PURIFICATION AND CHARACTERIZATION OF ALKALI-THERMOPHILIC LIPASE FROM *Pseudomonas aeruginosa* ECS3

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Abstract. Lipases are hydrolytic enzymes that are used in different biotransformation processes. This study aimed to purify and characterize lipase with suitable biochemical properties and to determine its compatibility with commercial detergents. The broth culture was centrifuged to obtain the crude lipase, which was purified by precipitating ammonium sulphate, ion exchange chromatography, and gel filtration chromatography. Some of the biochemical properties of the purified enzyme were determined using standard procedures. In addition, the compatibility of the purified lipase with some commercial detergents was evaluated. The purification of lipase from *P. aeruginosa* ECS3 gave the yield, specific activity, and purification fold as 4.35%, 2.40 µmol/min/mg, and 8.35, respectively. Purified lipase recorded 37.42 kDa on denaturing electrophoresis. The Michaelis-Menten constant (K_m), maximum velocity (V_{max}), maximum activity, and pH of the purified lipase were 14.79 µg/mL, 9.55 µmol/min, 50°C, and 8.0, respectively. K^+, Na^+, and Mg^{2+} stimulated the enzyme activity, while heavy metal ions inhibited the enzyme activity. Triglycerides improved the activity of *P. aeruginosa* lipase, while some organic solvents inhibited its activity. The purified enzyme was stable with some commercial detergents and therefore suggests the use of the enzyme as a detergent component. The results indicated that *Pseudomonas aeruginosa* ECS3 lipase has promising biochemical properties for use in the detergent industry.

Key words: characterization; detergents; lipase; *Pseudomonas aeruginosa*; purification.

INTRODUCTION

Enzymes called lipases catalyze the breakdown of ester bonds in triglycerides, releasing free fatty acids, glycerol, monoglycerides, and diglycerides [40]. Considering the market value of enzymes worldwide, almost 30% is made up of lipase, estimated at 400 million dollars in 2017. In 2023, this enzyme was projected to hit 590 million dollars. Furthermore, lipases are essential for fat and lipid metabolism in plants, animals, humans, and microbes [5, 27]. This important group of enzymes aids in the catalysis of synthetic and hydrolytic reactions. Lipases reverse hydrolytic reactions in micro-aqueous environments, leading to esterification and transesterification [40]. Some specific properties, like broad substrate specificity, selectivity, and superior stability, have given the use of lipases from microorganisms an advantage [40, 43]. Other endearing characteristics include ease of manipulation, increased yield, reduced production cost, short generation time, and ability to act on different substrates [33]. Bacteria, actinomycetes, or fungi produce microbial lipases. Microbial lipases, which are mostly glycoproteins, can be intracellular, extracellular, or membrane-bound [29]. Genus such as *Pseudomonas*, *Achromobacter*, *Burkholderia*, *Bacillus*, *Streptomyces* and *Arthrobacter* are the most common lipase producers [5]. Bacterial lipases are unique in their specific properties, enabling their classification into eight families [37]. Some methods used to produce this valuable enzyme include solid-state fermentation and submerged fermentation. In submerged fermentation, there is uniformity in the medium and it is easier to monitor various physicochemical parameters for maximum production [9]. Their ability to catalyze different reactions makes lipases suitable as biocatalysts in industries such as food, pharmaceutical, medical, detergent, cosmetic, dairy, leather, textile, pulp and paper, biodiesel, bioremediation, plastic, and wastewater treatment [12, 40]. As a result of the harshness of the chemical additives used in the detergent industry coupled with the toxicity of their waste product, it is necessary to source lipase with suitable biochemical and physicochemical properties for use in this industry. The continuous demand and search for active lipase enzymes to meet the increasing industrial demand required this research. In this study, lipase is produced by *Pseudomonas aeruginosa* ECS3, purified, and its biochemical properties are evaluated to incorporate the purified lipase in some known commercial detergents for removing oil stains.

MATERIALS AND METHODS

Maintenance of culture

Pseudomonas aeruginosa ECS3 (Accession number: PQ778810) was obtained from a previous work in the Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria.

Lipase production by *P. aeruginosa* ECS3

A pure culture of *P. aeruginosa* ECS3 was used for lipase production by sub-culturing the bacterium on nutrient agar plates. The basal medium for lipase production consisted of (% w/v): K_2HPO_4 0.09g; lactose 0.5g; yeast extract 0.5g; NH_4SO_4 0.1g; $CaCl_2$ 0.011g; Olive oil 1%, v/v, pH 8.0. The mixture was sterilized at 121°C for 15 min. One milliliter of 18 h old seed culture was used as an inoculum. The flask was inoculated and incubated at 35°C at a stirring speed of 120 rpm for 24 h as optimized by Ogunniran *et al*. [30]. The spent medium was centrifuged at 5000

rpm for 20 min at 4°C, and the resultant clear supernatant was used as crude enzyme solution.

Assay of lipase activity

The assay was carried out as described by slightly modifying the method of Lotrakul and Dharmsthiti [24].

Protein content determination

The total protein concentration of the enzyme solutions was determined using bovine serum albumin (BSA) as a standard, as described by Bradford [10].

Purification of lipase

Ammonium sulphate precipitation

A quantity of 950 mL of the crude enzyme was saturated to 60% with solid ammonium sulphate and kept overnight at 4°C. Subsequently, it was centrifuged at 10,000 ×g at 4°C for 20 min. The supernatant was discarded after confirming no lipase activity was present, and the pellet was collected and re-dissolved in 10 mL of buffer. The mixture was extensively dialyzed against 50 mM Tris-HCl, pH 8.0 at 4°C with changes in the buffer. Dialysate was assayed for lipase activity and protein concentration. The dialysate was concentrated in 4 M sucrose for further purification.

Ion–Exchange Chromatography in DEAE-Sephacel

For further purification of the concentrated dialysate solution, 15 mL of protein was carefully layered in an ion exchange column (2.5 x 40 cm) on DEAE-sephacel gel equilibrated with 50 mM Tris HCl, pH 8.0 at a flow rate of 60 mL/h. The column was eluted with the same buffer until no protein was detected in the flow-through fractions. The bound protein was eluted from the column and fractions were collected. The absorbance of the protein was measured at 280 nm using a UV spectrophotometer. The fractions showing the highest absorbance at 280 nm were analyzed for enzyme activity, and the fractions with the highest activity were pooled. The pooled fractions were concentrated with a 4 M sucrose solution [31].

Gel Filtration on Sephadex G-100

A quantity of 10 mL of the partially purified enzyme from ion exchange chromatography in a column (1.5 x 75 cm) on Sephadex G100. Then it was equilibrated with 50 mM Tris HCl buffer pH 8.0 and the same buffer was used to elute the column at a flow rate of 25 mL/h. Five milliliter (5 mL) fractions were collected, the absorbance of the fractions was read using a UV/Vis spectrophotometer at 280 nm, and lipase activity was determined. The fractions containing lipase activity were pooled and concentrated with a 4 M sucrose solution [31].

Determination of the molecular weight of purified lipase

The denaturing gel electrophoresis of purified lipase was determined on a 10% acrylamide gel, as described by Laemelli [22]. Protein markers used were: triosephosphate isomerase (26 kDa); lactic dehydrogenase (36.5 kDa); fumarase (48.5 kDa); pyruvate kinase (58 kDa); lactoferrin (90 kDa); β-Galactosidase (116 kDa); and macroglobulin (180 kDa). The purified enzyme solution (40 µL) was pipetted into an Eppendorf tube and mixed with 10 µL of loading dye; this was boiled for 1 min. The resolving gel consisted of 30% acrylamide, 1.5M Tris buffer pH 8.8, 10% SDS, 10 % ammonium per sulphate, and TEMED. The stacking gel solution comprised 30% Acrylamide, Tris buffer pH 6.8, SDS, 10 % ammonium per sulphate, and 3µL of TEMED. The purified enzyme solution (40 µL) was pipetted into an Eppendorf tube and mixed with 10 µL of loading dye; this was boiled for 1 min. Eight microlitres (8 µL) of the purified enzyme solution and 5 µL of the standard solution (standard proteins mixed with the loading dye) were separately applied into the wells of the slab gel. The electrophoresis was performed at 80 volts and 21 milliamps using the running buffer for 5 h. The gel was stained with 0.25% Coomassie Blue R-250 after electrophoresis. Subsequently, it was destained with the destaining solution. The relative mobility (R_m) of each protein band was obtained according to the method of Laemelli [22]. A standard curve was obtained by plotting the molecular weight of the standard protein against the corresponding relative mobilities (R_m). The subunit molecular weight value of purified lipase was obtained by interpolation of their R_m values.

Determination of the Michaelis-Menten constant and maximum velocity

The values of Michaelis-Menten's constant (K_m) and maximum velocity (V_{max}) were estimated from the Lineweaver Burk plot [23], using para-nitrophenyl palmitate as substrate. The purified lipase solution was incubated with various concentrations of p-NPP ranging from 0 to 10 mg/mL. In all cases, the enzymatic activity was assayed under temperature and pH optima.

Determination of optimum temperature and stability of purified lipase

The optimal temperature was determined by incubating the substrate and the purified lipase solution with the substrate at reaction temperatures ranging from 30 to 80°C. The thermostability study was investigated over 2 h (at 30 min intervals) by incubating the purified enzyme in 50 mM Tris-HCl buffer, pH 8.0 at different temperatures (30-70°C). The residual activity was measured as previously stated.

Determination of optimum pH and stability of purified lipase

The purified enzyme was incubated with the substrate at pH 3.0 to 10.0 using different buffer systems. For the thermostability study, the purified enzyme solution was pre-incubated in selected buffer solutions at 37°C for 2 h with periodic withdrawal of the enzyme every 30 min [7]. Then, the residual activity was measured as previously described.

Effect of metal ions on purified lipase

This was studied by pre-incubating the purified enzyme with different concentrations (1 and 5 mM) of chlorides of metal ions such as Ca^{2+}, Na^{+}, Ba^{2+}, K^{+}, Hg^{2+}, and Fe^{2+} and Mg^{2+} in a buffer solution. After 10

min of incubation, the p-NPP mixture was added, and the enzyme activity was measured.

Influence of organic solvents on purified lipase

Acetone, butanol, ethanol, carbon tetrachloride (CCl_4), petroleum ether, toluene, hexane, ethyl acetate, methanol, and formaldehyde at 1% concentration each were incubated with the purified enzyme at 30°C for 10 min. Enzyme activities were performed as previously stated.

Effect of surfactants, inhibitors, and oxidizers on purified lipase

This was studied by slightly modifying the method of Hu *et al*. [19], the reaction mixture containing the purified enzyme solution was incubated for 10 min with different surfactants, inhibitors, and oxidizers at a concentration of 1%. They are Triton X-100, ethlyene diamine tetraacetic acid (EDTA), tween 80, iodo-acetic acid (IAA), β-mercaptoethanol (C_2H_6OS), hydrogen peroxide (H_2O_2), sodium hypochlorite (NaClO), urea, and sodium dodecyl sulphate (SDS). The enzyme assay was performed as previously described.

Influence of triglycerides on purified lipase

The purified enzyme was incubated with inducers such as soya bean oil, coconut oil, vegetable oil, tributyrin, olive oil, Tween 80, and castor oil at 37°C for 10 min [14]. The enzyme assay was performed as modified by Alami *et al*. [3].

Detergent compatibility test

The following commercial detergents were used at a concentration of 7 mg/mL: Klin®, Ariel®, Good Mama®, Sunlight®, Waw®, Magik®, and Zip®. To simulate washing conditions, the liquid detergents (Mama Lemon®, Morning Fresh®, and locally made 1, 2, and 3) were diluted 100-fold [4]. The enzymes present (if any) were denatured by heating the detergents at 65°C for 1 hr. The detergent solution was mixed with the purified enzyme in a ratio of 1:1. The mixture was then incubated at 37°C for 30 minutes. Enzyme activity was determined by slightly modifying the method of Alami *et al*. [3]. The control consisted of enzyme diluted with tap water (1:1) without detergent [42]. Distilled water was used as blank.

RESULTS

Purification of lipase

At 60% saturation of the supernatant with ammonium sulphate, a recovery yield of 5.25% and a purification fold of 1.53 were obtained. After purification with the DEAE-Sephadex A50 ion and Sephadex G100, a recovery yield of 4.35 percent and a purification fold of 8.35 were obtained (Table 1). The purification was conducted severally to give maximum yield. Figure 1 shows the molecular weight with a distinct band of about 37.42 kDa on SDS-PAGE electrophoresis.

Optimal temperature and thermal stability

The optimum temperature for the activity of the purified enzyme was attained at 50°C with a corresponding lipase activity of 6.14 (µmol/min). A gradual increase in the enzyme activity was observed from 30°C to 50°C, followed by a gradual decrease. The least activity was observed at 80°C where only 32.7% of activity was retained (Fig. 2a). The stability study reveals the stability of the enzyme at 40°C for 1 h where 62.1% of its activity was retained. Activity decreased at temperatures above 60°C (Fig. 2b).

Optimal pH and pH stability

There was a gradual increase in the enzyme activity as shown in Figure 2c. Activity increased from pH 3 to 8, the optimal pH (100%) with a corresponding lipase activity of 6.68 (µmol/min). This shows the alkaline nature of the enzyme. About 78 and 79% of residual activity were retained at pH 9 and 10, respectively. However, enzyme activity was inhibited at the acidic pH of 3-5. Enzyme stability was achieved at pH 8 for 90 minutes, where 71.6% of its residual activity was retained. After 120 min, above 58.7% relative activity was retained at pH 8. However, about 85% of activity was lost after 120 min at acidic pH 5 (Fig. 2d).

Table 1. Summary of the purification profile of *Pseudomonas aeruginosa* ECS3 lipase

Step	Vol.	LA	PC	TLA	TP	SPA	Yield	Fold
Crude enzyme	950.0	2.56	8.88	2427.78	8431.25	0.29	100.00	1.00
Precipitation	45.3	2.81	6.38	127.34	288.79	0.44	5.25	1.53
Ion exchange	29.6	4.20	3.38	124.32	99.90	1.24	5.12	4.32
Gel	18.5	5.71	2.38	105.66	43.94	2.40	4.35	8.35

Vol: volume (mL); LA: lipase activity (µmol/min/mL); PC: protein content (mg/mL); TLA: total lipase activity (µmol/min/mL); TP: total protein (mg); SPA: specific activity (µmol/min/mg); Yield: %.

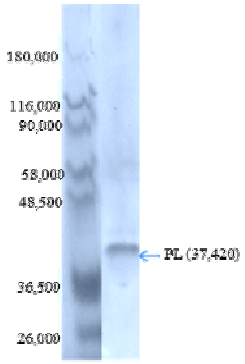

Figure 1. Gel plate of purified lipase from *P. aeruginosa* ECS3 on 10% acrylamide.

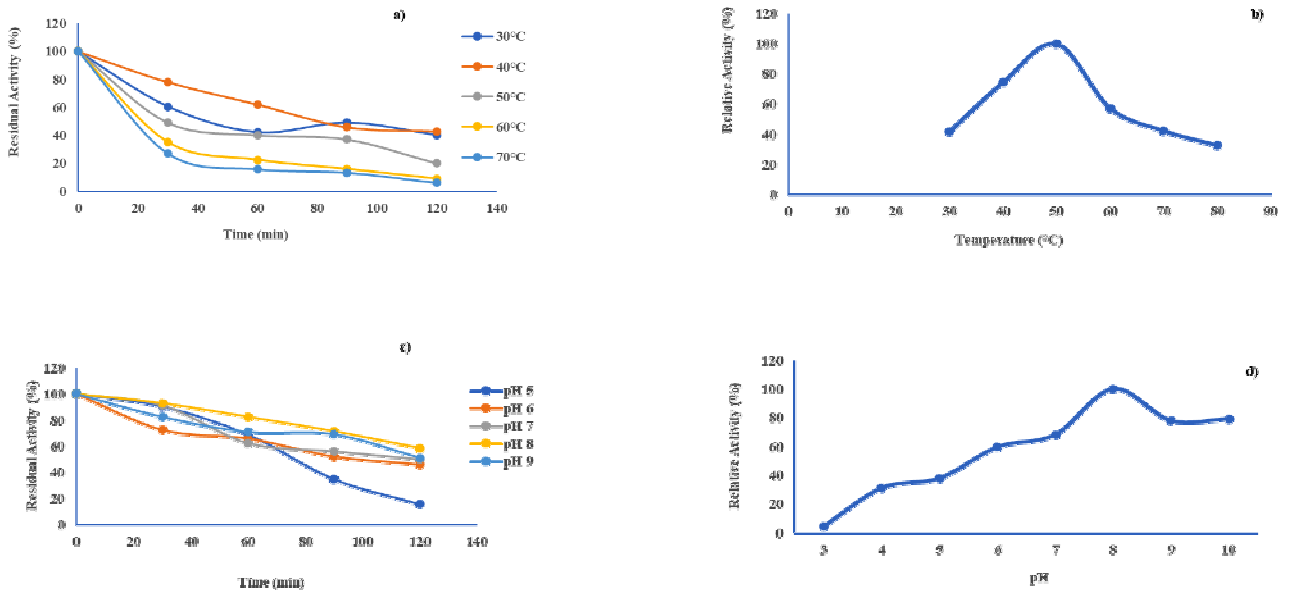

Figure 2. Effect of (a) temperature on activity of *Pseudomonas aeruginosa* ECS3 lipase (b)temperature on stability of *Pseudomonas aeruginosa* ECS3 lipase (c) pH on the relative activity of *Pseudomonas aeruginosa* ECS3 lipase (d) pH on stability of *Pseudomonas aeruginosa* ECS3 lipase

Kinetic constants of purified lipase

Kinetic studies from the Lineweaver-Burk plot revealed that K_m and V_{max} were 14.79 µg/mL and 9.55 µmol/min, respectively (Fig. 6).

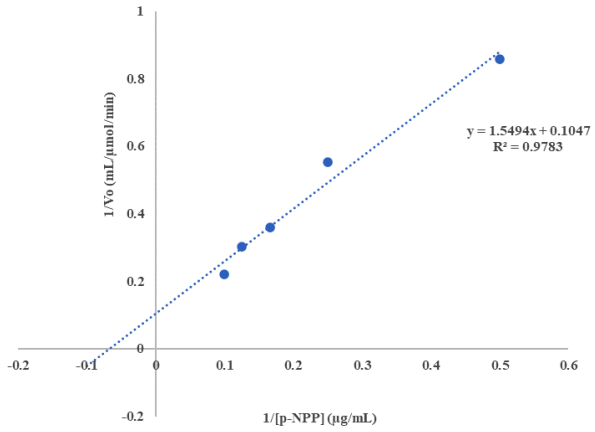

Figure 3. Lineweaver-Burk plot of *Pseudomonas aeruginosa* ECS3 lipase

Interaction of metal ions with purified lipase

The interaction of metal ions with the activity of *Pseudomonas aeruginosa* ECS3 lipase is shown in Fig. 4a. At 1 mM, the activity of *P. aeruginosa* ECS3 lipase was stabilized by K^+ ion with a relative activity of 100.68% corresponding with a lipase activity of 4.93 µmol/min while the control with 100% relative activity had a corresponding lipase activity of 4.9 µmol/min. At 5 mM, the activity was also elevated by K^+ ion with a relative activity of 108.62% corresponding with a lipase activity of 5.32 µmol/min while the control with 100% relative activity had a corresponding lipase activity of 4.9 µmol/min/. In addition, at a higher concentration of 5 mM, the enzyme activity was quite stable with Na^+, Mg^{2+}, and Ca^{2+} where relative activities of 90% (4.41 µmol/min), 92.97% (4.56 µmol/min), and 77.55% (3.8 µmol/min), were observed respectively. However, Ba^{2+}, Hg^{2+}, and Fe^{2+} decreased the enzyme activity by almost 89% (0.54 µmol/min), 100% (0), and 90% (0.51 µmol/min) at 5 mM.

Interaction of organic solvents with purified lipase

The addition of acetone, ethanol, toluene, n-hexane, ethyl acetate, and methanol to purified enzyme reduced relative activities to 67.18% (3.41 µmol/min), 69.37% (3.52 µmol/min), 69.37% (3.52 µmol/min), 73.09% (3.71 µmol/min), 64.55% (3.28 µmol/min), and 64.55% (3.28 µmol/min) respectively compared with the control (100%) which had an activity of 5.08 µmol/min (Fig. 3b). Furthermore, petroleum ether, butanol, and carbon tetra chloride (CCl_4) resulted to an enzyme loss of almost 81.2% (0.96 µmol/min), 63.5% (1.86 µmol/min), and 57.3% (2.17 µmol/min), respectively.

Interaction of oxidizers, inhibitors, and surfactants

P. aeruginosa ECS3 lipase was stable with Triton X-100 (3.71 µmol/min), Tween 80 (3.52 µmol/min), Urea (3.28 µmol/min), H_2O_2 (4.28 µmol/min), EDTA (4.66 µmol/min) and C_2H_6OS (3.18 µmol/min). In the presence of EDTA, an activity of about 98.82% (4.655 µmol/min) was recorded. Meanwhile, SDS, NaOCl, and IAA decreased enzyme activity to 20.28% (0.955 µmol/min), 21.70% (1.02 µmol/min), and 55.90% (2.63 µmol/min), respectively, compared with the control 100%, which had an activity of 4.71 µmol/min (Fig. 4c).

Interaction of triglycerides with purified lipase

All triglycerides used in this study enhanced lipase activity compared to the control (3.06 µmol/min) (Fig. 4d). Coconut oil had the highest activity (25.21 µmol/min) followed by tributyrin (24.79 µmol/min) and olive oil (20.14 µmol/min). Tween 80 which was the least, still had a relative activity of 170% (5.21 µmol/min).

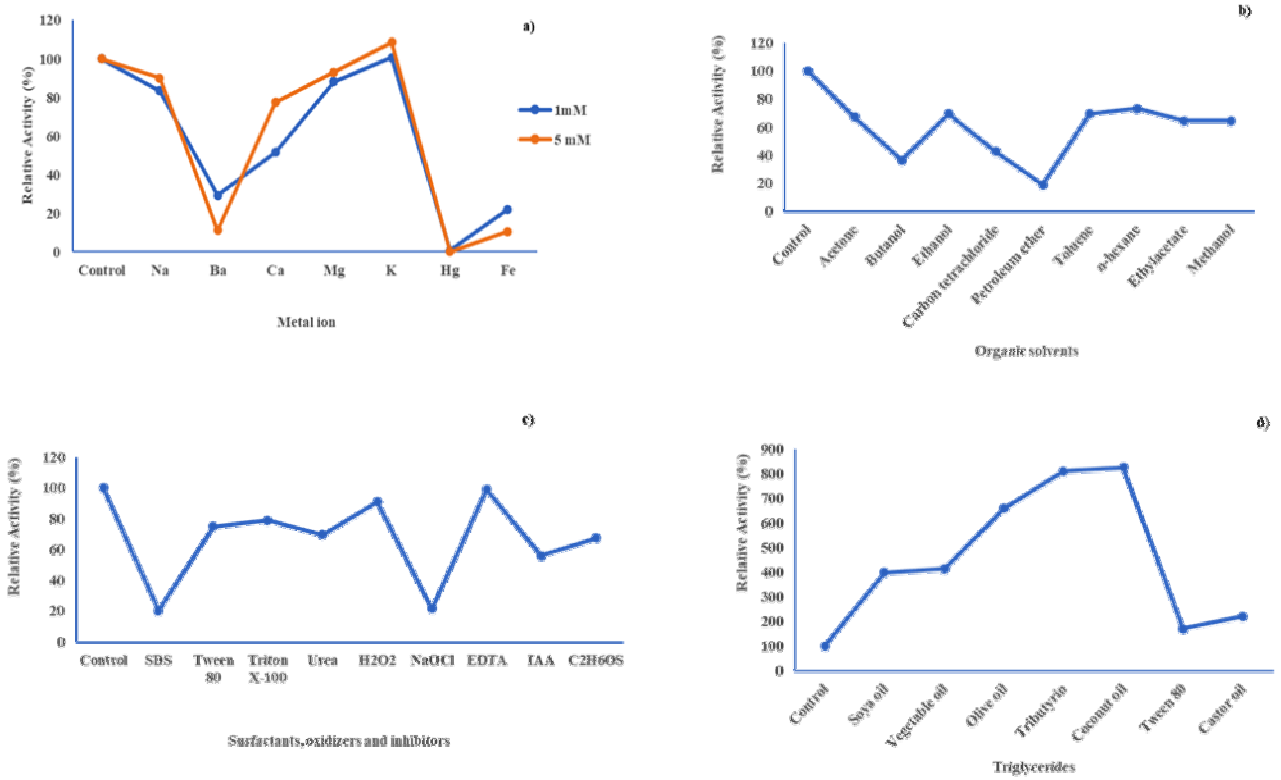

Figure 4. Effect of (a) Metal ion profile (b) organic solvents (c) surfactants, oxidizers, and inhibitors (d) triglycerides on *Pseudomonas aeruginosa* ECS3 lipase

Detergent compatibility of the detergent with the purified lipase

P. aeruginosa ECS3 lipase activity was highly stimulated by detergents such as Ariel®, Klin®, Waw®, Sunlight®, Canoe®, Morning Fresh® and locally made detergent 3, while enzyme loss was observed in the locally-made detergent 1 (Fig. 5).

DISCUSSION

One of the most important Gram-negative rods that can grow in different environmental conditions is *Pseudomonas aeruginosa* [1]. Adaptation to these conditions could be attributed to their ability to secrete alginate, an exopolysaccharide. The alginate helps the organism adhere to strong surfaces and protects against adverse conditions [17, 39]. *Pseudomonas aeruginosa* has diverse biotechnological applications because of its metabolic adaptability which has made it possible to be used as a model organism [11, 35]. It also serves as a good source of lipase enzymes with good potential in industrial applications [20].

The purification procedures gave a low yield mainly because of enzyme loss during the ammonium sulphate precipitation process. This low yield was catered for by purifying lipase several times to achieve the desired yield for the work. Purification is considered adequate due to increased specific activity and purification fold of 2.40 µmol/min/mg and 8.35, respectively. Similar methods were used to purify lipase of *Pseudomonas fluorescens* where a purification fold of 8.005 was achieved [36]. Mazhar *et al*. [26] also purified lipase from *Bacillus subtilis* PCSIR-N139 and subsequently obtained a purification

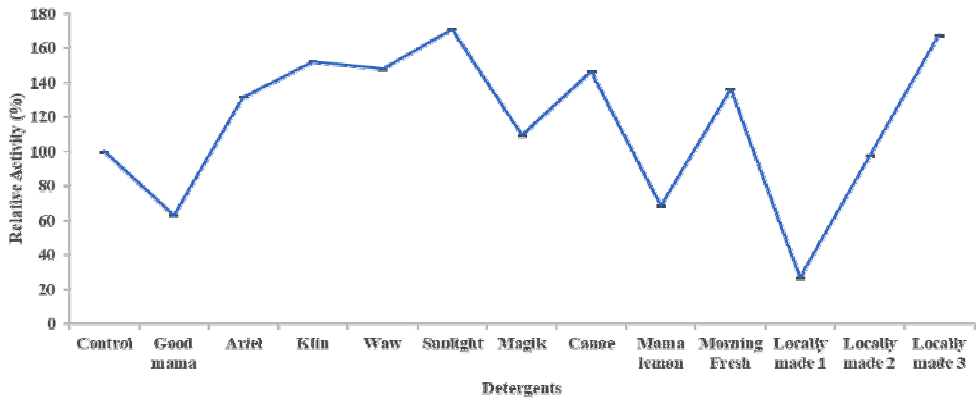

Figure 5. Activity of Lipase in the presence of commercial detergents

fold of 8.38. The purified lipase of *Pseudomonas aeruginosa* ECS3 had a 37.42 kDa molecular weight. Based on the position of the protein markers in related investigations, Poopola and Olateru [36] reported a molecular weight ranging between 35-50kDa for *Pseudomonas fluorescens* lipase. Previously, *Bacillus halotolerans* lipase had a molecular weight of 36 kDa [25]. The type of microorganisms or the method used can influence the varied molecular weights.

Enzyme structure and function are altered by the medium's pH, which impacts enzyme activity. The lipase activity seems to span a pH range of 7 to 10 where the optimum pH was attained at 8.0. Purified lipase was stable between pH 5 and 9. The best stability in terms of pH was attained at pH 8 for 90 min. *Anoxybacillus gonensis* UF7, which was characterized by Altinok *et al*. [6] attained its optimum pH 8. This also corroborates the optimal pH 8 by lipase produced from *Bacillus cereus,* as reported by Akhter *et al*. [2]. A purified lipase from *Pseudomonas reinekei* attained its stability between pH 5 and 9 [38].

Beyond the optimum temperature, denaturation occurred and this decreased the enzyme activity. Similarly, Phukon *et al*. [34] and Khudhair and Mohammed [21] reported an optimum temperature of lipase derived from *Pseudomonas helmanticensis* HS6 and *Bacillus licheniformis* 14T as 50°C. However, Facchini *et al.* [15] reported that two *Fusarium verticillioides* lipases were thermostable at 40°C. Temperature changes the enzyme reaction rate, modifying the protein's structure and function. The estimated K_m and V_{max} values of the purified enzyme were lower than the values reported by Mahnashi *et al.* [25]. In their investigation, they reported that *Bacillus halotolerans* lipase had a K_m of 9.37 mg/mL and a V_{max} of 41.66 IU / mL, respectively. The low K*m* value obtained in this study indicates that the enzyme has a high affinity for its substrate.

The potassium ion (K +) stimulated the activity of *Pseudomonas aeruginosa* lipase, suggesting its stabilizing effect on the enzyme. Enzyme stability was recorded in the presence of Na^+, Ca^{2+}, and Mg^{2+}. However, strong inhibition was exhibited by Hg^{2+} and Fe^{2+}. Similarly, Altinok *et al*. [6] in their investigation observed that *Anoxybacillus gonensis* UF7 lipase was active and stable with Ca^{2+} and Mg^{2+}. In addition, the report from Mazhar *et al*. [26] also highlighted that *Bacillus subtilis* PCSIR-N139 lipase was enhanced by Na^+, Mg^{2+}, K^+, and Ca^{2+}. Devi *et al*. [13] reported that the presence of Hg^{2+} reduced the stability of *Pseudomonas guariconesis* lipase, which agrees with this study. Depending on the effect or reaction triggered, metal ions can inhibit or enhance enzyme activity. Metal ions actively prevent denaturing and maintain the structure of the enzyme. Appreciable activities with organic solvents such as methanol, ethanol, toluene, n-hexane, ethyl acetate, and acetone were noticed. However, enzyme activity was reduced by carbon tetrachloride, butanol, and petroleum ether. Toluene and n-hexane enhanced the activity of *Penicillium crustosum* lipase as obtained in the investigations of Hasnaoui *et al.* [18]. Takó *et al.* [41] highlighted that *Rhizomucor mieei* NRRL5282 tolerated low concentrations of ethanol, propanol, isopropanol, and methanol. *Anoxybacillus gonensis* UF7 lipase was shown to be stable in methanol, as observed by Altinok *et al.* [6]. This suggests the usefulness of lipase in reactions like esterification and transesterification reactions.

The oxidizers, inhibitors, and surfactants used in this study enhanced lipase activity, except sodium dodecyl sulfate, sodium hypochlorite, and iodoacetate. In a related study, *Anoxybacillus gonensis* UF7 lipase was stable and active with Triton X-100 sodium dodecyl sulfate and Tween 80 [6]. Bakir and Metin [8] reported that EDTA enhanced activity, where 96% of activity was retained by *Anoxybacillus flavithermus* HBB134 lipase with 96%. Paitaid *et al.* [32] also stated that β-mercaptoethanol and EDTA did not significantly reduce lipase activity. *Bacillus cereus* lipase was inhibited by sodium dodecyl sulfate as reported by Akhter *et al*. [2]. Whereas, lipase from *Anoxybacillus flavithermus* HBB134 was inhibited by hydrogen peroxide and sodium hypochlorite as reported by Bakir and Metin [8]. The inhibition of enzymes by sodium dodecyl sulfate and sodium hypochlorite could be attributed to some modifications in the active site of the enzyme. The absence of metals at the active site could be responsible for its stability in EDTA, so it is not a metalloenzyme. The stability of the enzyme with various surfactants, oxidizers, and inhibitors suggests that the enzyme can be used in formulating detergent composition.

The stimulation of the purified lipase activity by triglycerides shows that the purified lipase exhibits broad substrate specificity. Mazhar *et al.* [26] revealed almond oil as the best substrate, while coconut oil had the least activity. According to Femi Ola *et al.* [16], olive oil was the preferred substrate for lipase activity, closely followed by groundnut oil. The lipase of *Pseudomonas aeruginosa* ESC3 was compatible with almost all detergents used in this study. The activities obtained with solid detergents seemed higher than those obtained with liquid detergents, but the enzyme was highly compatible with Klin®. It shows that it can withstand the harsh chemical additives present in commercial detergents. Devi *et al.* [13] observed the compatibility and stability of alkaline *Pseudomonas guariconesis* lipase with Henko, Ariel, Tide, Patanjali, and Surf Excel. Furthermore, the crude lipase of *Bacillus stratosphericus* was stable with three of the five detergents tested, as reported by Mohd Zin *et al.* [28]. For enzymes to be used as a detergent additive, it must be stable with other detergent components. The compatibility of *Pseudomonas aeruginosa* ECS3 lipase with commercial detergents is the first to be reported in the study area. This suggests that the enzyme obtained in this study could be used as a detergent additive.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article

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