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Comparative polymer biodegradation efficiency of an isolated *Acinetobacter* sp. with *Bravibacillus* sp. and *E. coli* by resting cells

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ABSTRACT

At a concentration of 4 g/L, an enteric polymer is utilized to target drug release in the small intestine and causes considerable toxicity in cells. Our ecology and ecosystem are also harmed by their non-biodegradable qualities. We isolated and identified polymer-degrading bacteria from industrial effluent in this work. The isolated strain's morphological, biochemical, and antibiotic sensitivities were also examined. The isolated strain was found to be gram-negative, round-shaped, and non-motile in morphological tests, while biochemical tests revealed it to be negative in starch agar and TSI but positive in methyl red, mannitol salt, simmon citrate, urea agar, and catalase test. The isolated strain was highly resistant to ciprofloxacin and vancomycin. The isolated bacterium was identified as *Acinetobacter* sp. by 16S rRNA sequencing. Additionally, *Acinetobacter* sp. strains of *Escherichia coli* and *Brevibacillus* sp. were used separately to observe the degradation of five synthesized non-biodegradable polymers (maleic acid propane-1,2 diol glycerol co-polyester, maleic acid phthalic acid propane-1,2 diol glycerol co-polyester, maleic acid phthalic acid butan-1,4 diol glycerol co-polyester, phthalic acid succinic acid propane-1,2 diol glycerol co-polyester, and phthalic acid succinic acid buten-1,4 diol glycerol co-polyester. The capacity of all three strains to degrade the above-mentioned polymers was greater than 75%. *E. coli*, for example, had a rapid disintegration rate but was responsible for human gastrointestinal and urinary tract infections. As a result, our isolated *Acinetobacter* sp. can be employed to degrade synthetic polymers.

INTRODUCTION

A polymer is a substance with a molecular structure that is made up of similar units and bonded together [1]. Polymers come in a variety of forms, including natural, synthetic, biodegradable, and non-biodegradable [2]. The enteric polymers have easy thermal processing, low price, and biodegradability. So, these enteric polymers can be utilized in the pharmaceutical and biomedical sectors [3]. Synthesis of newly designed polymers, the utilization of natural monomers, and chemical modification of current polymers are all viable options for achieving the stated objectives [4]. In today's polymer science research, the development of biodegradable polymers plays a critical role [5]. Polylactic acid, polyglycolic acid, and other linear network polyesters with citric acid and polyglycerol co-polyester are biodegradable and have been used in medicine, and agriculture [5–7]. Polyester biodegradation occurs as a result of enzyme action and chemical breakdown in living organisms [8]. For linear network polyester biodegradation, a variety of microorganisms are used where bacteria including *Enterobacter agglomerans*, *Serratia rubidaea*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Comamonas acidovorans*, *Corynebacterium* sp. etc. [9–11]. The indigenous microorganisms are responsible for polymer biodegradable illumination [12].

Acinetobacter sp. was identified as a synthetic polymer degrading bacteria [13]. Antibiotic resistance in bacterial strains means that many drugs are ineffective against the bacteria. So, sensitivity analysis is determining, so that isolated bacteria are highly resistant to which specific drugs [14].

An enteric coating is a polymer barrier that is added to oral medications to prevent them from dissolving or disintegrating in the stomach [15]. This aids in either shielding drugs from stomach acidity, insulating the stomach from the drug's negative effects, or releasing the drug after the stomach (typically in the upper intestine) [16]. Some drugs are sensitive to the acidic pH of the stomach and must be safeguarded from breakdown. Drug targeting can also be accomplished through the enteric coating (such as gastro-resistant drugs). Other drugs, such as anthelmintics, may require a high concentration in a specific area of the intestine [17]. The enteric coating can also be utilized as a research technique to measure drug absorption during trials [18]. The "delayed action" dosage form category includes enteric-coated medicines. From a pharmacological standpoint, the phrase "enteric coating" isn't accurate, because gastric resistance can also be achieved by including enteric polymeric systems into the dosage form's matrix. The most popular enteric-coated dosage forms include tablets, mini-tablets, pellets, and granules [19].

Recently, many advanced molecular culture-dependent techniques like library clones, LH-PCR (Length Heterogeneity Polymerase Chain Reaction), RISA (Rapid Interactive Structural Analysis), RT-Q-PCR (RT-Q-PCR Reverse Transcription Quantitative Real-Time PCR), RAPD (Random Amplified Polymorphic DNA), and RFLP (Restriction Fragment Length Polymorphism) have been developed and considered helpful tools for the isolation and identification of new bacterial strains. rDNA-dependent methods are rapid and reliable analyses of microbial cultures to identify microorganisms in comparison to traditional techniques. The 16s rRNA-specific molecular technique offers a better chance of identifying bacteria [20–22].

The polymer degradation is a major challenging issue for the environment due to their hazardous elements, which also pollute agricultural lands and reduce fertility. Some physiochemical recycling technologies are not environmentally friendly and cannot be used in all instances due to their high cost. So, in this study we isolated an ecofriend polymer degrading bacterial strain, and used morphological, biochemical, and molecular identification approaches to identify bacterial strain. Furthermore, synthetic polymer biodegradation capabilities were evaluated by resting cells using an estimated degradation rate (%) during the incubation period.

MATERIAL AND METHODS

Sample collection

Food industrial effluent was collected from Pran Agro Ltd. (latitude 24.838524°N and longitude 88.910172°E) Natore, Rajshahi, Bangladesh, and two other strains *E. coli* (Accession: NOLW03000003.1) and *Brevibacillus* sp. (Accession: MK517601.1) were collected from the Microbiology Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh. Five different synthesized co-polyester samples such, maleic acid propane-1,2 diol glycerol co-polyester (P-1), maleic acid phthalic acid propane-1,2 diol glycerol co-polyester (P-2), maleic acid phthalic acid butan-1,4 diol glycerol co-polyester (P-3), phthalic acid succinic acid propane-1,2 diol glycerol co-polyester (P-4), and phthalic acid succinic acid buten-1,4 diol glycerol co-polyester (P-5) were collected from Hybrid Engineering Material Lab, Department of

Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh.

Bacterial isolation and morphological characterization

The strain was isolated by using the LB agar plate method [23]. Various serially diluted samples were placed into agar plates and incubated for 24 h at 37°C. Then the chosen colony was cultured several times by the steak plate method. Morphological characteristics were examined and recorded according to the method described by Cheesbrough, 1984 [25] and motility was measured according to Jarrell and McBride, 2008 [26].

Biochemical test

The biochemical characterization of isolated strain was done according to the following method reported by Paul *et al.,* 2020 [23]. In biochemical characterization, Methyl Red, Catalase, MacConkey, Starch Agar, Mannitol Salt Agar, TSI (Triple Sugar Iron), Simmons Citrate Agar, and Urease tests were performed.

Antibiotic sensitivity test

Commercially available and frequently prescribed antibiotics such as Penicillin (10 units/disc), Amoxicillin (30 mcg/disc), Ciprofloxacin (5 mcg/disc), Erythromycin (15 mcg/disc), Gentamycin (10 mcg/disc), Vancomycin (30 mcg/disc), and Chloramphenicol (30 mcg/disc) were utilized in the antibiotic sensitivity test. This was accomplished by placing 10^9 CFU/mL of freshly cultured isolated bacterial strain on agar plates and inserting antibiotic disks into the plates. The plates were then incubated at 37°C for an overnight period. The zone was observed and quantified on a millimeter-scale after incubation.

Molecular identification

The genomic DNA was isolated using the method described by Cheng and Jiang, 2006, in which the 16S rRNA gene was amplified by PCR and sequenced for molecular identification of bacteria. For amplification, universal forward primer 27F – 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 1492R – 5′-GGTTACCTTGTTACGACT-3′ for amplification [27] were used. The amplified 16S rRNA gene fragment was purified and sequenced using the Sanger sequencing method [28], using the same primer used for PCR amplification. 16S rRNA gene sequences were sequenced and aligned by comparing them to other sequences from the gene bank database using the Basic Local Alignment Search Tool (BLAST) available from the website (www.ncbi.nlm.nih.gov/Blast) to identify bacteria [29].

Polymer degradation by bacterial resting cells

For this, 100 mL of nutrient broth medium containing 1 mg/L of synthetic co-polymers samples (Dissolved 3 mg of each sample in 0.6 ml DMSO (Dimethyl Sulfoxide) to get the concentration of 5μg μL^{-1} with 5% DMSO) in 250 ml Erlenmeyer flask was applied for culturing and incubating in MS medium at 37°C for 72 h. Every 12h, 24h, 36h, 48h, and 72h, 10 ml of an aliquot of the culture broth was collected and centrifuged at 5000

rpm for 5 min at 4°C. The supernatant was taken and analyzed by UV-visible Spectrophotometer (Analytical Jena, Germany) at a wavelength of 440 nm. The degradation rate was calculated according to the following formula [30].

$$\% \text{ of polymer degradation} = \frac{(Initial\ OD - Final\ OD)}{Initial\ OD} \times 100$$

Statistical analysis

For statistical analysis, this experiment was replicated three times for each biological sample. Duncan's Multiple Range Test (DMRT) was an analysis of the significance of each group data at P≤ 0.05 label of significance at one-way ANOVA in SPSS Statistics 26 software. Graph Pad Prism 8.0.2.263 was used for all figures preparation.

RESULTS

Morphological and biochemical identification

Morphological characteristics of the isolated strain are shown in Table 1. The isolated strain was distinguished by its nearly round, gram-negative, yellowish, raised, and entire smooth appearance. Biochemical features are shown in Table 2, which indicated that the isolated strain was positive for Methyl Red, Catalase, MacConkey, Mannitol salt, and Simmons citrate tests and negative for Starch agar, and TSI tests.

Table 1. Morphological characteristics of isolated bacterial strain.

Strain	Shape	Gram stain	Elevation	Pigmentation	Margin	Texture
Isolated	Almost round	(-)	Raise	Yellowish	Entire	Smooth

Table 2. Biochemical characterization of isolated bacterial stain.

Strain	MR test	Catalase test	MacConkey test	Starch test	Mannitol Salt test	TSI test	Simmons Citrate test	Urease test
Isolated Stain	(+)	(+)	(+)	(-)	(+)	(-)	(+)	(+)

Molecular identification

The isolated bacterial strain shared 95% of its DNA with *Acinetobacter* sp. and the constructed phylogenetic tree is shown in Figure 1a. Almost 1400 bp of targeted amp icons were amplified by PCR and are shown in Figure 1b.

Antibiotic sensitivity of isolate strain

The result showed that the isolated strain was susceptible to Chloramphenicol, Vancomycin, Gentamycin, and Ciprofloxacin but intermediate resistant to Erythromycin and resistant to Penicillin and Amoxicillin. The result of the antibiotic sensitivity test is shown in Figure 2.

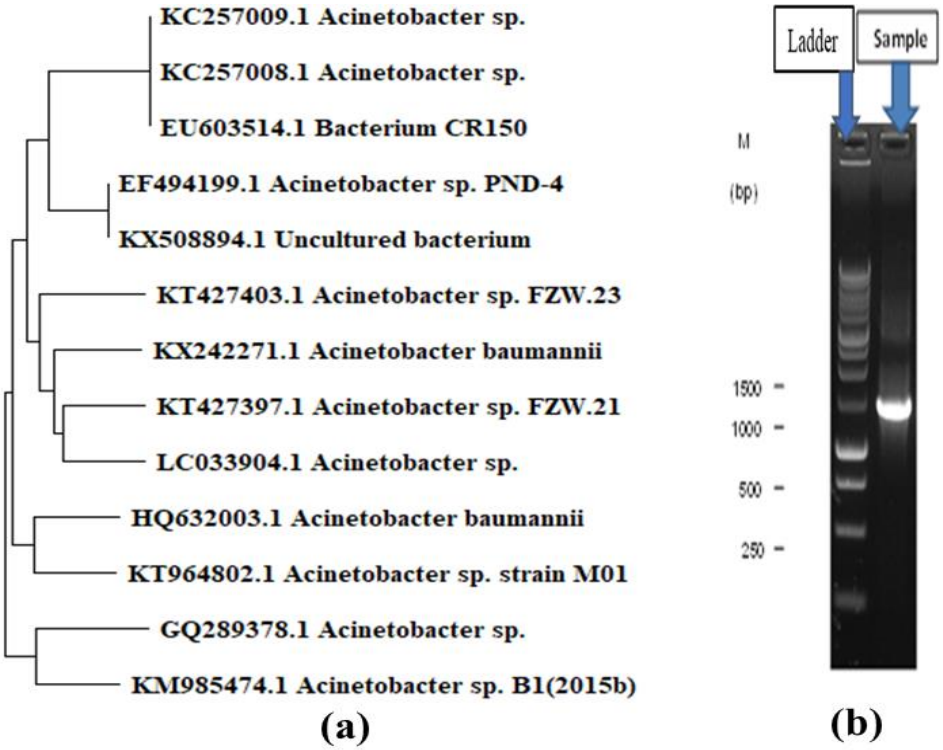

Figure 1. Phylogenetic tree analysis and agarose gel electrophoresis of 16S rRNA sequence of isolated bacterial strain. Here (a); indicate phylogenetic tree, and (b); indicate PCR amplified product on1.2% agarose gel.

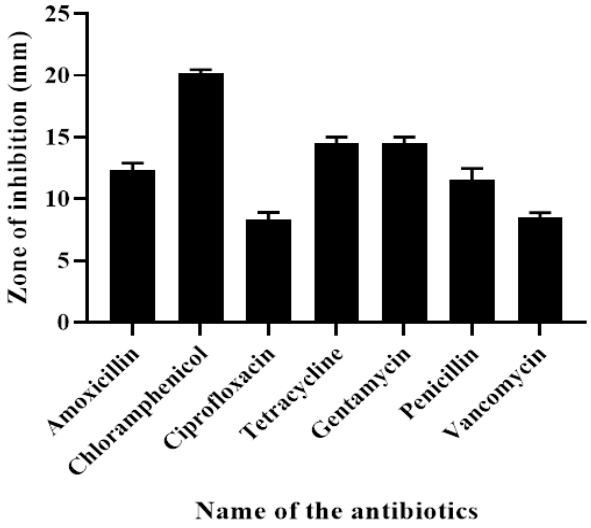

Figure 2. Antibiotic sensitivity test of isolate bacterial stain where Resistant=<10 mm; Intermediate =10-15 mm; Susceptible=>15 mm.

Synthesized polymer degradation

Various patterns for polymer degradation rate by growing cells of *Acinetobacter* sp., *E. coli*, and *Brevibacillus* sp. were observed, recorded, and detected and are shown in Figure (3-4) and Table 3. The degradation rate was measured by bacterial growing cells. In the case of maleic acid propane-1,2 diol glycerol co-polyester polymer degradation, *E. coli*, *Brevibacillus* sp., and *Acinetobacter* sp. showed 96.36 %, 95.97 %, and 97.02 %

degradation respectively whereas in Maleic acid phthalic acid propane-1,2 diol glycerol co-polyester *E. coli*, *Brevibacillus* sp., and *Acinetobacter* sp. showed 94.09 %, 92.86 %, and 89.15 % percent degradation respectively. and in maleic acid phthalic acid butan-1,4 diol glycerol co-polyester, *E. coli*, *Brevibacillus* sp., and *Acinetobacter* sp. showed 87.86 %, 82.12 %, and 77.95 % degradation after 72 hours of incubation. In Phthalic acid succinic acid propane-1,2 diol glycerol co-polyester *E. coli*, *Brevibacillus* sp., and *Acinetobacter* sp. showed 93.47 %, 83.50 %, and 75.75 % degradation and in Phthalic acid succinic acid buten-1,4 diol glycerol co-polyester polyester *E. coli*, *Brevibacillus* sp., and *Acinetobacter* sp. were showed 78.91 %, 77.77 %, and 73.90 % degradation respectively. These results suggested that all three strains degraded five synthetic polymers and *E. coli* showed the highest level of degradation. Both *Bravibacillus* sp. and *Acinetobacter* sp. also demonstrated considerable polymer breakdown after 72 hours of incubation.

Table 3. Comparative polymer degradation percentage of isolated *Acinetobacter* sp., *E. coli*, and *Brevibacillus* sp. by bacterial growing cells after 72 hours of incubation.

Name of polymers Samples	Degradation percentage (%) Mean ± SD		
	***Acinetobacter* sp.**	***E. coli***	***Brevibacillus* sp.**
Maleic acid propane-1,2 diol glycerol co-polyester (P-1)	97.87±1.13	96.90±2.71	95.25±1.20
Maleic acid phthalic acid propane-1,2 diol glycerol co-polyester (P-2)	89.15±4.50	94.09±1.49	92.86±1.21
Maleic acid phthalic acid butan-1,4 diol glycerol co-polyester (P-3)	77.95±1.90	87.86±3.12	82.12±5.35
Phthalic acid succinic acid propane-1,2 diol glycerol co-polyester (P-4)	75.75±1.81	93.47±2.03	83.50±8.86
Phthalic acid succinic acid buten-1,4 diol glycerol co-polyester (P-5)	73.90±4.70	78.91±4.00	77.77±0.96

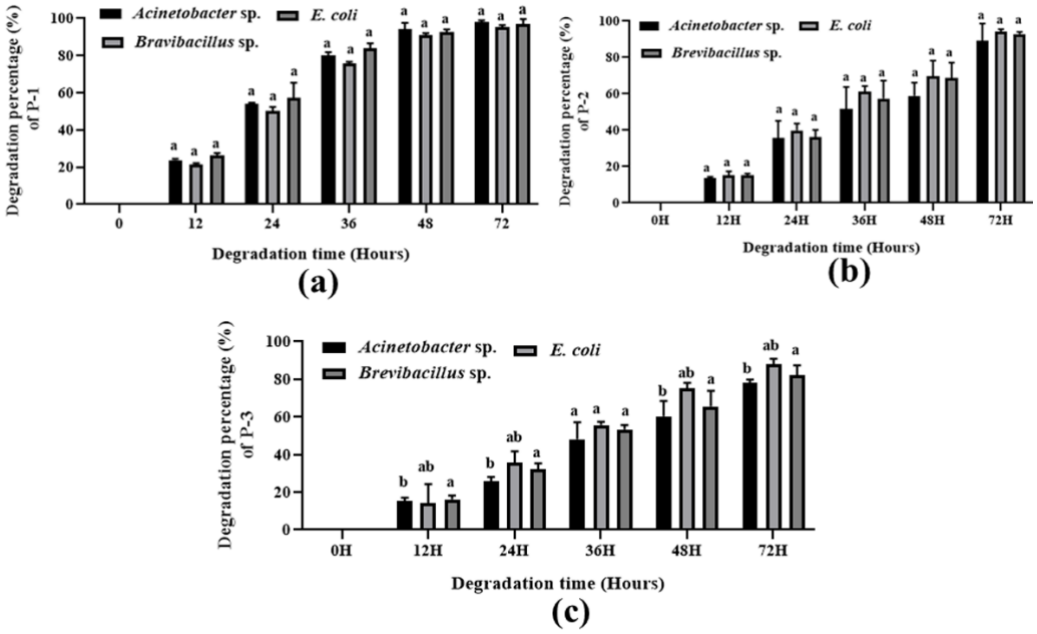

Figure 3. Degradation percentage of three bacterial stains by bacterial growing cells. Here (a); indicate maleic acid propen-1, 2 diol glycerol co-polyester degradation, (b); maleic acid phthalic acid propane-1, 2 diol glycerol co-polyester and (c) maleic acid phthalic acid butan-1, 4 diol glycerol co-polyester degradation. Different letters indicate significance differences between mean ± SD of replications (n=3) at a $P < 0.05$ significance level.

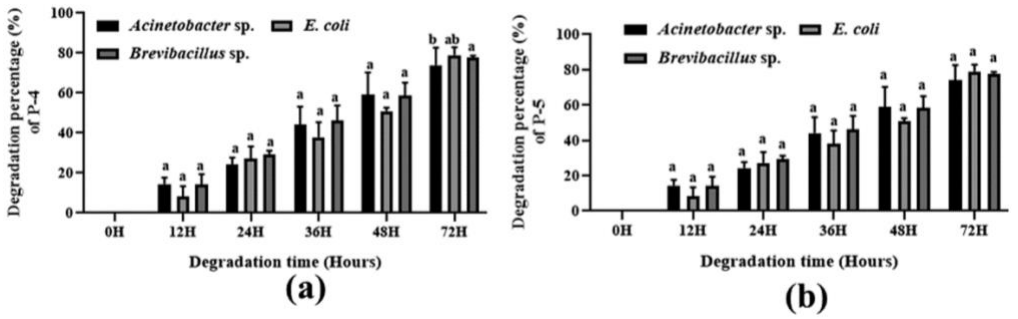

Figure 4. Degradation percentage of three bacterial strains by bacterial growing cells. Here (a); indicate phthalic acid succinic acid propane-1, 2 diol glycerol co-polyester degradation, and (b) phthalic acid succinic acid buten-1, 4 diol glycerol co-polyester degradation. Different letters indicate significance differences between mean ± SD of replications (n=3) at a $P < 0.05$ significance level.

DISCUSSION

Plastics are large-scale chemically generated long-chain polymers that have become an integral element of our society due to their low cost [32,33]. The rate of polymer deposition has accelerated dramatically in the last two decades, and it has also been imposed on the marine environment, destroying the marine ecosystem [33]. In the intestines of fish, birds, and marine mammals, it also creates obstructions. Moreover, entanglement with or ingestion of this trash has put hundreds of different species in jeopardy [34–36]. There are several physical and chemical ways of degrading polymers, but they are all quite expensive. As a result, there is a pressing need for low-cost, environmentally acceptable polymer breakdown processes. Bioremediation is a non-hazardous, cost-effective, and ecologically benign alternative approach for polymer breakdown. According to a survey on plastic garbage output in 60 major Indian cities, the country produces about 15,340 tons of plastic waste each day (Central Pollution Control Board (CPCB) New Delhi, India, 2013), which is tremendously hazardous to the environment.

In this investigation, we isolated a polymer-degrading bacterial strain from industrial effluent and performed morphological and biochemical characterization to confirm the isolate's identity. Methyl Red, Catalase, MacConkey, Mannitol salt, and Simmons citrate tests were positive for our isolated strain, whereas Starch agar and TSI tests were negative. According to a recent study, Acinetobacter sp. strains isolated from hospital units [37] had nearly identical biochemical properties, including being negative in mannitol and sucrose, H_2S (Hydrogen Sulfide) on TSI, nitrate reduction, and methyl red [37]. The isolate was susceptible to chloramphenicol, Vancomycin, Gentamycin, and Ciprofloxacin in an antibiotic sensitivity test, but intermediate resistance to erythromycin and resistant to penicillin and amoxicillin. According to a recent study, wastewater treatment plants are a rich source of antibiotic-resistant intestinal bacteria and genes that can be passed on to other bacteria in the environment [38].

Due to their high chemical inert complexity, only a tiny number of microbial organisms discovered on Earth are capable of digesting artificially manufactured polymers. *Brevibacillus borstelensis, Rhodococcus rubber, Bacillus circulans, B brevies, B pumilus, B cereus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Shewanella putrefaciens*, and *Nocardia asteroids* were among the bacteria recently recovered from industrial wastewater [39–46]. The capacity of bacteria to produce biofilm on polymer surfaces enhances polymer breakdown, and our isolated strain may have this potential. Both *E. coli* and *Brevibacillus* sp. showed significant degradation on chosen synthetic polymers in our study, while isolated *Acinetobacter* sp. also showed significant degradation. The

rate of Maleic acid propane-1,2 diol glycerol co-polyester polymer degradation was 97.02 %, maleic acid phthalic acid propane-1,2 diol glycerol co-polyester polymer degradation was 89.15 %, maleic acid phthalic acid butan-1,4 diol glycerol co-polyester polymer degradation was 77.95 %, phthalic acid succinic acid propane-1,2 diol glycerol co-polyester degradation was 75.75 % and phthalic acid succinic acid buten-1,4 diol glycerol co-polyester polyester degradation was 73.90 % by *Acinetobacter* sp.

Clostridium botulinum and *Alcaligenes faecalis* were previously identified as PCL degraders in a previous study [47]. Within 10 days, *P. lilacinus* D218 degraded the PCL by 10%, according to [48]. In PCL and PHB-containing media, *P. lilacinus* D218 produces PCL de-polymerases in addition to PHB de-polymerase. PCL de-polymerases were found to have the best activity at 30°C and pH levels between 3.5 and 4.5 [48]. [49] observed 92% degradation of 10 g PCL with particle size 125–250 m after anaerobic biodegradation at 55 °C with sludge (diluted 0.86% and undiluted 1.73%). In another study observed that the degradation of biodegradable bags was higher than polyethylene (PE) bags, with 100% degradation of the compostable material between 16 and 24 weeks [50]. Biofilm formation on the plastic bag surface after 15 days of exposure to the marine environment [51]. Microbial polymers degradation has recently been recorded as more economical and eco-friendlier than physiochemical methods. Bioremediation, especially using bacteria, is thus becoming an evolving and significant polymers treatment field.

CONCLUSIONS

Synthetic polymer production is expanding every day, causing pollution in the environment. The goal of this research is to find an ecofriendly bacterial strain that can biodegrade manmade polymers. Resting cells of this isolated *Acinetobacter* sp. displayed a substantial polymer degradation capacity. However, the study does not assess the cytotoxicity of the released components. It should be evaluated in the future, and specific enzymes from the strain should be identified for large-scale application.

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AUTHOR CONTRIBUTIONS

SA, GKP, and MSU conceived the idea and planned the research. SA and GKP also performed all experiments. GKP, SA, and SM prepared the manuscript. MSU supervised the research and MAS, and SZ revised the manuscript. All the authors read and approved the manuscript for publication. This research work receives no external funding.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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