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Research Article

Enzymatic biodegradation of low-density polyethylene sheet by *Proteus vulgaris JS01* isolated from a dumpsite

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Abstract

Low-density polyethylene waste poses enormous threat to ecosystem. Hence, an effective method of handling waste is expedient. In this study, the growth of *Proteus vulgaris* in a low-density polyethylene-based medium (LPM) was determined spectrophotometrically at 600 nm in a shaking incubator at pH 7.0, 37 °C, and 180 rpm. Laccase, esterase, and lipase activities produced by *Proteus vulgaris* in LPM were screened and their physicochemical properties were evaluated. Furthermore, the biodegradation efficiency of *Proteus vulgaris* on unmodified LDPE sheets was evaluated by scanning electron microscope (SEM). The results revealed optimum growth of *Proteus vulgaris* on the third day with an absorbance value of 0.215 ± 0.01. Laccase (5.96 U/mg), and lipase (3.12 U/mg) activities were optimum on the twelfth day while, esterase (4.70 U/mg) activity was highest on the tenth day. The physicochemical studies showed that laccase and esterase were optimum and stable at 50 °C and lipase was optimally active and stable at 60 °C. The optimum pH for laccase, esterase, and lipase activities were recorded at pH 5.0, pH 7.0, and pH 9.0 respectively. Lipase, esterase, and laccase activities were enhanced by Ca^{2+}, Mg^{2} , Cu^{2+}, and inhibited by Mn^{2+}, Hg^{2+} and EDTA. The SEM profile of the LDPE sheet after biodegradation revealed the presence of grooves, scrapings, and cracks. This study demonstrates that *Proteus vulgaris JS01* produces thermostable laccase, esterase, and lipase, indicating a strong potential for the biodegradation of various types of polyethylene.

1. Introduction

Proteus vulgaris is an opportunistic rod-shaped gram-negative bacterium belonging to the phylum of Proteobacteria of the family *Enterobacteriaceae. Proteus vulgaris* has been revealed to adapt and positively impact contaminated environments by involving in effective bioremediation of pesticides, herbicides, azo dyes, and heavy metals [1]. **Similarly,** crude oil, diesel, and kerosene have been reported to be actively degraded by *Proteus vulgaris* [2]. In addition, synthetic plastics (such as modified polyethylene) have been shown to be utilized as carbon sources for growth, and biodegraded by *Proteus vulgaris* [3]. Plastics are polymers that are synthesized from fossil resources. There are different types of plastics however, the commonly produced and utilized plastics include polyethylene terephthalate, nylon, polyethylene, and polyvinylchloride [4]. Among these plastics, polyethylene is the most extensively manufactured plastic polymer, with about 409.3 million tons produced in 2024 [4]. Polyethylene is also produced in different forms such as low-density polyethylene, linear low-density polyethylene, and high-density polyethylene [5].

Low-density polyethylene (LDPE) is the most

manufactured type of polyethylene, and accounts for about 50 % of the produced polyethylene [5]. LDPE is a thermoplastic made from ethylene monomers, and is used for manufacturing cups, bottles, containers, computer parts, caps and closures [5, 6]. It is the most flexible, durable, and extensively used type of polyethylene for industrial and domestic purposes. Unfortunately, excessive production and improper handling of the waste result in enormous amount of LDPE waste in terrestrial and aquatic environments [7]. In order to mitigate the waste generated from LDPE usage, recycling, burning, landfilling, and incineration are some of the methods adopted [8]. However, because these methods are costly, and release toxic chemicals into the environment they are not effective. Also, only about 12% of the waste is reported to be either incinerated or recycled, while the remaining waste is emptied into different landfills [4]. In addition, about 25,000 tons of LDPE waste was recorded to be dumped in landfills daily in the Metropolis of Akure, Ondo State, in 2024 [5]. Hence, an environmentally friendly method, such as biodegradation, is essential [9].

Biodegradation enmesh the application of biological emissaries such as plants, bacteria, and fungi which are readily available to break down LDPE waste with the release of CO_2, and H_2O as end products [10]. Biodegradation has evolved as a prominent and effective method for eradicating water and land pollution caused by LDPE waste [8, 11]. Bacteria and fungi including *Bacillus* [12], *Streptomyces* [13], *Pseudomonas* [14], *Aspergillus* [15], and *Penicillium* [16] have been studied and reported as effective bio-degraders of different types of modified LDPE. However, studies on the biodegradation of unmodified LDPE are limited [17]. Furthermore, despite the known capabilities of *Proteus vulgaris* in hydrocarbon degradation, its role in degrading unmodified LDPE remains poorly understood. Hence, this study investigated the biodegradative activity of *Proteus vulgaris* on unmodified LDPE sheets, and characterized the activities of laccase, esterase, and lipase produced in the biodegradation process.

2. Materials and methods

2.1. Preparation of low-density polyethylene (LDPE)

A bag of sachet water (10 kg) was purchased from the Jitkok water-producing company in Akure, Ondo State, Nigeria. The sachets were emptied of their water and cut open to air dry for 24 h in the laboratory. The sachets were confirmed to be low-density polyethylene (LDPE) by the Department of Chemistry, Elizade University, Ondo State, Nigeria. The LDPE sheets were manually minced and sieved to obtain a uniform particle size (0.6 mm). The LDPE sheets and particles were sterilized with 80% ethanol for 30 min [18].

2.2. Microorganism

A bacterium isolated from a local dump site in Akure, Ondo State, Nigeria, was used in this study. The bacterium was characterized based on morphological and biochemical methods, as shown in Table 1.

Table 1. Morphology and biochemical characterization of *Proteus vulgaris*.

Test	Result
Fermentation test	
Fructose	+
Arabinose	-
Urease	+
Lactose	-
Glucose	+
Galactose	+
Mannose	-
Maltose	+
Reactivity test	
Oxidase	-
Indole production	+
Catalase	+
Hydrogen sulfide	+
Casein reaction	-
Gram's reaction	-ve rod
Nitrogen reduction	-
Temperature optimum	37 °C
pH optimum	7.0

The isolate was identified as *Proteus vulgaris* based on 16S rRNA sequencing conducted at the Bioscience Centre, International Institute of Tropical Agriculture (IITA), Nigeria. *Proteus vulgaris* was maintained on nutrient agar slants and stored at 4°C.

2.3. Production of enzymes

Seed inoculum of *Proteus vulgaris JS01* was prepared

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by growing a loopful of slant *Proteus vulgaris* in 100 mL aseptic nutrient broth (seed culture) containing beef extract (0.30 % w/v), peptone (1.0 % w/v), NaCl (1.0 % w/v), and yeast extract (0.30 % w/v) at pH 7.0, 37°C and 180 rpm for 24 h in a shaking incubator (Stuart, UK). Subsequently, 100 mL of LDPE-based medium (LPM) was prepared using 1% (w/v) LDPE particles in a defined mineral salt medium containing KCl (0.04 % w/v), KH_2PO_4 (0.01 % w/v), $CaCl_2{\cdot}2H_2O$ (0.005 % w/v), $MgSO_4$ (0.01 % w/v), NH_4NO_3 (0.01 % w/v), $KH_2PO_4{\cdot}12H_2O$ (0.01 % w/v), NaCl (0.04 % w/v), and $FeSO_4{\cdot}7H_2O$ (0.0001 % w/v). Thereafter, five percent (v/v) inoculum of *Proteus vulgaris* was taken from the seed culture and transferred into the LDPE-based medium (LPM). The experiment was replicated thrice and incubated at 180 rpm, 37 °C and pH 7.0 for 30 days to study the growth and enzyme production from *Proteus vulgaris.* Fresh cultures of *Proteus vulgaris* were inoculated into freshly prepared LBM and incubated at 37°C, pH 7.0 and 180 rpm for the production of laccase, esterase, and lipase. The cultivation period was terminated by centrifuging the broths at 10,000 rpm for 20 min at 4°C. Lucid supernatants were retrieved and assayed for laccase, esterase, and lipase activities. Thereafter, the supernatants were used for enzyme characterization.

2.3.1. Laccase assay

Laccase activity was determined through the oxidation of 3.6 mM 2,2′-azino-di-[3-ethyl benzothiazoline-6-sulphonic acid] (ABTS) buffered with 0.2 M Sodium acetate buffer (pH 5) at 420 nm which was monitored for 5 minutes in a visible spectrophotometer. The reaction mixture (2 mL) contained 1 mL ABTS and 1 mL culture filtrate. One unit (U) of laccase activity was defined as the amount of laccase oxidizing 1 nmol ABTS per minute at pH 5.0 and 30 °C with a molar extinction coefficient for the ABTS radical cation (the reaction product) of ε420 nm = 36000 M^{-1} cm^{-1} [19].

2.3.2. Lipase assay

The activity of lipase was investigated using a substrate mixture that contained 0.5 mM p-NP palmitate (C16) in methanol, 50 mM Tris-HCl buffer (pH 8.0) and 0.1% Triton X-100. The reaction mixture contained 200 µL of substrate mixture and 20 µL of the crude supernatant was incubated at 37 °C for 1 h. Lipase activity was assessed by measuring the release of p-NP at an absorbance of 405 nm (ε405 ¼ 18,600 $nm^{-1}M^{-1}$). One unit (U) of enzyme activity was defined as the amount of enzyme required to release 1 nmol of p-NP per minute under the assay conditions [20].

2.3.3. Esterase assay

Esterase activity was quantified by measuring the release of p-NP butyrate in 50 mM tris-HCl buffer at pH 8.0. The reaction mixture contained 50 mM tris-HCl buffer (pH 8), 0.1% Triton X-100, and 0.5 mM p-NP butyrate (C4) in methanol. Thereafter, 600 µL was withdrawn into a sterile test tube and 100 µL of the supernatant was added, and incubated at 37 °C for 1 h. The release 1 nmol of p-NP in the reaction mixture was measured using a visible spectrophotometer at 405 nm (ε405= 18 mM^{-1} cm^{-1}). One unit (U) of enzyme activity was defined as the amount of enzyme required for the release of 1 nmol of p-NP per minute under the assay conditions [20].

2.4. Determination of protein content

Protein concentration was determined using the Bradford reagent, and bovine serum albumin (BSA) was used as the standard. A hundred (100)-fold diluted dye reagent pipetted into 5 uL of the sample solution was used for the assay. The mixture was then incubated at room temperature for 15 min to allow for proper color development. The absorbance was measured at 595 nm against a blank. The specific activities of the enzymes (laccase, esterase, and lipase) was expressed as U/mg protein [19].

2.5. Characterization of enzymes produced by Proteus vulgaris

2.5.1. Effect of temperature on enzyme activity and stability

The effect of temperature on laccase, esterase, and lipase activities were evaluated by incubating the assay mixture at 30 °C to 80 °C for 35 m after which the enzyme activities were recorded. Similarly, the stabilities of the enzymes were determined by measuring their residual activities, which is the percentage of activity retained post incubation. Their stabilities were assessed at 30 °C to 80 °C every 30 m intervals for 3 h of incubation, and their residual activities were determined using the earlier described enzyme activity assay.

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2.5.2. Effect of pH on enzyme activity and stability

The effect of pH on the activities of laccase, esterase, and lipase was determined at pH 3.0 – 11.0 (glycine-HCl (pH 3.0), sodium acetate (pH 5.0), Tris-HCl (pH 7.0) and glycine-NaOH (pH 9.0 and 11.0) using the standard assay method described earlier. pH stability was ascertained by incubating the enzymes at different pH values for 24 h. Residual enzyme activities was assessed using the standard enzyme activity assay.

2.5.3. Effect of metal ions and EDTA on enzyme activity

Metallic chlorides of selected concentrations (1, 5 and 10 mM) were used to investigate the effects of divalent cations (Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+} and Hg^{2+}) on laccase, esterase, and lipase activities. Ethylene diamine tetraacetic acid (EDTA) was also prepared (5 and 10 mM) to determine the effect of this metal-chelating agent on the crude enzyme. Each metallic chloride was added to the reaction mixture and incubated for 30 m at the optimum temperature and pH of the enzyme. Thereafter, enzyme activities was determined.

2.6. Biodegradation studies of LDPE sheet

Low density polyethylene (LDPE) (0.2 g) sheet of 3 X 2 cm was used for this study. The LDPE sheets were sterilized by soaking in 80% (v/v) ethanol and rinsed with sterile distilled water. The LDPE sheets were then introduced into 500 mL of *Proteus vulgaris* culture. Sterile nutrient medium containing only LDPE sheets was used as the control. The flasks were firmly corked with aseptic cotton wool covered with aluminum foil and incubated at pH 7.0 and 50 ^{o}C in a shaking incubator at 180 rpm for 30 days. All experimental setups were performed in triplicate (n=3). At the end of the experiment, the LDPE sheets were retrieved from the degradation setup, rinsed with sterile distilled water, and air-dried at ambient temperature until a constant weight was obtained. The sheets were subsequently analyzed using a Zeiss Evo MA variable pressure scanning electron microscope (SEM) (Carl Zeiss STM AG, Germany) [21].

2.7. Statistical analysis

Results were recorded as the mean ± standard deviation. Data were analyzed using one-way analysis of variance (typically $p < 0.05$). Means were compared using Duncan's multiple range test at the 5% significance level.

3. Results and discussion

3.1. Growth and enzyme production by Proteus vulgaris

The growth of *Proteus vulgaris* was spontaneous in low-density polyethylene-based media (LPM), as shown in Fig. 1a. An absorbance value of 0.143±0.03 was recorded at twenty-four hours (24 h) exposure, and increased to 0.215±0.01, which was the optimum growth absorbance value at seventy-two hours (72 h). Thereafter, the bacteria entered the death phase on the fourth day, which was the ninety-six hours of exposure (Fig. 1a). The growth of *Proteus vulgaris* shows its ability to utilize LDPE as a source of carbon, which is needed for cellular development, and other metabolic activities [13]. In addition, *Proteus vulgaris* grew under the mesophilic condition, because it was isolated from a dump site with an ambient temperature [17]. Furthermore, the activities of laccase, esterase, and lipase produced by *Proteus vulgaris* were determined (Figs. 1b-1d). The activities of these enzymes were screened because of the significant roles they play in the breakdown of polyethylene into monomers that are easily ingested by the microbial cells, and used for energy production with the released of carbon dioxide as the waste product [4, 13]. Interestingly, *Proteus vulgaris* produced 1.68 U/mg laccase activity on the first day. A daily increase in laccase activity was recorded until an optimum activity of 5.96 U/mg was obtained on the twelfth day (Fig. 1b). Laccase is an enzyme used for degrading lignin alongside manganese peroxidases [22]. However, it has been extensively studied because of its involvement in polyethylene biodegradation. The production of laccase activity by *Proteus vulgaris* in LPM shows its ability to form carbonyl groups necessary to weaken the LDPE structure during biodegradation. Previous study also reported laccase production by bacterial isolates used for modified LDPE bio-degradation [23]. Esterase activity was optimally produced on the tenth day with 4.70 U/mg specific activity (Fig 1c), while lipase activity was produced optimally on the twelfth day with 3.12 U/mg specific activity (Fig. 1d). *Proteus vulgaris* produced lipase and esterase activities because they

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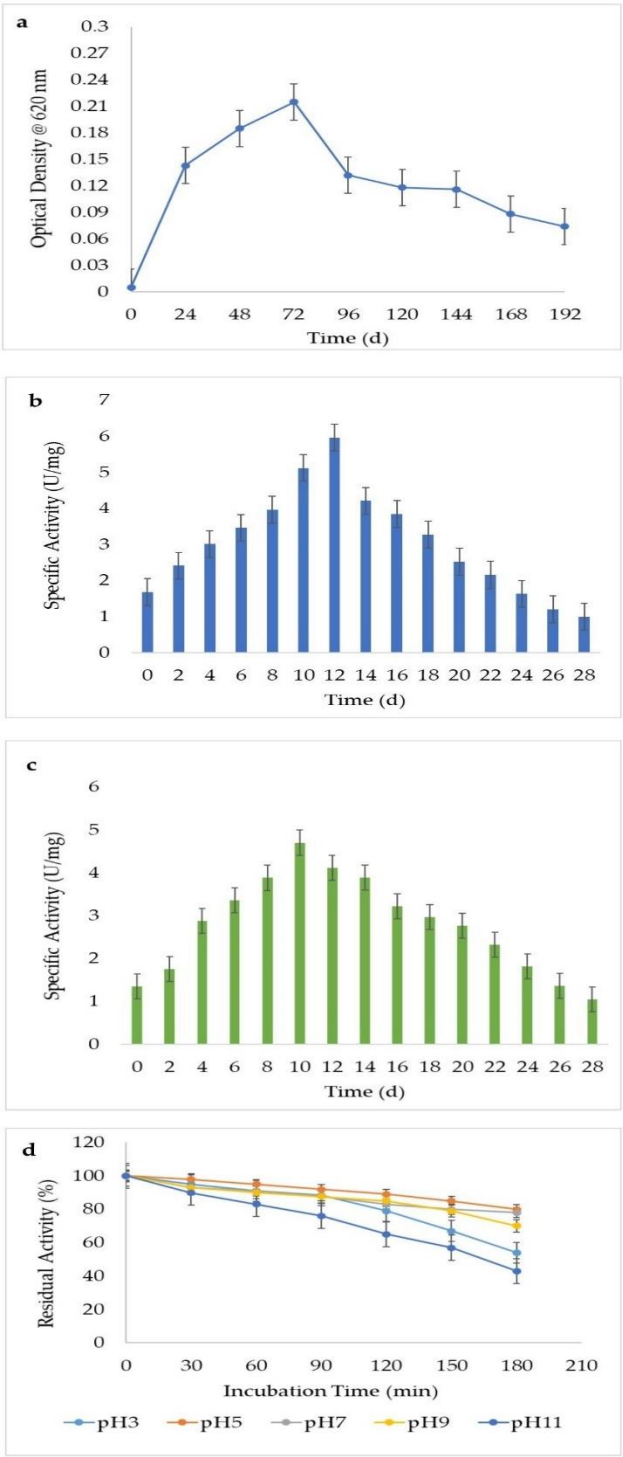

Figure 1. (**a**) Growth profile of *Proteus vulgaris* in LPM and Production of (**b**) Laccase, (**c**) Esterase and (**d**) Lipase from *Proteus vulgaris* (*Error bars* represent Mean ± standard deviation).

are essential for the hydrolysis, cleavage, and oxidation of ester bonds present in LDPE, which results in the breakdown of the LDPE polymer chain [12, 24].

3.2. Effect of pH on enzyme activity and stability

The effect of pH on the activity and stability of laccase, esterase, and lipase were studied because changes in the culture medium affect their activities, and substrate selectivity [24, 25]. The pH profile of laccase activity from *Proteus vulgaris* showed activity over a broad pH range (pH 3.0 to pH 11.0) (Fig. 2a). At pH 3.0, 60 % relative activity was observed and optimum laccase activity was obtained at pH 5.0. Similarly, laccase exhibited optimum stability at pH 5.0 with 80 % residual activity when incubated for 180 m at room temperature. Residual activities 54 %, 78 %, 70 %, and 43 % were obtained at pH 3.0, 7.0, 9.0, and 11.0, respectively (Fig. 2d). Laccase activity was optimum at pH 5.0 because an acidic pH enhances the oxidation of the substrate by laccase. Similarly, laccase was stable at pH 5.0 because the acidic pH maintained its stability, structural integrity, and catalytic potential [26]. Laccases with optimum activity at acidic pH have been reported to be efficient in the biodegradation of recalcitrant polymers [27].

Esterase produced by *Proteus vulgaris* also showed a wide pH activity between pH 3.0 to pH 11.0 with optimum activity at pH 7.0. At pH 3.0, relative esterase activity was 47.4 %, an increase of 59.2 % was recorded at pH 5.0, while a reduction to 40 % was obtained at pH 11.0 (Fig. 2b). Optimum esterase stability was likewise obtained at pH 7.0 with about 85 % residual activity when incubated for 180 min at room temperature. The residual activities of 54.3 %, 72 %, and 38.8 % were obtained at pH 5.0, pH 9.0, and pH 11.0, respectively (Fig. 2e). The activity and stability of esterase were optimum at pH 7.0 because neutral pH aids the production of hydrogen bonds, and salt bridges, which stabilizes esterase activity [28].

Lipase activity was optimum at pH 9.0 (Fig. 2c). The activity increased from pH 3.0 to 9.0, where the optimum activity was recorded. Similarly, lipase showed optimum stability at pH 9.0 with about 84% residual activity when incubated for 180 min at room temperature. Moreover, 55 %, 81 %, and 40 % residual activities were obtained at pH 5.0, 7.0, and 9.0, respectively (Fig. 2f). Lipase was optimally active at pH 9.0 because the alkaline buffer solution improved the reaction, and hydrolysis of bonds in the LDPE.

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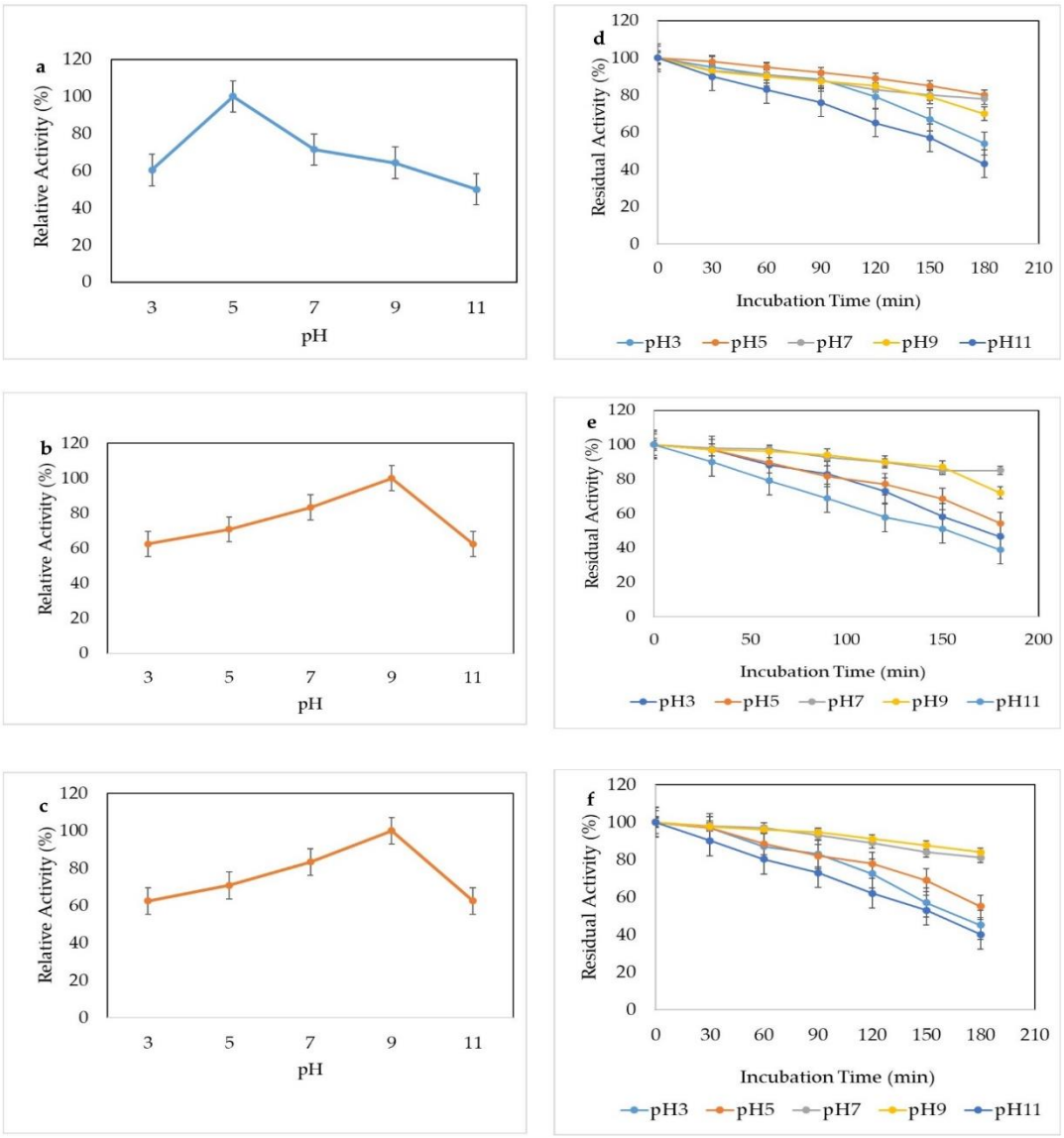

Figure 2. Effect of pH on the activity of (**a**) Laccase, (**b**) Esterase (**c**) Lipase and stability of (**d**) Laccase, (**e**) Esterase, and (**f**) Lipase produced by *Proteus vulgaris* (*Error bars* represent Mean ± standard deviation).

Lipase was stable at pH 9.0 because the lipase activity was maintained for a longer period at pH 9.0 [29]. A previous study also reported that lipase produced by bacterial isolates was effective in an alkaline medium [30]. Lipase purified from *B. methylotrophicus* PS3 was also used for polyethylene degradation and was reported to be stable at pH 7.0, and active between pH 7 to pH 9 [31].

3.3. Effect of temperature on enzyme activity and stability

The optimum temperature for laccase activity was obtained at 50 °C. Reduction in relative activity was recorded from 84 % to 52 % at 60 and 80 °C (Fig. 3a). After 3 h of incubation of enzyme solution at different temperatures, the enzyme was most stable at 50 °C retaining approximately 75% of its activity whereas 55 % residual activity was observed at 30 °C. (Fig. 3d). Laccase was active and stable at 50 °C because the temperature increased the kinetic energy and resulted in effective reactions between laccase and the substrate (LDPE) [32]. Laccase with 50 °C and 60°C optimum activities has also been observed [33, 34].

The effects of temperature on the esterase activity, produced by *Proteus vulgaris* are illustrated in Fig. 3b. The optimum temperature for esterase activity was

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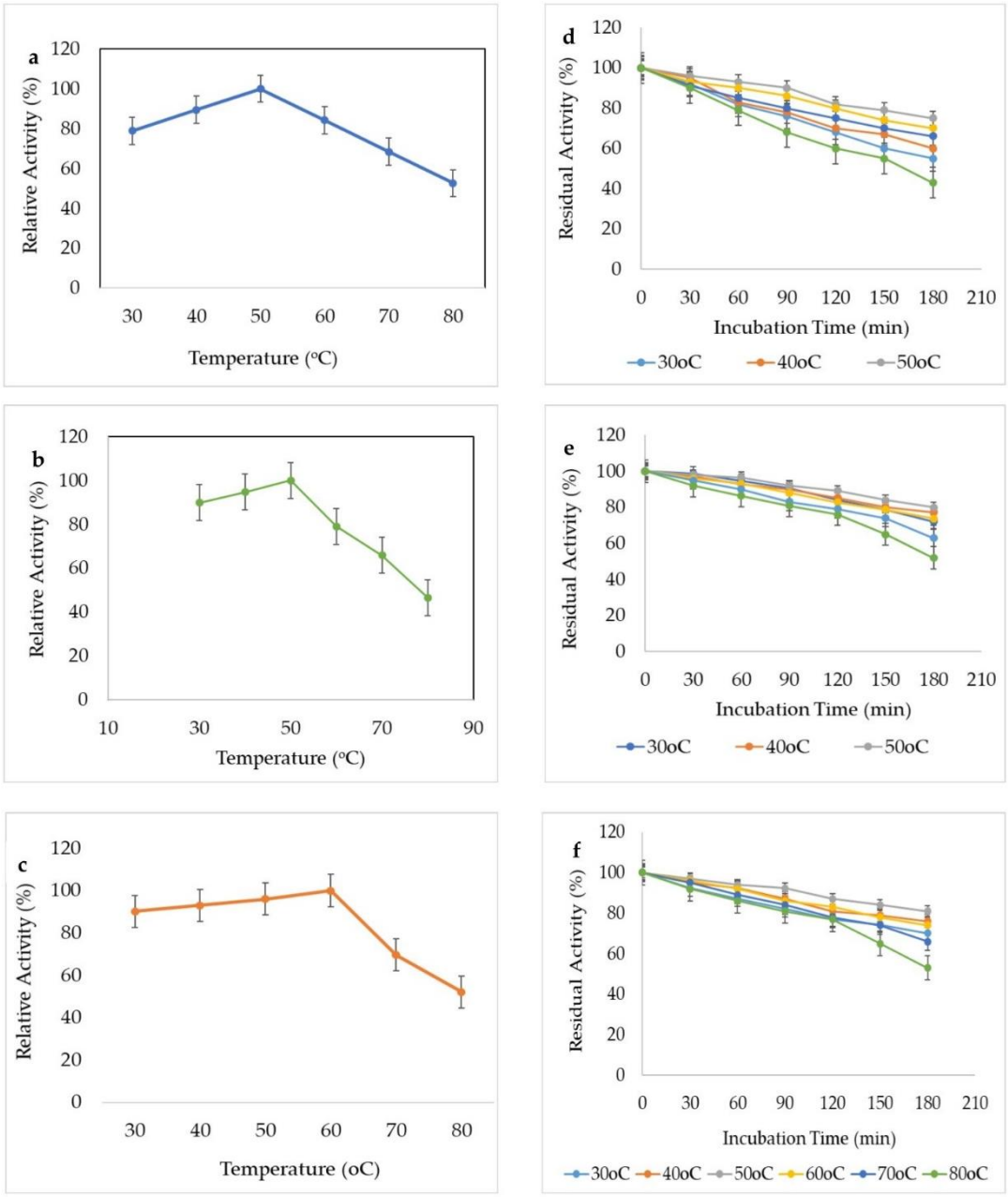

Figure 3. Effect of temperature on the activity of (**a**) Laccase, (**b**) Esterase (**c**) Lipase and stability of (d) Laccase, (**e**) Esterase, and (**f**) Lipase produced by *Proteus vulgaris* (*Error bars* represent Mean ± standard deviation).

also recorded at 50 °C. A gradual decline in relative activity was seen from 60 (79.1 %) to 80 °C (46.6 %) (Fig. 3b). After 3 h of incubation of esterase at different temperatures, the enzyme was most stable at 50 °C retaining approximately 80 % of its activity, while it showed 52 % residual activity at 80 °C (Fig. 3e). The produced esterase was active and stable at 50 °C because of the rigid thermophilic amino acid sequences in the structure that adapted to the temperature without being denatured [35].

The optimum temperature for the activity of lipase produced by *Proteus vulgaris* was recorded at 60 °C as shown in Fig. 3c. Lipase activity increased gradually with an increase in temperature within the range of 30 to 60 °C. A reduction in lipase activity was observed above at 60 °C (Fig. 3c). Lipase exhibited 66 % optimum activity at 70 °C and 53 % of its optimum activity was still retained at 80 °C. Lipase exhibited

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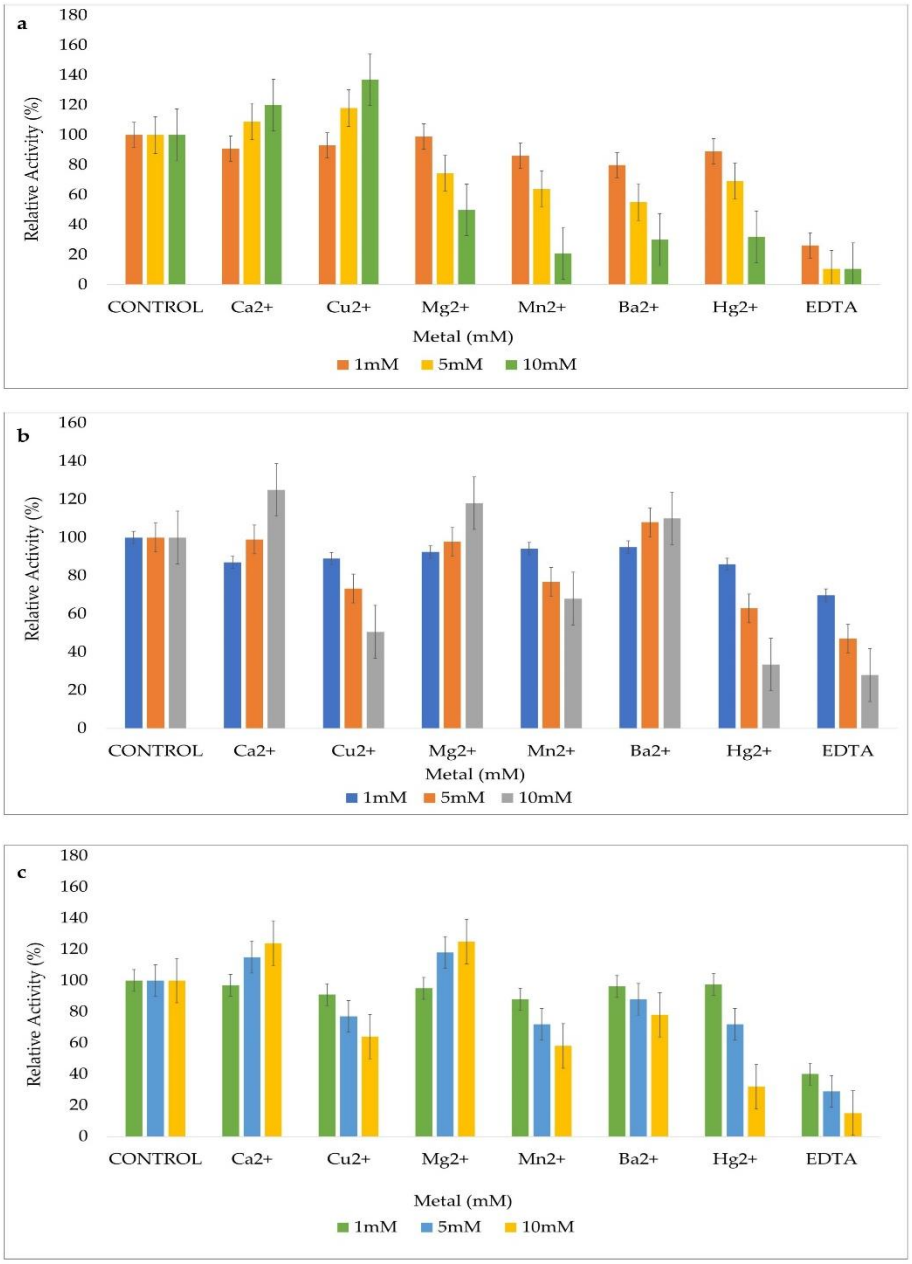

Figure 4. Effect of metal ions on the activity of (**a**) Laccase, (**b**) Esterase (**c**) Lipase produced by *Proteus vulgaris* (Error bars represent Mean ± standard deviation).

optimum stability at 50 °C with about 81% residual activity when incubated for 180 min at room temperature, while 74 %, 66 %, and 53 % residual activities were obtained at 60 70 and 80 °C, respectively (Fig. 3f). Lipase activity was optimum at 60 °C because the 3-dimensional structure of lipase was maintained at 60 °C, which enhanced maximum activity and stability [36]. The optimum temperature for lipase activity exposed to modified low-density polyethylene was also obtained at 50 °C [36]. Likewise, the temperature of lipase from *P. aeruginosa* EF2 used for polyethylene degradation was reported to be 50°C [37].

3.4. Effect of metal ions and EDTA on enzyme activity

Laccase activity produced by *Proteus vulgaris* was significantly enhanced by 10 mM Cu^{2+} (137 ± 0.02 %), and Ca^{2+} (120 ± 0.02 %) (Fig. 4a). The metal ions most especially Cu^{2+} increased laccase activity because they activated and stabilized the laccase structure by enhancing the conversion of oxygen to water, and

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increasing the oxidation reaction between laccase and LDPE [38]. Mg^{2+} and Cu^{2+} have been previously shown to increase the activity of laccase from *Bacillus spp.* [38]. In addition, Ca^{2+} enhanced laccase activity because it stabilizes the copper center of laccase enhancing its catalytic activity [39]. Laccase activity was lower in Mg^{2+}, Mn^{2+}, Ba^{2+} and Hg^{2+} solution and relative activities of 50 ± 0.02, 21 ± 0.02, 30 ± 0.02, and 32 ± 0.03 % were obtained, respectively. Laccase activity was also significantly reduced by 1 mM, 5 mM, and 10 mM EDTA (26 ± 0.03, 10.6 ± 0.02 and 10.6 ± 0.02 %, respectively). These metal ions (Ba^{2+}, Hg^{2+}, Mg^{2+}, and Mn^{2+}) reduced the laccase activity because they caused conformational changes when they bind to the active site, limited substrate binding, and finally inhibited laccase activity [17]. *Pseudomonas spp.* was also reported to produce laccase with reduced activity in Mn^{2+}, Ca^{2+}, and EDTA solutions [40].

Similarly, esterase produced by *Proteus vulgaris* increased in activity by 125±0.02 %, 118±0.01 %, and 110±0.02 % in 10 mM Ca^{2+}, Mg^{2+} and Ba^{2+} solutions (Fig. 4b), while there was a decrease in esterase activity in Mn^{2+}, Hg^{2+}, Cu^{2+} and EDTA solutions (68 ± 0.03, 33 ± 0.02, 50 ± 0.03, and 27 ± 0.02 %, respectively). Calcium ion (Ca^{2+}) increased esterase activity because it provided structural stability that enhanced the interaction of laccase with LDPE. Likewise, esterase activity was elevated by Mg^{2+} because it facilitated the oxidation at the binding site in the active site of esterase [41]. EDTA inhibited esterase activity because it limited the substrate affinity by binding to the metal ions present in the active site [22].

In addition, lipase activity was elevated by 10 mM Ca^{2+} (124.1 ± 0.02 %), and Mg^{2+} (125.1 ± 0.03 %) (Fig. 4c). The metal ions (Ca^{2+}, and Mg^{2+}) enhanced lipase activity because they stabilized the three-dimensional structure of lipase and maintained the active conformation crucial for catalytic activity [42].

There was a significant decrease in lipase activity in Cu^{2+} (64±0.02 %), Mn^{2+} (58.1 ± 0.02 %) and Hg^{2+} (32 ± 0.03 %) solutions because they bound to the enzyme active site, hindered substrate binding, and inhibited the enzyme's activity [42]. EDTA also chelated the activity of lipase and 15 ± 0.03 % relative activity was recorded.

3.5. Biodegradation study

In this study, the biodegradative activity of *Proteus vulgaris* on LDPE sheet was investigated using scanning electron microscopy (SEM), The results showed morphological changes such as the formation of holes, cracks, and scraps on the LDPE sheet. The alterations and disruptions of the LDPE structure are due to the enzyme activities produced by *Proteus vulgaris*, especially laccase, esterase, and lipase activities during the biodegradation process (Fig. 5).

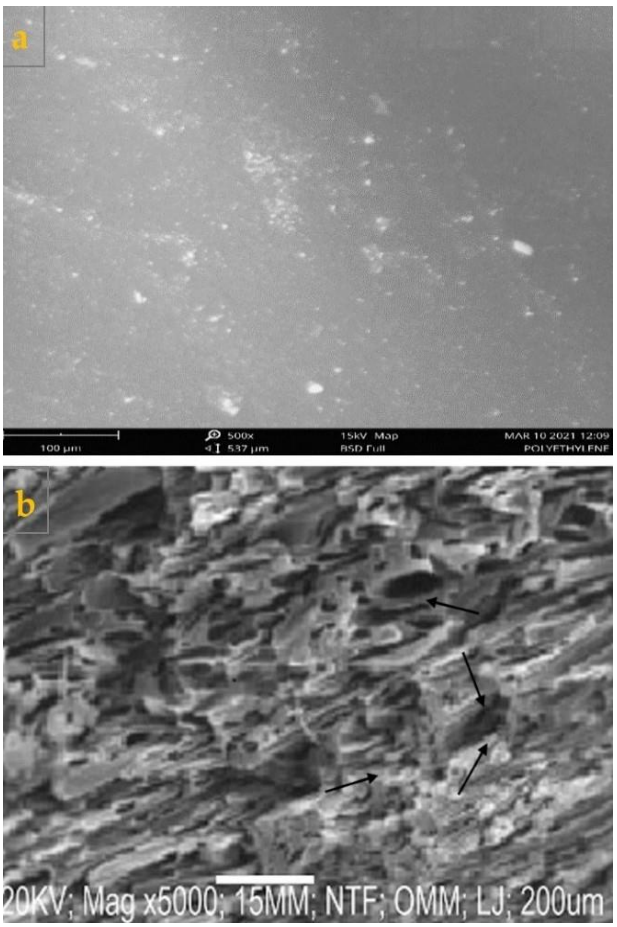

Figure 5. SEM micrograph of (a) Control and (b) LDPE biodegraded by *Proteus vulgaris* showing pits and cracks.

The alterations observed in the physical structure and appearance of LDPE sheet showed the biodegradation efficiency of *Proteus vulgaris.* Morphological changes were also observed on the surface of LDPE treated with *Microbulbifer hydrolyticus IRE-31* and *Bacillus pacificus* [43]. The results show that *Proteus vulgaris* has biodegradation activity on unmodified LDPE sheets.

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4. Conclusions

In conclusion, *Proteus vulgaris* produced thermostable laccase, esterase, and lipase. The enzymes showed activity and stability over a broad pH range. Optimum activity and stability were obtained for laccase at pH 5.0, esterase was optimum at pH 7.0, while lipase was optimum at pH 9.0. Laccase activity was enhanced by Ca^{2+}, and Cu^{2+}, esterase activity was increased by Ca^{2+}, $Ba^{2+}$and Mg^{2+}, and lipase activity was elevated by Ca^{2+}, and Mg^{2+}. However, EDTA chelated the activity of all the enzymes. After biodegradation over 30 days, the SEM micrograph of the LDPE sheet showed cracks, pits, and scrapings in the bio-degraded LDPE sheet when compared with the control. The results show the ability of *Proteus vulgaris* to produce thermostable enzymes, and biodegrade unmodified LDPE sheets *in vitro*. Hence, *Proteus vulgaris* can be explored for the biodegradation of different types of polyethylene.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conducted the research and wrote the manuscript, O.O.E.

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Availability of data and materials

The data used to support the findings of this study can be obtained from the corresponding author upon request.

Conflicts of interest

The author declares no conflict of interest.

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