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Research Article

Optimization Studies on Production and Activity of Lipase Obtained From *Acinetobacter Junii* VA2 Isolated from Pond Soil

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ABSTRACT
Lipases are enzymes that catalyze the hydrolysis of fats to glycerol and free fatty acids. In the current study, the optimization of various parameters for maximum production of lipase by *Acinetobacter junii* VA2 isolated from pond soil sample was carried out. Optimum lipase production was observed in a medium adjusted to pH6 containing Fructose (3g/L), Soya powder (5g/L), Beef extract (3g/L) and Castor oil (1mL/L), which was incubated at 30°C for 48h under shaker (120rpm) conditions. However, the optimum lipase activity (204.81U/ mL) was observed at a temperature of 30°C and pH9. It was also found to be stable at higher pH levels. Further characterization of lipase enzyme showed detrimental effects of various inhibitors, organic solvents, oxidizing agents, detergents and metal ions on its activity. The yield of lipase after purification of the enzyme by dialysis was found to be 510U/mL. Thus, our study provides a potential source of high yielding and alkali-stable lipase producer that may be suitable for industrial use after appropriate stabilization.

KEYWORDS: Lipase; *Acinetobacter junii;* Enzyme activity; Dialysis

INTRODUCTION

Lipases (glycerol ester hydrolases) are among the most important industrial enzymes used widely in organic synthesis and bio-transformations [1]. They occupy a prominent place among biocatalysts and have a wide spectrum of biotechnological applications [2]. Their ability is not restricted to hydrolyzation of ester bonds and trans-esterification of triglycerides in solutions. Instead, they can also synthesize ester bonds in non-aqueous media [3]. In addition to their role in synthetic organic chemistry, they find extensive applications in chemical, pharmaceutical, food and leather industries [4, 5]. Promising fields for the application of lipases also include the biodegradation of plastics [6] and the resolution of racemic mixtures to produce optically active compounds [7].

Lipases are distributed among higher animals, microorganisms, and plants in which they fulfill a key role in the biological turnover of lipids. They are required as digestive enzymes to facilitate not only the transfer of lipid from one organism to another but also in the deposition and mobilization of fat that is used as an energy reservoir within the organism. They are also involved in the metabolism of intracellular lipids and therefore, in the functioning of biological membranes [8].

Lipases have been extensively investigated with respect to their biochemical and physiological properties [8]. The main advantage of lipases over conventional Colgate-Emery (high-

temperature fat splitting) process is cleaner product due to a more specific reaction and a lower energy requirement. However, the lipase reaction systems are too complex, usually consisting of two immiscible phases; an aqueous phase with dissolved enzyme and an organic phase with the dissolved substrate [9]. The application of lipase has been deterred in these reaction systems by the high cost of enzymes, contamination of products with residual protein, slow reaction rate, and lack of an ideal emulsion reactor system to cater to the complex interfacial heterogeneous hydrolysis.

Consequently, the sourcing of enzymes is being expanded from the conventional animals and plants to microbial sources. More than 50 lipases have been identified, purified and characterized to date, which originates from these natural sources. In recent times, there is an enormous potential observed among microbial lipases. Microbial lipases are widely diversified in their enzymatic properties and substrate specificities, and are generally more stable than plant and animal lipases. They have come into prominence because of new and novel applications in organic chemical processing, detergent formulations and synthesis of bio-surfactants. They also find immense applications in the oleo-chemical, dairy, agrochemical, paper, nutrition, cosmetics and pharmaceutical industries. This arises from the fact that they are quite stable and active in organic solvents [2, 10], do not require co-factors [11], exhibit a high degree of enantio- and regio-selectivity [12], and possess a wide range of substrate specificity for the conversion of various unnatural substrates. One limiting factor is the shortage of microbial lipases that do not meet the industrial requirement currently. However, it may be overcome by enzyme immobilization techniques that will allow ease of handling and enzyme recovery [13, 14]. The development of technology for lipase production and synthesis of novel compounds using lipase-mediated reactions is expected to extend its applications into newer areas and will have a major impact on a range of industries [15].

The present study reports the optimized parameters for production of lipase enzyme by *Acinetobacter junii* VA2 isolated from pond soil.

MATERIALS AND METHODS

Isolation and screening

Soil samples collected from various sites i.e., oil refineries, ponds and lakes in Mumbai were used in the current study. These samples were inoculated in Nutrient Broth (NB) to propagate the possibly stressed organisms, and allowed to revive at 30°C for 24h. Following the revival stage, the organisms were isolated on sterile Nutrient Agar (NA) plates and incubated at 30°C for 48h. The isolated colonies were then spot inoculated on Gorodkowa's agar (Tributyrin agar) plates and incubated further at 30°C for 48h to screen for lipase producers [16]. The lipase production was confirmed by the appearance of zone of clearance around the spotted colonies. Similarly, the screening of lipase producers was also checked on Rhodamine B agar and Tween 80 agar plates [17].

Extraction of lipase and enzyme assay

The supernatant obtained after centrifugation (10,000rpm at 4°C for 20min) of fermented NB by test cultures, isolated in our study, was used as a crude enzyme for lipase assay [18]. Quantitative estimation of lipase in the crude enzyme was determined by colorimetric assay using p-nitrophenyl palmitate as the substrate. For this purpose, 0.3mL crude enzyme was mixed with 0.7mL of 5mM p-nitrophenyl palmitate prepared in iso-propyl alcohol, containing sodium deoxycholate, gum Arabic, Triton X-100 and tris HCl buffer (pH 8). The absorbance of the mixture was measured at 420nm after 1min. The reaction mixture containing heat-inactivated (100^0C for 10min) crude enzyme was used as a blank. The absorbance of the test supernatant against the blank was obtained and plotted on the standard graph of *p*-Nitrophenol (2-20mcg/mL) to estimate the amount of substrate converted.

One unit of lipase activity was calculated as the amount of lipase enzyme which liberates 1 µmole *p*-Nitrophenol from 4-Nitrophenyl-palmitate as substrate per minute under standard assay conditions.

Identification

The preliminary identification of the isolate showing maximum lipase production was done with the help of morphological, cultural and biochemical tests in our laboratory, as described in the Bergey's Manual of Determinative

Bacteriology, 8th edition [19]. Molecular confirmation of the strain was done by 16s rRNA sequence analysis carried out at Yaazh Xenomics Navi, Mumbai, India.

Optimization of culture conditions for maximum lipase production

For optimization of enzyme production, it is absolutely essential to study the optimum culture conditions for growth of the test organism. In view of this fact, we commenced our study by optimizing the growth media from among 8 commonly studied mediums used for lipase production. The media used in this study and its components (in g/L) are listed below.

1. PM-1 [Glucose (3), peptone (5), beef extract (3) and olive oil (1ml/L)] [20];
2. PM-2 [Peptone (5), yeast extract (3), tributyrin (1mL/L) and tween 80 (2ml/L)] [20];
3. PM-3 [Sucrose (3), beef extract (3), tryptone (5) and olive oil (1mL/L)] [20];
4. NB + olive oil [Peptone (10), meat extract (3), NaCl (5) and olive oil (10ml/L)] [20];
5. Maltose Peptone Yeast extract (MYP) medium + olive oil [Maltose (10), yeast extract (1), peptone (5), K_2HPO_4 (1), $MgSO_4.7H_2O$ (0.2), Na_2CO_3 (15) and olive oil (10mL/L)] [21];
6. Glucose Yeast extract Peptone (GYP) medium + olive oil [Peptone (10), yeast extract (5), dextrose (20) and olive oil (10mL/L] [22];
7. Medium A [Soluble starch (20), peptone (20), KH_2PO_4 (5), $(NH_4)_2SO_4$ (1), $MgSO_4.7H_2O$ (1), $(NH_2)_2CO$ (1)] [23];
8. Medium B [Peptone (5), yeast extract (5), NaCl (0.5), $CaCl_2$ (0.05) olive oil (10mL/L) [24].

In addition, the effect of different carbon, lipid and nitrogen sources on lipase production were studied by replacing these nutrients in the production medium with various substitutes listed in table 1.

The optimization of other cultural conditions was carried out by varying one parameter at a time while keeping the others constant. These varying parameters included incubation period (12, 24, 36, 48, 60 and 72h), aeration i.e., static or shaking, temperature (30°C, 35°C, 45°C, and 55°C) and pH (2-12). For studying these different parameters, a 2% inoculum (0.1 $O.D_{520nm}$) of test culture was inoculated in the medium and incubated at different temperatures under static or shaker conditions. At the end of incubation period, cell-free supernatant was collected and crude enzyme assay was carried out using a protocol described above.

Table 1: Nutrient sources and inhibitors used in the study

Sl. No.	Nutrient source	Substitutes used in the study
1	Carbon	glucose, maltose, lactose, sucrose, fructose, arabinose, mannitol, starch, galactose, xylose and citrate
2	Lipid	olive oil, castor oil, sunflower oil, groundnut oil, palm oil, coconut oil and tributyrin
3	Nitrogen	peptone, yeast extract, meat extract, beef extract, tryptone and soya powder
4	Inhibitors (0.1%)	Phenyl Methyl Sulphonyl Fluoride (PMSF), Ethylene Diamine Tetrachloro Acetic acid (EDTA), 2-mercaptoethanol, dithiothretol (DTT) and bile salt
5	Metal ions (0.1%)	Ca^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, K^+ and Na^+ in the form of chloride salt
6	Organic solvents (10%)	acetone, ethanol, methanol, 2-propanol and butanol
7	Surfactants (1%)	DS, Triton X -100 and commercial detergents (Tide and Wheel)
8	Oxidizing agent (0.5-2.0%)	Hydrogen Peroxide

Effect of different physicochemical parameters on lipase activity

After optimization of culture conditions for production of lipase, it is also crucial to study the enzyme activity under different physicochemical parameters. As described above, the enzyme activity was also carried out by varying one parameter at a time while keeping the others constant. These varying parameters included temperature (30-55°C with a 5°C interval) and pH (1-12). In addition, the effect of different substances on lipase production listed in table 1 was also studied.

For studying the effect of different compounds, the crude enzyme was pre-incubated with the inhibitors, solvents, metal ions, surfactants and an oxidizing agent, mentioned in table 1 at 30°C for 30min and the residual activity was measured by standard lipase assay.

Purification of enzymes

Ammonium sulfate precipitation

The crude enzyme obtained after optimization of physicochemical parameters was subjected to precipitation by using various saturation concentrations (40%-90%) of ammonium sulfate. The addition of salt was done with constant stirring on an ice bath. The lowest saturation concentration of ammonium sulfate that successfully precipitated the enzyme was used in further studies. The precipitate obtained using 100ml of crude enzyme was collected by centrifugation carried out at 5000rpm for 20min at 4°C. The precipitate pellet was dissolved in 5ml of Tris-HCl buffer (pH 9) to determine its protein content (biuret method) and enzyme activity (lipase assay). The enzyme was further purified by dialysis.

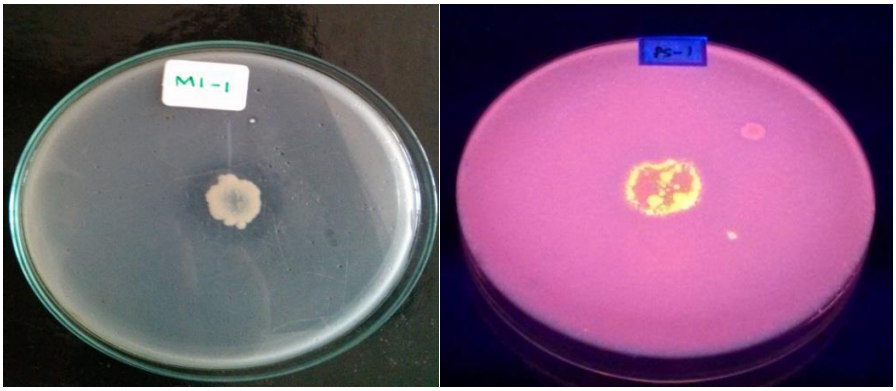
Dialysis

The dialysis tubing was cut and boiled for 10min in 2% Na_2CO_3 solution prepared in 1mM EDTA. It was then rinsed with distilled water and boiled in 1mM EDTA solution for 10min. The tubing was allowed to cool and stored at 4°C. Prior to use, it was washed thoroughly with distilled water. To carry out dialysis, the tube was filled with enzyme precipitate dissolved in buffer and sealed at both ends. The tubing was suspended in tris-HCl buffer and kept on ice bath overnight. The whole assembly was placed on a magnetic stirrer. On the next day, the dialysis tubing was punctured and the dialyzed enzyme was collected and assayed for its protein content and enzyme activity.

RESULTS

Isolation and screening of Lipase producers

The nine isolates obtained from soil samples were screened for extracellular lipase production on Gorodkowa's agar plates. Out of these, six isolates showed a zone of clearance on Gorodkowa's agar plates indicating lipase activity (Figure 1a). Three isolates produced fluorescence on Rhodamine B olive oil agar (Figure 1b) plates in addition to the zone of clearance on Gorodkowa's agar plates. The lipase activity of those three test isolates is indicated in figure 2. Among these, an isolate PS-1, obtained from pond soil sample showed maximum lipase activity and was selected for carrying out optimization studies.



(A) (B)

Fig 1: Detection of lipase activity on Gorodkowa's agar (A) and Rhodamine B olive oil agar (B)

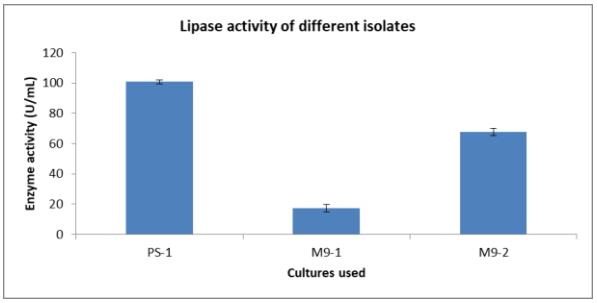


Fig 2: Lipase activity of test isolates

Identification

On the basis of morphological, cultural and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology, 8th edition, and by16S rRNA gene sequencing analysis, the PS-1 isolate was identified as *Acinetobacter junii* VA2.

Optimization of culture conditions for maximum lipase production

Figures 3-10 represents the observed lipase production by *Acinetobacter junii* VA2 under different cultural conditions. The maximum lipase activity (100-127U/mL) was found to occur /mL) as a substitute to peptone and tryptone respectively showed maximum lipase production.

in the PM-1 medium with pH6 when incubated at 30°C for 48h under shaker conditions. The lipase production was significantly increased in presence of carbohydrate source like 0.3% fructose (201.43 U/mL), followed by 0.3% maltose (200.00 U/mL), mannitol (189.92 U/mL) and sucrose (188.48 U/mL). Among the lipid sources, except for palm and coconut oil, all other sources showed an enzyme activity in the range of 189-201U/mL with castor oil (0.1%) exhibiting the maximum lipase yield (201.43U/ml). In addition, the combination of 0.5% soya powder (202.87 U/mL) and 0.3% beef extract (202.87 U)

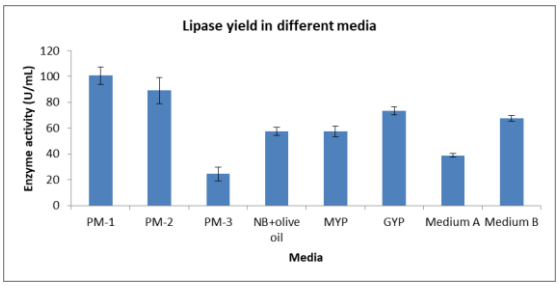


Fig 3: Lipase production in different media by *Acinetobacter junii* VA2

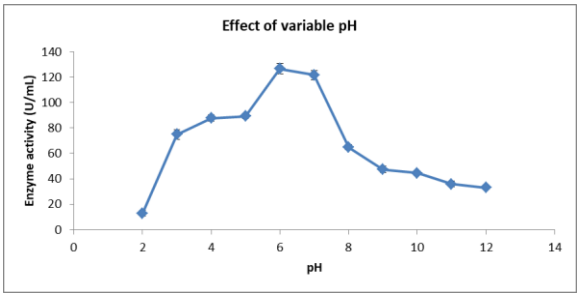


Fig 4: Effect of pH of the nutrient medium on the lipase production by *Acinetobacter junii* VA2

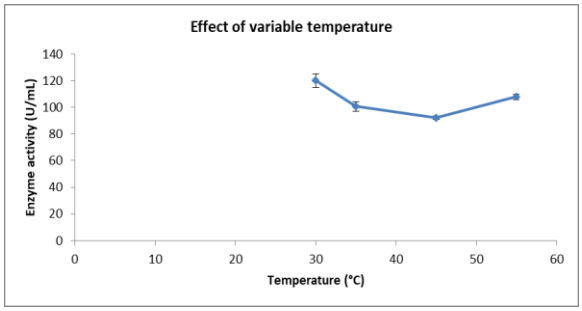


Fig 5: Effect of temperature of incubation on lipase production by *Acinetobacter junii* VA2

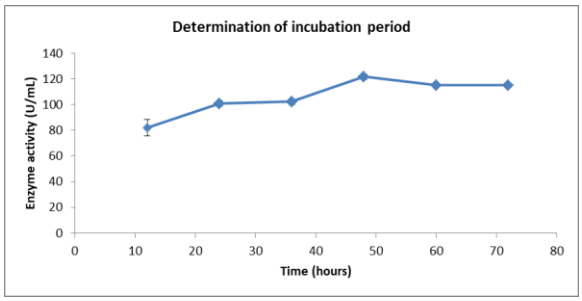


Fig 6: Effect of incubation period on lipase production by *Acinetobacter junii* VA2

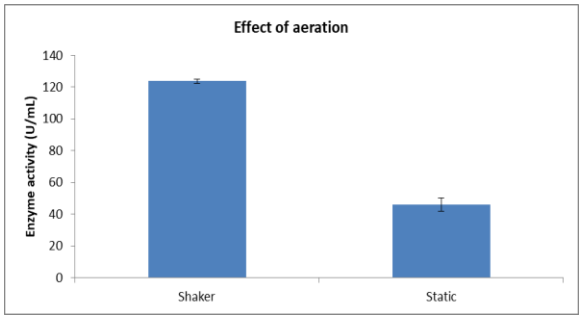


Fig 7: Effect of aeration on lipase production by *Acinetobacter junii* VA2

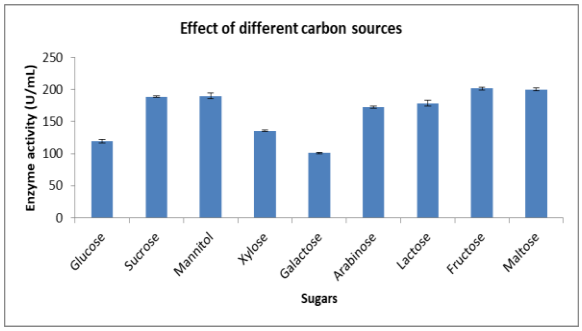


Fig 8: Effect of different carbon sources on lipase production by *Acinetobacter junii* VA2

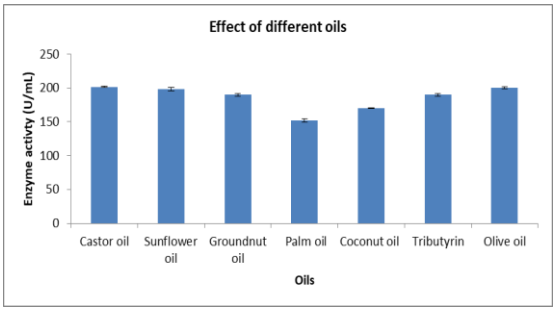


Fig 9: Effect of different lipid sources on lipase production by *Acinetobacter junii* VA2

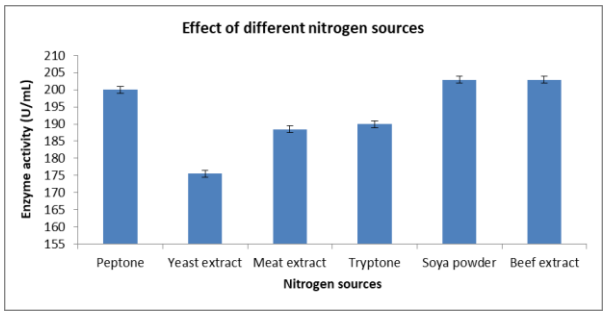


Fig 10: Effect of different nitrogen sources on lipase production by *Acinetobacter junii* VA2

Effect of physicochemical parameters on lipase activity

Figures 11 and 12 represent the enzyme activity of lipase. It was found to be most active at pH 9 (204.81 U/mL) and a temperature of 30°C (205.75 U/mL). Figures 13-17 show the effect of various test compounds on lipase activity. In the presence of inhibitors like PMSF, EDTA, 2-mercaptoethanol, bile salt and DTT a residual enzyme activity of 21.67%, 21.67%, 16.78%, 36.36% and 17.48% respectively was seen. Metal ions like Ca^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Na^{+} and K^{+} reduced the enzyme activity to 32.16%, 13.98%, 54.54%, 5.59%, 66.43% and 67.83% respectively. The stability of the enzyme was seen to be hampered in the presence of organic solvents. In the presence of acetone, butanol, ethanol, methanol, and 2-propanol, a residual enzyme activity of 29.37%, 25.17%, 29.37%, 26.57% and 25.87% was observed. Surfactants like SDS and Triton X-100 inhibited the activity of lipase, showing 6.29% and 13.98% residual activity respectively. Similarly, commercial detergents like Tide and Wheel reduced the enzyme activity to 34.26% and 36.36% respectively. The oxidizing agent also (H_2O_2) showed a negative effect on the activity of lipase. Increasing concentration of H_2O_2 declined the activity of

lipase, with 0.5%, 1.0%, 1.5% and 2.0% showing a residual activity of 39.86%, 39.16%, 24.96% and 32.16% respectively.

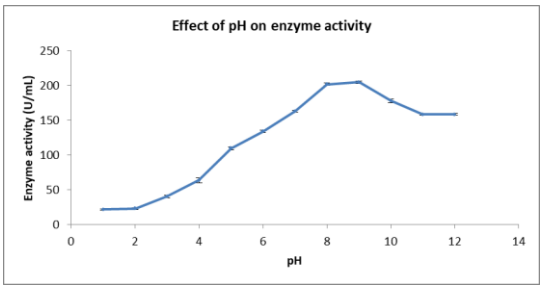


Fig 11: Effect of pH on the activity of lipase produced by *Acinetobacter junii* VA2

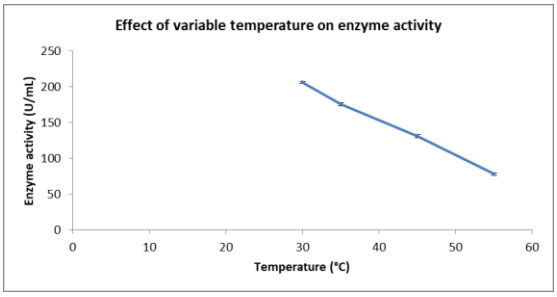


Fig 12: Effect of temperature on the activity of lipase produced by *Acinetobacter junii* VA2

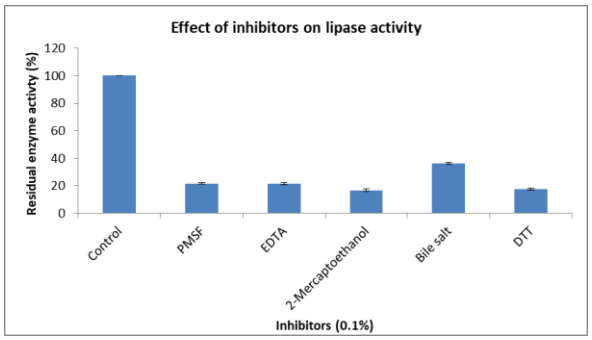


Fig 13: Effect of inhibitors on the activity of lipase produced by *Acinetobacter junii* VA2

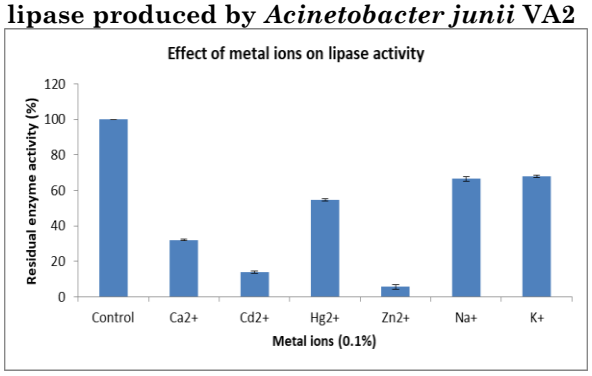


Fig 14: Effect of metal ions on the activity of lipase produced by *Acinetobacter junii* VA2

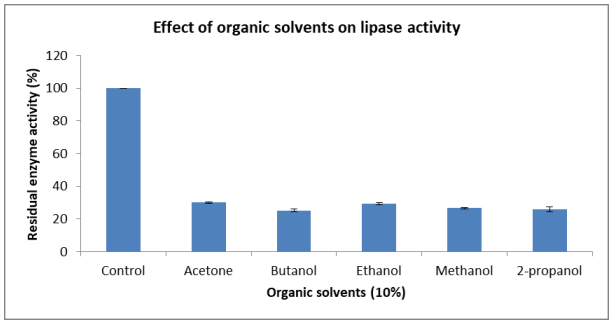


Fig 15: Effect of organic solvents on the activity of lipase produced by *Acinetobacter junii* VA2

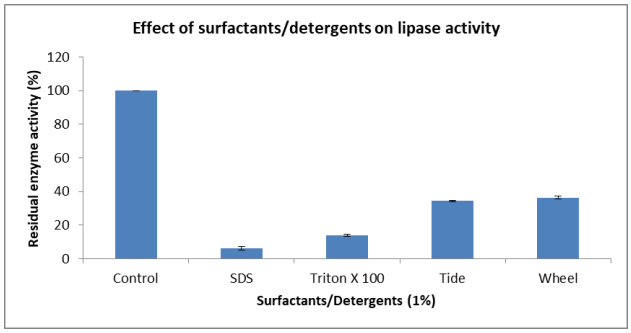


Fig 16: Effect of surfactants/detergents on the activity of lipase produced by *Acinetobacter junii* VA2

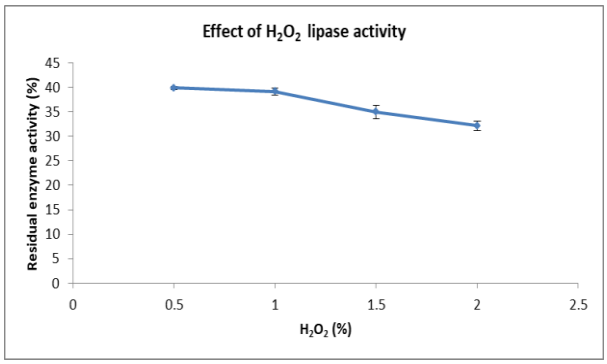


Fig 17: Effect of H_2O_2 on the activity of lipase produced by *Acinetobacter junii* VA2

Purification of the enzyme

The enzyme was precipitated at 50% saturation by ammonium sulfate. The precipitated enzyme, which was then desalted by dialysis, showed an activity of 510.76 U/mL. The specific activity of the purified enzyme was determined to be 37.55µmol $min^{-1}mg^{-1}$.

DISCUSSION

In the present study, various soil samples were analyzed for isolation of lipolytic organisms, and the most efficient lipase producer was identified as *Acinetobacter junii* VA2. Many strains of Acinetobacter have been previously reported to

produce extracellular lipases; for example, *Acinetobacter johnsonii*, *Acinetobacter baylyi* and *Acinetobacter calcoaceticus* [25, 26, 27].

The optimization studies revealed a direct correlation between certain parameters and production of lipase enzyme. In our study, the lipase production by *Acinetobacter junii* VA2 was optimum at 30°C in PM-1 medium (pH6) when incubated for 48h under shaker conditions. In a similar study, the optimum lipase production by *Acinetobacter junii* BK43 was obtained at pH6 and temperature 30°C after 24h of incubation, while *Acinetobacter junii* BK44 showed optimum lipase production at pH6 and temperature 25°C after 12h of incubation [25]. A recent study reported a high level of lipase production under submerged fermentation conditions at 37°C with pH 6.0 ± 0.5 by an unidentified bacterial strain [28]. In another study, Bacillus sp. SS2B1 showed maximum lipase production (0.1228 µg ml^{-1} min^{-1}) at pH7 and temperature 37°C [29]. In general, lipases are produced in the temperature range 20-45°C [30] and pH range 6-8 [31, 32]. However, the production of thermostable lipase from bacterial strains like Bacillus sp. strain WAI 28A45 has been described [33]. Similarly, optimum lipase activities at alkaline pH levels are also reported [34-40].

In addition to pH and temperature conditions, aeration is another important parameter essential for enzyme production. Shaker conditions are commonly used for the production of extracellular enzymes by aerobic organisms, as it enhances the aeration rate [41]. However, excessive aeration and agitation could lead to cell lysis and increased cell permeability due to abrasion by shear forces resulting in denaturation of enzymes [42, 43].

In our study, *Acinetobacter junii* VA2 showed increased production of lipase in PM-1 medium supplemented with fructose (3g/L), soya powder (5g/L), beef extract (3g/L) and castor oil (1mL/L). The optimum production of lipase by *P. fluorescens* SIK WI was reported previously in a medium (pH8.5) containing emulsified olive oil as the carbon source and tricaprylin as lipid source [44]. In another study, the presence of sucrose (3%) and yeast extract (5%) enhanced lipase production in the nutrient medium [28]. Fructose has been reported to have similar effects on the production of lipase by *Rotorua glutinis* [45]. Lipase production is also significantly influenced by other carbon sources, such as sugars, sugar alcohols, polysaccharides, whey, casamino acids and other complex substances [21, 39, 46]. Dharmsthiti et al. have reported earlier that addition of whey and soybean meal to the medium induces lipase production in *P. aeruginosa* LP602 [47]. Evidently, glucose has been reported to reduce the lipase production in several bacterial cultures [48, 49] with an exception of *Staphylococcus saprophyticus* [36]. The inducible nature of lipase enzymes is well documented [31, 47-53]. Hence it is necessary to incorporate inducers like tweens, triglycerols, fatty acids, hydrolysable esters, bile salts and glycerols in the medium for lipase production. It is also proposed that the chain length of the carbon source i.e the degree of unsaturation is a critical parameter for lipase production [57]. Kanwar et al. reported the production of lipase by Pseudomonas sp. G6 in the presence of n-alkane substrates, with the maximum production of about 25U/ml when n-hexadecane was used as the sole carbon source. Production was enhanced nearly 2-4 fold using tributyrin at a concentration of 0.05% in the production medium [54]. Maximum production of lipase has been reported by *Acinetobacter junii* and *Bacillus stearothermophilus* SB-1 in the presence of Tween 80 and olive oil as a lipid source respectively [25, 55]. In a comparative study carried out, a 2% palm oil was found to yield 12-fold more lipase than fructose supplemented medium [56]. In a similar study, glycerol tri-oleate proved to be a better carbon source as compared to oleic acid and glycerol tributyrate [57].

Besides the carbon source, the type of nitrogen source in the medium also influences the lipase yield in the production media [51]. Our present study indicated that supplementation of the nutrient medium with organic nitrogen sources like soy powder and beef extract resulted in high lipase production. Generally, microorganisms provide a high yield of lipase when organic nitrogen sources such as yeast extract, peptone or Tryptone are used; as observed in the case of various pseudomonads, thermophilic Bacillus sp. and *Staphylococcus haemolyticus* [13, 21, 34, 35, 40, 56, 58-60]. A study by Frerie et al. indicated that peptone contains certain cofactors and amino acids, which fulfill the physiological requirements for lipase biosynthesis [61]. Meher and Aruna (2009) also reported maximum lipase

production by *Staphylococcus saprophyticus* in the presence of 1% peptone [36]. Other studies reported, increase in lipase production by addition of 1% ammonium sulphate and 1% urea in case of *Penicillium aurantiogriseum* and a bacterial isolate SJ-15 respectively [30, 62]. In contrast to these studies, it was reported that lipase production was stimulated in *Rhdutorela glutinis* by addition of inorganic nitrogen such as ammonium phosphate 45].

In further studies, the extracellular lipase was standardized for maximum activity and the effect of various inhibitors was investigated. The lipase activity was found to be stable in the pH range of 9-12 and temperature range of 30-60°C. However, the presence of inhibitors and other compounds severely inhibited the lipase activity. Lipases from *Acinetobacter baylyi* and *S. hyicus* showed the highest activity at pH 8 and 8.5 respectively [27, 63]. In contrast to our study, lipases from *P. fluorescens* SIK W1, *Staphylococcus aureus* NCTC8530 and *S. epidermidis* RP62A were reported to show optimum activity under acidic conditions [64-66]. Wang et al.; (1995) reported production of a highly thermostable alkaline lipase by Bacillus strain A30-1 (ATCC 53841) in a medium containing yeast extract (0.1%) and ammonium chloride (1%) as nitrogen sources [34]. The partially purified lipase preparation had an optimal activity at the temperature of 60°C and the optimum pH was 9.5. This enzyme was stable to both hydrogen peroxide and alkaline protease [34].

Lipase inhibitors have been used in the study of structural properties of lipases. In view of pharmacological applications, lipase inhibitors are used for designing drugs for the treatment of obesity and acne. Unlike our study, there are several reports demonstrating bile salts as stimulatory agents in some microbial lipase production [67]. EDTA was found to be inhibitory to lipases obtained from *P. aeruginosa* 10145 and Bacillus sp. THL027 [30]. Co-factors are generally not required for lipase activity but divalent cations such as Ca^{2+} often stimulate the enzyme activity [65, 68]. However, there have been reports of structural stability provided by Ca^{2+} in enhancing the activity of lipases obtained from *Burkholderia glumae, Staphylococcus hyicus*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus warneri* [65, 69, 70]. In general, any enzyme activity is compromised in presence of heavy metals like Co^{2+}, Ni^{2+}, $Hg^{2+,}$ and Sn^{2+} and slightly inhibited by Zn^{2+} and Mg^{2+} [71]. However, the lipase from *Acinetobacter calcoaveticus* LP009 was stimulated by the presence of Fe^{3+} and its activity was reduced by less than 20% on addition of various other ions [47]. An increase in lipase activity (approximately 6-fold) and a shift in pH towards alkaline conditions were reported in presence of a Ca^{2+} ion in *Pseudomonas fluorescens* SIK W1. The lipase was inhibited by Hg^{2+}, Ag^{2+}, p-chloro-mercuri-benzoate, diethyl-pyrocarbonate and sodium dodecyl sulfate [44]. Lipases are diverse in their sensitivity to organic solvents [72]. Uttatree et al. reported a decrease in the activity of lipase produced by *Acinetobacter baylyi* and *A. calcoaceticus* LP009 in the presence of organic solvents [27, 47]. Contrary to our results and above findings, acetone, ethanol, and methanol significantly enhanced the lipase activity of *B. thermocatenulatus* [73]. In addition, the lipases from *P. aeruginosa* B11-1, Bacillus sp., B. *thermoleovorans* CCR11 and *Yarrowia lipolytica* showed a high stability in the presence of water-miscible organic solvents [74-77].

In addition to inhibitors, solvents, metal ions and other compounds, *Acinetobacter junii* VA2 also showed a decrease in enzyme activity in presence of several surfactants (SDS, Triton X-100) non-ionic detergents (Tide, and Wheel) and oxidizing agents (H_2O_2). In contrast, our previous study reported production of lipase enzyme by *Staphylococcus pasteuri* SNA59 that showed stable enzyme activity in presence of ionic, non-ionic and commercial detergents with the activity ranging from 80% to 120% [78]. A high activity of lipase in the presence of detergents has also been reported by other authors [36, 71, 79].

In further studies, partial purification of the enzyme was carried out using ammonium sulfate precipitation followed by dialysis. The lipase yield after dialysis was found to be 510U/mL. In a similar study, lipase enzyme was partially purified by 40–60% (w/v) ammonium sulfate precipitation method and the molecular weight was estimated to be 32-47 kDa by SDS-PAGE [28]. Chartrain et al. (1993) purified a lipase from *P. aeruginosa* MB5001 using a three-step procedure. The culture supernatant was concentrated by ultrafiltration, further purified by ion-exchange chromatography and gel

filtration. It showed a molecular mass of 29kDa as checked by SDS-PAGE [80]. Another extracellular lipase from *P. aeruginosa* KKA-5 was purified 518-folds by ammonium sulfate precipitation, hydroxyl appetite column chromatography and reported to be a 30kDa protein by SDS-PAGE [81]. Similarly, Bio-gel P-10 and Superose 12B chromatography have been employed previously for purification of 30kDa lipase from Pseudomonas sp. [37]. In another study, Ogino et al. reported the purification of lipase produced by *P. aeruginosa* LST-03 by ion-exchange and hydrophobic interaction chromatography achieving 34.7-fold purification [82].

CONCLUSION

The current study reports *Acinetobacter junii* as a potential source of microbial lipase. Although the enzyme activity was suppressed in presence of certain inhibitors used in our study, attempts can be made to adapt the enzyme for commercial applications. The other observed properties like stability to alkaline conditions and higher pH levels, in addition to the high yield of lipase obtained in this study indicates a promising application of the isolated bacteria which can be exploited on an industrial level for lipase production.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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