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Screening, Identification, Characterization and Production of Bacterial Lipase from Oil Spilled Soil

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A B S T R A C T

A bacterial strain isolated from oil spilled soil and identified as *Staphylococcus chromogenes* O1A by morphological, cultural, biochemical tests and 16S rDNA gene sequence analysis with maximum lipase activity (3.1) was screened on tributyrin medium. Maximum lipase production was observed at 48 h of growth (2.30 µM/min/mg), 37°C temperature (2.30 µM/min/mg) and pH 7.0 (7.96 µM/min/mg) with agitation of 130 rpm (3.02 µM/min/mg). Yeast extract (2.07 µM/min/mg) was found to be the best nitrogen source (1%). Lactose was used as a non Lipidic carbon source (1%) for optimum production of lipase (1.69 µM/min/mg). Of the natural oils, Sunflower oil was able to induce more lipase (3.87 µM/min/mg). Among the metal ions tested Ca^{2+} enhanced the lipase activity (1.67 µM/min/mg) and Zn^{2+} showed lower activity (0.28 µM/min/mg).The enzyme was found to be stable in 0.5 % solutions of different Surfactants such as SDS, Triton X-100 & Tween80. On the basis of optimized parameters the lipase activity in newly designed production medium 3 was found to be higher (18.18 µM/min/mg).

Introduction

The demand for microbial industrial enzymes has attracted much interest owing to their novel & multifold applications in a wide variety of processes. Lipases are triacylglycerol hydrolases (EC 3.1.1.3) that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids (Sharma *et al.*, 2001).

Currently bacterial lipases are of great demand because of potential industrial applications (Sirisha *et al.*, 2010). Lipases find their applications in various industrial sectors like processing of fats and oils, detergents and degreasing formulations, food processing, chemical and pharmaceuticals, paper mills, etc (Rubin and Dennis, 1997a,b; Kazlauskas and Bornscheuer, 1998; Masse *et al.*, 2001; Takamoto *et al.*, 2001).

The most suitable sources for lipase production are microbes including bacteria, fungi and yeast. These microorganisms can produce high quality lipases at lower cost and shorter time (Trichel *et al.*, 2010). Cost of lipase enzyme (food/ feed/ industrial grade) is US $ 10-30/kg & 200 tons/ month is its requirement in daily life.

Bacterial lipases are glycoproteins but some extracellular bacterial lipases are lipoproteins. Among bacteria, *Achromobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Chromobacterium* sp. (Godfredson *et al.,* 1990) have been exploited for the production of lipases. Staphylococcal lipases are lipoprotein in nature (Brune *et al.,* 1992). Many Staphylococci are able to produce extracellular lipases and some of them have been purified and their biochemical properties studied in detail (Oort *et al*., 1989; Gotz *et al*., 1985; Farrell *et al*., 1993; Lee and Iandolo, 1986; Talon *et al*., 1996; Oh *et al*., 1999; Simons *et al*., 1996; Jaeger *et al*., 1999; Van-Kampen *et al*., 2001; Pandey *et al*., 1999).

In order to get the highest yields of lipase, the optimal growth conditions were studied (Linefield *et al.,* 1990). Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors, such as temperature, pH, nitrogen and carbon sources, presence of lipids, inorganic salts, agitation and dissolved oxygen concentration (Brune and Gotz, 1992; Aires-Barros *et al.,* 1994; Jaeger *et al.,* 1994; Kim *et al.,* 1996).

Various methods of lipase assay have been classified as; Titrimetry, Interfacial tensiometry, Spectroscopy, Chromatography, Immunochemistry and Conductimetry (Beisson *et al.,* 2000; Kulkarni, 2002) of these methods titrimetry is the simplest method which was used in our studies also.

The purpose of the present study was to screen potential lipase producing bacteria from various samples and optimize the production of lipase by isolated and identified strain as *Staphylococcus chromogenes* O1A.

Materials and Methods

Screening of Lipase Producing Bacteria

Collection of Samples

Various 17 samples from diverse sources, such as oil contaminated soils of vegetable oil processing factories, oil packing industries & selling shops, auto garage soil, domestic waste water (sewage), slaughter house soil, spoiled coconut water, milk & milk cream etc. were collected in and around the city of Indore of Madhya Pradesh, India for the isolation of potential lipase producing bacteria.

Enrichment and Isolation of Lipolytic Bacteria

Lipolytic bacteria were screened by enrichment culture technique from 17 diverse samples. These samples were enriched by inoculating in Tributyrin broth medium flask (50 ml) containing 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (v/v) Tributyrin pH 7.0 & 9.0 and incubated at 37˚C for 48 hrs. After incubation, a loopful of growth obtained from each enriched culture sample was isolated on Tributyrin agar medium plates (Lawrence *et al.,* 1967) (pH 7 & 9) by sector plate method and incubated at 37˚C for 48 hrs.

Screening of the Isolates for Lipase Activity

Lipolytic organisms were screened by qualitative plate assay. The lipolytic activity of isolated colonies were observed by spot inoculation on Tributyrin agar medium plates and incubated at 37˚C for 48 hrs (pH-7 & 9) and zone of clearance was observed due to hydrolysis of tributyrin by lipase. The alkalotolerant nature of isolates was determined by growing each isolate of pH- 7 Tributyrin agar medium plate to pH- 9

Tributyrin agar medium plate & vice versa and incubating at 37˚c for 48 hrs. The organisms producing maximum zone of hydrolysis around the colony were selected for further study. Pure cultures of the isolate were maintained on minimal medium agar slants containing 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 0.5% (w/v) NaCl, 2.0% (w/v) agar pH 7.0 at refrigerated temperature and were sub cultured every month.

Characterization and Identification of Selected Isolate

The bacterial isolate, O1A (pH-7) isolated from oil spilled soil from vegetable oil packing factory of Indore region of Madhya Pradesh, found to produce maximum zone of hydrolysis (Table-1) around the colony was selected and was studied for its morphological, cultural, physiological, biochemical characteristics and 16S rDNA gene sequence analysis. The colonial characteristics of the isolate were studied on Tributyrin agar medium plate (pH- 7).

Morphological Characteristics

The size, shape and arrangement of the cells were studied by Gram's staining technique.

Standard Bacteriological Characterization

The physiological characteristics included the growth experiments to check the effect of various parameters on growth of bacterial isolate (O1A). The growth experiments at pH 4.4-12, growth at various NaCl concentrations (0.5%-10%), growth at various Sucrose concentrations (0.5%-10%) and at various temperatures (15-55˚C) were performed in Tributyrin broth medium by inoculating the inoculum (1%) and incubated at 37˚C for 24 hrs. The growth of the organism (O.D.) was determined spectrophotometrically at 660 nm.

Identification

The taxonomic status of the selected bacterium O1A was identified following the criteria laid down by Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). The biochemical tests such as indole production from tryptophan, methyl-red and Voges–Proskauer tests, Simmons' citrate utilization test, urea hydrolysis, production of H_2S from cysteine, various sugar fermentation tests, catalase and oxidase activity, Nitrate reduction tests were examined.

The isolate was further identified up to species level and confirmed on the basis of 1500bp of 16S rDNA gene sequence analysis by Merck Millipore, Bangalore, India.

Molecular Characterization and Identification of the Bacteria

DNA Preparation and PCR Amplification

Genomic DNA was isolated from the culture using DNA Extraction Solution, Cat No. 612104680501730. Using consensus primers, 16S rDNA fragment was amplified using *Taq* DNA Polymerase. Primers used for PCR amplification were the Forward primer 5'-AGAGTTTGATCMTGGCT CAG-3' and Reverse primer 5'-ACGGYTA CCTTGTTACG ACTT-3'. Amplification process was carried out in 50 µl of reaction mixture containing ~20ng Genomic DNA, 1.0µl dNTP mix (2.5mM each), 100ng Forward Primer, 100ng Reverse Primer, 1X Taq Buffer A (10X), 3U Taq Polymerase enzyme and glass distilled water to make up the volume 50 µl. Thermal cycler was programmed as denaturation at 94˚C for 5

min followed by subsequent 35 cycles of denaturation at 94˚C for 30 sec, annealing at 55˚C for 30 sec, extension at 72˚C for 2 min with final extension at 72˚C for 5 min. The PCR product was analyzed on 1.0% agarose gel along with Step Up^{TM} 500bp DNA ladder.

16 S rDNA Sequencing and Data Analysis

Sequencing analysis was performed on a 1500bp by PCR product. The PCR product was cloned into TA vector and sequenced using The T7 forward read, 13BG read (internal primer) & SP6 (reverse primer) and were checked for the overlap to get the similarity and then compile the whole sequence with results obtained. This compiled sequence uploaded in NCBI Basic Local Alignment Search Tool BLAST with nucleotide filtering option to get the 10 closest homologs. A distance matrix was generated using the Kimura-2-Parameters. The phylogenetic analysis was performed using CLUSTAL W program (Thompson *et al.,* 1997) and multiple sequence alignment. The phylogenetic trees made using Neighbour Joining method (Saitou and Nei, 1987) with alphabet size 4 and length size 1000.

Characterization of the Isolate O1A for Lipase Activity

The isolate (O1A) showing the maximum zone of clearance was selected for further analysis. The extracellular lipase produced by O1A a soil isolate was characterized for optimum temperature, optimum pH, and various carbon and nitrogen sources.

Lipase Assay

The selected bacterium (O1A) was assayed for extracellular lipase production using titrimetric method using olive oil as a substrate. Olive oil (10% v/v) was emulsified with gum Arabic (5% w/v) in 0.1M Tris-HCl buffer with pH 7.0. 0.1 ml of cellular extract/partially purified lipase was added to the emulsion and incubated for 30 min. at 37˚C. The reaction was stopped and fatty acids were extracted by addition of 2.0 ml of acetone. The amount of fatty acid liberated was estimated by titrating with 0.05M NaOH until pH 10.5 using phenophathelin as indicator. Amount of NaOH required to achieve end point (colorless to pink) was recorded (Jensen, 1983). One unit of lipase activity is defined as the amount of enzyme required to hydrolyse µmol of fatty acids from triglycerides.

Lipase Activity

$$(\mu M/min/ml) = \frac{\text{Volume of alkali consumed} \times \text{Strength of alkali} \times 1000}{\text{Volume of sample} \times \text{Time in min}}$$

One unit (U) of lipase activity is equal to one µmol of free fatty acid liberated per min per ml using the assay condition.

Specific activity was determined as enzyme unit per mg of total protein concentration. Protein concentration was determined by Lowry's method (Lowry *et al.,* 1951).

Enzyme Unit (µM/min) = Fatty Acids Liberated (µM) / Time of Incubation (in min)

Specific Activity (µM.min-1.mg-1) = Enzyme Units / Protein Concentration

Optimization of Media Parameters for Lipase Production by Isolate O1a

Influence of Incubation Period on Lipase Activity

To study the effect of time course of lipase production, 500-ml Erlenmeyer flasks each containing 100 ml of Tributyrin broth medium containing yeast extract, NaCl, Peptone and 1% (w/v) olive oil was

inoculated with 1% of inoculum and incubated at 37˚C in orbital shaker at a rotary speed of 130 rpm for 5 days (120 hours). The crude broth was harvested, aseptically, at every 12 hours interval by high speed cooling centrifugation at 10,000 g for 30 min at 4˚C. The supernatant collected was used as crude enzyme solution and was assayed for enzyme activity.

Influence of Temperature on Lipase Activity

For selecting optimum temperature for lipase production by isolate O1A, the incubation temperatures varying from 22˚C - 42˚C were selected, keeping the remaining parameters same, except the incubation period as standardized above.

Influence of pH on Lipase Activity

Effect of pH on lipase action was analyzed by substituting the buffer in reaction mixture with the different buffers for different pH. Acetate buffer for pH – 4, 5; Phosphate buffer for pH – 6, 7, 8; & pH – 9, 10 was adjusted by adding Na_2CO_3. Thus pH from 4.0 to 10.0 was scanned for determining the optimal pH for lipase production by the isolate O1A, keeping other parameters unchanged except for the incubation time and temperature, as optimized.

Influence of Agitation Speed on Lipase Activity

To determine the optimum agitation speed for the maximum production of lipase by isolate O1A, the isolate was cultured in orbital shaking incubator at varying rotary speed from 110-160 rpm at 37˚C for 48 hrs.

Influence of Different Lipidic Carbon Sources (Oils) on Lipase Activity

To evaluate different Lipidic C-sources for maximum lipase production by the isolate O1A, olive oil (1% w/v) present in the culture media was replaced with different oils like palm oil, sunflower oil, mustard oil, soybean oil, coconut oil, groundnut oil, castor oil, tributyrin and ghee, with the respective final concentration of 1% (w/v). The other parameters were as per their respective optimized value.

Influence of Different Non Lipidic Carbon Sources (Sugars) on Lipase Activity

Effect of Non Lipidic Carbon sources on the lipase production was analysed with different Carbon sources glucose, mannose, xylose, mannitol, fructose, lactose, sucrose, maltose, molasses at a concentration of 1% (w/v) were added into the production medium on a rotary shaker (130 rpm) and incubated at 37˚C for 48 hrs and the enzyme was assayed.

Influence of Different Nitrogen Sources on Lipase Activity

Different organic nitrogen sources like peptone, yeast extract, beef extract, gelatin, casein, soy meal, corn steep liquor, tryptose and inorganic nitrogen sources like ammonium sulphate, ammonium hydrogen phosphate, urea, and sodium nitrate were added to the broth at a final concentration of 1 % (w/v). Remaining parameters were unaltered.

Influence of Different Concentration of Oil on Lipase Activity

To study the effect of different concentration of olive oil, the culture media flasks with different percentage of oil 1, 3, 6, 9, 12, 15 % were inoculated with 1% of inoculum and incubated at 37˚C for 48 hrs in a rotary shaker (130 rpm) and the enzyme was assayed.

Influence of Different mineral salts on Lipase Activity

Screening for the optimum mineral salts was conducted using the lipase production medium containing either of the mineral salts viz., magnesium sulphate ($MgSO_4$), manganese sulphate ($MnSO_4$), copper sulphate ($CuSO_4$), zinc sulphate ($ZnSO_4$), iron sulphate ($FeSO_4$), calcium chloride ($CaCl_2$), calcium carbonate ($CaCO_3$) at a concentration of 0.02 % were inoculated with 1% of inoculum and incubated at 37˚C for 48 hrs in a rotary shaker (130 rpm) and the enzyme was assayed.

Influence of Surfactants on Lipase Activity

Various surfactants viz., Tween 80, and Triton X-100 (0.5%, v/v); SDS (0.5%, w/v) were incorporated in the production medium. Rest of the parameters was kept unaltered and checked for lipase assay.

Design of Production Media for maximum yield of Lipase Activity

On the basis of optimization of media parameters for Lipase production by Isolate O1A, various media were designed to get the maximum yield of lipase enzyme activity.

Composition of Fermentation Media (gram/100ml) are, Medium 1: Sun flower Oil–2 ml; Yeast Extract–1gm; NaCl–0.5gm; Lactose–1gm; $MnSO_4$-0.02gm; K_2HPO_4-1.07gm; KH_2PO_4-0.52gm; D/W – 100 ml; pH– 7.0 (Phosphate buffer). Medium 2: Olive Oil–2 ml; Yeast Extract–0.3gm; NaCl–0.5gm; Peptone-0.5gm; D/W–100ml; pH–7.0. Medium 3: Sun flower Oil–1 ml; Yeast Extract–0.3gm; NaCl–0.5gm; Peptone-0.5gm; Lactose–1gm; $CaCl_2$-0.02gm; K_2HPO_4-1.07gm; KH_2PO_4-0.52gm; Tween80- 0.5ml; D/W–100 ml; pH–7.0 (Phosphate buffer). Medium 4: Sun flower Oil–1 ml; Yeast Extract–0.3gm; NaCl–0.5gm; Peptone-0.5gm; Lactose–1gm; $CaCl_2$-0.05gm; K_2HPO_4-1.07gm; KH_2PO_4-0.52gm; Tween80- 1ml; D/W–100 ml; pH–7.0 (Phosphate buffer).

These media were inoculated with 1% of inoculum in 500-ml Erlenmeyer flasks each containing 100 ml of medium and incubated at 37˚C in orbital shaker at a rotary speed of 130 rpm for 48 hours. The crude broth was harvested, aseptically, by high speed cooling centrifugation at 10,000 g for 30 min at 4˚C. The supernatant collected was used as crude enzyme solution and was assayed for enzyme activity.

Results and Discussion

Screening of Lipase Producing Bacteria

Enrichment, Isolation and Screening of Lipolytic Bacteria

72 Lipolytic bacterial isolates were screened by enrichment culture technique from 17 diverse samples. Out of these 24 isolates were found to be growing well at pH 7& 9. Among the 24 Isolates, O1A (pH-7) showed maximum zone of hydrolysis around colony (Fig-1) and was also able to grow at pH-9 with maximum lipase activity (3.12) (Table-1) which shows its alkali tolerant nature and was selected for further studies.

Characterization of Selected Bacterial Isolate

Morphological and Cultural Characterization of Selected Isolate

The morphological and cultural studies of selected isolate O1A were performed. The isolate O1A was found to be gram positive cocci. The colonial characters of isolate

O1A were medium, round, even, regular, low convex, smooth, opaque, orange pigmented.

Physiological and Biochemical Characterization

Isolate designated as O1A was studied further for their physiological characters. The isolate O1A was able to grow up to pH 9.2 which shows its alkali tolerant nature. Optimum temperature for growth was 37˚C and was able to tolerate up to 7.5% salt and 5% sucrose concentration. It was negative towards citrate utilization, indole test, MR-VP tests, H_2S production, urea hydolysis and oxidase. The strain could reduce nitrate weakly and was catalase positive.

Molecular Characterization and Identification of the Bacteria

The strain showing maximum zone of hydrolysis was designated as O1A. Using consensus primers, the ~ 1.5 kb 16S rDNA fragment was amplified using *Taq* DNA Polymerase by PCR technique (Fig-2). The physiological analysis of this strain using its 16S rDNA sequence shows that strain O1A had highest homology (99.9%) with *Staphylococcus sp. Ch*DC B592 (accession no. KF733731.1). The biochemical characteristics as well as phylogenetic trees made using Neighbour Joining method (Saitou and Nei, 1987) suggested that the isolate O1A was close to *Staphylococcus chromogenes* (Hajek *et al.*, 1987) which was earlier named as *Stapylococcus hyicus subsp. Chromogenes* (Devriese *et al.*, 1978). Hence this strain was identified as *Staphylococcus chromogenes* O1A.

Optimization of Media Parameters for Lipase Production by *Staphylococcus chromogenes* O1A

Influence of Incubation Period on Lipase Activity

The effect of incubation time on lipase production revealed that maximum lipase production 2.30 µM/min/mg for *Staphylococcus chromogenes* O1A was found to be at 48 hours of incubation. The activity gradually decreased after 48 hours (Fig-3).

Influence of Temperature and pH on Lipase Activity

The Study of the effect for the optimization of temperature on lipase production showed that the bacteria produce lipase in wide range of temperature from 22°C to 42°C. The optimum temperature for lipase enzyme production was at 37°C (2.30 µM/min/mg) (Fig-4) and the enzyme production was affected and decreased after increase of temperature above 37°C to 42 °C. It was also noted that the lipase enzyme production was ceased at temperature 22°C.

It was observed from the results that the bacterium is capable of producing lipase from initial pH 4.0 to pH 10.0. The enzyme production varied considerably from 0.181 to 7.96 µM/min/mg. The bacteria *Staphylococcus chromogenes* O1A has optimum lipase production at pH 7.0 (7.96 µM/min/mg) (Fig-5). However it was noted that the lipase production was declined with increase in pH from pH 7.0 to pH 10.0 but was able to produce lipase towards alkaline pH which shows its alkalotolerant nature.

Influence of Agitation Speed on Lipase Activity

Agitation at 110 rpm to 130 rpm enhanced the lipase production. The optimum agitation speed for the production of lipase by the bacteria was 130 rpm (3.02

µM/min/mg). The rate of agitation speed above 130 rpm led to decrease in the enzyme production ((Fig-6).

Influence of Lipidic (Oils) and Non Lipidic Carbon Sources (Sugars) on Lipase Activity

It was inferred from the results that the maximum lipase production of the natural oils, sunflower oil was able to induce more lipase (3.87 µM/min/mg) followed by mustard oil and olive oil while optimizing the process for Lipidic C-source (Fig-7).

Among the Non Lipidic C-sources, it was reported that 1 % lactose was the best carbon source for lipase production (1.69 µM/min/mg) followed by moloasses (1.488 µM/min/mg) by *Staphylococcus chromogenes* O1A (Fig-8).

Influence of Different Nitrogen Sources on Lipase Activity

Among the different organic nitrogen sources, yeast extract (2.07 µM/min/mg) enhanced lipase production followed by beef extract (1.83 µM/min/mg) by *Staphylococcus chromogenes* O1A while inorganic nitrogen sources were found to be poor for lipase production (Fig-9).

Influence of Different Concentration of Oil on Lipase Activity

Production of lipase by *Staphylococcus chromogenes* O1A gradually increased from 1%-12% and was found to be maximum at 12% olive oil concentration (2.50 µM/min/mg) in the production medium while it got reduced at 15% of olive oil concentration (0.8 µM/min/mg) (Fig-10). But 1-2% of oil was sufficient to induce lipase production.

Influence of Different mineral salts on Lipase Activity

Among the metal ions tested Ca^{2+} enhanced the lipase activity (1.67 µM/min/mg) followed by Mn^{2+} (0.92 µM/min/mg) & Zn^{2+} showed lower activity (0.28 µM/min/mg), while Mg^{2+}, Cu^{2+} & Fe^{2+} inhibited its activity (Fig-11).

Influence of Surfactants on Lipase Activity

In order to determine the effect of surfactant at a conc. of 0.5 % in production medium, SDS (2.27 µM/min/mg) was shown to enhance lipase production after 48 hrs of incubation which was followed by Triton X-100 (2.0 µM/min/mg) & Tween80 (1.72 µM/min/mg) (Fig-12).

Lipase Activity in designed production medium of isolate O1A

The specific lipase activity of isolate O1A in designed production medium 1 was found to be (3.55 µM/min/mg), in medium 2 (11.89 µM/min/mg), and medium 3 (18.18 µM/min/mg), medium 4 (5.52 µM/min/mg) (Fig-13). Results indicates that medium 3 was found to increase the lipase activity maximally which was used further for production and purification of lipase enzyme.

Bacterial lipases are mostly extracellular and are greatly influenced by the type and concentration of carbon and nitrogen sources, the culture pH, the growth temperature, and the dissolved oxygen concentration (Elibol and Ozer, 2001). Many strains of staphylococci have been reported previously which produce extracellular lipases e.g. *Staphylococcus aureus, S. caseolyticus, S. epidermidis, S. haemolyticus, S. hyicus, S. warneri, S.*

xylosus (Jaeger *et al.*, 1999; Volpato *et al.*, 2008; Simons *et al.*, 1998; Oh *et al.*, 1999; Van Kampen *et al.*, 1998; Khoramnia *et al.*, 2010).

Staphylococcus chromogenes O1A, the isolate reported in this study, also produces extracellular lipase in 48 hrs. The results are in good accordance with Kumar *et al.*, 2012. Maximum lipase activity was observed at 37°C temperature and pH 7.0 by *Staphylococcus chromogenes* O1A. Similar results were reported for staphylococcus sp. (Sirisha *et al.*, 2010). Various *Pseudomonas* species were found to be mesophilic (Dharmasthiti and Kuhasuntisuk, 1998, Dong *et al*. 1999; Kulkarni and Gadre, 1999, Rashid *et al.*, 2001, Kanwar *et al.*, 2002). This finding supports the data by Veerapagu *et al.*, 2013 that the optimum temperature for lipase enzyme production by *Pseudomonas gessardii* was also at 37°C.

Most bacterial species are able to produce greater amounts of lipase at pH 6.5 to 7.0 (Dharmsthiti *et al.*, 1998; Gao *et al.*, 2004; Joseph *et al.*, 2006). *S. xylosus* lipase remained active at a pH range of 6-10 (Khoramnia *et al.*, 2010). The bacteria *Pseudomonas gessardii* has optimum lipase production at pH 7.0 (Veerapagu *et al.*, 2013). Our results were found to be similar accordingly that is pH 7.0 adjusted by phosphate buffer during lipase production by *Staphylococcus chromogenes* O1A.

Table # 1

COMPARATIVE ANALYSIS OF LIPASE ACTIVITY BY ISOLATES (pH-7.0)

S. No.	Designation of Isolates	Zone of lipid hydrolysis (mm)	Growth zone (mm)	Lipolytic activity *
1	O1A	25	8	3.12
2	O7A	21	11	1.90
3	O8A	20	10	2.00
4	O13A	19	10	1.90
5	O1B	18	8	2.25
6	G4A	20	8	2.50
7	G5A	21	12	1.75
8	S3A	15	7	2.14
9	OC	15	8	1.87
10	C1	19	10	1.90
11	St1	18	10	1.80
12	St2	19	10	1.90

* Lipolytic activity = Hydrolysis zone (mm): growth zone (mm)

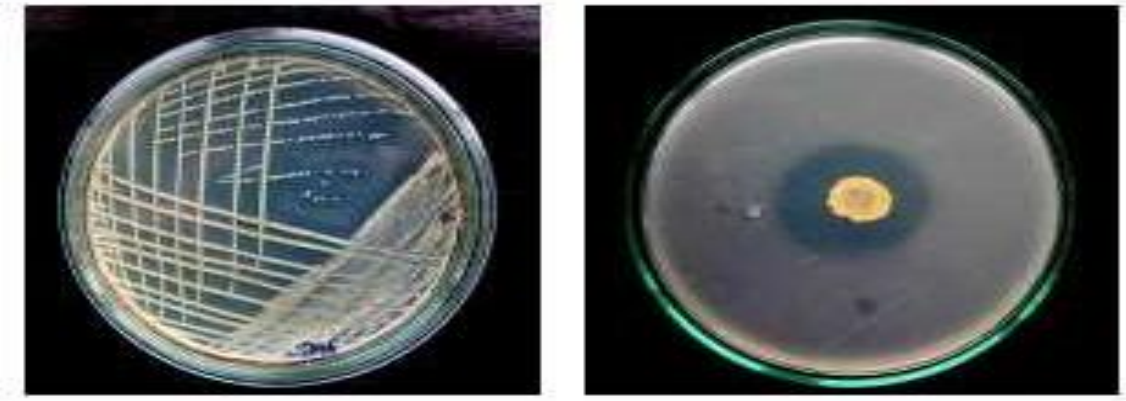

Fig.1 Zone of hydrolysis by lipase producing *Staphylococus chromogenes* O1A on Tributyrin agar plate

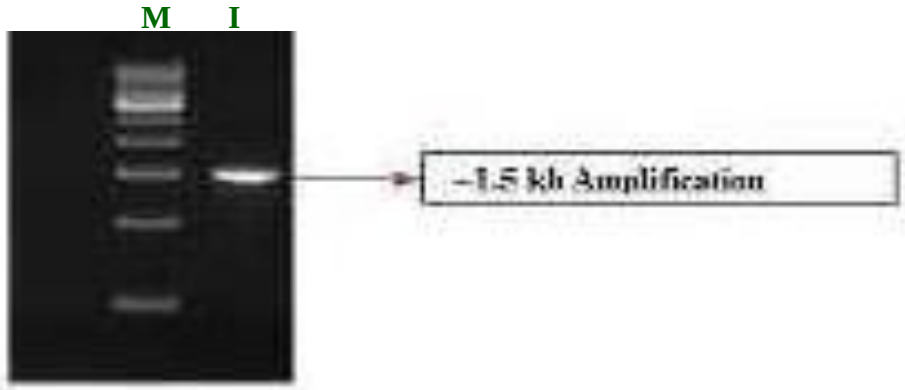

Fig.2 Lane Description

Lane-1 PCR Amplification of – Staphylococcus chromogram O1A

Lane-2 StepUpTM 500bp DNA ladder (Cx#612657970501730)

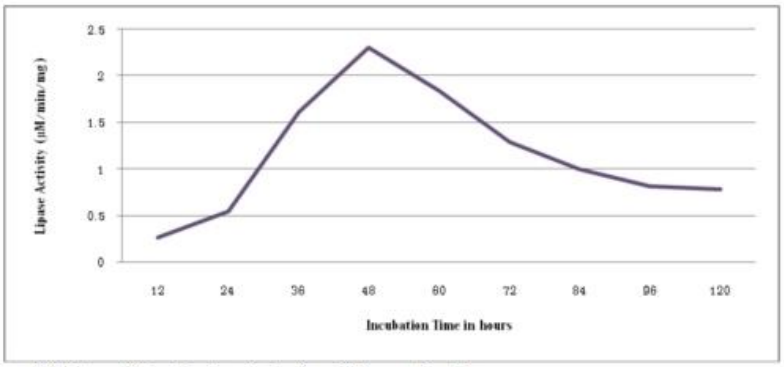

Fig- 3: Effect of Incubation Period on Lipase Activity

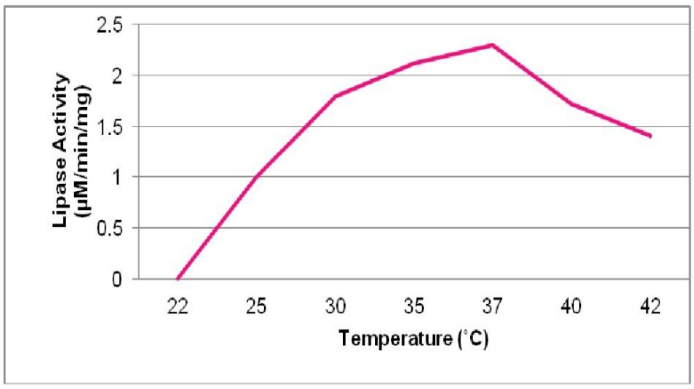

Fig- 4: Effect of Temperature on lipase Activity

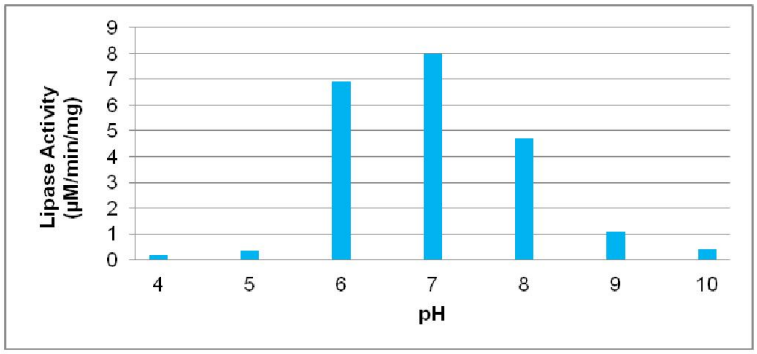

Fig- 5: Effect of pH on Lipase Activity

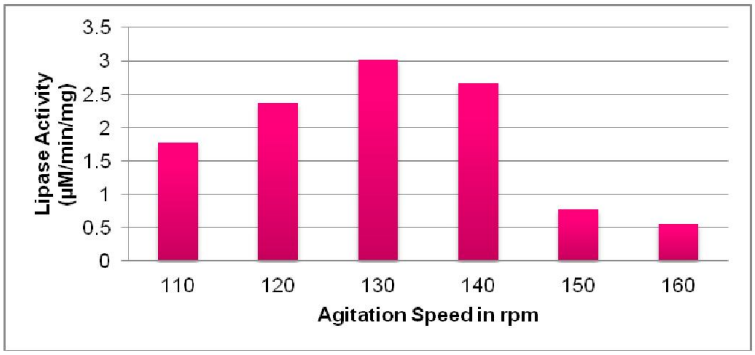

Fig- 6: Effect of Agitation Speed on Lipase Activity

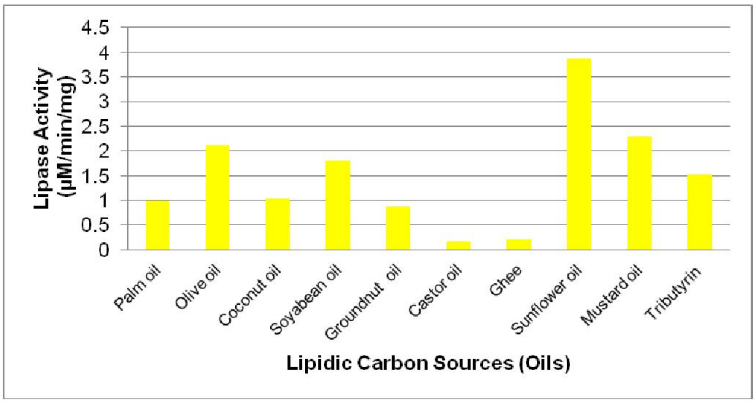

Fig- 7: Effect of Lipidic carbon Sources on lipase Activity

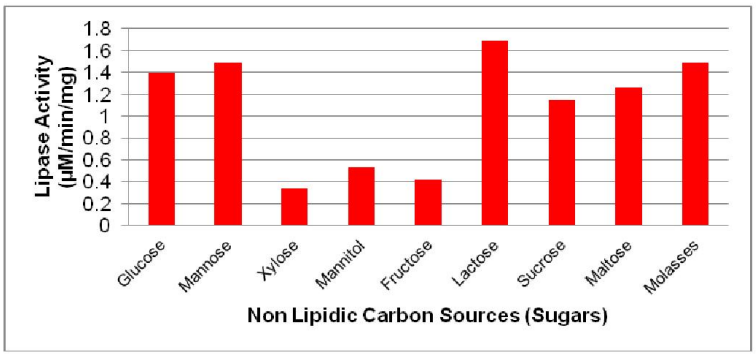

Fig- 8: Effect of Non Lipidic carbon Sources on lipase Activity

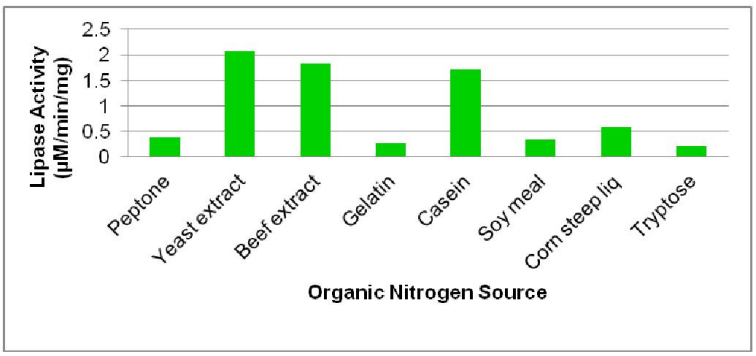

Fig- 9: Effect of Organic Nitrogen Sources on lipase Activity

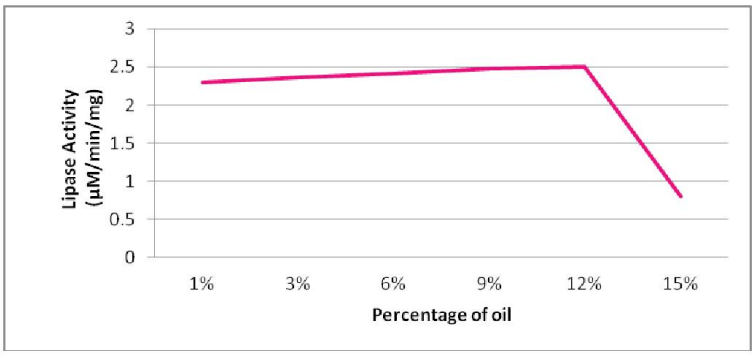

Fig- 10: Effect of Different Concentration of Oil on lipase Activity

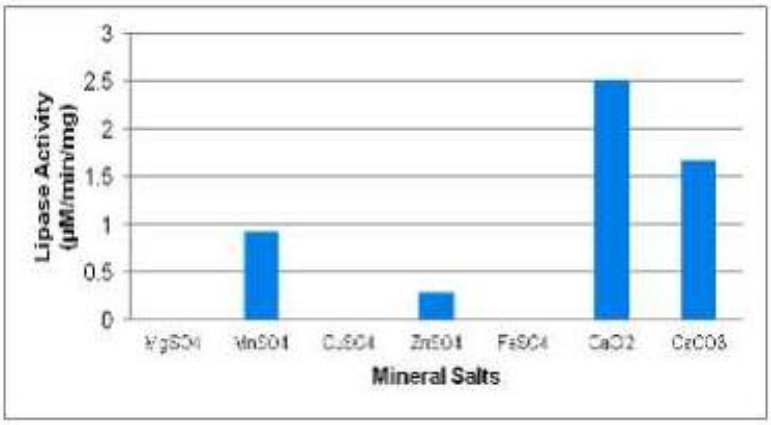

Fig- 11: Effect of Mineral Salts on lipase Activity

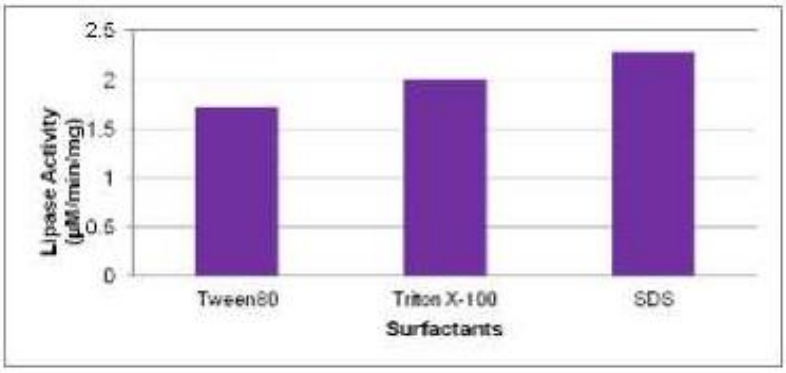

Fig12: Effect of Different Surfactants on lipase Activity

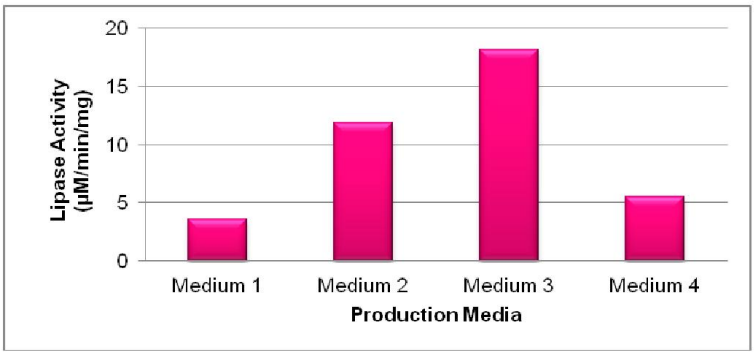

Fig- 13: Effect of Different Production Media on lipase Activity

It was clear from the results that agitation is required for proper mixing of oil and medium along with bacterial culture for the production of lipase (Veerapagu *et al.*, 2013).

Lipidic C-source that is sunflower oil (1 %) induced more lipase production by *Staphylococcus chromogenes* O1A than Non Lipidic C-sources (1 % lactose). Lipidic carbon sources seem to be generally essential for obtaining a high lipase yield. Most bacterial lipases are generally induced in medium that contains the proper fatty acids and oils (Joseph *et al.*, 2006; Immanuel *et al.*, 2008; Kiran *et al.*, 2008). Other carbon sources such as sugars, polysaccharides, whey, casamino acids and other complex sources influences its production significantly (Dharmsthiti and Kuhasuntisuk 1998; Ghanem et al. 2000; Rashid et al. 2001). Natural oil like palm oil was found to be best carbon source for *Staphylococcus* sp. (Sirisha *et al.*, 2010) and mustard oil for *Pseudomonas* sp. (Tembhurkar *et al.*, 2012). Among the different carbon sources used, olive oil was found to be the most suitable carbon source (Senthilkumar *et al.*, 2008, Omar *et al.*, 2010, Mishra *et al.*, 2011, Kumar *et al.*, 2012).

Besides carbon source, the type of nitrogen source in the medium also influences the lipase titers in production broth (*Ghosh et al., 1996*). *Staphylococcus chromogenes* O1A released maximum lipase when organic nitrogen sources like Yeast Extract and Peptone were used in the production medium 3 and poor yield with inorganic nitrogen sources. Generally, microorganisms provide high yields of lipase when organic nitrogen sources are used, such as peptone and yeast extract, which have been used for lipase production by various thermophilic *Bacillus sp*. (viz. B. alcalophilus, Bacillus sp. RSJ1) (Ghanem *et al.*, 2000, Sharma *et al.*, 2002b), and *P. aeruginosa* KKA-5 (Sharon *et al.*, 1998) and by *Staphylococcus xylosus* also (Khormania *et al.*, 2010). In some cases meat extract and yeast extract was found to be the best carbon source for Staphylococcus spp (Tembhurkar *et al.*, 2012, Kumar *et al.*, 2012). Sharon *et al.*, (1998) reported a lipase of *P. aeruginosa* KKA-5 that retained its activity in presence of Ca^{2+} and Mg^{2+} but was slightly inhibited by Mn^{2+}, Cd^{2+}, and Cu^{2+}. In our results Ca^{2+} enhanced the lipase activity of *Staphylococcus chromogenes* O1A while Mg^{2+}, Cu^{2+} & Fe^{2+} inhibited its activity. The effect of various metal ions on *S. epidermidis* lipase activity was reported that enzyme needed calcium as a cofactor for catalytic activity (Simons *et al.*, 1998). Metal

cations, particularly Ca^{2+}, play important roles in influencing the structure and function of enzymes, and calcium-stimulated lipases have been reported (Khattabi *et al.*, 2003). It has been demonstrated that the activity of Staphylococcal lipases may depend on the presence of Ca^{2+} ions (Rosenstein and Gotz, 2000). The lipase activity of *S. xylosus* increased maximum about 3 times at the Ca^{2+} concentration of 10 mM however, Mosbah *et al.*, (2005) reported 1.9 times increase with 2 mM Ca^{2+} concentration. It has been reported that the lipases from *Staphylococcus hyicus* (Rosenstein and Gotz, 2000; Tiesinga *et al.*, 2007) contain a Ca^{2+}-binding site which is formed by two conserved aspartic acid residues near the active-site, and that binding of the Ca^{2+} ion to this site dramatically enhanced the activities of these enzymes. These data supports our results that Ca^{2+} ions enhance lipase activity.

Among the different lipase inducers tested, Tween 80 produced a great level of extracellular lipase (Anbu et al. 2011). The same results were observed when Tween 80 (0.5%) was used in production medium 3 during the lipase production by *Staphylococcus chromogenes* O1A.

The isolate O1A, characterized as *Staphylococcus chromogenes* O1A, has shown a broad range of pH (6-10) and temperature (25-42˚C). The newly designed production medium 3 increased the yield of lipase enzyme. It can be used as a potential bacterial source of lipase and due to alkali tolerant nature of bacterium; lipase enzyme can be used in detergent formulation and also in various industrial applications.

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