Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i4.3175

Research Article

Biodegradation of Unpretreated Low-Density Polyethylene (LDPE) by Thermophiles Isolated from Paku Hot Springs Water and Sediment in Sarawak, Borneo, Malaysia

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ABSTRACT

Plastic pollution has emerged as a significant environmental concern nowadays, necessitating innovative solutions for its mitigation. Hot springs, while traditionally valued for their health and relaxation benefits, also present unique environments that may harbour thermophile bacteria species capable of degrading plastic polymers. The study aimed to screen thermophiles from Sarawak hot springs for the biodegradation of potential low-density polyethylene (LDPE) film and evaluate their efficiency in degrading the plastic in 30 days. Water and sediment samples were collected from each pond of the Paku hot spring in Sarawak, Borneo, Malaysia, in triplicate. The thermophiles were cultivated in an enriched medium supplemented with 0.5% (w/v) PE powder at 55°C for 5 days. Colony morphology and Gram staining were carried out. Screening of isolates for LDPE biodegradation was conducted using the BATH test and, clear zone assay. Additionally, the efficiency of the isolates for 30 days of LDPE biodegradation was evaluated using the pH change, bacteria growth observation, and weight loss method. A total of 96 thermophilic isolates were cultured, 11 isolates exhibited hydrophobicity levels above 30%, and 7 isolates showed clear zone formation. After the biodegradation process, the pH was slightly decreased to pH 6.5. The bacterial colonies were Gram-positive (3) and Gram-negative (4) short rods. Isolates SPK(W)M1(1), SPK(SD)P1(2), and SPK(SD)P1(3) resulted in increased of growth (OD_{600} 0.06 ± 0.02, OD_{600} 0.08 ± 0.01 & OD_{600} 0.1 ± 0.02, respectively). The highest growth absorbance OD_{600} was shown by isolate SPK(SD)P1(3), while isolate SPK(W)M1(1) showed the highest LDPE film weight reduction of 10 % (0.45 ± 0.05 g). The thermophiles SPK(SD)P1(3) and SPK(W)M1(1) are potentially to be used to biodegrade LDPE plastic. The preliminary study offers insight into microbial biodegradation mechanisms; further research and advanced sequencing techniques are necessary for a thorough analysis of the metabolic pathways involved.

Key words: Bacteria, biodegradation, hot spring, low-density polyethylene, thermophiles

INTRODUCTION

Anthropogenic activities and the expansion of urban areas significantly exacerbate global pollution, especially through the extensive utilization and improper management of plastics. The ubiquity of plastic pollution adversely affects a wide range of ecosystems worldwide, posing threats to human health, wildlife, and their natural environments. Despite initiatives aimed at minimizing plastic waste, its accumulation is on the rise, with predictions suggesting that emission rates could quadruple by 2025 (Lebreton & Andrady, 2019). Global production of plastics is set to rise to around 590 million metric tons by 2050, up from the 445.25 million metric tons estimated for 2025. Plastic pollution inflicts considerable economic harm on the global marine ecosystem, with damages valued at a minimum of $13 billion per year, as stated by the United Nations year 2014 in UNEP Yearbook 2014 (United Nations, 2014). Should the current trends in plastic consumption continue, projections indicate that 12 billion tons of plastic waste will be destined for landfills and natural settings by 2050 (MacLeod *et al*., 2021).

The plastics industry has played a pivotal role in fostering a disposable culture by persuading the public of the necessity and acceptability of single-use plastics. Today, the absence of single-use plastics seems unfathomable, as these items are deeply embedded in our culture of convenience, fast-paced living, and on-the-go consumption. To effectively combat this crisis, we must implement systemic transformations, including the adoption of a circular economy and the enactment of responsible plastic management practices. Although plastic represents a mere 10% of urban refuse by weight, it constitutes up to 85% of marine debris, primarily stemming from terrestrial sources (Rhodes, 2018). Notably, China, Indonesia, the Philippines, Vietnam, and Sri Lanka emerge as the principal contributors to plastic pollution, accounting for 56% of the global plastic waste output (Rhodes, 2018). This pollution manifests locally through discarded waste along roadways, coastlines, riverbanks, and urban waterways.

Article History
Accepted: 25 September 2025
First version online: 21 December 2025
Cite This Article:
Khairul, S.R., Toh, S.C., Lingoh, A.D. & Leong, S.S. 2025. Biodegradation of unpretreated low-density polyethylene (LDPE) by thermophiles isolated from Paku Hot Springs water and sediment in Sarawak, Borneo, Malaysia. Malaysian Applied Biology, 54(4): 78-88. https://doi.org/10.55230/mabjournal.v54i4.3175

The primary challenge associated with plastics lies in their disposal within landfills and indiscriminate release into the environment, particularly when considering that a significant proportion of plastics manufactured is polyethylene (PE). Particularly, low-density polyethylene (LDPE), alongside its high-density counterpart, presents a formidable environmental challenge due to its resilience and widespread use in packaging for its insulating properties (Parker, 2019; Plastic Expert, 2022). LDPE is proven to be durable and flexible, maintaining stability between 50 and 85°C (Molgroup, 2017). The overwhelming majority of monomers utilized in plastic production originate from fossil hydrocarbons. Common plastics in use today lack biodegradability, leading to their accumulation in landfills and natural ecosystems instead of breaking down. Consequently, the only definitive method to dispose of plastic waste involves destructive thermal processes, such as combustion or pyrolysis, which break down the plastic materials at high temperatures. This approach highlights the urgent need for sustainable alternatives in plastic manufacturing and waste management practices. While initiatives such as cleanups and awareness campaigns can address smaller-scale pollution, improper management results in plastics breaking down into microplastics, thus exacerbating environmental degradation (MacLeod *et al*., 2021). Research into microbial biodegradation offers a glimmer of hope, particularly focusing on the slow biodegradation rates of polyethylene (PE) and LDPE, even after pre-treatment processes (Dey *et al*., 2020).

Synthetic polymers generally remain non-biodegradable until they are fragmented into smaller particles of low molecular weight, at which point they can become digestible by microorganisms (Dey *et al*., 2020). This process highlights the critical steps of polymer degradation necessary for enabling microbial assimilation and underscores the challenge of plastic waste management. Biodegradation of plastic polymers involves fragmentation by microbial intervention, followed by polymer breakdown into oligomers or monomers as well as fatty acids, ketones, aldehydes, and alcohols by enzymatic attack and free radicals, uptake of these small products inside the microbial cells, and lastly utilization of those molecules in cellular metabolism and ultimately production of gases and water (Silva *et al*., 2023). Montazer *et al*. (2020) have demonstrated that microbial degradation of LDPE presents a promising approach, with recent studies showing that the biodegradation of LDPE waste using specific microbial strains offers a feasible solution. Previously polyethylene biodegradation was proven effectively using *Rhodococcus ruber* (Sivan *et al*., 2006), *Bacillus subtilis* (Vimala & Mathew 2016), *Brevibaccillus borstelensis* (Hadad *et al*., 2005), *Kocuria palustris* (Harshvardhan & Jha 2013), *Pseudomonas aeruginosa* (Kyaw *et al*., 2012), *Klebsiella pneumoniae* (Awasthi *et al*., 2017), *Arthrobacter pittobacter* (Zhang *et al*., 2022), *Micrococcus luteus* (Gupta *et al*., 2022), *Bacillus tropicus* (Samanta *et al*., 2022), *Streptomyces albogriseolus* (Shao *et al*., 2019), and *Nocardia asteroids* (Bonhomme *et al*., 2003). Many studies on biodegradation have been conducted using microbes from different sources, such as seawater, soil, compost, and sludge (Alvarez-Zeferino *et al*., 2015).

Thermophiles, microorganisms that thrive in high temperatures, show promise in hastening the decomposition of polyethylene. Toh *et al*. (2023) isolated *Amnoxybacillus* spp. and *Geobacillus* spp. from two Sarawak, Borneo hot springs, which were able to synthesize enzymes and have the potential to be thermostable and used for other industry applications. Nonetheless, comprehensive data on the effectiveness of thermophilic microbes in breaking down LDPE is limited, indicating a crucial area for further investigation. Skariyachan *et al*. (2018) revealed the potential for polymer degradation by thermophilic microbial consortia, including *Brevibacillus* sp. and *Aneurinibacillus* sp., which were isolated from sewage treatment plants and waste management landfills. These consortia have shown effectiveness in degrading films and pellets made of LDPE, high-density PE, as well as polypropylene. Further support for the biodegradation of LDPE by extremophiles, particularly thermophiles, comes from several studies. Zahari *et al*. (2021) reported that two thermophilic microorganisms, *Bacillus subtilis,* and *Candida tropicalis*, exhibited significant growth and plastic degradation after a 49-day incubation period. *B. subtilis* showed optimal growth in week 5 with colony-forming units (CFUs) of 8.9×10^8 for LDPE and 9.1×10^8 for starch-blended polyethylene (SBP), while *C. tropicalis* peaked at week 4 with 9.6×10^8 CFUs for both materials. *Atanasova et al*. (2021) also highlighted that extremophiles facilitate plastic degradation under extreme conditions by producing enzymes at a higher rate, which softens the plastics and disrupts their mechanical integrity. This mechanism suggests that biodegradation by extremophiles, whether in unique environmental settings or waste treatment facilities, represents a viable strategy for mitigating the accumulation of plastic waste.

The study was conducted as a preliminary study toward the development of microbial LDPE bioremediation by evaluating the potential of thermophiles isolated from Borneo hot springs. The application of thermophilic bacteria in plastic biodegradation, especially in Sarawak, remains unexplored. Thus, this study aims to isolate the potential LDPE-degrading thermophiles from Paku hot spring, Sarawak, Borneo, and investigate their effectiveness in 30 days of LDPE biodegradation.

MATERIALS AND METHODS

Sampling site

The study was conducted at Paku Hot Spring, located in the Bau district of Sarawak, Borneo (GPS: 1°25'06.5"N 110°11'55.5"E) (Figure 1), a recreational site for residents of the nearby Serian neighborhood. There are 3 ponds with the water temperatures ranging from 37.9 to 40.0 ˚C with a noticeable but mild sulfur odor.

Sample collection and processing

The collection of water and sediment samples was undertaken in accordance with Leong *et al*. (2018). Three water samples (500 mL each) and three sediment soil samples (20 g each) were collected aseptically at three random locations from each heat source and the discharge region using a sterile Schott bottle and a thermos. Within 12 hr, the samples were labelled and brought to the laboratory to limit environmental changes for further investigation.

Polyethylene (PE) powder preparation

Research-grade polyethylene granules (Sigma-Aldrich, USA) were used in this study. The LDPE granules were melted using xylene (Biolab, Malaysia) to produce powder according to Torre *et al*. (2018). After PE powder was obtained, it was rinsed using 70% (v/v) ethanol (Biolab, Malaysia) for 30 min. The PE powder was rinsed three times to ensure there was no xylene left on the PE powder. The PE powder was then dehydrated overnight in an oven at 55°C.

Fig. 1. Sampling location at Paku hot spring, Sarawak, Borneo (Google Map).

Low-density polyethylene (LDPE) film preparation

The LDPE films were cut into 40 mm × 40 mm square sheets, disinfected using 75% ethanol, and subsequently air-dried. These prepared sheets were then utilized as the sole carbon source in the subsequent biodegradation experiments.

Isolation of LDPE-degrading thermophilic bacteria

Isolation of the thermophilic bacteria was performed according to Balasubramanian *et al*. (2010) with modifications. Customise carbon-free synthetic medium (CFSM) was prepared with chemical (Sigma-Aldrich, USA) as: 1.0 g/L ammonium nitrate (NH_4NO_3), 1.0 g/L of magnesium sulfate ($MgSO_4$.7H20), 0.2 g/L of potassium phosphate, dibasic (K_2HPO_4), 1.0 g/L of calcium chloride dihydrate ($CaCl_2$.2$H_2$0), 0.1 g/L of potassium chloride (KCl), 0.15 g/L of Yeast extract (Difco, Malaysia), and 1.0 mg/L of each of the following micronutrients: ferrous sulfate hexahydrate ($FeSO_4$.6$H_2$0), zinc sulfate heptahydrate ($ZnSO_4$.7$H_2$0, and manganese(II) sulfate ($MnSO_4$) and 0.5% (w/v) PE powder was added respectively to the CFSM as the sole carbon source for the bacterial growth. 1 mL of hot spring water (10 cm depth) and 1 g of sediment sample were each separately added to 9 mL of CFSM medium and incubated for 5 days at 55°C. Following enrichment, the CFSM medium was serially diluted and spread-plated onto CFSM agar plates. These plates were then incubated at 55°C for an additional five days. Thermophilic bacteria isolated from these cultures were preserved in 20% (w/v) glycerol stock as working stocks for further analysis.

Screening of potential LDPE-degrading thermophiles

Bacterial Adhesion to Hydrocarbons (BATH) Test

BATH test was performed according to Farid *et al*. (2021) with modifications. The overnight thermophilic bacteria grown culture was centrifuged at 10,000 rpm for 10 min three times and washed with PUM (phosphate-urea-magnesium) buffer. The pellet was resuspended in 2 mL of PUM buffer to an absorbance of 0.7 at 400 nm. The 0.3 mL of n-hexadecane (Nacalai Tesque, Japan) was added and incubated at 37°C for 10 min. After vortexing, incubation for phase separation was carried out at 37°C for 1 hr, where the absorbance of the aqueous phase was recorded at 400 nm. The surface hydrophobicity (percent) was calculated upon the following Equation 1:

Hydrophobicity % = (OD of bacteria suspension-OD of aqueos phase)/(OD of the initial suspension) × 100 (Equation 1)

Clear zone assay

The thermophilic bacteria were screened using a clear zone assay, with modifications based on the method described by Soud (2019). Each thermophilic bacterial culture was streaked onto CFSM agar plates supplemented with 0.5% (w/v) PE powder. Sterile liquid CFSM broth served as the negative control. After incubation, the agar plates were treated with approximately 0.1% (w/v) Coomassie Blue R-250 solution for 20 min. Subsequently, the plates were rinsed, and a mixture of 20% methanol and 10% acetic acid was applied to the plates for 5 min. This rinsing and staining procedure was repeated three times to ensure complete removal of unbound Coomassie Blue. The presence of clear zones was then assessed to indicate areas of PE degradation.

Low-Density Polyethylene (LDPE) biodegradation study

The 30-day LDPE biodegradation study using thermophilic bacteria was carried out based on the methodology described by Dang *et al*. (2018), with certain modifications. Optimal concentrations of standardized bacterial cultures (1 mL) were inoculated into 100 mL of sterile CFSM medium contained in flasks. As the sole carbon source, 1 g of sterile LDPE sheets, optimized previously for their concentration (w/v), was added to each flask. Throughout the biodegradation study, flasks containing bacterial cell-CFSM medium supplemented with LDPE sheets served as the treatment, and flasks with non-inoculated CFSM medium alone acted as the reference blank. These inoculated flasks were incubated for 30 days at a constant temperature of 55°C and pH 7 in triplicate. To ensure adequate nutrient supply while excluding the carbon source, 15 mL of fresh sterile CFSM medium was added to each flask at 15-day intervals. At the end of the biodegradation process, LDPE films and 15 mL of CFSM medium were randomly collected from the flasks for subsequent analytical analysis.

Biodegradation efficiencies analysis

Observation of pH changes

The effectiveness of biodegradation was evaluated based on the metabolic activity of bacterial growth in CFSM broth. The changes in pH were measured following the method described by Leong *et al*. (2022). The pH of the bacterial suspension in CFSM broth was recorded at the start (day 0), midpoint (day 15), and end (day 30) of the experiment. Non-inoculated CFSM broth served both as the negative control and the reference blank.

Observation of thermophiles growth

The growth of the thermophiles during the biodegradation process was measured according to Yan *et al*. (2020). The bacterial growth was determined by monitoring the cell optical density at 600 nm (OD600) for days 0,15, and 30. Non-inoculated CFSM served as the negative control and reference blank, respectively.

Weight loss analysis for LDPE

Evaluation of the thermophile's biodegradability was performed through weight loss measurement for the LDPE films as described by Ji *et al*. (2024). The LDPE film was recovered on days 15 and 30 of the biodegradation process. In order to precisely quantify the dry weight, 2% sodium dodecyl sulfate (SDS) (Merck, Germany) solution was used to wash the film in order to get rid of the bacteria that had colonized the LDPE surface. The LDPE film was also thoroughly dried in an oven set to 55°C for a whole night after being cleaned three times using 70% ethanol and distilled water. As controls, LDPE film-containing cultures cultured for 30 days in CFSM medium without microorganisms were employed. The following calculation was used to compute the weight loss of the LDPE film (Equation 2):

$$\text{Weight loss (\%)} = (\text{Initial weight} - \text{Final weight})/\text{Initial weight} \times 100 \qquad \text{(Equation 2)}$$

Molecular Characterization of LDPE Degrading Bacteria

To identify the accurate genotypic identification of the isolated thermophilic bacteria, 16S rRNA gene sequencing was conducted according to Toh *et al*. (2023) with modifications. The bacterial isolates' genomic DNA was extracted from freshly grown colonies using a heat shock method. The 16S rRNA gene was PCR-amplified with bacterial universal primers 27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 519R 5'-GWATTACCGCGGCKGCTG-3' under the following cycle conditions: 95°C for 1 min, 30 cycles of 94°C for 30 sec, 55°C for 60 sec, and 72°C for 90 sec, and a final extension at 72°C for 10 min. The samples were cooled to a temperature of 4°C. The amplified products were observed using a 1.0% agarose gel in TBE buffer at a voltage of 90 V and a current of 400 mAh for a duration of 30 min. The 100 base pair DNA ladder (Promega, United States) was utilized. The PCR product, after purification, was submitted to Apical Scientific Sdn. Bhd. (Malaysia) for DNA sequencing. The results were matched by comparing the product with other sequences in the NCBI database using BLAST on the website (http://www.ncbi.nlm.nih.gov).

Statistical analysis

All quantitative data were subjected to analysis of variance (ANOVA) using SPSS version 23. A test of least significant differences was used to separate means. The Tukey test was used to analyze the mean comparison between the data. Differences between means were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Enrichment and isolation of potential LDPE-degrading thermophiles

Bacteria capable of biodegrading polyethylene (PE) were isolated by enriching cultures in CFSM broth containing 0.5% PE powder as the sole carbon source, incubated at 55°C for 24 hr (Tao *et al*., 2023). All CFSM plates exhibited significant microbial growth. Aquatic microorganisms are well-documented as potential plastic-degrading bacteria, with many such bacteria successfully isolated from seawater. Examples include *Rhodococcus* sp. C-2 and *Lysobacter maris* sp. from China and Korea (Rong *et al*., 2024), *Stenotrophomonas* sp. and *Achromobacter* sp. from a disposal site (Dey *et al*., 2020), and *Nostoc carneum* from domestic sewage water (Sarmah & Rout, 2019). Additionally, *Pseudomonas mendocina* ABF786, isolated from marine environments, has been identified as one of the significant breakdown plastic-degrading microorganisms (Jadhav *et al*., 2024).

The potential application of thermophilic biodegradation techniques to enhance plastic degradation is a topic of ongoing research. Wu *et al*. (2023) suggest that these techniques, which improve enzyme accessibility, could yield more effective results compared to mesophilic microorganisms. Higher temperatures cause more biodegradation for the plastic, which is induced by these hydrolase enzymes by bacteria such as *Bacillus* sp. BCBT21 (Suresh *et al*., 2025). In contrast, mesophilic microbes operate at lower temperatures, where plastic polymers under mesophilic conditions, the polymer chains remain rigid and less susceptible to degradation (Nguyen *et al*., 2024). In this study, a total of 96 potential LDPE-degrading thermophilic bacteria were isolated from water and sediment samples collected at Paku hot spring, Sarawak. Most of the colonies exhibited a milky white appearance.

Screening of Potential LDPE-degrading thermophiles analysis

BATH test

The Bacterial Adhesion to Hydrocarbons (BATH) test, depicted in Figure 2, examined microbial interactions with LDPE film, revealing that logarithmic-phase cells possess hydrophobic characteristics. Microbial adhesion to substrates is essential for

substrate utilization and is influenced by factors such as attachment forces, substrate properties, and microbial traits (Zheng *et al*., 2021). In our study, BATH test results ranged from 0% to 45%, with 11 thermophiles showing over 30% hydrophobicity, indicating strong adherence to LDPE film. Low hydrophobicity has been linked to poor biofilm formation (De Souza *et al*., 2019). This test demonstrated the binding efficiency of bacterial cells to organic hydrocarbons, highlighting the ability of enriched microbes to adhere to polyethylene surfaces (Sarker *et al*., 2020). These findings suggest that BATH test results showing over 30% hydrophobicity indicate effective bacterial adherence to LDPE film, aligning with the influence of cell hydrophobicity on microbial adhesion to various surfaces as discussed by Krasowska and Sigler (2014).

Similarly, Duddu *et al*. (2015) reported that thermophilic *Streptomyces coelicoflavus* NBRC 15399T exhibited an 11% reduction in turbidity at the lowest concentration and approximately a 30% reduction at the highest concentration. Additionally, the biodegradation of untreated LDPE by *Stenotrophomonas* sp. and *Achromobacter* sp., isolated from waste dumpsites and drilling fluids, showed that all treated samples exhibited more than 80% hydrophobicity, while the untreated sample had only 11.11% hydrophobicity (Dey *et al*., 2020). Furthermore, two extremophiles, *Nesiotobacter exalbescens* and *Bacillus vietnamensis*, isolated from saltpan water samples in India, exhibited similar hydrophobicity percentages of 30% (Rafiq *et al*., 2018). Thus, the thermophiles isolated in this study demonstrate significant potential for degrading LDPE.

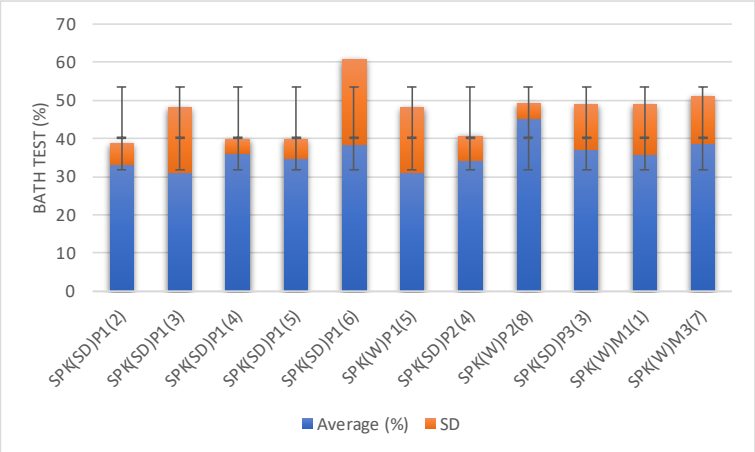

Fig. 2. Mean percentages (%) of Bacterial Adhesion to Hydrocarbons (BATH) test tested on 11 thermophiles isolated from Paku Hot Spring water (*n*=3).

Clear zone

The 11 thermophiles that exhibited hydrophobicity levels above 30% were further assessed using the clear zone assay to determine their potential for degrading LDPE plastic film. Upon inoculation with these thermophiles, a clear halo formed and expanded over time, typically within 1 to 5 days. This observation indicates that thermophiles can effectively depolymerize PE on CFSM agar at a notable rate. The results of the clear zone assay were documented to evaluate the biodegradation of PE by thermophilic bacteria, as summarized in Table 1. Figure 3 illustrates the formation of clear halo zones around thermophilic cultures. Out of the 11 isolates, only 7 exhibited a distinct and noticeable clear zone visible to the naked eye. These 7 thermophiles were selected as candidate strains for subsequent biodegradation experiments.

The criterion for selecting the most effective PE degrader involved assessing the ratio of the clear zone size to the colony size. A higher clear zone ratio typically indicates greater degrading activity. This approach aimed to identify isolates demonstrating superior PE degradation capabilities, aligning with findings reported by Li *et al*. (2023). Similar results were observed in other studies, such as those by Rafiq *et al*. (2018), where two halophilic bacteria (*Nesiotobacter exalbescens* and *Bacillus vietnamensis*) isolated from saltpan water samples also exhibited clear zones during LDPE degradation testing. Additionally, Charnock (2021) reported comparable findings, noting that the thermophiles *Thermobifida fusca* and *Thermobifida alba* showed complete clearing zones after 24 hr of incubation on LDPE agar.

Table 1. Clear zone assay result for 11 thermophiles isolated from Paku hot spring, Sarawak, Borneo

No.		Clear Zone Formation	
	Isolates	Yes	No
1	SPK(SD)P1(3)	√	
2	SPK(SD)P2(4)		√
3	SPK(SD)P1(2)	√	
4	SPK(W)M3(7)		√
5	SPK(SD)P1(5)	√	
6	SPK(SD)P1(6)		√
7	SPK(W)M1(1)	√	
8	SPK(SD)P1(4)	√	
9	SPK(SD)P3(3)	√	
10	SPK(W)P2(8)	√	
11	SPK(W)P1(5)		√

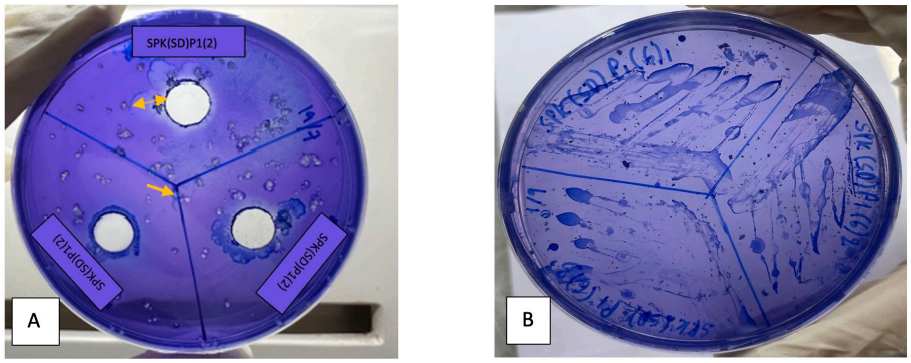

Fig. 3. The clear zone was conducted by streaking 11 samples of thermophilic bacteria on PE agar. A: presence of clear zone (arrow); B: No clear zone.

Thermophiles colony morphology

Four thermophilic bacteria were Gram-positive bacteria, while three were Gram-negative bacteria. The study identified all the isolates as short rods (Table 2). The colonies were smooth, circular, and milky white.

Thermophiles colony morphology

Four thermophilic bacteria were Gram-positive bacteria, while three were Gram-negative bacteria. The study identified all the isolates as short rods (Table 2). The colonies were smooth, circular, and milky white.

Table 2. Gram staining result of 7 thermophiles isolated from samples collected at the Paku Hot Spring, Sarawak

Isolates	Gram stain	Shape
SPK(W)M1(1)	-	Rod
SPK(SD)P1(2)	+	Rod
SPK(SD)P1(3)	-	Rod
SPK(SD)P1(4)	+	Rod
SPK(SD)P1(5)	-	Rod
SPK(SD)P2(8)	+	Rod
SPK(SD)P3(3)	+	Rod

Gram stain: -: Negative; +: Positive

Biodegradation of LDPE Sheets by thermophilic bacteria

The 30-day LDPE biodegradation study revealed that the pH of the CFSM medium decreased from an initial pH of 7 to a slightly acidic pH of 6.5 to 7.0, as shown in Table 3. Changes in pH are critical in plastic degradation, as they influence hydrolysis reactions and microbial activity during the biodegradation process. The pH reduction suggested potential metabolic shifts in the enriched cells. Al-Jailawi *et al*. (2015) reported optimal growth occurring at pH 6.5, which aligns with the final pH observed in this study for the thermophiles SPK(W)M1(1), SPK(SD)P1(2), and SPK(SD)P1(3) after 30 days.

Table 3. Mean value of the pH recorded after 30 days of the LDPE biodegradation process using the selected three thermophiles isolated from Paku Hot Spring water, Sarawak

Thermophilic Isolates	pH measurement	
	Before treatment	After treatment
SPK(W)M1(1)	7	6.5 ± 0.08
SPK(SD)P1(5)	7	7.0 ± 0.06
SPK(SD)P1(4)	7	7.0 ± 0.05
SPK(SD)P3(3)	7	7.0 ± 0.01
SPK(W)P2(8)	7	7.0 ± 0.03
SPK(SD)P1(2)	7	6.5 ± 0.06
SPK(SD)P1(3)	7	6.5 ± 0.05
Control	7	7.0 ± 0.00

± standard deviation (*n*=4)

Ahmed *et al*. (2018) noted that pH changes significantly influence the degradation rate of plastics due to their impact on hydrolysis reactions, which alter the environment's acidity or basicity. The breakdown products of polymers further modify pH levels, thereby affecting degradation rates and microbial growth dynamics. Additionally, the softening temperature of polymers plays a crucial role in enzymatic breakdown efficiency. Specific enzymes, such as those produced by fungi like *Aspergillus flavus* and *Aspergillus niger*, exhibit varying affinities for breaking down different types of polyesters, particularly those derived from diacid monomers with 6 to 12 carbon atoms (Ahmed *et al*., 2018). Shifts in pH from neutral to acidic are likely to influence the metabolic activity of enriched microbial cells (Duddu *et al*., 2015). The fragmentation and depolymerization of LDPE result in the

formation of smaller molecular weight products such as fatty acids and organic acids, contributing to pH reduction (Karamanlioglu *et al*., 2017).

The study demonstrated that all samples exhibited microbial utilization of LDPE as the sole carbon source, evident from the decrease in medium salinity and increase in absorbance. Table 4 recorded the bacterial growth absorbance (OD_{600}) after 30 days of the LDPE biodegradation process. The OD_{600} of the CFSM medium showed a significant change in medium turbidity, indicating possible cell growth following 30 days of incubation. Results showed that thermophilic isolates exhibited decreasing mean growth absorbance of $OD_{600,}$ ranging from 0.06 to 0.1. Isolate SPK(SD)P1(3) showed the highest absorbance (OD_{600} 0.1 ± 0.02); isolates SPK(W)M1(1) reported the lowest as OD_{600} 0.06 ± 0.02. The control set showed low absorbance. Visible growth and turbidity in the CFSM medium post-incubation confirmed the successful enrichment of LDPE-degrading microorganisms.

As per Haider *et al*. (2019), microorganisms adhere to the plastic surface and establish a biofilm as part of the biodegradation process. These microorganisms release extracellular enzymes that break down the polymer, resulting in shorter chains and various oligo-, di-, and monomers that are utilized by the microorganisms. The increase in growth absorbance suggests microbial breakdown of LDPE (Chatterjee *et al*., 2010). However, there was a trend of gradually decreasing absorbance until 32 hr into the biodegradation process, followed by an increase during further biodegradation. The decrease in bacterial growth absorbance suggested possible cell lysis without further nutrient addition, as explained by Zhou *et al*. (2021). A similar bacterial growth trend was observed by Dey *et al*. (2020), where LDPE biodegradation tested with *Stenotrophomonas* sp. and *Achromobacter* sp. showed a significant increase in growth absorbance, indicating possible cell growth following 100 days of incubation.

Table 4. Mean value of the bacteria growth (OD600) recorded after 30 days of the LDPE biodegradation process using selected 3 thermophiles isolated from Paku Hot Spring water, Sarawak

Thermophilic Isolates	bacteria growth (OD600)
SPK(W)M1(1)	0.06 ± 0.02
SPK(SD)P1(5)	0.00 ± 0.00
SPK(SD)P1(4)	0.00 ± 0.00
SPK(SD)P3(3)	0.00 ± 0.00
SPK(W)P2(8)	0.00 ± 0.00
SPK(SD)P1(2)	0.08 ± 0.01
SPK(SD)P1(3)	0.1 ± 0.02
Control	0.00 ± 0.00

± standard deviation (*n*=4)

The weight of the LDPE films was measured to evaluate the degradation potential of the thermophiles. Kumar Sen and Raut (2015) explained that LDPE plastic, being a high molecular weight polymer, requires assessment of weight changes as a crucial step in evaluating its degradation. The mean weight loss (g) of the LDPE plastic sheets after 30 days of biodegradation by the three selected thermophiles is shown in Table 5. The mean weight reduction observed after 30 days of incubation ranged from 0.45 g to 0.49 g. Among the thermophiles, SPK(W)M1(1) demonstrated the highest weight reduction of 10% (0.45 ± 0.05 g) for the tested LDPE film, whereas no weight loss was observed in the control flask. The other two thermophiles showed only slight weight reductions of about 2% for the tested LDPE film.

Table 5. Mean value of the weight loss (g) for the LDPE plastic sheet recorded after 30 days of the LDPE biodegradation process using 3 thermophiles isolated from Paku Hot Spring water, Sarawak

Thermophilic Isolates	Weight loss of the LDPE film (g)		Weight reduction (%)
	Before treatment	After treatment	
SPK(W)M1(1)	0.5	0.45 ± 0.05	10
SPK(SD)P1(5)	0.5	0.50 ± 0.00	0
SPK(SD)P1(4)	0.5	0.50 ± 0.00	0
SPK(SD)P3(3)	0.5	0.50 ± 0.00	0
SPK(W)P2(8)	0.5	0.50 ± 0.00	0
SPK(SD)P1(2)	0.5	0.49 ± 0.00	2
SPK(SD)P1(3)	0.5	0.49 ± 0.00	2
Control	0.5	0.50 ± 0.00	0

± standard deviation (*n*=3)

Thermophiles that adhere to LDPE polymer can cause initial weight gain, while the degradation of the polymer results in weight loss. The extent of LDPE film degradation through biological processes is directly related to its surface area, as biodegradation typically starts at the outer layers of the polymer material, gradually breaking it down and reducing its weight. Thus, a larger surface area of LDPE film typically results in more significant weight loss over time due to biodegradation.

In this study, the thermophile SPK(W)M1(1) showed a weight reduction of 10% for the tested LDPE film after 30 days of incubation, which is lower compared to the 30% weight reduction reported by Hadad *et al*. (2005) for polyethylene biodegradation by the thermophilic bacterium *Brevibacillus borstelensis* at 50°C over the same period. The weight reduction percentages observed for SPK(W)M1(1) were also lower than those reported by Gajendiran *et al*. (2016), who documented a 35% weight loss of LDPE films after 90 days of incubation with *Aspergillus clavatus* strain JASK1, and by Nademo *et al*. (2023), who reported a 23.87% weight loss of LDPE films after 60 days of incubation with *Methylobacterium* sp. Abdullah *et al*. (2022) reported a 22.9% weight loss for LDPE films biodegraded by various *Aspergillus* species isolated from waste disposal soil. Similarly, low weight changes were observed for thermophiles SPK(SD)P1(2) and SPK(SD)P1(3), which are consistent with the findings of Zahari *et*

al. (2021), who reported 3.2% and 4.6% weight loss of LDPE films after 49 days of biodegradation using thermophiles *Candida tropicalis* and *Bacillus subtilis*. Dey *et al*. (2020) estimated an 8% weight loss of LDPE beads incubated with *Stenotrophomonas* sp. and *Achromobacter* sp. isolated from waste dumpsites and drilling fluids over a 100-day biodegradation process.

Essentially, the bacteria used for the 30-day LDPE biodegradation process primarily indicate structural and surface changes (Zhang *et al*., 2022) and the formation of additional carbonyl groups (Li *et al*., 2020). The study linked the pH decrease to the fragmentation and depolymerization of LDPE, resulting in smaller molecular weight products. This was a preliminary estimation of degradation since the bacteria present in the samples utilized polyethylene as the sole carbon source, leading to the observed weight reduction of the plastic (Kyaw *et al*., 2012).

LDPE degrading bacteria identification

The seven LDPE-degrading bacteria were characterized molecularly using 16S rRNA gene sequencing (Table 6). *Anoxybacillus* sp. were successfully identified through molecular sequencing analysis.

Table 6. The 16S rRNA sequencing homology search (BLAST) results of the three LDPE-degrading thermophilic bacteria isolated from Paku Hot Spring, Sarawak

Thermophilic Isolates	Source	Bacterial species	Size(s) bp	Homology (%)	Accession Number
SPK(W)M1(1)	sediment	*Anoxybacillus* sp.	545	100	OQM45369.1
SPK(SD)P1(2)	water	*Anoxybacillus* sp.	545	100	OQM45369.1
SPK(SD)P1(3)	water	*Anoxybacillus* sp.	545	100	OQM45369.1

The thermophiles SPK(SD)P1(3) and SPK(W)M1(1) are potentially to be used to biodegrade LDPE plastic. The genus *Anoxybacillus* sp. is a relatively new genus of Gram-positive bacteria that was proposed in the year 2000 (Pikuta *et al*., 2000). Notably, emerging genomic and biochemical studies have highlighted *Anoxybacillus* sp. as a promising source of thermostable enzymes, including for the degradation of complex polymers. For instance, *Anoxybacillus* sp. has been shown to utilize lignin as a sole carbon source and produce hyperthermostable alkaline laccase, expanding its relevance in biodegradation applications beyond traditional polysaccharide substrates such as starch, cellulose, and xylan (Al-kahem Al-balawi *et al*., 2017). This aligns with our findings, further emphasizing the potential of *Anoxybacillus* strains in the biodegradation of persistent polymers such as LDPE. The successful identification of *Anoxybacillus* sp. among thermophilic isolates with LDPE degradation potential highlights the relevance of this genus for plastic biodegradation under elevated temperatures. Coupling molecular identification with functional assays and advanced characterization will enable the development of robust microbial solutions for mitigating plastic waste.

CONCLUSION

The study demonstrated that three short rod-shaped, Gram-negative thermophilic bacterial isolates from Paku Hot Spring, Sarawak, Borneo (SPK(W)M1(1), SPK(SD)P1(2), and SPK(SD)P1(3)) possess the potential to degrade LDPE. These isolates exhibited hydrophobicity levels above 30% and formed clear zones, indicating their ability to adhere to and degrade LDPE. After the biodegradation process, the pH of the medium changed to slightly acidic (pH 6.5). Among the isolates, SPK(SD)P1(3) showed the highest growth absorbance, while SPK(W)M1(1) exhibited the highest LDPE film weight reduction of 10% (0.45 ± 0.05 g). These findings suggest that the thermophilic bacteria SPK(SD)P1(3) and SPK(W)M1(1) have potential applications in LDPE plastic biodegradation. This preliminary study offers valuable insights into microbial biodegradation mechanisms, although further optimization is required to achieve significant degradation levels.

ACKNOWLEDGEMENTS

Department of Animal Science and Fishery, UPM Sarawak, is highly acknowledged for providing the research facilities. This research study was supported by the Bintulu Development Authority grant (Grant number: 6300964).

ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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