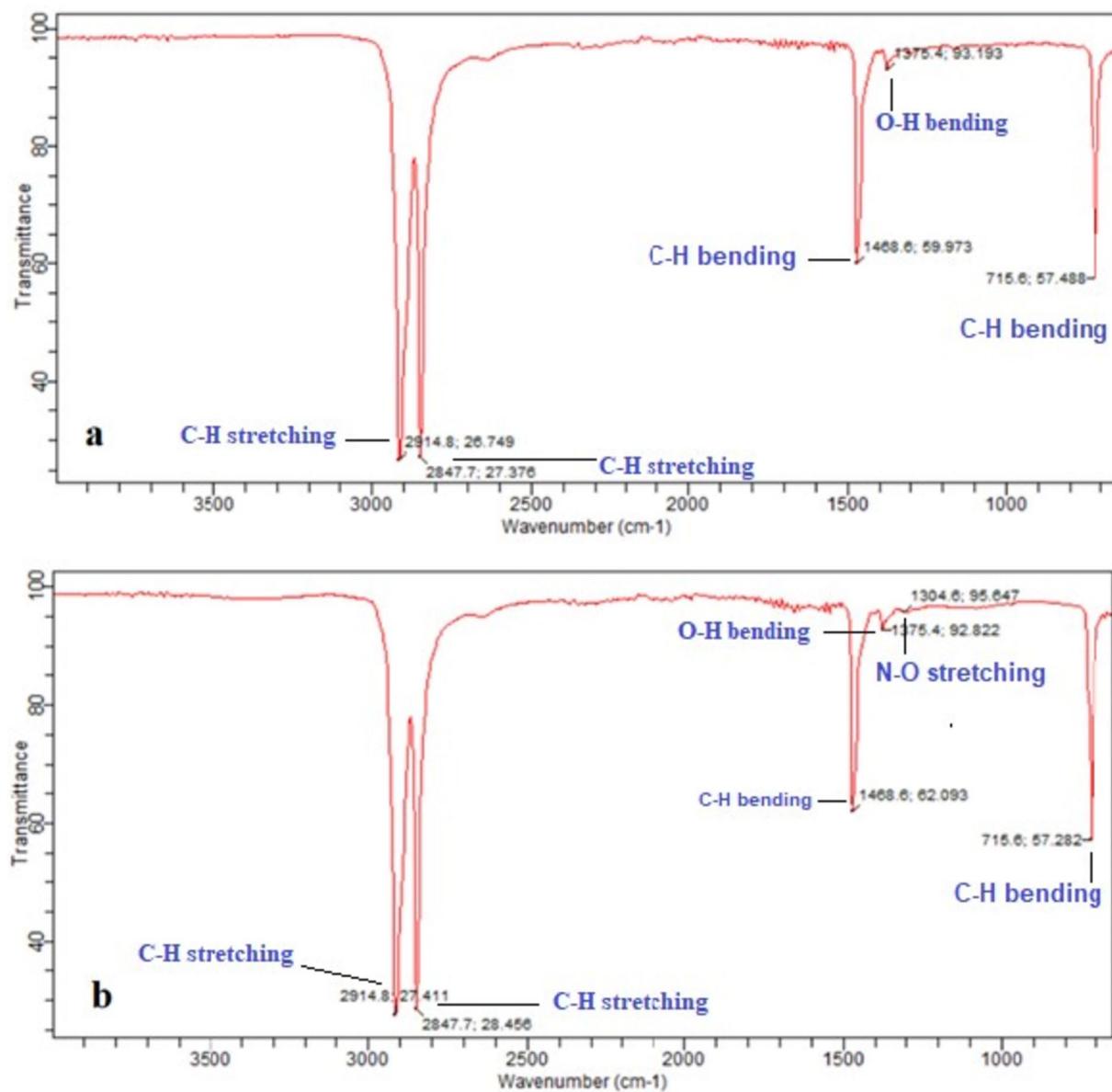


Fig. 18 FTIR spectroscopy of HDPE beads (**a**-control; **b**-Treated with *PM S1*)

28 °C than at 34 °C, which was shown to be less favorable were displayed in (Fig. 10). *PM S1* were shown to grow significantly at 28 °C, showing that this is the ideal temperature for their development. Identified mealworm gut bacteria were kept surviving in Nutrient broth without the use of any growth supplements, but when LDPE, HDPE, and PS was added as a carbon source, the bacteria exponentially increased as the concentration of LDPE, HDPE, and PS (low increased from 0.5%, 1%, and 1.5%) as shown in (Fig. 11).

Assessing the biodegradation

After incubation for 14 days, the LDPE, HDPE, and PS beads inoculated with *PM S1* were observed. The result shows that biofilm formation over the surface of the beads, and the change in media color were due to the growth of microbes.

PM S1 colonization over bead surface

A group of organisms observed adhering to a solid surface in an extracellular matrix that the organism produces itself

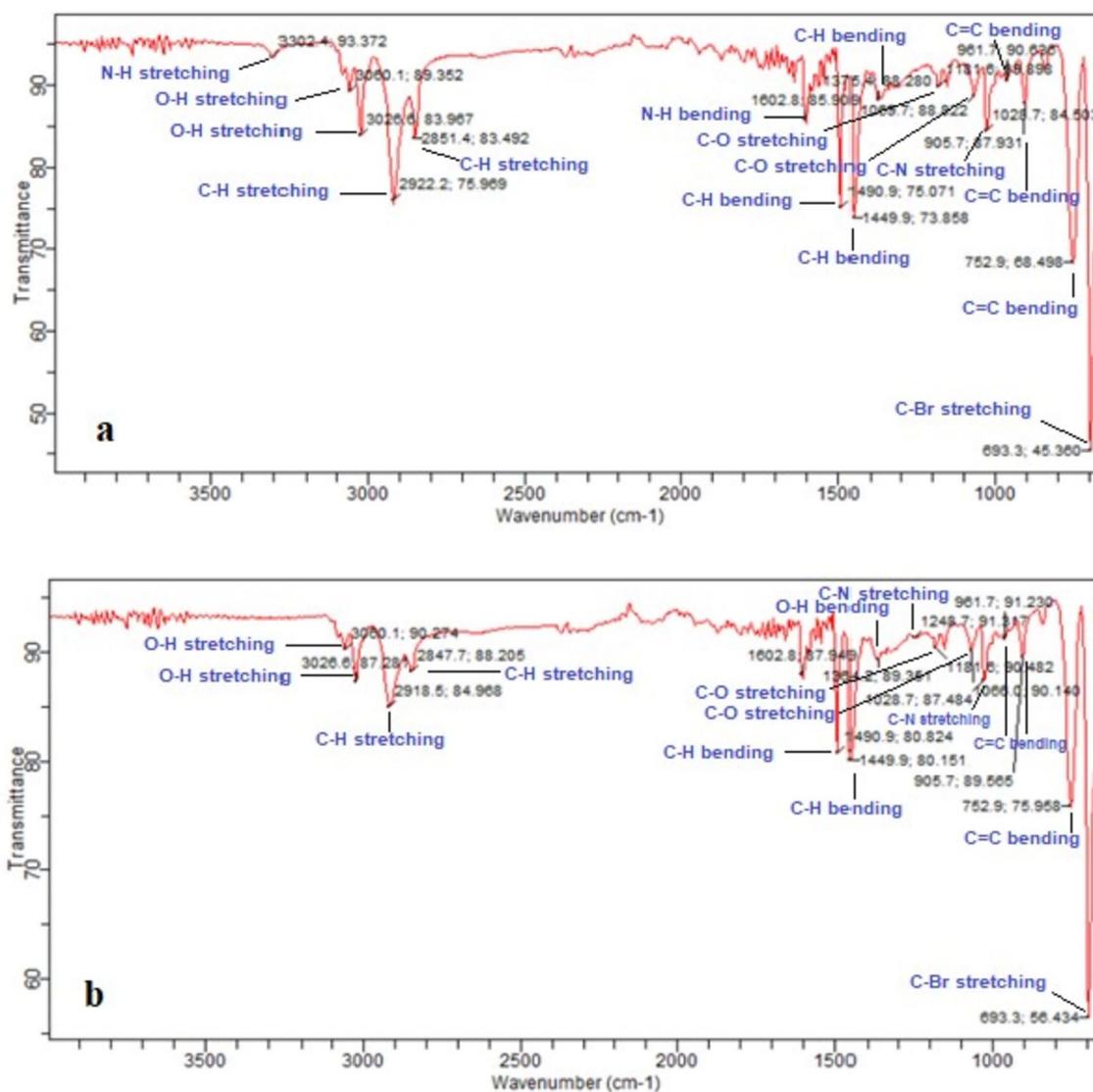


Fig. 19 FTIR spectroscopy of PS beads (**a**-control; **b**-Treated with *PM S1*)

is known as a microbial biofilm. If bacterial biofilms are produced on the surface of LDPE, HDPE, and PS by deteriorating microorganisms, PE and PS biodegradation becomes more efficient. The study delivered that the LDPE, HDPE, and PS beads weight gradually increased LDPE, HDPE, and PS bead weight due to the formation of a biofilm layer on the surface of beads after 15 and 30 days of incubation at controlled conditions, respectively, by inoculating with *PM S1* as shown in (Figs. 12 and 13). There is no weight gain of the beads due to no biofilm formation in the control (uninoculated) flask. When the weight of the beads increases with biofilm formation and Percentage of colonization also increases. It increases due to the inoculation of microbes. When compared to uninoculated (without microbe) the hydrophobic properties of PE and PS often disrupt the development of biofilm. The release of extracellular molecules

such as polysaccharides, proteins, and biosurfactants, as well as motility, are factors that contribute to the creation of biofilm. However, the most crucial element is cell surface hydrophobicity, which enhances bacterial attachment potential and contributes to biofilm formation.

Weight loss studies

Initial weight, final weight, and percent of weight loss by *PM S1* at the duration of 2, 4, and 6 months were represented in (Table 2). The maximum weight loss of LDPE, HDPE, and PS beads was observed by a nutrient broth inoculated with bacteria and the result showed that the bead weight gradually decreased (8.42, 8.77, and 8.61), (7.75, 8.18, & 7.93) and (6.39, 7.14, and 6.81), respectively, at the 2nd, 4th, and 6th month of incubation. The

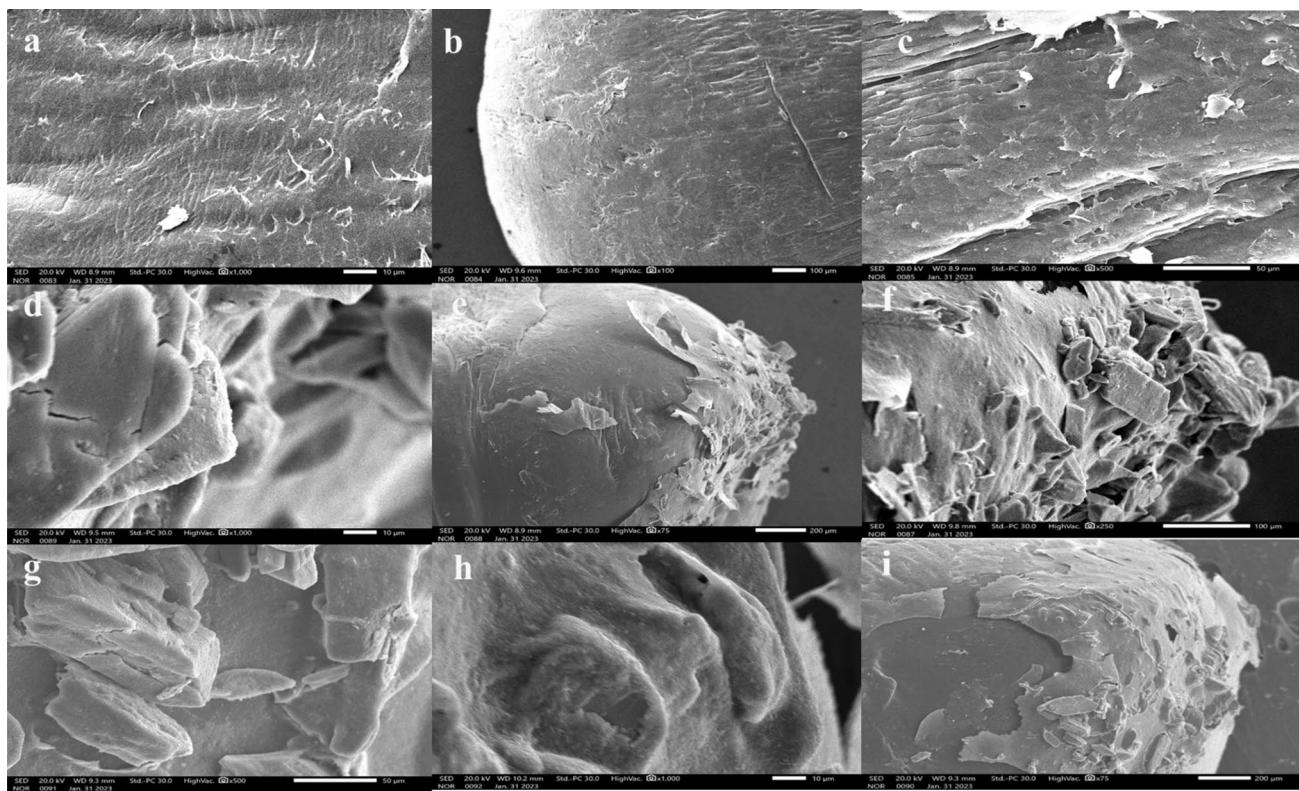


Fig. 20 **a, b, c**—LDPE control where no degradation occurs; **d, e, f, g, h, i**—surface erosion formation, breakdown occurs over surface of LDPE beads it confirms degradation when inoculated with *PM S1*

results clearly showed that the weight loss occurred when inoculated with *PM S1* when compared to uninoculated (without microbe) and the percentage loss is 36.1%, 31.9%, and 28.6% of LDPE, HDPE, and PS beads, respectively, as shown in (Fig. 14). Jayan et al. (2023) reported that the *Bacillus cereus NJD1* strain obtained from a long-term dumpsite, exhibited its natural capacity to breakdown the polymer by achieving a weight loss of about 43% in 100-micron LDPE films over a short period of 120 days without pre-treatments. Mohan et al. (2016) investigated the efficacy of newly isolated *Bacillus* strains to degrade brominated high-impact PS (HIPS). Degradation with *Bacillus* strains showed a weight loss of 23% of HIPS film in 30 days. The percentage of weight loss was noted that the inoculation of microbes delivers losses in weight when compared to control.

BOD

BOD test is also known as Biochemical oxygen demand which measures the dissolved oxygen consumed by microorganisms while assimilating and oxidizing the organic matter under aerobic conditions at certain temperature over specific period of time. BOD was found when inoculated with *PM*

S1 showed that LDPE (7.4 mg/l), HDPE (7.2 mg/l and PS (6.7 mg/l) after one month of incubation. This shows that the *PM S1* consumes the dissolved oxygen.

Effect of carbon and hydrogen

Elemental analysis of the LDPE, HDPE, and PS was done to determine the percentage of carbon and hydrogen after degradation. After an incubation period of 6 months with the respective *PM S1* concerning the percentages of both carbon and hydrogen were the percentage loss of 32.57%, 27.96%, and 18.57% of carbon and hydrogen were found to be 37.82%, 27.97%, and 20.46% of LDPE, HDPE, and PS beads, respectively, as shown in (Figs. 15 and 16).

FTIR of beads

To determine the appearance of newly formed functional groups or their removal, FTIR spectroscopy is used. Therefore, this approach may be used to identify degradation products, chemical moieties including branches, co-monomers, and unsaturation that are integrated into polymer molecules, as well as the presence of additives like antioxidants. Control spectra of beads (not treated with microbes) displayed several peaks reflecting the complex nature of the LDPE bead

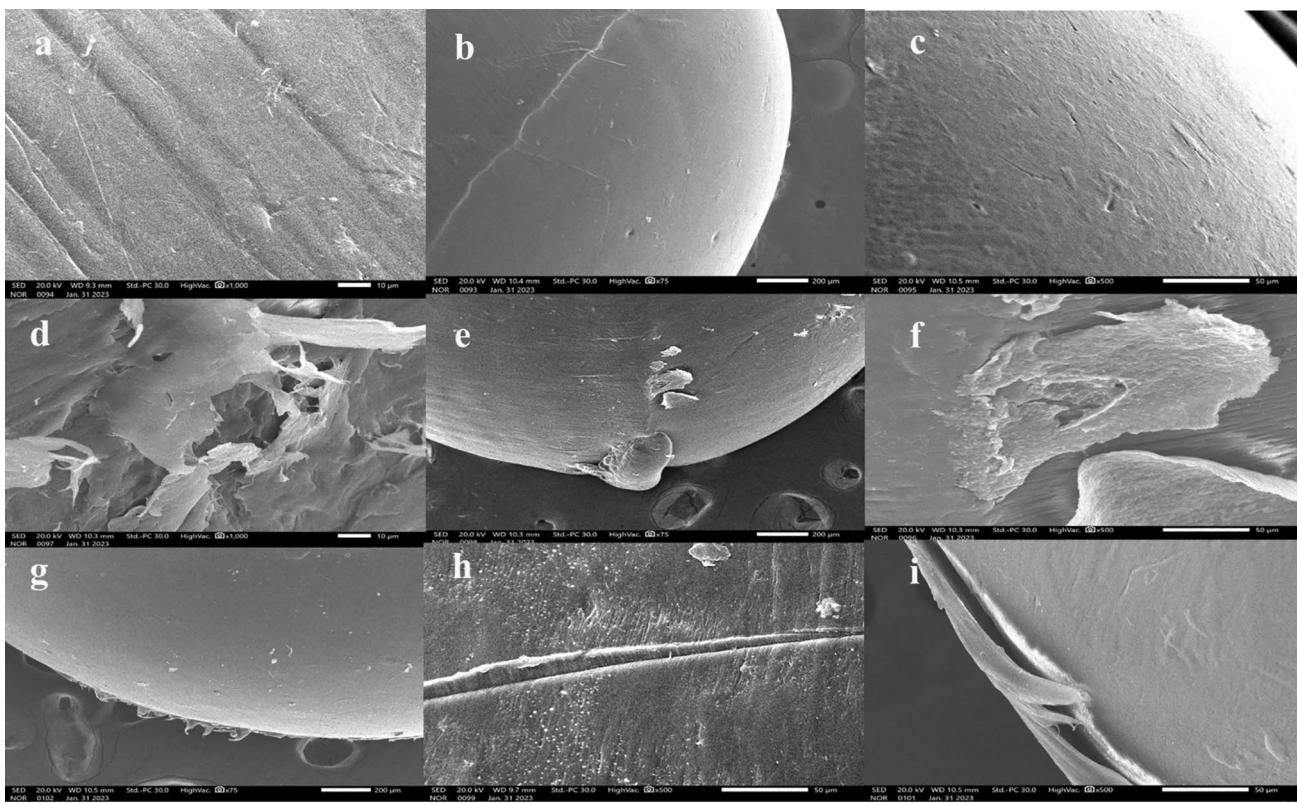


Fig. 21 **a, b, c**—HDPE control where no degradation occurs; **d, e, f, g, h, i**—surface erosion formation, breakdown occurs over surface of HDPE beads, it confirms degradation when inoculated with *PM S1*

(Fig. 17)(a); HDPE bead (Fig. 18)(a), and PS bead (Fig. 19)(a). Occurrence of variation in the intensity of bands in different regions when LDPE, HDPE, and PS beads are treated with *PM S1*, were analyzed and represented in (Fig. 17)(b); (Fig. 18)(b), and (Fig. 19)(b). To confirm the occurrence of biodegradation, by fluctuation of functional groups, indicating the conformational change on the bead surface.

SEM of beads

Due to changes in the pH, loss of carbon and hydrogen, loss of weight in beads and the difference in the absorption spectra provide solid evidence of polymer biodegradation, the structural changes that occur on the surface of LDPE, HDPE, and PS beads were elucidated by SEM. A smooth surface appears on the surface of the LDPE bead control (Fig. 20)a–c, HDPE bead control (Fig. 21)a–c and PS bead control (Fig. 22)a–c and there is no appearance of cracks over the bead surface. In the case of treated with *PM S1* strain, the surface of beads shows several cracks, minute holes and adhesion of microbes over the beads indicating the microbe utilize the PE and PS bead as carbon sources as shown in (Figs. 20, 21 and 22) -d, e, f, g, h, i. This SEM

analysis confirms that the *PM S1* will be having more degrading capacity.

Sturm test

Carbon dioxide production confirmed the degradation of LDPE, HDPE, and PS beads by *PM S1*. CO_2 was evolved by LDPE (5.05 g/l), HDPE (4.26 g/l) and PS (3.91 g/l) as shown in Table 3.

Conclusion

As the accumulation of plastic waste increases in the environment, there requires stepping up the biodegradation strategy for a change towards sustainable plastic waste management. The present investigation reveals that *Pries-tia megaterium* S1(ON024787) exhibiting biodegradation capabilities over PE and PS beads and it was evident from physical and structural changes over period of 180 days. The isolate thrives well in pH (6.5), temperature (28°C), and at 1.5% LDPE/HDPE/PS concentration. After thirty days of colonization over the beads found to be near 50% (LDPE 52.47%, HDPE 49.26%, and PS 48.11%). by weight loss

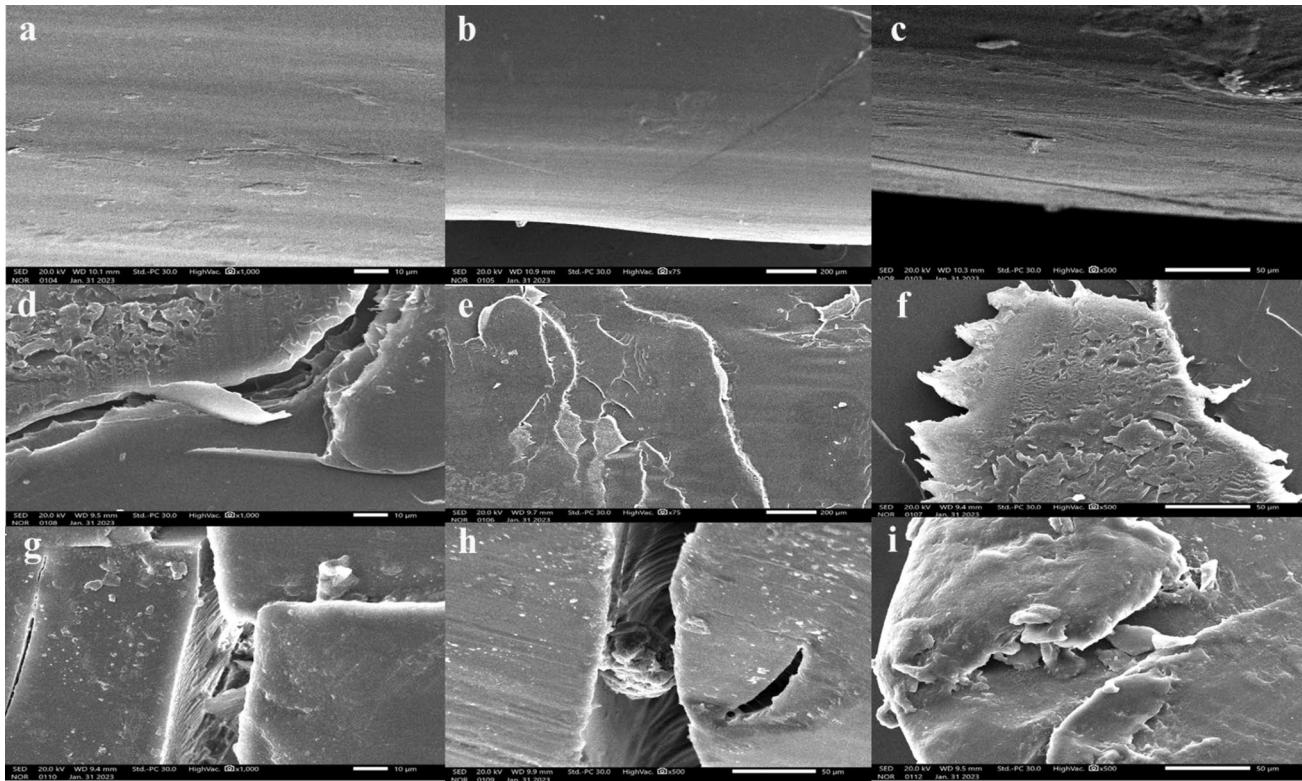


Fig. 22 **a, b, c**—PS control, where no degradation occurs; **d, e, f, g, h, i**—surface erosion formation, breakdown occurs over surface of PS beads, it confirms degradation when inoculated with *PM S1*

Table 3 Carbon dioxide evolution

Name of the beads	Carbon dioxide evolution (g/l)		
	Uninoculated	Treated with PM S1	CO ₂ produced after biodegradation
LDPE	16.40	21.45	5.05
HDPE	16.40	20.66	4.26
PS	16.40	20.31	3.91

studies at 6th-month, there is a significant weight loss of LDPE (36.1%), HDPE (31.9%), PS (28.6%) compared to control, and this has been supported with studies on Biological Oxygen Demand and CO₂ evolution. Physical changes are clearly visible in FTIR and SEM studies on the surface of beads.

Author contributions KA—study conception and draft Manuscript preparation; RP—revised and finalized the manuscript; RE and SB—finalize the data and edited the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Data availability The information provided in this research is accessible upon request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Dr. R. Parthasarathi reports financial support was provided by the Annamalai University, Faculty of Agriculture. Dr. R. Parthasarathi reports on a relationship with the Annamalai University, Faculty of Agriculture, including employment.

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