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Investigation of Lipase Producing Bacteria from Oil Contaminated Soil and Characterization of Lipase

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Abstract

Hydrolytic enzymes such as lipases have emerged as key enzymes in a broad array of biotechnological industries due to their multifaceted characteristics. Many of the lipases that are currently in use in industry are of microbial origin. The aim of the present study was to isolate and identify lipase producing bacteria from oil contaminated soil and subsequent optimization of their culture conditions to maximize lipase production. Lipase producing bacterium *Burkholderia* sp. was isolated and identified by morphological studies, biochemical methods and 16S rDNA sequencing method. pH, incubation period, temperature, carbon source, nitrogen source and substrate concentration were studied to determine the optimum culture conditions for enzyme production. Media optimization studies showed that culture condition and media composition should be at pH 6, 30 °C, 48 hours of culture in a medium containing 2% olive oil as the main carbon source and yeast extract as the main nitrogen sources to maximize the production of lipase of *Burkholderia* sp. The crude enzyme exhibited hydrolytic activity in a wide range of temperatures (30 – 50 °C) and pH values (6–12), with an optimal temperature at 40° C and optimal pH at 8 with para-nitrophenyl palmitate as the substrate. Metal ions such as Cu^{2+}, Mn^{2+} and Zn^{2+} inhibited lipase activity. The enzyme preferably acted on olive oil as a substrate. The results are a clear indication that *Burkholderia* sp. has a capacity to produce lipases and can be considered as a potential candidature for biotechnological applications.

Keywords: 16S rDNA sequencing, *Burkholderia* sp., Lipases, pNPP assay

1. Introduction

Scientists paid several attempts to isolate lipase enzyme from microbes, with the primary focus on its role in biotechnological processes such as food, detergent, cosmetic and biomedical industries. Lipases, also known as triacylglycerol acyl hydrolases (EC 3.1.1.3), mainly catalyze the total or partial hydrolysis of ester linkages of glycerides at the oil-water interface [1]. In addition to hydrolysis activity, lipases exhibit esterification, inter-esterification, aminolysis, and alcoholysis activity, all of which contribute to a wide range of applications [2]. Lipases are secreted by many microorganisms, including bacteria, yeast, and fungi that have been domiciled in a diverse range of environments, including oil-contaminated soil, vegetable oil mill effluent, industrial dumping sites, dairies and so on [3]. After proteases and carbohydrases, lipases are the third most commercially produced enzymes, accounting for more than one-fifth of the worldwide enzyme market due to their broad substrate specificity, stability, ease of gene manipulation and bulk production [4]. In addition to physicochemical parameters like temperature and pH, the composition of the medium, for example, carbon sources, nitrogen sources and their concentrations, has a significant impact on the synthesis of extracellular lipases from bacteria [3]. Bacterial lipases offer a considerable potential for diverse applications in various industrial contexts. Therefore, the present study was conducted to isolate lipase-producing bacteria from oil-contaminated soil and to optimize their lipase production by assessing production medium conditions and composition.

2.1 Isolation of lipase producing bacteria

Oil contaminated soil samples were collected from areas in and around the University of Peradeniya. A 1 g of soil from each sample was stirred in 10 ml of sterile distilled water. The water phase was serially diluted up to 10^6 fold and 1 µl of each dilution was spread on minimal medium containing (g/L) Olive oil 10; K_2HPO_4, 1.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; NH_4NO_3, 1.0; $FeCl_3 \cdot 6H_2O$, 0.05; $CaCl_2$, 0.02; peptone, 2.0 and Agar, 15.0 with pH adjusted to 6.8. Plates were incubated at 37 °C for up to 48 hours.

2.2 Screening lipase producing bacteria

Bacterial isolates were assessed for lipase production on phenol red and Tween 20 media. Phenol red media was prepared as follows: 0.01% (w/v) phenol red, 0.1% (w/v) $CaCl_2$, 1% (v/v) olive oil, 2% (w/v) agar and pH adjusted to 7.3-7.4 with 0.1 N NaOH. Five-millimeter wells were bored into the phenol red plates. Wells were filled with 100 µl of

cell-free culture supernatant which contains crude enzyme. Cell-free culture supernatant was obtained by centrifuging bacterial culture broths at 15,000 rpm for 15 minutes. To prepare the bacterial culture broths, the minimal media (without agar) detailed above was inoculated with bacteria and incubated at 37 °C for up to 48 hours. Plates were incubated at 37 °C for 24 hours. Color change from pink to yellow around the wells was considered as an indication of lipase activity. Tween 20 plates were prepared as follows (g/L): 10 g peptone, 5 g NaCl, 0.1 g $CaCl_2 \cdot 2H_2O$, 20 g agar and 10 ml (v/v) Tween 20. The bacterial isolates were point inoculated onto the Tween 20 plates and incubated at 37 °C for 48 hours. A white precipitation around the colony was indicative of lipase activity.

2.3 Enzyme production

For the enzyme production, the 5 selected isolates were cultured in flasks agitated at 150 rpm at 37 °C for 48 hours in a minimal medium prepared by adding olive oil as the main carbon source. Media contained (g/L) Olive oil 10; K_2HPO_4, 1.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; NH_4NO_3, 1.0; $FeCl_3 \cdot 6H_2O$, 0.05; $CaCl_2$, 0.02; peptone, 2.0 with a pH adjusted to 6.0. The olive oil was emulsified in 1.0% (v/v) Triton X100. Cell free culture supernatant was obtained by centrifugation of culture broths for 15 min at 15,000 rpm. This served as the crude enzyme for the subsequent assays.

2.4 Lipase assay

Lipase activity was measured by determining the amount of p-nitrophenol released from p-nitrophenyl palmitate (p-NPP). The substrate solution was made by mixing 30 mM p-NPP in acetone (10 ml) with a second solution consisting of, 0.1 g gum Arabic, 0.4 ml Triton X100 and 90 ml distilled water. The crude enzyme (0.1 ml) was added to 1 ml of substrate solution and 0.5 ml of 50 mM Tris-HCl (pH 8). The solution mixture was incubated at 40 °C for 15 min. The reaction was terminated by adding 0.5 ml of ethanol and acetone mixture (1:1, v/v) and diluted to 4 ml using distilled water. Absorbance was measured spectrophotometrically using Shimadzu-1800UV double-beam UV/VIS spectrophotometer against a blank at 410 nm. The amount of p-nitrophenol released was calculated using the standard curve. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µM of p-nitrophenol per minute.

2.5 Morphological characterization

For studying morphology of the isolate, bacteria were grown on minimal media for 48 hours at 37 °C and morphological features such as colony shape, colony size, colony color, opacity and colony margin were recorded. For the microscopic characterization, Gram staining procedure was followed for bacterial culture grown for 48 hours on minimal media. Biochemical characteristics of the isolate were determined by the standard methods described in Bergey's Manual of Determinative Bacteriology [5].

2.6 Molecular identification

The isolate was identified by 16S rDNA sequencing method. Genomic DNA extraction was done by the standard phenol chloroform method described by Psifidi *et al*. [6]. Extracted DNA was amplified by the polymerase chain reaction (PCR) using 16S rRNA universal primers 786F: 5′-GATTAGATACCCTGGTAG-3′ and 1387R: 5′-GGGCGGWGTGTACAAGGC-3′. The 30 µL PCR mixture was composed of 2 µL of DNA template, 15 µL of PCR Master Mix (GoTaq® Green Master Mix), 0.5 µL of each primer (10µM) and 12 µL of nuclease free water. Thermocycler program was set to an initial denaturation at 95 °C for 15 min. followed by 30 cycles each of 95 °C for 45 sec, 52 °C for 1 min, 72 °C for 1 min, and finally, 72 °C for 10 min. Amplified DNA was visualized using agarose gel electrophoresis. The PCR product was sequenced by dideoxynucleotide chain termination method using Applied Biosystems® 3500 Series Genetic Analyzer. The generated sequences were aligned by MEGA version 7.0 software and resulted consensus sequence was compared with those in the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov) by using the BLAST (blastn) program (https://blast.ncbi.nlm.nih.gov). A phylogenetic tree was constructed by the neighbor joining method in MEGA 7.0 software with the bootstrap analysis value based on 1000 replicates to confirm the identity of the bacterial species of LDB-1 isolate.

2.7 Optimization of culture conditions for maximal lipase production

To enhance the ideal production of the lipase enzyme, different physiological parameters were tested. To determine the effect of pH, the culture media with pH adjusted to values ranging from 3-12, were inoculated and incubated at 37 °C for 24 hours. Similarly, the effect of temperature was evaluated by inoculating bacteria to the culture medium at five different temperatures (30 °C - 70 °C). To determine the effect incubation period, samples were withdrawn from cultures periodically at 24 - hour intervals. Five carbon sources: olive oil, coconut oil, vegetable oil, almond oil and glucose were used to find the optimal carbon source for enzyme production. The effects of NH_4NO_3, peptone, yeast extract, glycine and tryptone as nitrogen sources on enzyme production were examined. To determine the effect of substrate concentration, the culture media adjusted to values ranging from 0.25-10% olive oil were examined.

2.8 Characterization of lipase enzyme

Optimal temperature for lipase activity was determined by incubating enzyme reaction mixture at different

temperatures in the range of 30 ºC - 90 ºC in 50 mM Tris-HCl buffer at pH 6.

Effect of pH on lipase activity was determined by performing enzyme assay in different buffers with pH ranging from 3-12. To study the effects of metal ions on enzyme activity, crude enzyme was pre-incubated with 1% solution of $CaCl_2$, $CuSO_4$, $MnCl_2$, $MgCl_2$, KCl, NaCl and $ZnSO_4$ in 50 Mm Tris-HCl (pH 8) at 37 ºC for 1 hour. Substrate specificity of the enzyme was determined using olive oil, coconut oil, sunflower oil and vegetable oil. The lipase activity was measured using the titrimetric method using the following procedure. The substrate emulsion mixture was prepared by adding 20 ml of substrate solution to 80 ml of 10% gum arabic. The reaction mixture contained 3 ml of substrate emulsion mixture, 1 ml of 200 mM Tris-HCl buffer pH 7.2, 2.5 ml of deionized water and incubated for 5 min at 37 °C. Then 1 ml enzyme extract was added and incubated for exactly 30 min. The reaction was stopped by adding 3 ml of 95% ethanol. The solution was mixed with 4 drops of 0.9% (w/v) phenolphthalein indicator solution and the titration was performed with 0.05 N NaOH. Blank assays were conducted by adding enzyme just before titration. The amount of fatty acids liberated was determined by calculating the equivalents of NaOH required to achieve the titration endpoint using the following equation.

$$\mu\text{mol fatty acid/ml (U)} = [(\text{ml NaOH for sample} - \text{ml NaOH for blank}) \times N \times 1000] / V$$

Where:

U= µmol of fatty acid released/ml
N= The normality of the NaOH titrant used
V= Total volume of reaction mixture used (ml)

One unit of lipase activity was defined as the amount of enzyme which produces 1 µmol of fatty acids per min under assay conditions.

2. Results and Discussion

In the present study, nine isolates were obtained based on the ability to grow on selective media. Five of isolates LDB-1, LDB-2, LDB-3, LDB-4 and LDB-5 gave color change and clear zone formation around the colonies in phenol red agar plates (Figure 1A) and Tween-20 agar plates (Figure 1B) respectively. Detection of fatty acids release from triacylglycerols or fatty acid esters is the main principle behind these approaches [7]. Among other pH-based indicators, phenol red is considered the most sensitive indicator of its high reproducibility with a sensitivity level reported to be 0.5 enzyme unit (U) on a chromogenic agar plate and 5 µg amount of lipolytic enzyme by zymography [8]. Phenol red is a pH indicator dye with an endpoint at pH 7.3 – 7.4. The liberation of fatty acids into the medium from lipolysis causes a slight decrease in the pH, resulting in the color change from pink to yellow. This plate assay method has been widely employed by many researchers due to its sensitivity and distinct visualization of the results [7,9]. Another commonly used plate assay screening method used by researchers is the precipitation test employing Tween 20 as the principal substrate. The fatty acids released from hydrolysis of Tween 20 bind with the calcium present in the media, and visibly precipitate as calcium salts, around the colony. Tween 20 is favored as a substrate for detecting bacterial lipolytic activity due to its ability to promote optimal contact between cells and/or enzymes and substrate [10]. The use of Tweens has recently been challenged because it can be degraded by esterases (carboxylic ester hydrolase, EC 3.1.1.1), resulting in false-positive results in lipase screening assays. However, Tweens are still preferred as lipase substrates in screening assays because of their ready availability and clear visibility of results [7]. Lipase producing potential of isolates was assayed using p-nitrophenyl palmitate as the substrate and LDB-1 which showed the highest absorbance at 410 nm was chosen for further studies.

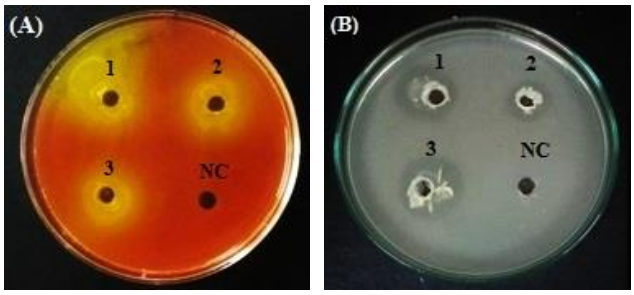

Fig. 1. Screening of LDB-1 isolate for lipolytic activity with phenol red as pH indicator (A) and Tween 20 agar plate with salt precipitates (B); cell free culture supernatant of LDB-1 isolate was loaded into wells 1-3, NC, negative control (culture supernatant without bacterial inoculation)

3.1 Bacterial isolate identification and characterization

The colony morphology of the LDB-1 isolate was observed. The colony was small, rod-shaped, white, the edge was observed to be smooth and the surface was wet. The microscopic study revealed the Gram-negative nature of LDB-1 bacterial strain (Figure 2).

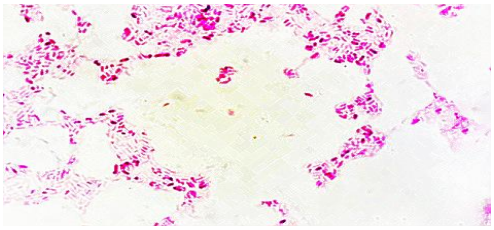

Fig. 2. Gram staining of LDB-1 isolate

Biochemical characteristic of the isolate were determined by the standard methods described in Bergey̓s manual [5]. Voges-Proskauer test, fermentation tests (mannitol, sucrose, and glucose), catalase, citrate and urease tests were positive; and methyl red, nitrate reduction and H2S production tests were negative. Standard biochemical approaches fail to identify bacteria at the species level. Therefore, further

identification, LDB-1 bacterial isolate was subjected to DNA Sequencing.

3.2 Molecular identification and phylogenetic tree construction

16S rRNA sequence of the LDB-1 showed 99.35% homology with *Burkholderia* sp. strain B20003 16S ribosomal RNA gene, partial sequence. ID: MT626032.1.

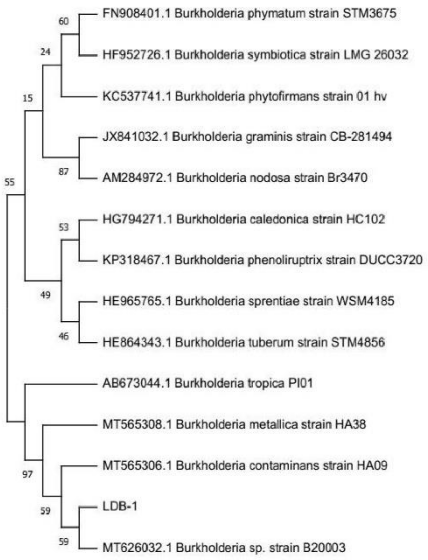

Fig. 3. The phylogenetic tree based on the sequence of LDB-1 isolate and reference strains belonging to genera *Burkholderia*.

LDB-1 isolate clusters with a *Burkholderia* sp. strain B20003 in the neighbor-joining phylogenetic tree (Figure 3). However, due to the average bootstrap values, moderately supporting the nodes, the reliability of the phylogenetic tree is not very high. This could be due to the high similarity among the sequences. Further taxonomic analysis methods, such as DNA barcoding and Multilocus Sequence Analysis (MLSA), are desirable for identification of this species.

3.3 Optimization of culture conditions for optimum lipase production

3.3.1 Effect of Incubation period

Considerable lipase activity was observed in the early exponential growth phase and the maximum lipase activity was attained after 72 hours (0.070 Uml^{-1}) during the late growth phase (Figure 4-A). The decline of the lipase activity after 96 hours could be due to the limitation of olive oil as it provides protection for lipase to avoid digestion by proteases in the external environment or change of pH of the medium or the repression by fatty acids accumulated from lipolysis [11,12,13].

3.3.2 Effect of temperature

Considerable enzyme activity was observed around 30 °C to 50 °C and the optimum enzyme production was at 30 °C. A noticeable reduction of enzyme activity was observed above 50 °C (Figure 4-B). Temperature is a major parameter that influences the secretion of extracellular enzymes by changing the physical properties of the cell membrane. Slight change in growth temperature can have a major impact on enzyme synthesis [14]. These results are consistent with earlier reports on optimum temperatures of *Burkholderia pyrrocinia* B1213 (50 °C) [15] and *Burkholderia cepacia* (40 °C) [16]. The enzyme activity drastically decreased after an increase in temperature to above 50 °C The decline implies the denaturation and thermal instability of the enzyme.

3.3.3 Effect of pH

The LDB-1 isolate is capable of producing lipase enzyme across a range of pH levels, from pH 3 to pH 12. The enzyme activity was considerably high at pH 6 to pH 8 and the optimum lipase activity was observed at pH 6 (0.076 Uml^{-1}) (Figure 4-C). pH is a major factor which influences cell growth and metabolic activity of an organism. Each microbe has a unique optimum pH. The enzyme activity being significantly high at pH 6 to pH 8 indicate that the bacteria can tolerate neutral or alkali environments. The results agree with previous pH optima of various *Burkholderia* strains including *Burkholderia unamae* pH ranging from 4.5 to 7.1 [17], *B. pseudomallei* pH ranging from 6.5 to 7.5 [18] and *Burkholderia cepacia* optimum pH of 9 [19]. Extreme and low culture pH such as 3 has repressed the lipase production. Previous studies have reported that neutral and alkaline media are more favourable for *Burkholderia* sp than acidic medium [19].

3.3.4 Effect of carbon source

Among the various carbon sources used, olive oil exhibited the maximum enzyme activity (0.115 Uml^{-1}) followed by vegetable oil, coconut oil and almond oil. Glucose utilization indicated a depressed lipase activity (Figure 4-D). Lipase is an inducible enzyme, and its expression is known to be altered by the carbon sources. Among the various carbon sources used, olive oil exhibited the maximum enzyme activity. Olive oil is composed of mainly oleic acid that accounts for 65–85% of total fatty acid content [23]. High oleic acid content has been reported to enhance the lipase activity [24]. Olive oil is known to be one of the best inductors of lipase production by not only *Burkholderia* sp, but also many other microbial species [25]. Since the market price of olive oil is moderately high, vegetable oil is likely to decrease production cost and chosen to be a versatile option for industries related to lipase production. Glucose utilization resulted in depressed lipase activity, which could be due to the catabolite repression by readily available carbon source in the medium [15].

3.3.5 Effect of substrate concentration

2% of substrate (olive oil) yielded a maximum enzyme activity (Figure 4-E). The increasing concentration of substrate increased the lipase activity, but it decreased by increasing the amount after 2% of concentration. This may be attributed to the feedback inhibition.

3.3.6 Effect of nitrogen source

Maximal lipase activity was observed when yeast extract was used as the nitrogen source (0.102 Uml^{-1}) followed by tryptone (0.099 Uml^{-1}) and peptone (0.097 Uml^{-1}) respectively (Figure 4-F). Bacteria in general express high enzymatic activity when organic nitrogen sources are used. In many cases, peptone, yeast extract and tryptone are considered as a common inducer for lipase production. Peptone was reported as the best nitrogen source for lipase production as it releases NH_4^+ ions in to the medium and stimulates the growth and elevates the enzyme production rate [26]. In contrast, reports of Thakur *et al.* [27] showed that peptone does not influence the lipase production of *Pseudomonas stutzeri* MTCC 5618. Other studies have also shown that the addition of organic nitrogen sources into growth media increases the production of lipase enzymes, including *Candida viswanathii* [21], *Micrococcus* sp. [28] and *Pseudomonas stutzeri* [27]. Many organic nitrogen sources provide additional nutritional factors such as vitamins, amino acids, and cofactors to the cells which are highly conducive to the growth of microbial cells and lipase production. Considerably high lipase production has been reported with inorganic nitrogen sources such as ammonium nitrate and ammonium chloride [29].

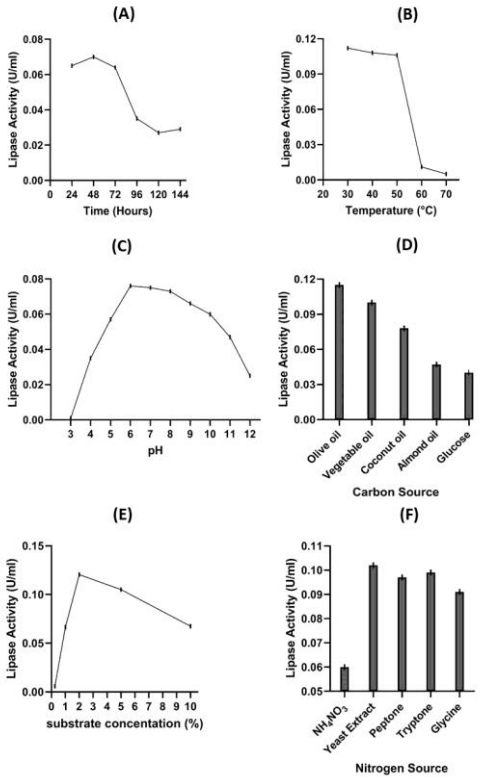

Fig. 4. Optimization of various culture parameters for maximum lipase activity (A) Incubation period, (B) Temperature, (C) pH, (D) Carbon Source, (E) Substrate concentration, (F) Nitrogen Source.

3.4 Optimization of enzyme reaction conditions

3.4.1 Effect of temperature

The enzyme activity was determined at various temperatures ranging from 30 °C to 90 °C. Considerable enzyme activity was observed around 30 °C to 50 °C and the maximum enzyme activity was observed at 40 °C. A significant reduction of enzyme activity was observed after 60 °C (Figure 5-A). According to Ungcharoenwiwat *et al.* [30], extracellular lipase from *Burkholderia* sp. EQ3 was active in the temperature range of 35–55 °C with the maximum activity at 30 °C. Moreover, the optimal temperature of lipases from *B. cepacia* ST 200 and *B. multivorans* AH-130 was 45 °C and 55 °C respectively, whereas *Burkholderia* sp. HY-10 exhibited 50% activity at 60 °C [30]. Many bacterial lipases are reported to have an optimum activity below 70 °C, except *Cohnella* sp. A01 lipase studied by Golaki *et al.* [31] that showed maximum activity at 70 °C. Recently, from moderate thermophilic isolates, several lipases have been purified, mainly representatives of the genus *Pseudomonas* [32,33] and *Bacillus* [34]. Minimum optimum temperature of 10 °C was reported by Salwoom *et al.* [35] for cold adapted *Pseudomonas* sp. LSK25 lipase. Psychrophilic lipases are making a mark in industries due to their high potential use in biotechnology industries.

3.4.2 Effect of pH

The enzyme activity was significantly high at pH 6 to pH 12 and the optimum lipase activity was observed at pH 8 (0.0727 Uml^{-1}) (Figure 5-B). A higher activity at alkaline pH is evident from the results. This result is in accordance with other *Burkholderia cepacia* lipases such as *Burkholderia* sp. GXU56 (pH 8), *Burkholderia* sp. HY-10 (pH 8.5) *B. cepacia* S31 (pH 8.5–9) [36] and *Burkholderia cepacia* RQ3 (pH 9) [16]. Newly purified Lipase SL-4, isolated from *Burkholderia ubonensis*, exhibited optimum activity at pH 8.5 [36]. Extracellular alkaline lipases are found to be more prominent than acidic lipases. Specifically, commercially available lipases from various microbes including *Pseudomonas* sp. BUP6, (pH 7–9 with the optimum activity at pH 8.2) [32], *Pseudomonas aeruginosa* HFE733 (pH 7–8.5) [37], and *Candida albicans* (CaLIP10) (pH 8.0) [22] are reported to have an alkaline stability. In contrast, for lipase from *Rhizopus oryzae* R1, highest activity was recorded at pH 6 [38]. Lipase is a proteinaceous enzyme. The ionized state of amino acids of a protein can be changed due to pH alterations and the ionic bonds responsible for maintaining the three-dimensional structure of the enzyme can be

disturbed leading to the changes in protein function thus affecting the enzymatic activity [39].

3.4.3 Effect of metal ions

The strongest inhibition of the enzyme was observed with Cu^{2+}, Mn^{2+} and Zn^{2+} while the highest enzymatic activity was observed when Mg^{2+} ions are present (0.0527 Uml^{-1}) (Figure 5-C). The ability of an enzyme to tolerate various metal ions is important not just for understanding its mechanism but also for commercial applications. Metal ions are known to be implicated in lipase catalyzed reactions by causing fatty acids to generate their respective metal salts at the oil–water interface, thus allowing the enzyme to act on oil molecules [40]. Lipase of *Burkholderi pyrrocinia* B1213 was activated by Mg^{2+}, Al^{3+}, Mn^{2+}, and Fe^{3+} ions [15]. Fe^{2+}, Mg^{2+} and Ba^{2+} ions were reported to enhance the lipase activity of *Burkholderia gladioli* while it was inhibited by Zn^{2+} and Fe^{3+} [41]. In *Burkholderia cepacia*, Mn^{2+}, Co^{2+} and Ca^{2+} ions sustained enzymatic activity while it was inhibited by the presence of Fe^{2+}, Hg^{2+} and Al^{3+} [42]. Ca^{2+} is considered as the most suitable metal ion for lipase activity. Addition of Ca^{2+} ions has reported to increase the lipase activity of *Staphylococcus caprae* NCUS6 by 1.66 folds with respect to control [40]. It is reported to enhance the lipase activity by stabilization of the whole three-dimensional structure. Thermostability of the lipase was increased in presence of Ca^{2+} ion [43]. Calcium-dependent thermostabilization has been exhibited by *Bacillus circulans* and *B. thermoamylovorans* lipases [44,45]. This could be due to the restriction of conformational plasticity of specific regions of the protein [46]. Similar enzymatic inhibition by Zn^{2+} and Cu^{2+} was observed in *Burkholderia gladioli* Bsp-1 [47]. It has been reported by Zhu *et al.* [47] that at a concentration of 10 mM, transition metal ions might strengthen the interactions between the ions and the surface amino acids of charged side-chain radicals. This could affect the ionization of specific amino acid residues, which can lead to the instability of the enzyme due to ion toxicity. In contrast, reports of Yao *et al.* [48] showed that Mn^{2+} had an activation effect on *Burkholderia ambifaria* YCJ01.

3.4.4 Effect of substrate specificity

As shown in Figure 5-D olive oil exhibited the maximum substrate specificity and *Burkholderia* sp. was able to hydrolyze all-natural lipid substrates. Many literature citations report the use of synthetic substrates such as para-nitrophenyl esters for the measurement of lipase, which yield a colored product upon hydrolysis [40, 48,49]. Not many recent works have reported using natural substrates for substrate specificity. However, Lopes *et al.* [50] has noted the high activity of *Geotrichum* sp. lipase in natural substrates than synthetic substrates. In contrast, *F. oxysporum* displayed a high specific lipase activity in the presence of synthetic substrates [50].

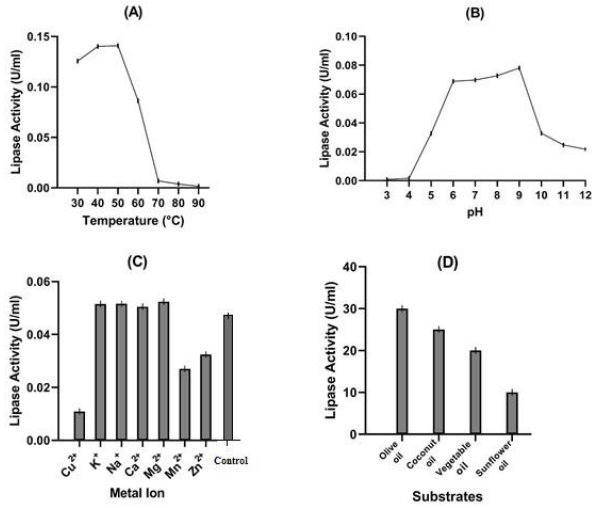

Fig. 5. Optimization of various enzymatic parameters for maximum lipase activity (A) Temperature, (B) pH, (C) Metal Ions, (D) Substrate Specificity

4 Conclusion

In summary, to maximize the enzyme production of *Burkholderia* sp, the media condition and composition should be at pH 6, 30 °C, 48 hours of incubation with yeast extract and 2% olive oil as the main nitrogen and carbon sources respectively. Furthermore, enzyme characterization results showed that lipase from *Burkholderia* sp. is an alkaline lipase. It is also relatively active in the pH range of 6–12 and temperature range of 30–50 °C. The presence of Mg^{2+} stimulated the lipase activity, whereas Ca^{2+}, Mn^{2+} and Zn^{2+} ions repressed the activity of enzyme. Of coconut oil, sunflower oil and vegetable oil, lipase showed the highest activity on 2% olive oil as the main natural substrate. Utilizing lipolytic bacteria has the potential to offer an eco-friendly and cost-effective option for remediating polluted soils and wastewater treatments. It is important to conduct further experiments at both laboratory and large-scale levels to explore the possible industrial uses of this enzyme.

Conflicts of Interest

No conflicts of interest are disclosed by the authors.

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