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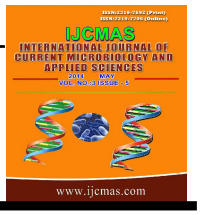

Original Research Article

Optimization studies on production and activityof lipase obtained from *Staphylococcus pasteuri* SNA59 isolated from spoilt skin lotion

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A B S T R A C T

A Lipase producing bacterium was isolated from spoilt skin lotion and identified as *Staphylococcus pasteuri* SNA59 by morphological, cultural, biochemical tests and 16S rRNA sequence analysis. Maximum production of lipase (49.62U/ml) by this isolate (1% of 0.3OD_{530nm}) was achieved in optimized Maltose Peptone Yeast Extract (MPY) medium containing Maltose (1%), Peptone (0.5%), Yeast Extract (0.1%), $MgSO_47H_2O$ (0.04%), K_2HPO_4 (0.1%), Na_2CO_3 (3%) and glycerol (1%), pH 10 incubated on a shaker 120 rpm, at 30^0C for 48h. The lipase enzyme showed stability in the pH range of 7-12and a temperatures range of 30-60^0C with maximum activity at pH 10 and 45^0C.The enzyme was found to be stable in most of the solvents used;however, 2-propanol was found to inhibit enzyme activity by 51%. Metal ionslike Ca^{2+} enhanced the lipase activity slightly whereas Zn^{2+} significantly inhibited the enzyme with the residual activity of 30%. The Enzyme was found to be stable in 1% solutions of different surfactants such as Tween 80, Triton X-100 and 1% commercial detergent Wheel powder.At 0.1% concentration, the inhibitorsEDTA, PMSF, bile salt and 2-mercaptoethanol moderatelyinhibited the lipase with residual activity of 60–96%.The Enzyme retainedits activity in 1.5% H_2O_2indicating it to be bleach-stable.The present findings show that the staphylococcal lipase is alkali- and detergent- stable and hence, has a potential to be used in the detergent industry.

Keywords

Staphylococcus pasteuri SNA59, lipase; 16S rRNA sequence analysis

Introduction

Lipases have recently emerged as key enzymes in the swiftly-growing biotechnology sector and are defined as triacylglycerol acylhydrolases (E.C. 3.1.1.3) that catalyze the hydrolysis of fats to glycerol and free fatty acids. They occur widely in animals, plants and many microorganisms. Lipase-producing microorganisms such as bacteria, yeast and fungi have been found in diverse habitats such as industrial wastes, premises of vegetable oil-processing factories, dairies and soil contaminated with oil (*Sztajer et al., 1988*). Extracellular microbial lipases are high in demand due to their thermostablility, stereo-specificity and lower energy consumption than conventional methods. Hence, they have

received much attention with respect to their potential use in various industries such as food, chemical, dairy, detergent, cosmetic, tanning, pharmaceutical (diagnostics) and especially in biodiesel production(*Saxenaet al., 1999;Svendsen, 2000; Margesin et al., 2002; Gupta et al., 2004a; Jaeger and Eggert, 2002; Kulkarni and Gadre,2002; Nelson et al., 1996; Marek and Bednarski,* 1996).

Although a number of lipase-producing bacterial sources are available, only a few are commercially exploited as wild or recombinant strains (*Jaeger et al., 1994; Palekar et al., 2000*). Of these, the important ones are: *Achromobacter, Alcaligenes, Arthrobacter, Bacillus, Burkholderia, Chromobacterium* and *Pseudomonas* (*Jaeger et al., 1994; Pandey et al., 1999*; *Beisson et al., 2000*).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*). It appears that the mature forms of Stapylococcal lipases are homologous with identities ranging from 48 to 99%. In contrast to most other lipases, some staphylococcal lipases are distinguished by their extremely broad substrate specificity and tendency to formaggregates (*Tysky et al.,1983; Gotz et al.,1998; Simons et al., 1998;Jurgens and Huser,1981; Jurgens et al.,1981; Kotting et al.,1983*). Finding of new lipase producers followed by theiroptimization to improve and increase the efficiency of the lipase production without increasing the cost are very important.

This study focuses on isolation of lipase producing bacterium from spoilt skin lotion that is expected to have higher lipase producing variants.This strain is identified as *Staphylococcus pasteuri* SNA59. The culture conditions for maximum lipase production by this strain were identified and assay conditions for optimum activity were alsoinvestigated.

Materials and Methods

Enrichment, isolation, screening and identification

Bacteria were isolated by enrichment from various samples,viz. soils, spoilt coconutoil and skin lotion. Enrichment was carried out by adding 1 g or 1ml of sample to 100ml of Nutrient Broth medium (1 % Peptone, 0.3% Meat extract, 0.5% NaCl,1% Olive oil, pH 7.2). All theflasks were incubated at 30^0C for 72h on shaker-incubator at 120rpm. Subculturing (2% v/v) was carried out three times at 72h intervals. Finally, 1 ml from each flask, appropriately diluted, was spread on Nutrient Agar containing 1% olive oil. These plates were incubated at 30°C for 24 h and the colonies were picked and maintained on Nutrient Agar slants of same composition at 4°C.All the isolates were checked for lipase activity qualitatively on Tributyrin agar(*Lawrence et al., 1967*)and Rhodamine B-olive oil agar plate (*Kouker and Jaeger,1986*).The lipase-producing bacteria were identified by the presence of azone of clearance on Tributyrin agar and an orange fluorescent halo on Rhodamine B-olive oil agar seen under UV lightafter 48 h incubation at 30°C.The isolate giving maximum lipase production was identified by using morphological and biochemical appropriate and culture tests as per Bergey's Manual of Determinative

Bacteriology, 8th edition (*Buchanan and Gibbons, 1975)*. Further confirmation of the strain's identity by 16S rRNA sequence analysis was carried out atSciGenom Labs Pvt Ltd. Kerala, India.

Extraction and assay of lipase

Lipase activity was assayed by modified method of Winkler and Stuckmann (*1979*). Briefly, the crude enzyme was obtained by centrifuging the culture at 10,000 rpm at 4°C for 20 minutes (*Joshi et al., 2006*). This crude enzyme (0.75 ml) was mixed with 0.5mM of 4-nitrophenylpalmitate substrate prepared in isopropyl alcohol and 1.95 ml of50mM Phosphate Buffer (pH 7.2) incubated at 30°C for 30 minutes. After incubation the reaction mixture was kept in ice bath for 5 minutes to stop the reaction and 150 µl of Triton X-100 was added to the mixture. It was centrifuged at 10,000 rpm for 25 minutes and the absorbance of the supernatant was measured at 420 nm using a spectrophotometer. The reaction mixture containingheat-inactivated crude enzyme (100^0C for 10 min) instead of the active culture supernatant 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 (2-20µg/ml) to obtain the amount of substrate converted. One unit of lipase activity is the amount of lipase enzyme, whichliberates 1 µmole *p*-Nitrophenol from 4-Nitrophenylpalmitate as substrate per minute under standard assay conditions.

Optimization of culture conditions for maximum lipase production

Five different media viz. Nutrient broth, Maltose Peptone Yeast extract (MPY) medium (*Ghanem et al., 2000*), Glucose Yeast extract Peptone (GYP) medium (*Esakkiraj et al., 2010*), Medium A (*Dutta and Ray, 2009*) and Medium B (*Kumar et al., 2005*) were investigated for maximum lipase production by the selected isolate.

One loopful of 30 h-old culture of the isolate grown onan agar slant of MPY medium with 1% olive oil was transferred (1% of inoculum of 0.3O.D_{530nm}) into a separate 250 ml Erlenmeyer flask containing 50 ml MPY medium containing 1% olive oil, 1% maltose, 0.5% peptone, 0.1% yeast extract, 0.1% K_2HPO_4, 0.02% $MgSO_4.7H_2O$ and 1.5% Na_2CO_3in 100ml of distilled water pH 7.2 and incubated on a rotary shaker at 30^0C and 120 rpm for 48 h. The supernatant of the culture was assayed as described above for lipase yield.This medium was used for further study of various parametersfor maximum lipase production by the selected isolate.

The culture conditions (aeration, incubation period, temperature,pH, carbon, nitrogen source,oils and various mineral salt concentrations) were optimized for maximum lipase productionby the selected isolate.The effect of aeration on lipase production was studied by incubating one culture flask onshaker (120rpm) and other under static condition. Lipase production was determined at various time intervals such as 0, 24, 36, 48, 60 and 72 h on shaker (120rpm). The optimum culture temperature for lipase production was determined in the range of 30^0C to 55^0C with 5^0C interval on shaker (120rpm) for 48 h.The optimum pH for lipase production was determined for the culture grown in MPY medium at apH range from 4 to 12 (1N NaOH and 1N HCl were used for adjusting pH of medium). The effect of different carbon sources on lipase production was checked by adding 1% w/vGlucose, Galactose, Lactose, Fructose, Maltose, Sucrose, Arabinose, Xylose,

Mannitolor Starch to the MPY medium. Of these,0.5-2% w/vMaltose was tested at increments of 0.5 %.Different oils (1% v/v of each) such as Olive Oil, Coconut Oil, Tributyrin, Sunflower Oil, Almond Oil, Mustard Oil, Soya Bean Oilas well asGlycerol, were checked for the maximum lipase production. In addition, 0.5-2% glycerolwas tested with increment of 0.5% v/v concentration. The effect of different nitrogen sources on lipase production was studied by replacing peptone with various organic 0.5% w/v nitrogen sources like Yeast extract, Meat extract, Casein and Casitone, as well as inorganic nitrogen sources like Urea, Ammonium chloride, Ammonium sulphate, Ammonium nitrate, Potassium nitrate and Sodium nitrate.Also,yeast extract in the MPY medium was replaced with0.1% w/v of organic and inorganic nitrogen sources mentioned above.Of these nitrogen sources,Peptone 0.5-3%(0.5%increment) andYeast extract 0.05 -0.3% (0.05% increment) weretested formaximum lipase production.The optimal salt concentrations (w/v) ofK_2HPO_4 (0.05-0.3% with 0.05% increment), $MgSO_4.7H_2O$ (0.02-0.1% with0.02% increment) and Na_2CO_3(0.5-4% with 0.5% increment)weredetermined for highest lipase yield.

Effect of different physicochemical parameters on lipase activity

Lipase enzyme from the new isolate was assayed to determine the optimum conditions of temperature, pH, and salt concentration. 1 ml of the inoculum ($0.3OD_{530nm}$) was cultured in 100ml of optimized Maltose Peptone Yeast Extract (MPY) medium containing Maltose (1%), Peptone (0.5%), Yeast Extract (0.1%), $MgSO_4$ (0.04%), K_2HPO_4 (0.1%), Na_2CO_3 (3%) and Glycerol (1%), at pH 10 and 30^0C for 48 h, with shaking at 120rpm.. The cell-free culture supernatant was obtained by centrifugation at 10,000 rpm, 4°C for 20 minutes and was used in the assays. The optimal temperature was determined at a 5^0C interval from 25^0 to 60^0C. The lipase was assayed at various pH ranging from 5 to 11 in the following buffer systems: 0.1M Acetate buffer (pHrange 4-6), 0.1M Phosphate buffer (pHrange 7-8) and 0.1M Glycine-NaOH buffer (pHrange 9-12). The effect of various inhibitors such as (0.1% v/v or w/v) Phenyl Methyl Sulphonyl Fluoride (PMSF), Ethylene Diamine Tetrachloro Acetic acid (EDTA),2-mercaptoethanol and bile salt were investigated**.** Various metal ions such as Ca^{2+}, Cd^{2+}, Hg^{2+}, Mg^{2+} Mn^{2+}, Zn^{2+}, $K^+$and Na^+in the form of chloride salt (0.1% w/v) were tested on lipase activity.The presence of organic solvents (10% v/v) such as acetone, benzene, cyclohexane, ethanol, methanol, 2-propanol and butanol on lipase activity was also investigated. The surfactants (1% v/v) such as SDS, Tween 80, Triton X - 100 and commercial detergents (1% w/v of Surf Excel, Tide, Wheel and Ariel) were tested on the lipase. To check the effect of an oxidizing agent on lipase activity, different concentrations (0.5-2.0%)of Hydrogen Peroxide wasused. The crude enzyme was pre-incubated with the above-mentioned respective inhibitors, solvents, metal ions and surfactants (with control) for 30mins at 30^0C. The residual activity (%) was measured by standard lipase assay.

Results and Discussion

Enrichment, isolation, screening and identification

The soil, spoilt butter and skin lotion samples were enriched in Nutrient broth containing 1% olive oil, streaked on agar plates containing the same medium and

incubated at 30^0C for 48 h. Total 7 isolates were obtained and were screened for extracellular lipase activityby observing the zone of hydrolysis around the colonies on Tributyrin agar plates (fig. 1A). This was confirmed on all the isolates on RhodamineB agar plates as seen from the orange fluorescent halo around the colonies when the plates were observed under UV light(fig. 1B). The isolate designatedLL3-1 exhibited maximum lipase activity in Nutrient Broth containing 1%Olive Oil as shown in Figure 2, and hence, was selected for further optimization studies. On the basis of morphological, cultureand biochemical characteristicsaccording to Bergey's Manual of determinative Bacteriology, 8th edition and by16S rRNA gene sequencing analysis, the LL3-1 isolate was identified as *Staphylococcus pasteuri* SNA59.

Optimization of culture conditions for maximum lipase production

From the results shown in Figure 3, it was found that MPY medium facilitated maximum lipase yieldby *Staphylococcus pasteuri* SNA59, and hence was used throughout the study.Lipase productionwas observed starting from 12 h of growth(18.93U/ml),till it reached itsmaximum in 48 h (39.04U/ml)and then declined with longer incubation times (Figure 4).

As shown in theFigure 5, shaker culture at 120rpm gave maximum lipase yield (39.09U/ml) than the culture incubated at static conditions (27.47U/ml). As indicated in Figure 6,30°C supported optimum lipase yield(39.14U/ml) and lipase production decreased as temperature increased.Lipase production was observedto be minimum at pH 8 (6.99U/ml) but was maximum at pH 10(45.12U/ml) and interestingly, significant yield was also seen at pH 11 and 12 (Figure 7).

Lipids can be a source of carbon for lipase- producing organismsbut addition of carbohydrate further enhances lipase production.As shown in Figure 8, maximum lipase yield by *Staphylococcus pasteuri* SNA59 was observed in MPY medium with 1%Maltose (45U/ml), followed by Starch (38U/ml), Lactose and Mannitol (35U/ml and 34 U/ml respectively)althoughCitrate did not induce lipase production. The following oils which were used in MPY medium at 1% concentration,viz. Olive Oil, Coconut Oil, Tributyrin, Sunflower Oil, Almond Oil, Mustard Oil, Soya Bean Oil and Glycerol, the maximum yield of lipase (45 U/ml) was shown by Glycerol,followed by Soya Bean Oil (36.88U/ml) (Figure 9).The supply of organic nitrogen sources like Peptone (0.5%) resulted in 46.9U/ml of lipase production whereas 0.5% of other organic andinorganic nitrogen sources showed production of lipase in the range of 33U/ml to 45U/ml and 25U/ml to 43U/ml respectively(Figure 10).

Similarly, Yeast Extract (0.1%) exhibited 47.49U/ml of lipase yield while 0.1% other organic and inorganic nitrogen sources yielded lipase in the range of 25U/ml to 36U/ml and 27U/ml to 42U/ml respectively (Figure 11). As indicated in Table 1, other media components such asK_2HPO_4 (0.1%), $MgSO_4$, $7H_2O$(0.04%) and Na_2CO_3 (3%)increased lipaseproduction.When various concentrations of Maltose, Glycerol, Peptone and Yeast Extract were used in the medium,Maltose (1%), Glycerol (1%), Peptone (0.5%) and Yeast Extract (0.1%) exhibited maximum lipase yield (Table 2).

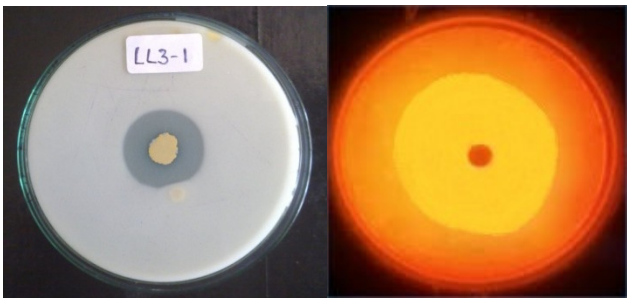
Effect of different physicochemical parameters on lipase activity

The lipase enzyme from the culture supernatant of optimized MPY medium was assayed in the reaction mixture containing 0.75ml of crude enzyme, 0.5mM 4-Nitrophenylpalmitate substrate in isopropyl alcohol and1.95 ml of 50mM phosphate buffer (pH 7.2) at 30^0C for 30min.

Optimization of lipase activity was carried out on the basis of different parameters. The lipase enzyme was found to be more active in the alkaline range than in the acidic range,with an optimum at pH 10 (Figure 12). The lipase enzyme showed maximum activity at 45°Cand retained more than 60% of the maximum enzyme activity at 60°C (Figure13). In an attempt to further characterize the lipase enzyme; different inhibitors were tested at 1 % (w/v) concentrations. Results presented in the Figure 14 showed that with PMSF, EDTA and 2-Mercaptoethanol and Bile Salt, 96%, 81%, 87% and 60% lipase activity was retained respectively. Among the metal ions tested, Ca^{2+}enhanced the lipase enzyme activity by109% while Zn^{2+}inhibited its activity by 30% (Figure 15).Organic solvents like Benzene, Cyclohexane, Butanol, 2-Propanol, Ethanol, Methanol and Acetone (at 10% v/v) preserved 79%, 71%, 85%, 49%, 75%, 61% and 81% of the lipase activity respectively(Figure 16).The lipase enzyme displayed stability in the presence of various surfactants and detergents used. More than 80% of the enzyme activity was retained by the enzyme after treatment with SDS, and commercial detergents like Surf Excel and Tide, whereas, Tween80, Triton X100 and Wheel were found to stimulate the relative enzyme activity by 120%, 117% and 105% respectively(fig.17).The oxidizing effect of hydrogen peroxide (H_2O_2)was studied on the lipase activity using three different concentrations of H_2O_2. The results showed that the lipase enzyme activity was enhanced at all the three concentrations tested, with 1.5% H_2O_2 giving maximum lipase activity (fig.18).

Bacterial lipases are mostly extracellular and their production is influenced by nutritional and physicochemical factors, such as temperature, pH, nitrogen, carbon sources, inorganic salts, agitation and dissolved oxygen concentration (*Gupta et al., 2004b*).Many strains of staphylococci have been reported previously which produce extracellular lipases. Some of them have been purified and their biochemical properties studied in detail e.g. *Staphylococcus hyicus, S. aureus, S. epidermidis,S. warneri, S. haemolyticus*and*S. xylosus* (*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*).

Staphylococcus pasteuri SNA59, the isolate reported in this study, also produces extracellular alkaline lipase in MPY medium with 1% olive oil.Sugihara et al. (*1991*) used 1% olive oil in the culture medium for the enrichment of lipase producers. Olive oil was employed as the carbon source for producing lipase from *A. radioresistens* and *Bacillus stearothermophilus* SB-1 (*Liu and Tsai, 2003; Bradoo et al., 1999*). The major factor for the expression of lipase enzyme hasbeen the carbon source. Since lipases are by-and-large inducible enzymes and are thus generally produced in the presence of a lipid source such as oil or any other lipidic substrate such as



A B

Fig.1 Detection of lipase activity on Tributyrin agar (A) and Rhodamine B agar plate (B) by the isolate LL3-1

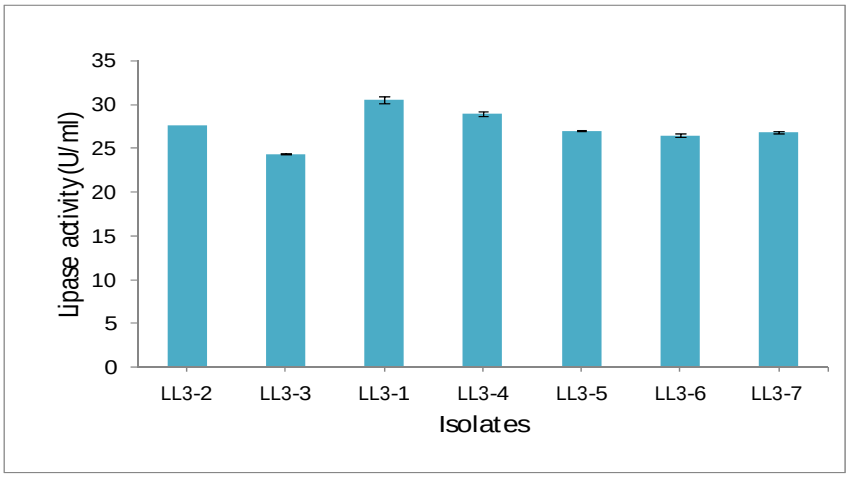


Fig.2 Lipase activity of 7 different isolates

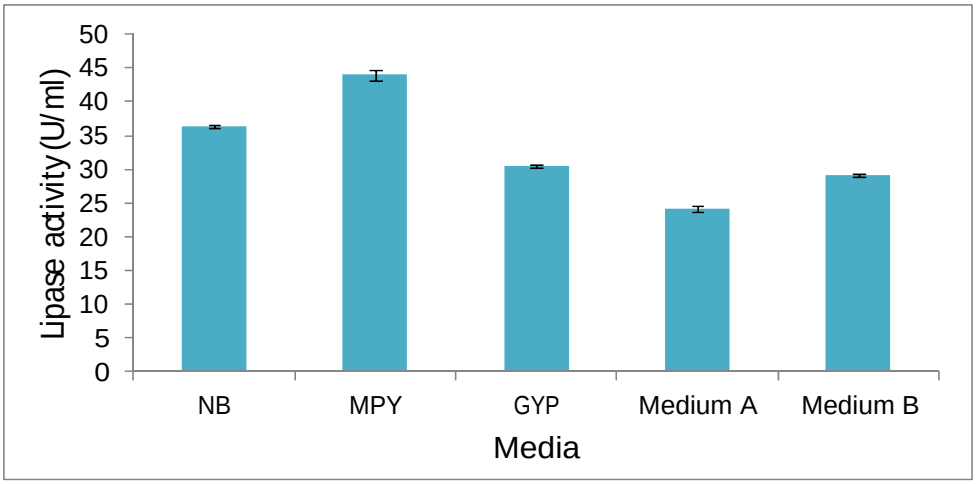


Fig.3 Effect of different media on lipase production by *Staphylococcus pasteuri* SNA59

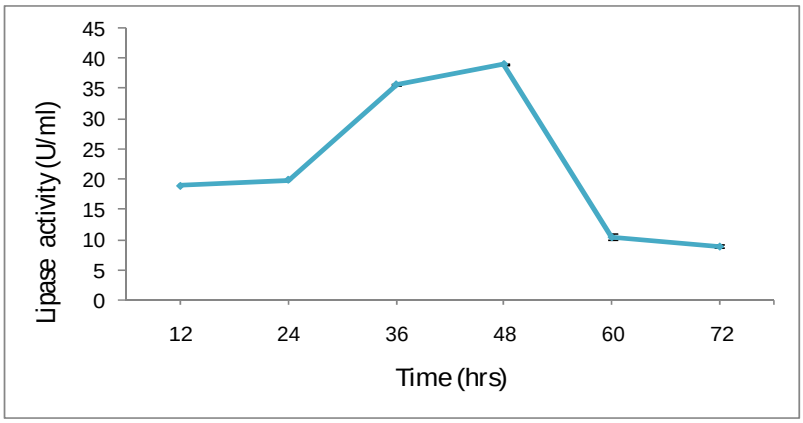


Fig.4 Effect of incubation period on lipase production by *Staphylococcus pasteuri* SNA59

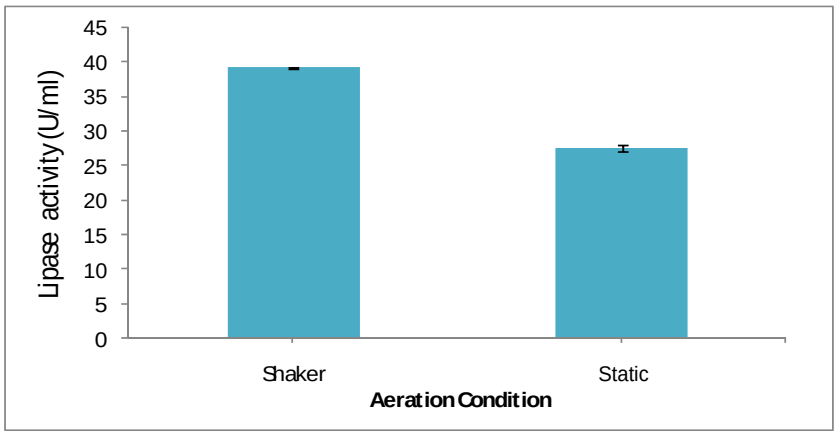


Fig.5 Effect of aeration on lipase production by *Staphylococcus pasteuri* SNA59

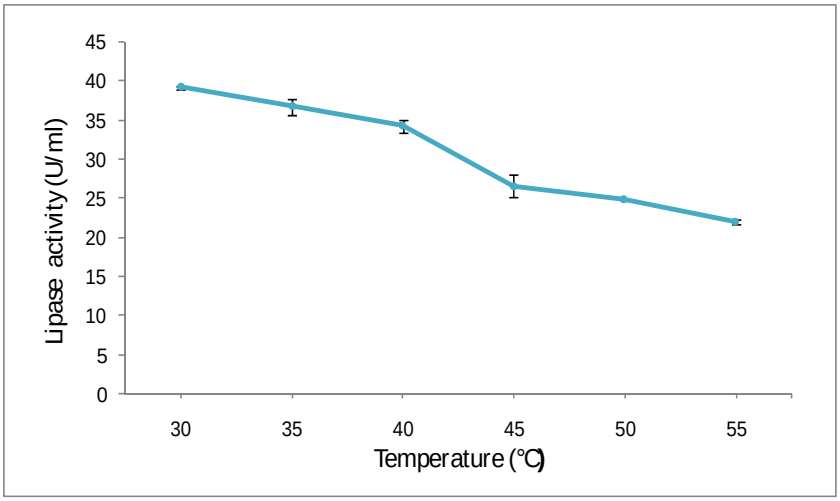


Fig.6 Effect of different incubation temperatures on lipase production by *Staphylococcus pasteuri* SNA59

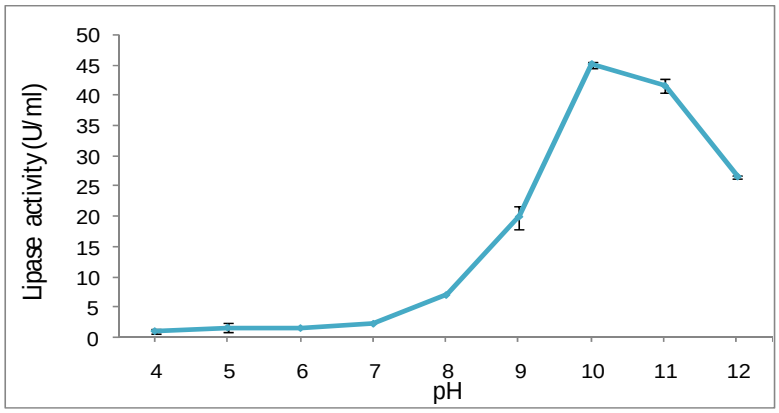


Fig.7 Effect of different pH of the growth medium on lipase production by *Staphylococcus pasteuri* SNA59

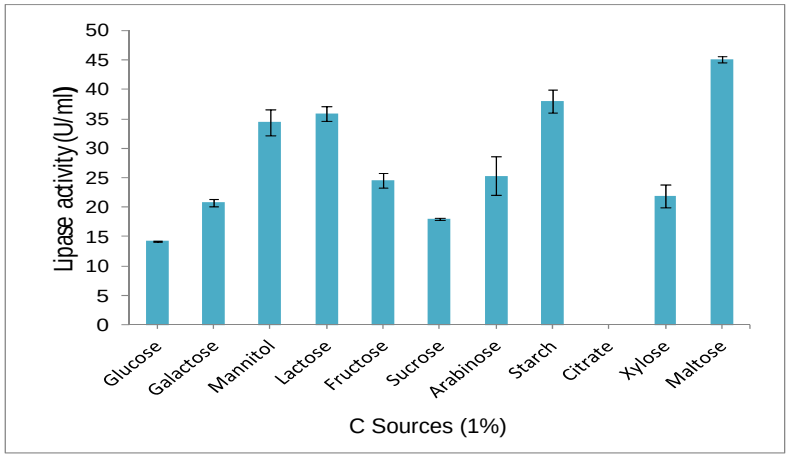


Fig.8 Effect of different carbon sources on lipase production by *Staphylococcus pasteuri* SNA59

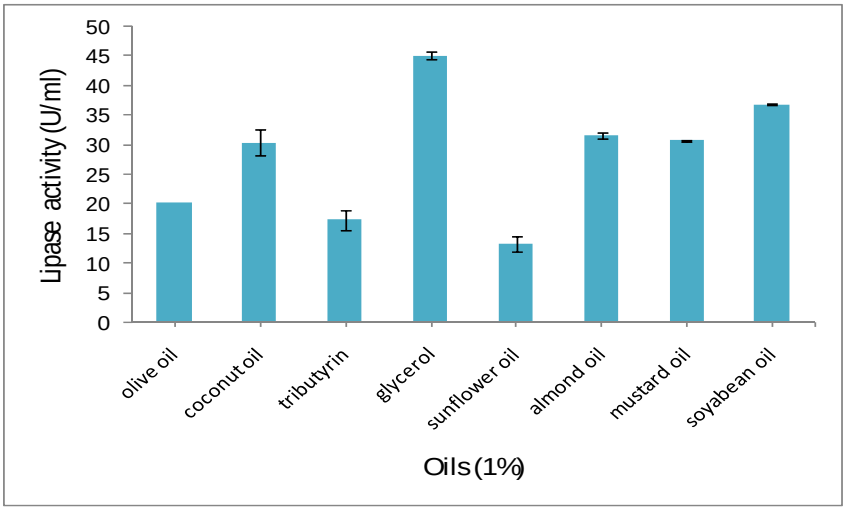


Fig.9 Effect of different lipid sources on lipase production by *Staphylococcus pasteuri* SNA59

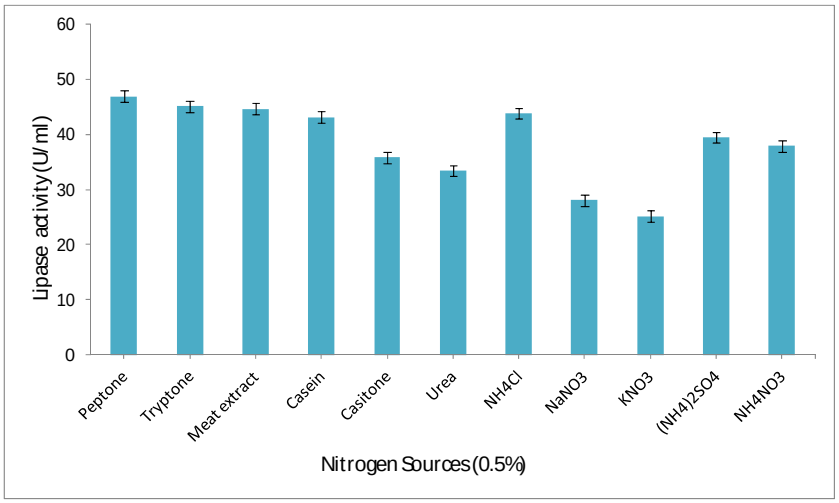


Fig.10 Effect of different nitrogen sources (0.5%) on lipase production by *Staphylococcus pasteuri* SNA59

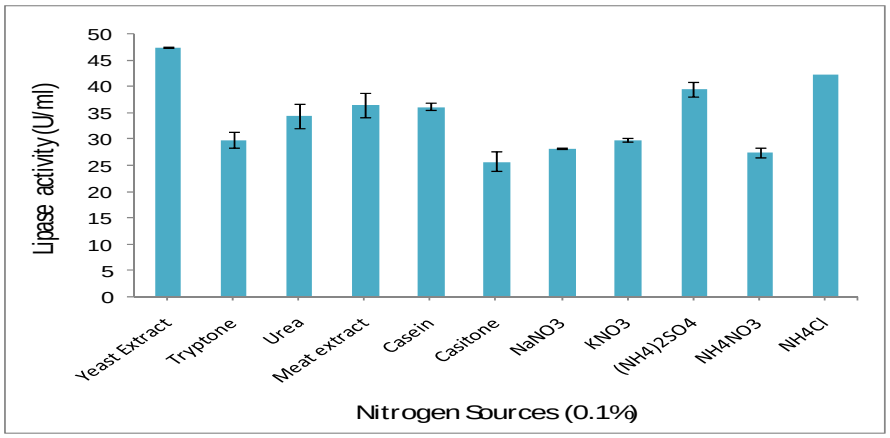


Fig.11 Effect of different nitrogen sources (0.1%) on lipase production by *Staphylococcus pasteuri* SNA59

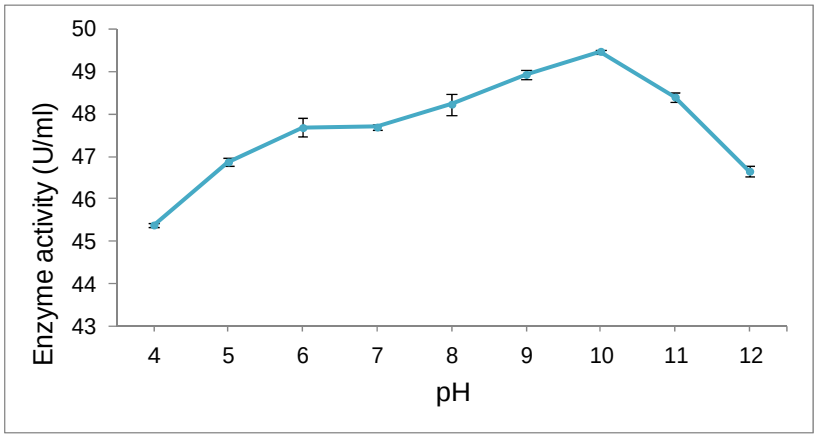


Fig.12 Effect of different pH on activity of the lipase of *Staphylococcus pasteuri* SNA59

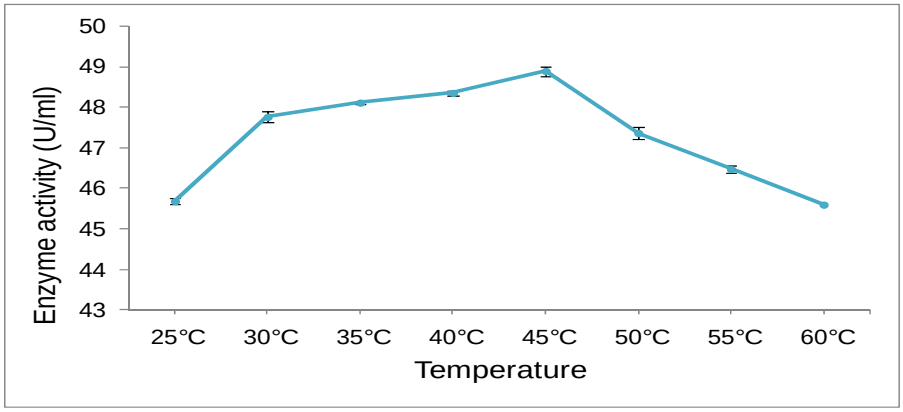


Fig.13 Effect of reaction temperature on activity of lipase of *Staphylococcus pasteuri* SNA59

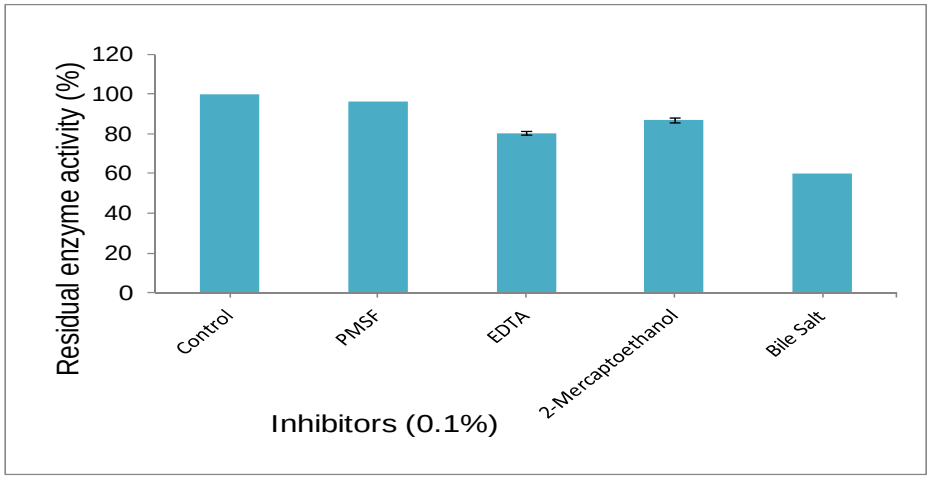


Fig.14 Effect of different inhibitors on lipase the activity of *Staphylococcus pasteuri* SNA59

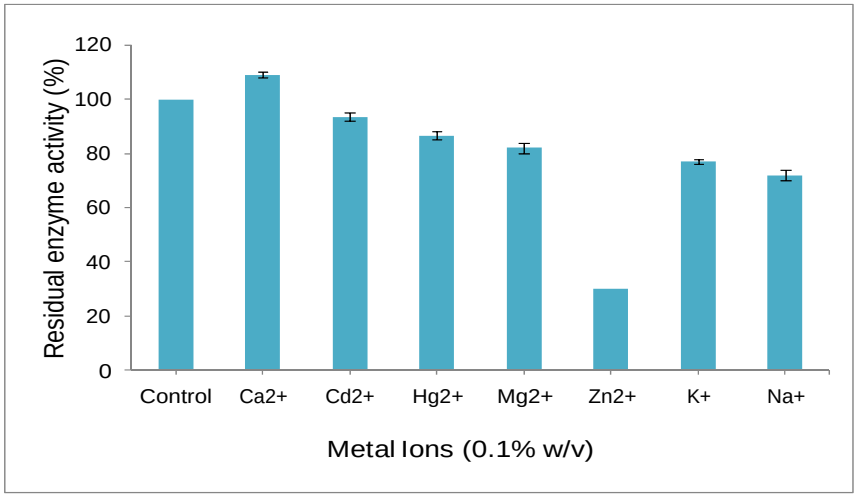


Fig.15 Effect of different metal ions on the lipase activity of *Staphylococcus pasteuri* SNA59

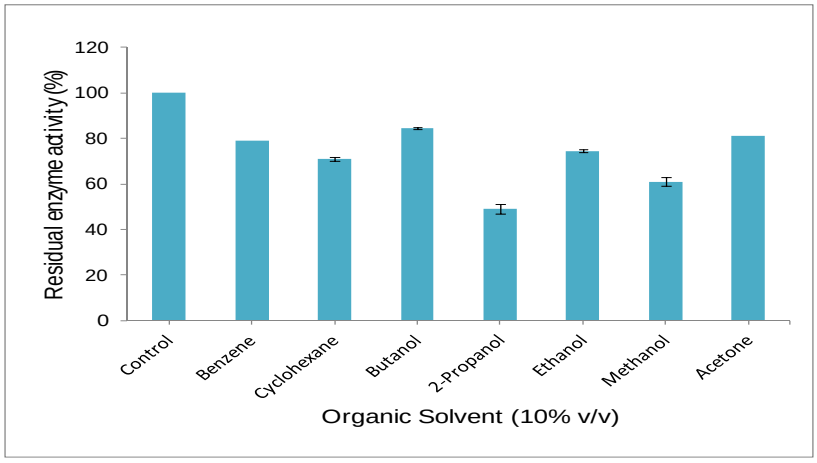


Fig.16 Effect of different organic solvents on the lipase activity of *Staphylococcus pasteuri* SNA59

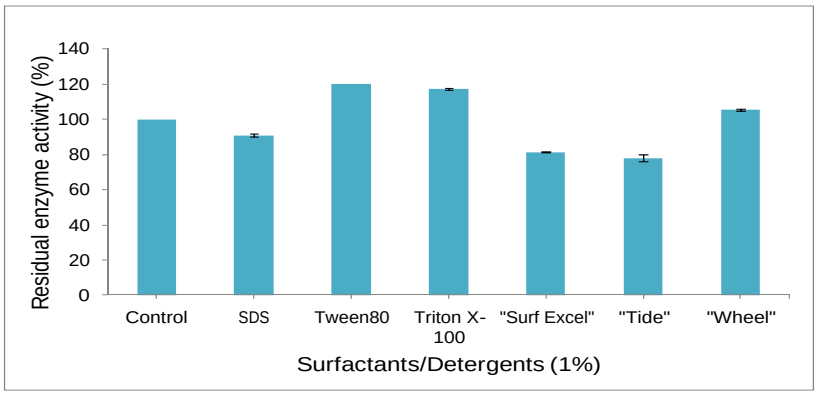


Fig.17 Effect of different surfactants/detergents on the lipase activity of *Staphylococcus pasteuri* SNA59

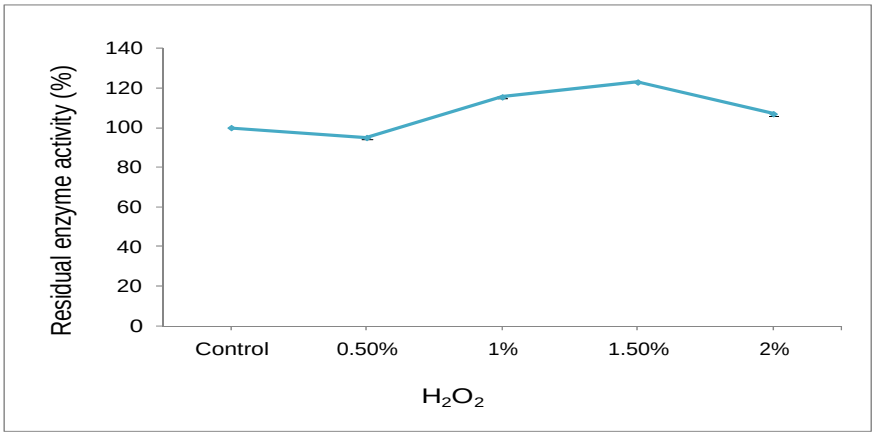


Fig.18 Effect of different concentrations of hydrogen peroxide on lipase activity of *Staphylococcus pasteuri* SNA5

Table.1 Effect of different mineral salt concentrations on lipase production by *Staphylococcus pasteuri* SNA59

K_2HPO_4 (%)	Lipase Activity U/ml	Na_2CO_3 (%)	Lipase Activity U/ml	$MgSO_4,7H_2O$ (%)	Lipase Activity U/ml
0.05	19.64	0.5	No activity	0.02	47
0.1	45.36	1	No activity	0.04	47.44
0.15	20.87	1.5	45.6	0.06	45.66
0.2	15.56	2	47.48	0.08	46.13
0.25	10.43	2.5	48	0.1	44.4
0.3	7.02	3	49		
		3.5	48.77		
		4	48		

Table.2 Effect of different concentrations of peptone, yeast extract, maltose and glycerol on lipase production by *Staphylococcus pasteuri* SNA59

Peptone (%)	Lipase Activity U/ml	Yeast extract (%)	Lipase Activity U/ml	Maltose (%)	Lipase Activity U/ml	Glycerol (%)	Lipase Activity U/ml
0.5	48.41	0.05	34.1	0.5	37.26	0.5	37.14
1	44.79	0.1	47.34	1	45.36	1	45.66
1.5	38.46	0.15	44.55	1.5	22.74	1.5	29.42
2	28	0.2	38.28	2	18.33	2	25.14
2.5	17.52	0.25	30.71				
3	12.44	0.3	22.1				

triacylglycerols, fatty acids, hydrolyzable esters, tweens, bile salts and glycerol (*Rathi et al., 2001; Lotti et al., 1998; Ghosh et al., 1996; Dharmsthiti et al., 1998; Shirazi et al., 1998; Bradoo et al., 2002*).

The presence of 1% maltose in the MPY medium induced maximum lipase production by *Staphylococcus pasteuri* SNA59. Lipase production is significantly influenced by other carbon sources, such as sugars, sugar alcohols, polysaccharides, whey, casamino acids and other complex substances (*Gilbert et al., 1991; Lotrakul and Dharmsthiti, 1997a; Ghanem et al., 2000; Rashid et al., 2001*). Ghanem et al. (*2000)*had earlier reported that addition of

Starch or Maltose to themedium induced lipase production in *Bacillus alcalophilus andPseudomonas aeruginosa* LP602 in Whey and Soybean Meal-containing media (*Dharmasthiti and Kuhasuntisuk, 1998*).However, Glucose in GYE broth inhibited lipase production by *Staphylococcus pasteuri* SNA59, perhaps by catabolic repression. Similar results have been reported with other lipase-producing organisms where a high Glucose concentration reduced the lipase production (*Satomura et al., 1958; Yamada et al., 1963; Chen et al., 1992; Dharmsthiti and Kuhasuntisuk, 1998; Lotrakul and Dharmsthiti, 1997a*).

Besides the carbon source, the type of nitrogen source in the medium also influences the lipase yield in the production broth (*Ghosh et al., 1996*). *Staphylococcus pasteuri* SNA59released maximum lipase when organic nitrogen sources like Yeast Extract and Peptone were used in the MPY medium and lesser yield with inorganic nitrogen sources. Generally, microorganisms provide high yields of lipase when organic nitrogen sources are used, such as Yeast Extract with Peptone or Tryptone as in the case of various pseudomonads, thermophilic *Bacillus sp*. and *Staphylococcus haemolyticus* (*Wang et al., 1995; Khyami-Horani, 1996; Pabai et al., 1996; Oh et al., 1999; Ghanem et al., 2000; Lanser et al., 2002; Sharma et al., 2001; Sugihara et al.,1991; Sharma et al., 2002a).* According to Ferie et al. (1997) peptone contains certain cofactors and amino acids, whichfulfilthe physiological requirements for lipase biosynthesis. Lima et al. (*2003*) found that lipase production by *Penicillium aurantiogriseum* was stimulated with Ammonium Sulfate. One exception was reported where *Rhdutorela glutinis* requires organic nitrogen source for good growth, but inorganic nitrogen such as Ammonium Phosphate and organic nitrogen source like Urea for lipase production (*Papaparaskevas et al.,1992*). Urea was found to increase lipase production from a bacterial isolate SJ-15 (*Gupta et al., 2004b*).

Staphylococcus pasteuri SNA59 produced lipase in late log phase. Lipases are released throughout bacterial growth, while peaking by the late log phase. Incubation periods ranging from few hours to several days are reported for maximum lipase production by bacteria (*Gupta et al., 2004a*). Zhen-Qian and Chun-Yun (*2009*) found that *Enterobacter agglomerans*yields maximum lipase after 48 hthat decreases thereafter due to cessation of thesynthesis along with rapid inactivation of existing enzyme. This finding was also supported in *Pseudomonas sp.* 7323 (*Zhang and Zeng, 2008*). An incubation period of 12 h was optimum for lipase production by *Acinetobacter calcoaceticus* and Bacillus sp. RSJ1, 16 h for *B. thermocatenulatus*, 72 h for *P. fragi* and 96 h for *P. fluorescens* BW 96CC (*Mahler et al., 2000; Sharma et al., 2002b; Schmidt-Dannert et al., 1997; Pabai et al., 1996; Dong et al., 1999*).

Shaker conditions are usually used for the production of extracellular enzymes by aerobic organisms as it enhances the aeration rate by increasing the dissolved oxygen. In the present study aeration has been found to increase lipase production. A similar result was reported by El-Shafei and Rezkallah (*1997)* in case of *Bacillus sp*. On the contrary, high levels of aeration decreased lipase production in *Staphylococcus carnosus* (*Genovefa et al., 1994*).

The lipase production by *Staphylococcus pasteuri* SNA59 was optimumat 30^0C and decreased with the highertemperature, perhaps due to reduced growth rate or lower survival. This finding supports the data by Zhang and Zeng (*2008*) in *Pseudomonas sp.* 7323. It has been observed that, in general, lipases are produced in the temperature range 20–45°C (*Gupta et al., 2004a*).

The maximum lipase production by *Staphylococcus pasteuri* SNA59 was at alkaline pH 10. Largely, bacteria prefer pH around 7.0 for best growth and lipase production, such as in the case of *Bacillus* sp. (*Sugihara et al., 1991*), *Acinetobacter* sp. (*Barbaro et al., 2001*) and *Burkholderia* sp. (*Rathi et al., 2001*). However, maximum activity at pH 9 has been observed by an improved strain of *Pseudomonas aeruginosa* MTCC 10,055 (*Bisht et al., 2012*) and at pH 10 by*Arthrobacter* sp. BGCC#490 (*Sharma et al., 2009*).Thus, *Staphylococcus pasteuri* SNA59 isolated from a spoilt skin lotion was shown to be a good lipase producer.It was grown in MPY medium containingmaltose (1%), peptone (0.5%), yeast extract (0.1%), $MgSO_4$,$7H_2O$ (0.04%), K_2HPO_4 (0.1%), Na_2CO_3 (3%) and glycerol (1%), pH 10 incubated on a shaker (120 rpm) at 30^0C for 48h to obtain maximum lipase yield. Lipase enzyme fromthe culture supernatant of optimized MPY medium grown with *Staphylococcus pasteuri* SNA59 was assayed in the reaction mixture containing 0.5mM of 4-Nitrophenylpalmitate substrate in Isopropyl Alcohol, 0.75ml of crude enzyme and1.95 ml of 50mM phosphate buffer (pH 7.2) at 30^0C for 30min.

The lipase enzyme from *Staphylococcus pasteuri* SNA59 showed maximum activity at pH 10. Generally, bacterial lipases have neutral (*Dharmsthiti et al., 1998; Dharmsthiti and Luchai, 1999; Lee et al., 1999*) or alkaline pH optima (*Schmidt-Dannert et al., 1994; Sunna et al., 2002; Joshi et al., 2006; Lotrakul and Dharmsthiti, 1997b; Vargas et al., 2004*).For *S. hyicus*, a pH optimum of 8.5 has been reported (*Oort et al., 1989*). *Bacillus alcalophilus* lipase remained stable at pH 10–11 (*Ghanem et al., 2000*). Exceptions reported include*P. fluorescens* SIK W1 lipase, which has an acidic optimum at pH 4.8 (*Andersson et al., 1979*). *Staphylococcus aureus* NCTC8530 and *S. epidermidis* RP62A are active over a broad pH range, with an optimum around pH 6 (*Simons et al., 1996; Simons et al., 1998*) and both lipases are stable under acidic conditionsand are inactivated at pH values above 10. This preference of acidic conditions is quite unusual among bacterial lipases, which in most cases exhibit their highest activities at alkaline pH.

The optimum temperature of 45^0C was observed for activity of lipase from *Staphylococcus pasteuri* SNA59 and similar results were reported for lipase from *Staphylococcus xylosus* (*Mosbah et al., 2005*). Bacterial lipases generally have temperature optima in the range 30–60°C (*Lesuisse et al., 1993; Wang et al., 1995; Dharmsthiti et al., 1998; Litthauer et al., 2002; Dharmsthiti and Luchai, 1999; Lee et al., 1999; Oh et al., 1999; Sunna et al., 2002*).

Various inhibitors were used to understand the aminoacids involved in the activity of the lipase enzyme from *Staphylococcus pasteuri* SNA59.In the presence of PMSF, EDTA and 2-Mercaptoethanol, lipase activity of *Staphylococcus pasteuri* SNA59 was retained indicating that enzyme was neither a Serine lipase, a

metallo-lipase nor a Cysteine lipase (*Sharma et al., 2002b*). EDTA does not affect the activity of most lipases (*Oh et al.,1999; Rosenstein and Gotz, 2000; Simons et al.,1996; Simons et al., 1999*). However, it is inhibitory to lipases from *P. aeruginosa* 10145 and *Bacillus* sp. THL027 (*Gupta et al., 2004a*). Bile salts inhibited lipase activity in our studies,whichcorroborates with the results of Watanabe et al. (*1977*). In contrast,Sodium Deoxycholate and Sodium Taurocholate increased the activity of lipase obtained from *Bacillus cereus* C7 (*Dutta and Ray, 2009*).

In general, lipase activity is inhibited drastically by heavy metals like Co^{2+}, Ni^{2+}, $Hg^{2+}$and $Sn^{2+}$and slightly inhibited by Zn^{2+} and Mg^{2+} (*Patkar and Bjorkling, 1994*). This contrasted with our results where Zn^{2+}inhibited the lipase enzyme activity of *Staphylococcus pasteuri* SNA59 to the extent of 70% while Cd^{2+}, Mg^{2+}, Hg^{2+} had very little effect (less than 10%). A number of lipases produced from other microorganisms were found to be Ca^{2+} dependent (*Kambourova et al., 2003; Lee et al., 2006; Kim et al., 2000; Ma et al., 2006*). Our results showed that while the enzyme was not Ca^{2+} dependent, it slightly enhanced the activity. A structural role for calcium in enhancing the activity of staphylococcal lipases was reported (*Rosenstein and Gotz, 2000*). *Staphylococcus hyicus, S. aureus, S. epidermidis* and *S. warneri* require Ca^{2+} for full enzymatic activity (*Simons et al., 1996*). This result supports the previous finding by Mori et al. (*2009*). One proposed the functionof Calcium to be the formation of salts of the released fatty acids in order to remove them from the reaction equilibrium to circumvent product inhibition (*Hiol et al., 2000; Shah and Wilson, 1965*). It has also been hypothesized that Ca^{2+} might be directly involved in catalysis. Recently, it was shown that Ca^{2+} is most probably necessary for stabilizing the three-dimensional structure of the lipase during catalysis (*Simons et al., 1996*). However, in the crystal structure of *Burkholderia glumae* lipase, the calcium binding site was localized far from the active site (*Noble et al., 1993*). This supports the hypothesis that Ca^{2+} rather sequesters fatty acids discussed above.

The lipase activity of *Staphylococcus pasteuri* SNA59 was not greatly affected by organic solvents like Acetone, Butanol, Benzene, Ethanol andCyclohexane,while Isopropanol and Methanol caused 51% and 39% inhibition respectively. In fact, lipases are diverse in their sensitivity to organic solvents *(Nawani et al., 1998*).Acetone, Ethanol and Methanol enhanced the lipase activity of *B. thermocatenulatus* (*Schmidt-Dannert et al., 1994*), whereas Acetone and Hexane were inhibitory for *P. aeruginosa* YS-7 lipase and Hexane for *Bacillus* sp. lipase (*Sugihara et al., 1991*). The lipases from *P. aeruginosa* B11-1 **(***Ogino and Ishikawa, 2001*), *Bacillus* sp. **(***Torres and Castro, 2004*), *B. thermoleovorans* CCR11 **(***Castro-Ochoa et al., 2005*) and *Yarrowia lipolytica* (*Wang et al., 2009*) showed a high stability in the presence of water-miscible organic solvents. It is likely that a thin layer of water film remains bound to the enzyme molecules in water-miscible organic solvents, allowing retention of the native conformation of the enzymes (*Wang et al., 2009*). Lipase from *A. calcoaceticus* LP009 was highly unstable with various organic solvents (*Dharmsthiti et al., 1998*). Stability in organic solvents is desirable in synthesis reactions. Most of the enzymes are inactivated or denatured in organic solvents whereas lipases which are active in organic solvents have

potential industrial applications. Employing lipases for bioconversions in organic solvents is advantageous, and hence activity and stability in solvents are considered novel attributes of lipase (*Gaur et al., 2008*).

Staphylococcus pasteuri SNA59 lipase was quite stable in the presence of ionic, non-ionic and commercial detergents with the activity ranging from80% to 120%.Non-ionic detergents have often been proposed to stimulate extracellular lipase production by some microorganisms, due to their potential ability to increase cell wall permeability and /or to release cell-bound enzymes (*Corzo and Revah, 1999*). However, their efficiency is (strong and weak are used when describing quantitative parameter) highly strain-dependent, and sometimes can even inhibit the lipase activity (*Nascimento and Campos-Tataki, 1994*). The stability in surfactant and bleach oxidants is desirable for lipases in detergent formulations. Similar results are reported by many investigators (*Iizumi et al., 1990; Patkar and Bjorkling, 1994*). Surfactants facilitate the access of substrate to the enzyme by stabilizing the interfacial area where catalytic reaction of lipase takes place (*Singh and Banerjee, 2007*).

It was observed that *Staphylococcus pasteuri* SNA59 lipase under study can tolerate upto 2% Hydrogen peroxide and hence is expected to be of high commercial value. Bleach stability is a desirable property and is not very common among lipasesbut may be achieved by site directed mutagenesis (*Outtrup et al.,1995;Tsuchiya et al.,1992*) or protein engineering (*Boguslawski and Shultz, 1992; Wolf et al.,1996*). Rathi et al. (*2001*) studied lipase stability in presence of oxidizing agents like Hydrogen Peroxide, Sodium Perborate, and Sodium Hypochlorite at 1% w/v or v/v. Gulati et al. (*2005)* reported bleach stability of a novel alkaline lipase by *Fusarium globulosum* to 0.1 M Hydrogen Peroxide and Sodium Perborate.H_2O_2 is known to be a strong oxidizing agent and it mediates inactivation of proteins and other macro molecules. Therefore the enzyme showing extreme stability towards the oxidizing agents are of immense significance for detergent industries because peroxides are common ingredients of modern bleach-based detergent formulations. Commercial detergents contain bleaching agents such as Sodium Percarbonate which liberate hydrogen peroxide that oxidizesMethionine and Tyrosine residues of enzymes thereby inactivating them.

The characteristics of an ideal detergent enzyme are broad specificity, stability at high pH, temperature, ability to withstand oxidizing and chelating agents, and high specific activity **(***Saisubramanian et al., 2008***)**. *Staphylococcus pasteuri* SNA59 lipase discussed here has shown the properties best suited for use as a detergent additive and has properties similar to alkaline lipases under use. Therefore, a*Staphylococcus pasteuri* SNA59 enzyme preparation could be considered as a potential candidate for use as a cleaning additive in detergents to facilitate the release of lipid stains.

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