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Original Research

Isolation and Optimization of Lipase-Producing *Staphylococci* for Industrial Applications

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ABSTRACT
Lipases are an extremely significant group in the biotechnologically related enzymes. Lipases have massive applications in various industries that include detergent, dairy, food, and pharmaceutical industries. Lipase producing bacteria were isolated from different oil contaminated sites using tributyrin agar as the media. All the isolates belonged to the genera Staphylococci. The isolates with highest lipolytic activity were selected by using titrimetric assay. The lipolytic activity of 2S, 10S and 12S was more noticeable than other isolates. The effects of different parameters like temperature, pH and incubation time on lipolytic activity were assessed. The isolates showed maximum activity at 30°C 2S and 12S displayed least activity at 45°C and 10S displayed least activity at the lowest provided temperature that was 25°C. Incubation time of 20-30 minutes was effective in increasing the enzyme activity considerably. 25 minutes was shown to decrease the lipolytic potential of 10S and 45 minutes of isolate 2S and 12S. Thin layer chromatography was also employed for the visualization of fatty acids bands. The isolates were identified as *Staphylococcus* species through 16S rRNA gene sequencing. Such isolates can be of industrial importance due to their ability to produce industrially important enzymes.

1. INTRODUCTION

Man's use of enzymes can be dated to ancient times. Today these enzymes play boundless roles in the industries. Enzymes are manipulated industrially to achieve the desired outcomes It would not be wrong to say that without these biological catalysts industries would literally be lost as they are involved from the minutest to the leading complex process taking place in the industries. Lipases are an important class of industrially imperative and potent enzymes. Lipases are triacylglycerol hydrolases (Gupta *et al.*, 2004) that catalyze the hydrolysis of triacylglycerols to glycerol and fatty acids respectively. They (Triacylglycerol, EC 3.1.1.3) have many uses in various industries like food industry, detergent industry, pharmaceuticals manufacturing, oleochemical and fat industry, textile industry, paper and pulp industry, cosmetic production etc (Hasan *et al.*, 2006) (Aly *et al.*, 2012). Lipolytic enzymes also play significant role in the production of single cell proteins (Aly *et al.*, 2012), biodegradable polymers (Linko *et al.*, 1998), and biodiesel production (Shah *et al.*, 2004).Lipases are acquired by plants, animals as well as microorganisms. However, whatever may be their source, their functions remain the same, that is, to break down lipids

for energy production. Lipids are the richest energy yielding source. Lipases are procured by plants, animals and microorganisms. Lipases that are obtained from microbial sources are considered superior due to their high yields. Microbes can be cultured more economically, and mainly due to their stability as compared to their animal or plant counterparts and ease of genetic manipulation. Lipases that are obtained from microbial sources are viewed as better due than their exceptional returns and high yield supply and yield are not inclined to occasional varieties. Microbial lipases can be cultured and refined all the more financially and basically because of their strength when contrasted with their animals or plant partners and ease of genetic manipulation (Gupta *et al.*, 2004).

A wide range of bacterial and fungal species produce lipases which hydrolyze esters of glycerol with ideally long-chain unsaturated fats. Certain central lipase-producing bacterial genera include *Bacillus*, *Pseudomonas*, *Staphylococcus* and *Burkholderia (*Gupta *et al*., 2004) As far as the distinct and specific species of the lipolytic bacteria is concerned, the activity has been reported in *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Propionibacterium granulosum*, *Acinetobacter calcoaceticus*, *Achromobacter lipolyticum*, *Corynebacterium acnes*, *Myxococcus xantus*, *Staphylococcus aureus*, *Streptococcus lactis*, and *Lactobacillus* sp (Sztajer *et al.*, 1988) .Fungal lipase activity has been reported in *Pencillium*, *Geotrichum*, *Rhizopus*, *Mucor*, *Rhizomucor* and *Aspergillus genera* (Treichel *et al*., 2010; Griebeler *et al*., 2011)

Within the class of microbial obtained lipases, the lipases of bacterial origin are considered superior because of many reasons such as being less generation time of the bacteria, better activity thermostability, ease of genetic manipulation, simpler screening procedures as well as the simple nutritional requirements of the bacteria (Gupta *et al.*, 2004) .

Lipases are usually extracellular glycoprotein enzymes with molecular weight that is between 20 and 60kDa. The high molecular weight lipases may form multimeric units or aggregates depending on the condition. As most lipases are glycoproteins, they contain 2-15% carbohydrates. With the advancement of time, the primary structure of the lipases from fungal, mammalian and bacterial lipases has now been successfully determined and contains around 270-641 amino acids (Hari Krishna and Karanth, 2002).

Lipases works best at neutral (Dharmsthiti and Kuhasuntisuk, 1998; Dharmsthiti and Luchai, 1999; Lee *et al.*, 1999; Gupta *et al.*, 2004) or alkaline pH(Schmidt-Dannert *et al.*, 1994; Sidhu *et al.*, 1998; Kanwar and Goswami, 2002). However exceptions to this range is *Pseudomonas fluorescensme SIK W1 enzyme (*Andersson et al., *1979).* Lipase produced from *Pseudomonas gessardii* works at an acidic pH (Ramani *et al.*, 2010).The lipases of *Bacillus stearothermophilus SB- 1, B. licheniformis* SB-3 and *B. atrophaeus* SB-2 work finest over an expansive pH range around 3 to 12 (Bradoo *et al.*, 2002; Gupta *et al.*, 2004) *Pseudomonas aeruginosa KM 110* is active over a pH range of 7 to 10 (Mobarak-Qamsari *et al.*, 2011). In case of temperature, bacterial lipases show maximum activity over a vast range of temperatures between 30°C to 60° C rendering them mesophilic as well as thermophilic (Dharmsthiti *et al.*, 1998; Litthauer *et al.*, 2002) . However, there are also studies that have reported lipase activity below and above this range (Dharmsthiti and Luchai, 1999; Gupta *et al.*, 2004).

At the moment lipases are of colossal importance due to their numerous applications. The aim of the study was to isolate, screen and consequently characterize bacterial lipases with high lipolytic activity and then optimize

their growth and activity parameters for maximum benefit.

2. MATERIALS AND METHODS

2.1 Sample Collection

Oil-contaminated soil samples were collected from three sampling sites the confectioner's shop and two automobile workshops in Lahore with the help of a sterile spatula in a clean and sterile polythene bag and then transported into the laboratory 24 hours with caution and care so that the soil samples are not contaminated from any other sources.

2.2 Isolation and Screening of the Lipolytic Bacteria

The three soil samples were subjected to serial dilutions and spread on prepared tributyrin plates and incubated for 72 to 96 hours at 37°C. The colonies which exhibited a clearing zone proceeded for further experimentation.

2.3 Biochemical Characterization

The isolated strains were tested for catalase and oxidase test after Gram staining.

2.4 Selection of Best Isolates for Lipase Production

The isolates proficient of lipase production were further screened to isolate the best possible lipase-producing bacteria based on the titrimetric method. Lipase activity was determined by combining the protocols of Sagar *et al.* (2013) and Bhavani *et al.* (2012). A pre-culture for the production medium of lipase was prepared by inoculating 10ml of l broth followed by incubation at 37°C at 120 rpm. Tryptic soy broth supplemented with 1% olive oil was seeded with the pre-culture followed by incubation at 37°C for 16 hours at 120 rpm. The culture was centrifuged at 5,000 rpm at 4°C for 15 minutes. Lipase activity was determined in the supernatants of the cultures. One ml of the supernatant was added to a mixture of 10% olive oil and 5% gum acacia (in 50mM phosphate buffer saline). This was followed by incubation at 37°C for 15 minutes. A mixture of ethanol and acetone (1:1) was added to stop the reaction. Titration was performed using 0.05M NaOH and the appearance of pink color was taken as the endpoint. The lipase activity was calculated using the following formula reported Bhavani *et al.* (2012)

$$(VS - VB) \times N \times 1000 / S$$

VS represents the volume of 0.05M NaOH used in the titration of the enzyme and substrate cocktail (ml), *VB* represents the volume of 0.05M NaOH used in the titration of the Control cocktail (ml) (substrate only). *N* represents the concentration of the NaOH solution (0.05M); *S* represents the volume of substrate cocktail solution used (10ml). One unit (U) of lipase enzyme is defined as the amount of enzyme required to liberate 1µmol of fatty acids from triglycerides.

2.5 Effect of pH, Temperature, and Incubation Time on Lipase Activity

The preculture of all the isolates was prepared and then transferred into Tryptic soy broth supplemented with 1% olive oil. The cultures were incubated at 37°C on shaking for 16 to 24 hours. The cultures were centrifuged at 4°C and cell free supernatant was obtained. A cocktail mixture of olive oil, phosphate buffer, gum acacia and 1 ml of the supernatant of the isolate was made. The pH, temperature of the incubation and the incubation time of the assay were varied. Reactions were stopped with ethanol and acetone solution (1:1) and then titrated against 0.05M NaOH to assess the effect of different physical parameters on the lipase activity.

2.6 DNA Extraction

DNA was isolated by using Favorprep Tissue Genomic DNA Extraction Mini Kit by

following the manufacturer's instructions. This was followed by gel electrophoresis. Polymerase Chain Reaction (PCR) was performed with program: initial denaturation at 94ºC for 3 minutes, denaturation at 94ºC for 45 seconds, annealing at 50ºC for 60 seconds, extension at 72ºC for 90 seconds, and final extension at 72ºC for 1 minute, in the Thermal Cycler (Advance Primus 96). PCR products were confirmed by gel electrophoresis using a DNA ladder.

2.7 Thin Layer Chromatography

The bacterial isolates were pre-cultured in L-broth for 24 hours at 37°C. Next the pre-cultures were inoculated in Tryptic soy broth supplemented with 1% olive oil and incubated at 37°C for 24 hours as shaking cultures. Then the cultures were centrifuged at 4°C. The obtained supernatants were spotted on silica gel TLC plate and the plate was run in a TLC tank with chloroform and methanol solution (2:1) as the running buffer. The running buffer was chosen for thin layer chromatography was according to the protocol of Kupke and Zeugner (1978) with a slight modification. The plate was allowed to run until the solvent reached 1/4th of the TLC plate. The plate was then removed and air dried followed by observation under UV at two wavelengths 254 nm and 366nm.

2.8 Phylogenetic Analysis

Gel cleaned PCR products from bacterial isolates was sent to Macrogen (Korea) for 16s rRNa gene sequencing. Sequences were classified using 16S ribosomal RNA sequences database of NCBI. Neighbour joining tree was made in MEGA 5.0. The reliability of the constructed tree was measured by 100 replicates of boot strap method.

3. RESULTS

Soil samples were collected from different oil contaminated sites such as the confectioners' shop and automobile workshops. A total of 13 isolates were obtained all screened on tributyrin agar and colony morphology was recorded as shown in table 1. Initial biochemical tests revealed the biochemical characteristics of the isolates as shown in table 2. The optimum temperature was 37°C whereas the optimum pH was 7 (Figure 1).

Table 1. Colony Morphology for the Isolates for the Isolates

SR.NO	FORM	ELEVATION	MARGINS	PIGMENT	SIZE
1S	Circular	Flat	Entire	Off-White	Pinpoint
2S	Circular	Raised	Entire	Off- white	Medium
3S	Circular	Flat	Entire	Off-White	Small
4S	Circular	Raised	Entire	Off-White	Small
5S	Circular	Raised	Undulate	Off-White	Medium
6S	Circular	Flat	Entire	Off-White	Small
7S	Circular	Raised	Undulate	Off-White	Small
8S	Circular	Flat	Entire	Off-White	Small
9S	Circular	Raised	Entire	Off-White	Small
10S	Circular	Raised	Entire	Off-White	Small
11S	Circular	Flat	Entire	Off-White	Pinpoint
12S	Circular	Flat	Entire	Off-White	Small
13S	Circular	Flat	Entire	White	Pinpoint

Table 2. Biochemical Characterization of Isolates

SR.NO	WET MOUNT	GRAM STAINING	CATALASE TEST	OXIDASE TEST
1S	Cocci	Positive	Positive	Negative
2S	Cocci	Positive	Positive	Negative
3S	Cocci	Positive	Positive	Negative
4S	Cocci	Positive	Positive	Negative
5S	Cocci	Positive	Positive	Negative
6S	Cocci	Positive	Positive	Negative
7S	Cocci	Positive	Positive	Negative
8S	Cocci	Positive	Positive	Negative
9S	Cocci	Positive	Positive	Negative
10S	Cocci	Positive	Positive	Negative
11S	Cocci	Positive	Positive	Negative
12S	Cocci	Positive	Positive	Negative
13S	Cocci	Positive	Positive	Negative

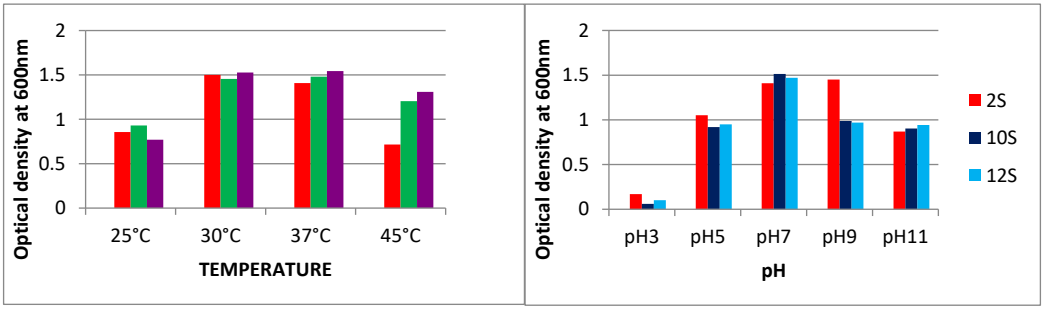

Figure 1 Comparative Analysis of Growth Pattern of Isolates at 2S, 10S and 12S at Different Temperatures

3.1 Effect of Temperature on Lipase Activity

Lipase activity of the lipase from our isolates 2S, 10S and 12S demonstrated best activity at 30°C. 2S gave lowest activity at 37°C and highest activity at 30°C. The lipase activity of 2S at 25°C and 45°C was round about. The activity same with only little of 2S at 25°C was a little higher than at 45°C difference. 10S like 2S also displayed highest activity at 30° but unlike 2S its lowest activity was recorded at 25°C. This strain shows considerable activity at 37°C. Isolate 12S also displayed its maximum activity at 30°C and the rest of the activity trend for 12S was identical to 2S (Figure 2).

3.2 Effect of pH on Lipase Activity

High activity was observed in case of pH 3 followed by pH 5 then 7 and so on. The volume of NaOH in this case corresponds to the amount of acid present in the cocktail rather than the fatty acids liberated by the enzyme (effect of pH cannot be determined accurately). The graphs (Figure 3) show this trend. For all the isolates 2S, 10S and 12S highest activity is exhibited by the enzyme at the most acidic pH and lowest at the most basic pH. This is because much of the base is consumed to neutralize the acidity of the cocktail at a high acidic pH. On the other hand, almost no volume of NaOH was consumed at a high basic pH because the reaction mixture itself had high

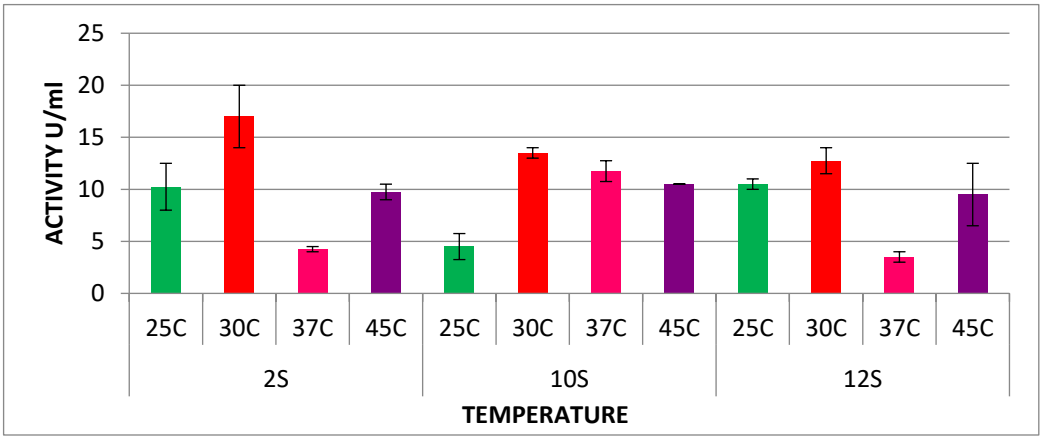

Figure 2. Comparative analysis of lipase activity of isolates 2S, 10S and 12S at different temperatures

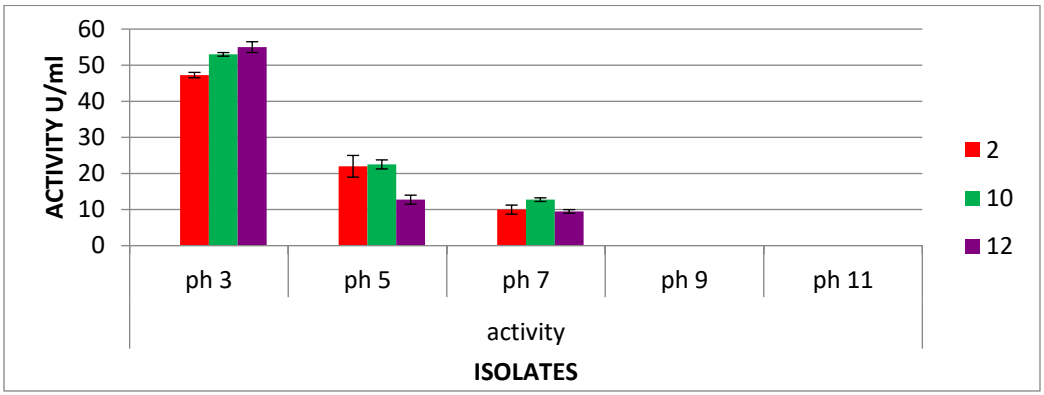

Figure 3. Comparative analysis of lipase activity of isolates 2S, 10S and 12S at different Ph

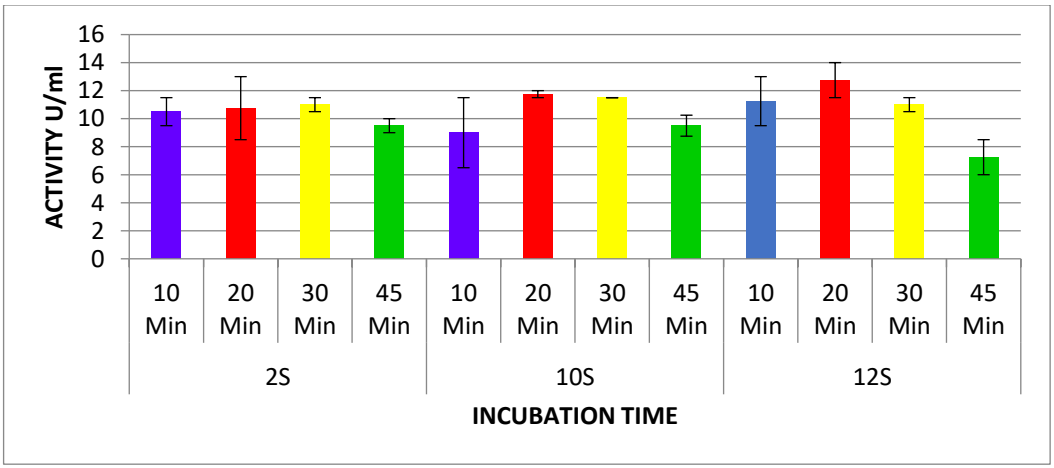

Figure 4. Comparative analysis of lipase activity of 2S, 10S and 12S at different incubation times

amount of NaOH to bring the pH at 9 or 11. The true enzyme activity was overshadowed in this scenario hence proper lipolytic potential at varying pH could not be determined (figure 3).

3.3 Effect of Assay Incubation time on Lipase activity

20-30 minutes were found as the ideal incubation temperatures at which the isolates show their maximum activity. 2S show maximum at 30 minutes followed by 20 minutes, 10 minutes and then 45 minutes respectively. 10S displayed maximum activity at 20-30 minutes like 2S then 45 minutes and least activity was observed for 10S at 25minutes. 12S exhibits least activity at 45 minutes and highest like the rest three strains at 20-30 minutes. Results are depicted in bar graph (Figure 4)

3.4 DNA Extraction

DNA was extracted and gel was run for half an hour at 100 volts. Samples were loaded in duplicates. Bands were obtained for isolates 2S, 10S and 12S in wells 7, 8, 9, 10, 11 and 12 respectively. The yield in wells 8, 9 and 11 was more than in other wells. Wells 8, 9 and 11 had duplicates of sample 2S, 10S and 12S.

3.5 Polymerase Chain Reaction

DNA obtained was subjected to PCR in a thermocycler (PCR machine). Samples were loaded in duplicates in the wells 3, 4, 5, 7, 8 and 9 and bands were obtained for isolate 2S, 10S and 12S respectively.1.5 kb ladder was loaded in well 6. Figure 4 shows the gel for PCR. High yield was obtained for each isolate. The PCR products were gel purified and sent to Macrogen (Korea) for 16S rRNA sequencing.

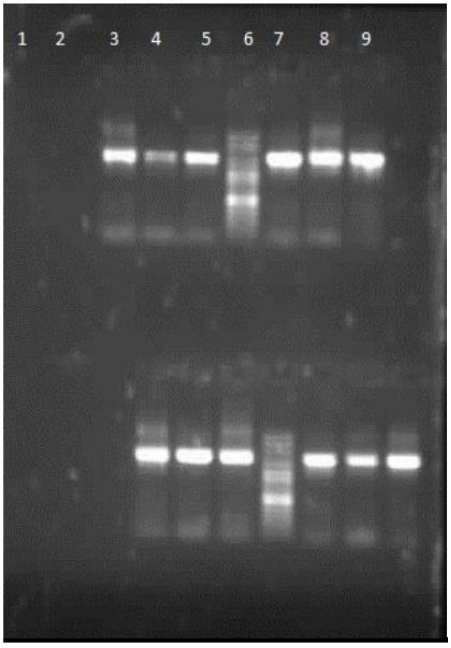

Figure 5. Agarose gel for PCR

3.6 Phylogenetic Analysis of Bacterial Isolates

DNA was extracted from the bacterial isolates and was sent for 16S rRNA sequencing. Sequences of the isolates were compared to their respective nearest homologs and no anomaly was found. 2S and 12S were identified as *Staphylococcus aureus* after 16S rRNA sequencing and 10S as *Staphylococcus hemolyticus* (Figure 6).

3.7 Thin Layer Chromatography

The chromatogram was observed under UV illuminator under two wavelengths 254nm and 366nm. Fatty acids bands were observed in the lane of isolate 2S, 10S and 12S which produce lipase. No bands were observed in the lane where control was spotted this means that without the inoculum there are no lipases and substrate is not broken down therefore no band was observed in the lane where control was spotted. The results are shown in Figure 7.

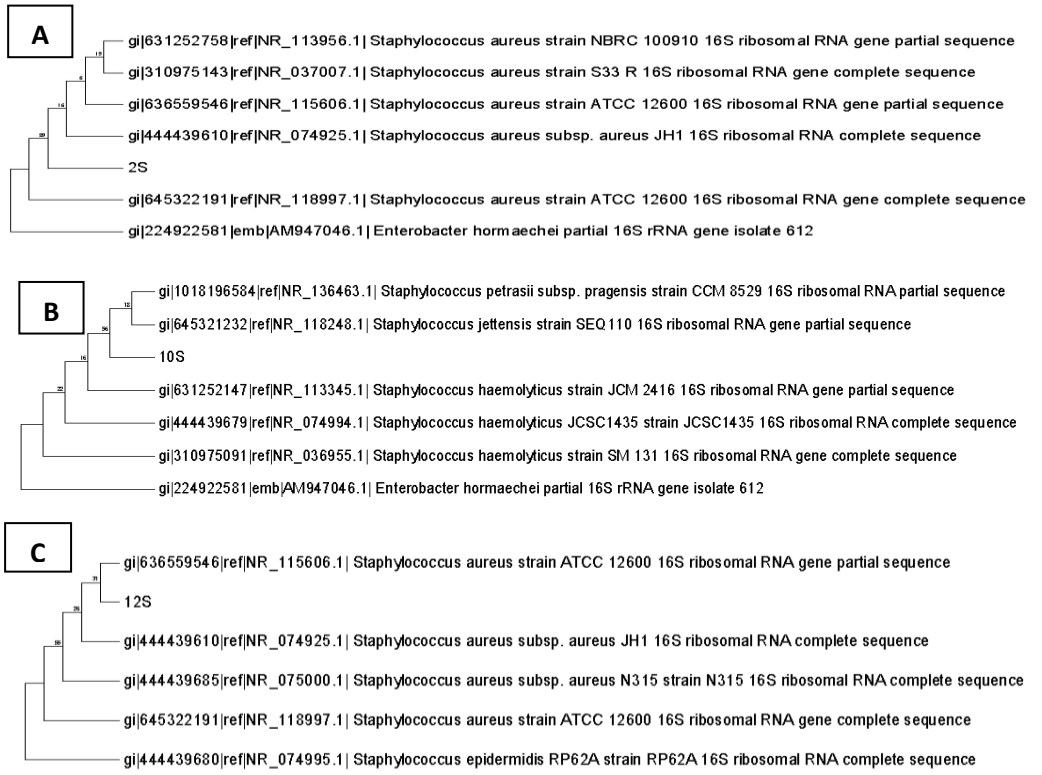

Figure 6. A: Phylogenetic tree of the isolate 2S. **B**: Phylogenetic tree of the isolate 10S. **C:** Phylogenetic tree of the isolate 12S

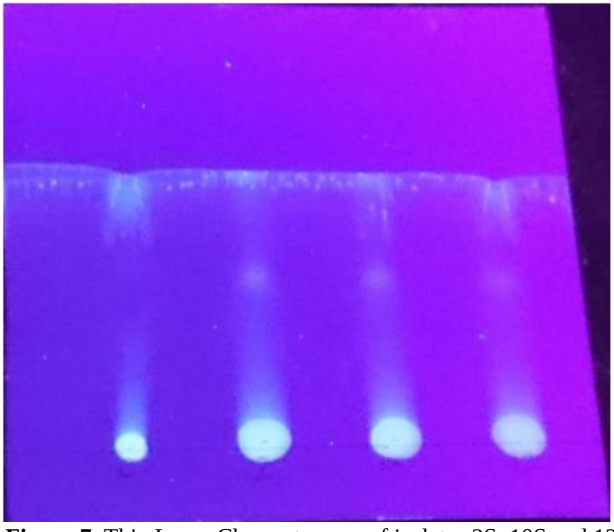

Figure 7. Thin Layer Chromatogram of isolates 2S, 10S and 12S

4. DISCUSSION

The use of lipases in industries is increasing with advancement in technology each day. In this study a total of 13 isolates were obtained from oil contaminated sites by using tributyrin. Further 3 best isolates were selected for further experimentation after assessing their lipase activity titrametrically. The three best isolates in accordance with staining and biochemical testing were Gram positive cocci which means they belonged to the genus *Staphylococcus*. Such Gram positive bacteria that are lipase producers have been reported by Sagar *et al.* (2013). Different *Staphylococcus* species that are lipase producing have also been reported by Oh *et al.* (1999) and Kalyani and Saraswathy (2014). The titrimetric technique employed the phenomenon of the neutralization of the free fatty acids released after the process of lipolysis has taken place and the volume of the base consumed signposts the degree of lipolysis (Shukla and Gupta, 2007; Aly *et al.*, 2012). All the isolates displayed activity within a range of 1.75U/ml-5U/ml.

Lipase activity in the range of 1.5 to 6.9IU/ml has been reported by Aly *et al.* (2012). Isolate 10S exhibited the best activity of 5U/ml followed by 12S and 2S with activities 4.5U/ml and 4.33U/ml respectively. All the isolates could be cultured best at neutral pH and a temperature range of 30°C to 37°C. However, isolates also exhibited some growth at 45°C.

Isolate 2S showed high optical densities at pH 7 and 9 which means that it can be cultured under both alkaline and neutral conditions. Alkaline Staphylococcal species having stability over a wide range of pH (9-13) have been reported by (Cherif *et al.*, 2011a) . Furthermore alkaline and thermophilic staphylococcal lipase have also been reported by Cherif *et al.* (2011b) To assess the effect of temperature, pH and incubation time on the lipase activity the conditions of the cocktail mixture were varied. Conditions of the cocktail mixtures were varied because the enzyme was present in the mixture that was prepared for titration for the deduction of free fatty acids after lipolysis in the form of added supernatant so once the parameters around the cocktail mixture was varied the enzyme will be affected directly and hence, we could infer that how physical parameters like temperature pH and incubation time affect the lipase activity. Temperature and incubation time effects were recorded conveniently. The trends obtained are depicted in the graphs. However, the effect of H^+ ions or pH could not be evaluated by varying the pH of the cocktail mixture because the amount of NaOH consumed to reach the endpoint corresponded to the acid or base in the cocktail mixture used to adjust its pH instead of fatty acids produced by the lipase using olive oil as the substrate. More volume of NaOH is consumed at pH3 because large amount of HCl was added in the cocktail mixtures to adjust the pH of the cocktail mixture to check how hydrogen ions concentration affects the lipase activity. As the figure shows the volume of NaOH consumed decreases as the pH increases and at pH 9 and 11 no volume of NaOH is consumed and the end point is reached without the addition of NaOH from the burette. Hence titrametrically it could not be deduced of how pH affects lipase activity in our study. Thin layer chromatography was done to visualize the bands of fatty acids. Mixtures with and without enzymes and olive oil as the substrate were prepared and spotted on the TLC plate so that the pattern of lipolysis in presence of olive oil by our isolated bacteria could be visualized. No bands were observed where there was no enzyme. Bands were observed of mixtures that had enzymes and substrate. The bands were the outcome of the lipolytic action of the enzyme in the presence of oil. Hence, the selected species identified as *Staphylococcus* species displayed lipase activity can therefore be used for several industrial applications.

5. CONCLUSION

In conclusion, this study highlights the potential of lipase-producing *Staphylococci* bacteria for various industrial uses. The isolates 2S, 10S, and 12S exhibited significant lipolytic activity. Temperature, incubation time, and pH are vital for enhancing enzyme efficiency. Thin-layer chromatography further clarifies the composition of fatty acids, offering an understanding of the functioning of lipolytic enzymes. These results highlight the importance of identifying microbial sources for an increased production of lipase followed by the optimization of conditions for industrial processes.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Aly, M. M., Tork, S., Al-Garni, S. M. and Nawar, L. 2012. Production of lipase from genetically improved *Streptomyces exfoliates* LP10 isolated from oil-contaminated soil. *African Journal of Microbiology Research,* 6:1125-1137.

Andersson, R., Hedlund, C. and Jonsson, U. 1979. Thermal inactivation of a heat-resistant lipase produced by the psychrotrophic bacterium *Pseudomonas fluorescens*. *Journal of Dairy Science,* 62:361-367.

Bhavani, M., Chowdary, G., David, M. and Archana, G. 2012. Screening, isolation and biochemical characterization of novel lipase producing bacteria from soil samples. *International Journal of Biological Engineering,* 2:18-22.

Bradoo, S., Rathi, P., Saxena, R. and Gupta, R. 2002. Microwave-assisted rapid characterization of lipase selectivities. *Journal of biochemical and biophysical methods,* 51:115-120.

Cherif, S., Mnif, S., Hadrich, F., Abdelkafi, S. and Sayadi, S. 2011a. A newly high alkaline lipase: an ideal choice for application in detergent formulations. *Lipids in health and disease,* 10:1.

Cherif, S., Mnif, S., Hadrich, F., Abdelkafi, S. and Sayadi, S. 2011b. Strategy for improving extracellular lipolytic activities by a novel thermotolerant *Staphylococcus* sp. strain. *Lipids in health and disease,* 10:1.

Dharmsthiti, S. and Kuhasuntisuk, B. 1998. Lipase from *Pseudomonas aeruginosa* LP602: biochemical properties and application for wastewater treatment. *Journal of Industrial Microbiology and Biotechnology,* 21:75-80.

Dharmsthiti, S. and Luchai, S. 1999. Production, purification and characterization of thermophilic lipase from Bacillus sp. THL027. *FEMS microbiology letters,* 179:241-246.

Dharmsthiti, S., Pratuangdejkul, J., Theeragool, G. and Luchai, S. 1998. Lipase activity and gene cloning of *Acinetobacter calcoacetlcus* LP009. *The Journal of general and applied microbiology,* 44:139-145.

Griebeler, N., Polloni, A. E., Remonatto, D., Arbter, F., Vardanega, R., Cechet, J. L., Di Luccio, M., de Oliveira, D., Treichel, H. and Cansian, R. L. 2011. Isolation and screening of lipase-producing fungi with hydrolytic activity. *Food and Bioprocess Technology,* 4:578-586.

Gupta, R., Gupta, N. and Rathi, P. 2004. Bacterial lipases: an overview of production, purification and biochemical properties. *Applied microbiology and biotechnology,* 64:763-781.

Hari Krishna, S. and Karanth, N. 2002. Lipases and lipase-catalyzed esterification reactions in nonaqueous media. *Catalysis Reviews*, 44:499-591.

Hasan, F., Shah, A. A. and Hameed, A. 2006. Industrial applications of microbial lipases. *Enzyme and Microbial technology*, 39:235-251.

Kalyani, N. and Saraswathy, N. 2014. Production of extracellular lipase by a new strain *Staphylococcus aureus* NK-LB37 isolated from oil contaminated soil. *African Journal of Biotechnology*, 13.

Kanwar, L. and Goswami, P. 2002. Isolation of a *Pseudomonas* lipase produced in pure hydrocarbon substrate and its application in the synthesis of isoamyl acetate using membrane-immobilised lipase. *Enzyme and Microbial technology*, 31:727-735.

Kupke, I. R. and Zeugner, S. 1978. Quantitative high-performance thin-layer chromatography of lipids in plasma and liver homogenates after direct application of 0.5-µl samples to the silica-gel layer. *Journal of Chromatography B: Biomedical Sciences and Applications*, 146:261-271.

Lee, D.-W., Koh, Y.-S., Kim, K.-J., Kim, B.-C., Choi, H.-J., Kim, D.-S., Suhartono, M. T. and Pyun, Y.-R. 1999. Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. *FEMS microbiology letters*, 179:393-400.

Linko, Y.-Y., Lämsä, M., Wu, X., Uosukainen, E., Seppälä, J. and Linko, P. 1998. Biodegradable products by lipase biocatalysis. *Journal of biotechnology*, 66:41-50.

Litthauer, D., Ginster, A. and van Eeden Skein, E. 2002. *Pseudomonas luteola* lipase: a new member of the 320-residue *Pseudomonas* lipase family. *Enzyme and Microbial technology*, 30:209-215.

Mobarak-Qamsari, E., Kasra-Kermanshahi, R. and Moosavi-Nejad, Z. 2011. Isolation and identification of a novel, lipase-producing bacterium, *Pseudomnas aeruginosa* KM110. *Iranian journal of microbiology*, 3:92.

Oh, B.-C., Kim, H.-K., Lee, J.-K., Kang, S.-C. and Oh, T.-K. 1999. *Staphylococcus haemolyticus* lipase: biochemical properties, substrate specificity and gene cloning. *FEMS microbiology letters*, 179:385-392.

Ramani, K., Kennedy, L. J., Ramakrishnan, M. and Sekaran, G. 2010. Purification, characterization and application of acidic lipase from *Pseudomonas gessardii* using beef tallow as a substrate for fats and oil hydrolysis. *Process Biochemistry*, 45:1683-1691.

Sagar, K., Bashir, Y., Phukan, M. M. and Konwar, B. 2013. Isolation of lipolytic bacteria from waste contaminated soil: A study with regard to process optimization for lipase. *International Journal Of Scientific & Technology Research*, 2.

Schmidt-Dannert, C., Sztajer, H., Stöcklein, W., Menge, U. and Schmid, R. D. 1994. Screening, purification and properties of a thermophilic lipase from *Bacillus thermocatenulatus*. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid metabolism*, 1214:43-53.

Shah, S., Sharma, S. and Gupta, M. 2004. Biodiesel preparation by lipase-catalyzed transesterification of Jatropha oil. *Energy & Fuels*, 18:154-159.

Shukla, P. and Gupta, K. 2007. Ecological screening for lipolytic molds and process optimization for lipase production from Rhizopus oryzae KG-5. *Journal of Applied*

Sciences in Environmental Sanitation, 2:35-42.

Sidhu, P., Sharma, R., Soni, S. and Gupta, J. 1998. Production of extracellular alkaline lipase by a new thermophilicBacillus sp. *Folia microbiologica*, 43:51-54.

Sztajer, H., Maliszewska, I. and Wieczorek, J. 1988. Production of exogenous lipases by bacteria, fungi, and actinomycetes. *Enzyme and Microbial technology*, 10:492-497.

Treichel, H., de Oliveira, D., Mazutti, M. A., Di Luccio, M. and Oliveira, J. V. 2010. A review on microbial lipases production. *Food and Bioprocess Technology*, 3:182-196.