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Production of a Novel Thermophilic and Solvent-Tolerant Lipase by *Streptomyces* sp.: Influence of Organic Solvents

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Abstract: A thermophilic *Streptomyces* sp. producing an extracellular alkaliphilic lipase was selected after screening of 30 bacterial isolates obtained from a water sample of a hot-spring. The *Streptomyces* sp. designated STL-D8 produced a maximum zone of hydrolysis on the tributyrin (0.5 % v/v) agar plate during primary screening. This bacterial isolate produced 16 U/ml of lipase in the broth containing DMSO (5 % v/v) at 55°C and pH 8 in 48 h. Supplementation of production broth with cottonseed oil (2 %, v/v) enhanced the lipase activity to 17.8 U/ml. Carbon and nitrogen sources were optimized and a mild increase in lipase activity (18.9 U/ml) was achieved with glucose (0.4 % v/v) added to the production broth. The lipase production by *Streptomyces* sp. further increased in the presence of yeast extract (0.4 % w/v; 19.6 U/ml) and $NaNO_3$ (0.4 % w/v 24.4 U/ml). Thus a production broth containing cottonseed oil, glucose, yeast extract and $NaNO_3$ was used to optimally produce extracellular lipase by *Streptomyces* sp. in the presence of selected solvents (dimethyl sulfoxide (DMSO), diethylphathalate (DEP), toluene, xylene, *n*-pentane, *n*-hexane, *n*-heptane, methanol, ethanol, *n*-propanol, isobutanol and *n*-octanol) added to the broth at 10-40 % (v/v) concentrations. The lipase production was least affected by DMSO, *n*-pentane and *n*-hexane (40 % v/v). The lipase production occurred frequently at higher solvent concentration and both the hydrophobic nature (Log *P* value) as well as the nature of groups in the organic solvent(s) appear to affect the cell growth and lipase production by *Streptomyces* sp.

Key words: *Streptomyces* sp., solvent tolerance, medium optimization, extracellular lipase, dimethylsulfoxide.

Introduction

Biotransformation in organic solvents has emerged as an area of organized research and industrial growth, accompanied by the synthesis of products of chemical and pharmaceutical values. Water is the major life supporting solvent but unfortunately it is poor medium for most of the chemical reactions at industrial scale, primarily because of low solubility of uncharged organic compounds and high reactivity of water as nucleophile. From the processing and economic point of view the high boiling point and low vapour pressure of water results in the expensive purification steps to separate the product(s) from an aqueous based biotransformation system. Finally unwanted side-reactions such as hydrolysis, recemization, polymerization and decomposition often occurs in water that limit many of reactions of interest in enzymatic synthesis. All these limitation lead to use of enzymes in the non-aqueous solvents and replaced the water as bulk medium. Despite various limitations the organic solvent-tolerant bacteria are capable of thriving in the presence of toxic solvents such as benzene, toluene, xylene and alkanes [1-4]. Thus for last three decades solvent-tolerant bacteria are being explored for their potential applications in industrial and pharmaceutical biotechnology [5-6]. Alkanes, alcohols, benzene, and dimethylsufoxide are some of the major organic solvents used in the industry for enzyme-

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catalyzed reactions. DMSO has been widely used for the lipase catalyzed esterification reactions for the synthesis of ferulate esters [7- 12]. Lipases are not only stable in these toxic solvents but are also capable of catalyzing many synthetic reactions in these organic solvents.Thus lipases are considered to be most promising tools for biocatalysis in non-aqueous systems [13-14]. The solvent tolerant organisms have been reported from *Bacillus, Rhodococcus, Staphylococcus* and *Arthrobacter* sp. [15-17]. Exploring the microbes capable thriving at diverse pH range, high temperature, highsalt contentand in the presence of toxic organic solvents that too without losing their activities is a challenging area of non-conventional enzymology. In order to use the microbial lipase(s) for hydrolysis, esterification or other applications, it is essential to produce the purified enzyme at high concentration/ amount and to determine its biochemical properties. Hydrophobic solvent molecules can interact with hydrophobic amino acid residues present in the 'lid' that covers the catalytic site of the lipase, thereby maintaining the lipase in its open conformation [18]. The enzyme activity is enhanced without the 'lid' covering the active site. High thermal stability of enzymes is considered to be positively correlated with stability in the presence of organic solvents [19]. From the processing and economic point of view the high boiling point and low vapour pressure of water results in the expensive purification steps to separate the product(s) from an aqueous based biotransformation. Thus keeping in mind all these facts, a thermophilic and solvent-tolerant lipase producing bacterial isolate was isolated from a water sample of a hot-spring. The objective of the current work was to optimize lipase production by a thermophilic and solvent tolerant strain of *Streptomyces*sp. STL-D8 by optimizing the parameters like medium pH, temperature, carbon, nitrogen source and to check the lipase production in the presence of various toxic solvents at increasing concentrations.

Material and methods

Chemicals

The chemicals that included $NaNO_3$, K_2HPO_4, KCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $(NH_4)_2SO_4$ (S.D. Fine-Chem, Ltd. Hyderabad); *p*-nitrophenyl-palmitate (*p*-NPP), Tris-HCl [Sigma Aldrich USA; dimethyl sulphoxide (DMSO), diethyl phthalate (DEP), toluene, xylene, *n*-pentane, *n*-hexane, *n*-heptane, methanol, ethanol, *n*-propanol, isobutanol and *n*-octanol (Merck Ltd. Mumbai, India) were procured from commercial suppliers and were of analytic grade and were used as received.

Isolation of thermophilic solvent tolerant lipase producing bacterial strain

The isolation of lipase producing bacterial strain was performed by 10-fold serial dilutions of the hot-spring water sample on tributyrin (0.5 %; v/v) agar plates [20]. Bacterial colonies showing clear zones around them were carefully picked and transferred onfresh sterile agar plates for further purification. The colony forming units (cfu) appeared in the plated containing tributyrin agar medium were kept at 5°C. The extracellular lipase producing cfu were identified according to cell morphology, Gram staining and spore formation. Screening of isolated bacteria for their lipolytic activity was carried out in tributyrin (0.5 %; v/v) broth at pH 8.0. The mineral-based (MB) broth contained (g/L), 3.0 $NaNO_3$, 0.1 K_2HPO_4, 0.5 KCl, 0.5 $MgSO_4.7H_2O$, 0.01 $FeSO_4.7H_2O$, 5.0 yeast extract and 1.0% (v/v) cottonseed oil (pH 8). The seed culture was prepared by inoculation of 50 ml of broth with a loopful of culture followed by incubation for 18 h at 55°C under shaking (150 rpm).

Characterization ofbacterial strain STL-D6 by 16SrRNA sequencing

DNA isolation from the bacterial strain STL-D6 was done and its DNA quantitation analysis was performed onagarose gel electrophoresis. Amplification of the target DNA template was attempted by using universal 16S rRNA polymerase chain reaction (PCR) primers forward primer: 5'-ACAAGCCCTGGAAACGGGT-3' and reverse primer 5'-ACGTGTGCAGCCCAAGACA-3'. The amplicon was electrophoresed on 1 % agarose gel and visualized under UV. The amplification was performed with an initial denaturation step of 3 min at 94°C and then 35 cycles were developed (60 sec denatur-

ation at 94°C, 30 sec, at 59°C for primer annealing and 60 sec at 72°C for primer extension) and kept at 72°C for complete extension. A dendrogram analysis was performed with tree-joining method involving 10 nearest neighbors presenting the maximum similarity to genus *Streptomyces.* The genetic analysis also showed the high GC content (59.88 %) of *Streptomyces* that reflected its thermophilic nature (Figure 1).

Lipase assay

Lipase in the cell free broth was assayed by a colorimetric method using *p*-NPP as a chromogenic substrate [21]. The reaction cocktail contained 80 µl of *p*-NPP (10 mM, *p*-NPP prepared in isopropanol) stock solution and 10 µl of enzyme. The final volume of this reaction mixture was made to 3 ml with 0.05 M Tris buffer, pH 8.5 containing gum acacia (0.1 % w/v). The test tubes were incubated for 10 min at 55°C under continuous shaking in a water-bath. Appropriate control without enzyme was included with each assay. Reaction was stopped by keeping reaction mixture at -20°C for 7 min. The absorbance of *p*-nitrophenol released was measured at 410 nm (Shimazdu UV/Visible spectrophotometer, Japan). The unknown concentration of *p*-nitrophenol released was determined from a reference curve of *p*-nitrophenol (2-14 µg/ml final concentrations in 0.05 M Tris buffer, pH 8.5 for lipase assay). Each of the assays was performed in duplicates and ± Standard deviations (SD) were presented. The protein was assayed by a standard method [22].

Optimization of culture conditions and reaction parameters for assay of extracellular lipase

Optimization of carbon sources

Effect of organic carbon sources

The mineral based broth (50 ml taken in 250 ml Erlenmeyer flask) supplemented with 1 % (v/v) of the selected oil (Soya bean oil, cottonseed oil, mustard oil, olive oil, ricebran oil and control (without oil) was inoculated with 10 % (v/v) of 18 h old seed culture of *Streptomyces* sp. STL-D8. The inoculated flasks were incubated at 55±1°C for 48 h and broth was sampled at periodic intervals to determine the amount of extracellular lipase produced by the selected microbe. The broth was rendered cell free by centrifugation at 10,000 x g for 10 min at 4°C, and the supernatant was used for the assay of lipase and protein content. The lipidic source giving optimal production of lipase in the MB broth was further used at different concentration and an optimum concentration was selected for subsequent experiments.

Effect of inorganic carbon sources

To evaluate the effect of carbohydrates (Glucose, fructose, D-galactose, xylose, ribose, dextrose, sucrose and maltose), on extracellular lipase production by *Streptomyces* sp., each of the carbohydrates was separately added (0.4 % v/v) in the production broth containing DMSO (5 % v/v). The broth in each case was inoculated with 10 % (v/v) of 18 h old inoculum and flasks were

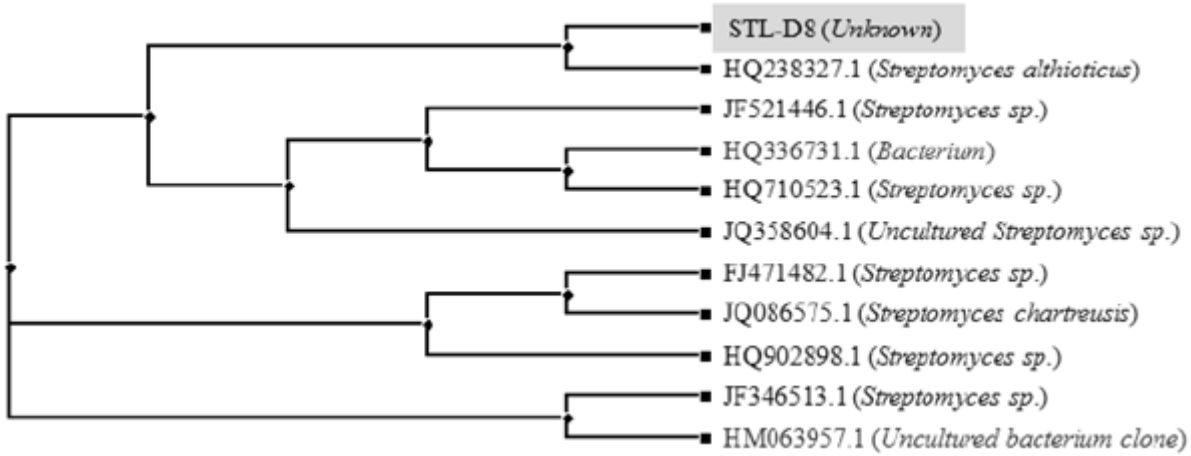


Figure 1. Dendrogram analysis involving 10 nearest neighbors presenting the maximum similarity to *Streptomyces althioticus*

incubated at 55±1°C under shaking. The amount of lipase produced after 48 h was assayed in the cell free broth. The inorganic carbon source giving optimum lipase production with different concentrations and an optimum concentration was selected for subsequent studies.

Optimization of nitrogen sources

***Effect of organic nitrogen (complex) source on lipase production by Streptomyces* sp.**

The effect of organic nitrogen sources *viz.* beef extract, malt extract, casein (fat free), casein, peptone, soybean meal, tryptone and yeast extract (1.0 % w/v) on lipase production *Streptomyces* sp. was studied by using the conditions mentioned above. The lipase activity in the culture broth was determined in the presence of each of the complex organic sources. The nitrogenous source giving highest lipase activity in the inoculated broth was added to the broth in the subsequent experiments. Production broths containing 1% (v/v) cottonseed oil and various concentrations of the optimized nitrogen source (0.1-1.0 %, w/v) were prepared with pH 8.0 ±0.2. The broths dispensed in flasks (50 ml in 250 ml capacity flask) were rendered sterile by autoclaving. Each of the flasks (in triplicate) was inoculated (10 %, v/v) aseptically with 18 h old *Streptomyces* sp. seed culture. The flasks were incubated at 55 ±1°C under continuous shaking (150 rpm) up to 48 h. The culture broth was aseptically drawn and rendered cell free to assay protein and lipase activity.

Effect of inorganic nitrogen (salts) source on lipase production

A 18 h old seed culture (10 %, v/v) was inoculated in sterile broth containing anyone of the selected inorganic nitrogen sources [ammonium acetate, ammonium ferrous sulfate, ammonium dihydrogen phosphate, ammonium nitrate, ammonium ferric chloride, urea, sodium nitrate, ammonium oxalate and ammonium sulphate (0.2 % w/v nitrogen concentration)] along with optimized concentration of the carbon source in each of flasks. The flasks were incubated at 55±1°C under continuous shaking (150 rpm). The culture broth was aseptically withdrawn and rendered cell free to assay protein and lipase.

Cumulative effect of optimized culture conditions as a function of initial pH and temperature

The production broth with optimized carbon and nitrogen sources and DMSO (5 %; v/v) was calibrated to final pH of 7.0, 7.5, 8.0, 8.5, 9.0 9.5 and 10.0 to determine cumulative effect of all the selected medium constituents on extracellular lipase production by *Streptomyces* sp. The production broth was autoclaved at 1.1 bar for 20 min at 121°C in the capped flasks. This broth was inoculated with 10 % (v/v) of 18 h old seed culture and incubated under shaking at 55±1°C for 48 h. The inoculated MB broth was harvested at 48 h by centrifugation (10,000 X g for 10 min at 4°C; SIGMA 3K30, Germany). The supernatant was filtered through Whatman filter paper No. 1. This enzyme preparation was termed as crude lipase. The lipase produced by *Streptomyces* sp. in various batches was recorded. Effects of cultivation temperature (25, 35, 45, 55, 65 and 75°C) on the cell growth and extracellular lipase production of *Streptomyces* sp.were studied. The extracellular lipase produced in the broth was assayed after 48 h post inoculation at above mentioned cultivation temperatures.

Effect of different organic solvents at varied concentrations

The MB broth with optimized carbon, nitrogen sources optimized temperature and pH was prepared with supplementation of each organic solvents (DMSO, DEP, toluene, xylene, pentane, *n*-hexane, *n*-heptane, methanol, ethanol, *n*-propanol, iso-butanol and *n*-octanol) at 10-40 %, v/v; separately in 250 ml Erlenmeyer's flask. All organic solvents used were sterilized through 0.22 um membrane filter before their addition to the sterile broth. The broth was inoculated with 10 % (v/v) of 18 h old seed culture and incubated under shaking at 55±1°C for 48 h. The lipase produced by *Streptomyces* sp. in the presence of different organic solvents at varied concentrations was recorded.

Influence of DMSO on the growth and lipase production by *Streptomyces* sp.

The solvent-tolerance property of *Streptomy-*

ces sp. is one of the critical parameter to be conquered in organic synthesis. Following inoculation, when the culture reached its exponential phase (24 h) the DMSO was added after 6 h interval *i.e*. at 30, 36, 42 and 48 h to study its effect on the production of extracellular lipase by *Streptomyces* sp. STL-D8.

Results

Isolation of thermophilic solvent-tolerant lipase producing bacteria

The isolated strain of *Streptomyces* sp. STL-D8 obtained from a hot-water spring produced extracellular lipase with a maximum activity of 37.6 U/ml at 65°C in the presence of 10 % (v/v) of DMSO. In our study, among 30 cfu obtained after primary screening on the tributyrin agar plates, the cfu/ colony (STL-D8) with the maximum clear zoneformation was selected and was adapted to grow in an optimized mineral based broth supplemented with organic solvents. The bacterium was found to be Gram-positive, rod shaped, spore-forming giving creamish white colonies with irregular edges and filamentous growth. Molecular characterization by 16 SrRNA sequencing (Figure 1) and subsequent dendrogram analysis showed the highest genetic homology of the bacterial strain STL-D8 to *Streptomyces althioticus* (Figure 1).

Optimization of lipase production and assay

When 10 % (v/v) inoculum of 18 h old seed culture was employed in the production broth, 16 U/ml of enzyme was produced at 55±1°C in compassion to the broth inoculated with 2, 4, 6, 8, 12 or 14 % (v/v) of inoculum (data not shown). The production broth containing DMSO (5 % v/v) when inoculated with 10 % (v/v) of 18 h old seed culture produced optimal amount of lipase at 48 h post inoculation at 55±1°C, under continuous shaking (150 rpm).

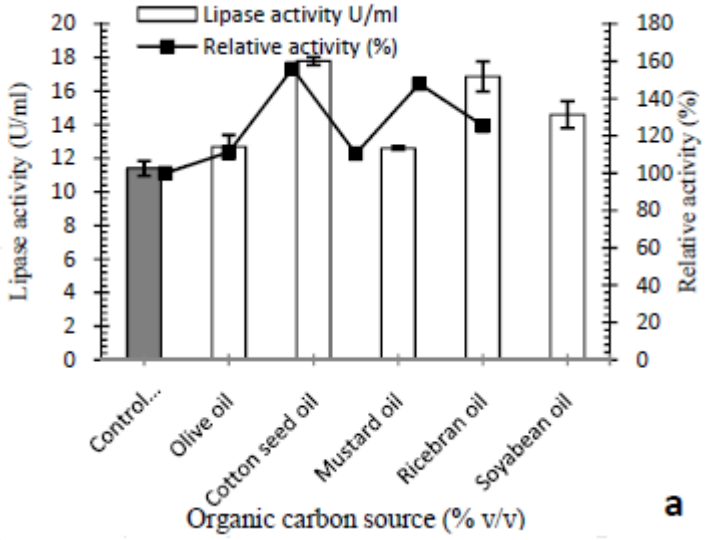
Optimization of carbon sources

Effect of organic carbon sources

The mineral based broth (50 ml taken in 250 ml Erlenmeyer flask; DMSO 5 % v/v) supplemented with 1 % (v/v) of the selected oils (Soya bean oil, cottonseed oil, mustard oil, olive oil andrice bran oil) and control (without oil) was inoculated with 10 % (v/v) of 18 h old seed culture. Among all the commercially available oils, supplementation of cottonseed oil to the production broth gave optimal production of extracellular lipase (17.8 U/ml) as compared to control (11.4 U/ml; Figure 2 A). When the concentration of cottonseed oil was varied (1 to 10 %; v/v) in the production broth, a maximal amount of lipase 18 U/ml was recorded when 2 % (v/v) cottonseed oil was added to MB broth. Thus presence of cottonseed oil (2 %; v/v) in the MB broth was considered optimum for further studies.

Effect of inorganic carbon sources (carbohydrates)

Among the selected carbohydrates added to the



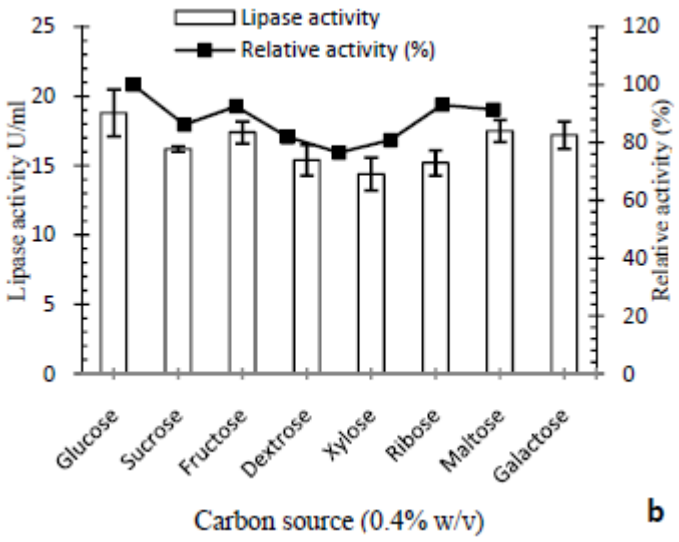


Figure 2. Effect of organic (a; 2 % v/v) and inorganic carbon (b; 0.4 % w/v) sources on lipase production by *Streptomyces* sp. STL-D8

MB broth,glucose yielded the maximum production of lipase (18.9 U/ml). The level of lipase produced at 0.4 % (18.9 U/ml) and 0.6 % (18.7 U/ml) glucose supplemented in broth was more or less than same (Figure 2b).

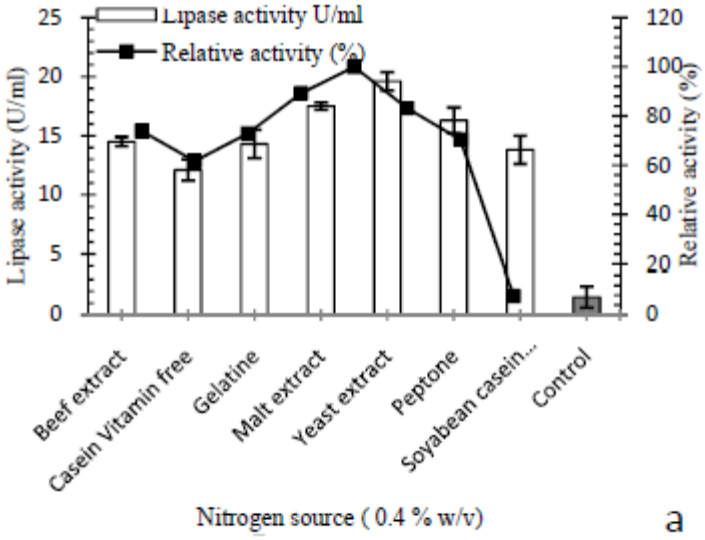
Optimization of nitrogen sources

Effect of organic nitrogen source on lipase production by Streptomyces sp.

The effect of a few selected organic nitrogen sources on lipase production was studied by using the optimized conditions mentioned above. Among the nitrogenous sources, yeast extract gave the highest lipase activity (19.6 U/ml) in the broth containing 2 % (v/v) cottonseed oil and 0.4 % (v/v) glucose at pH 8.0 ±0.2 (Figure 3a). After optimizing the yeast extract concentration in the broth, more or less a similar lipase activity at could be attained with 0.4 % (19.6 U/ml) and 0.5 % (19.5 U/ml) yeast extract.

Effect of inorganic nitrogen (salts) source on lipase production

Amongst broths containing DMSO (0.5 % v/v) and anyone of the selected inorganic nitrogen sources, the maximum lipase activity (24.4 U/ml) was recordedin the presence of sodium nitrate (0.3 %, w/v; Figure 3b).



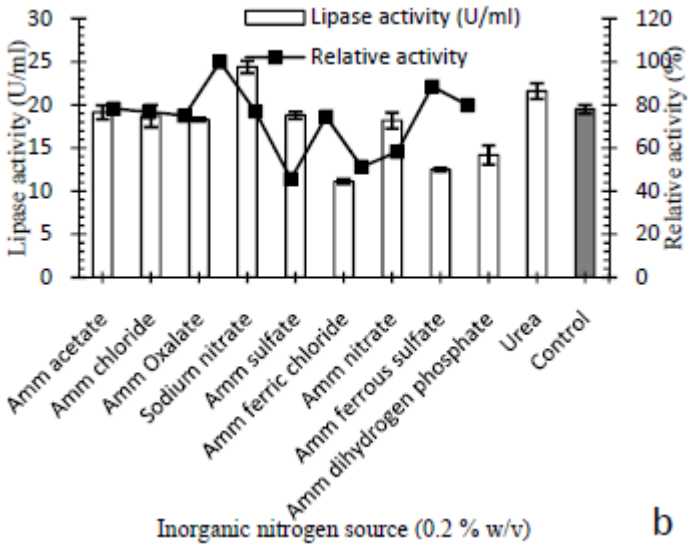


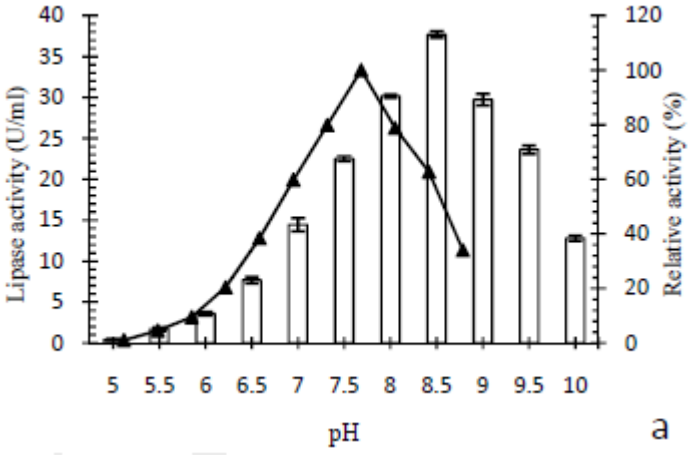
Figure 3. Effect of organic (a; 0.4 % w/v) and inorganic nitrogen (b; 0.2 % w/v) sources on lipase production by *Streptomyces* sp. STL-D8.

Cumulative effect of optimized culture conditions as a function of initial pH and temperature

The broth containing optimized concentration of carbon sources; cottonseed oil (2 % v/v) and glucose (0.5 % w/v), and nitrogen sources; Sodium nitrate (0.4 % w/v) and 0.5 % (v/v) yeast extract along with DMSO (5 %, v/v) was set to varying pH (7.0 -10.0) to determine the optimum pH that might yield the maximal lipase in the production broth.The maximal lipase activity (37.6 U/ml) was recorded at 55°C at pH 8.5 (Figure 4a). Further when the broth contacting optimized constituents at pH 8.5 was incubated at different temperatures (25-75°C), the maximum extracellular lipase activity was observed at 65°C.

Effect of type and concentration of organic solvents

The MB broth containing optimized carbon, nitrogen sources and optimized temperature and pH was prepared with added organic solvents at selected concentration (10, 20, 30 and 40 % v/v), separately. The *Streptomyces* sp. survived at all the tested concentration of solvents but also produced good amount of lipasein DMSO (31.8 U/ml), DEP (31.6 U/ml), *n*-pentane (32.1 U/ml) and xylene (33.18 U/ml) at 10% (v/v) concentration as compared to the control (34.4 U/ml; Figure 5). Interestingly, the lipase produced by *Streptomyces* sp. was active in selected solvents at 40 % (v/v) concentration. However, alcohols like ethanol, methanol and *n*-propanol showed a propor-



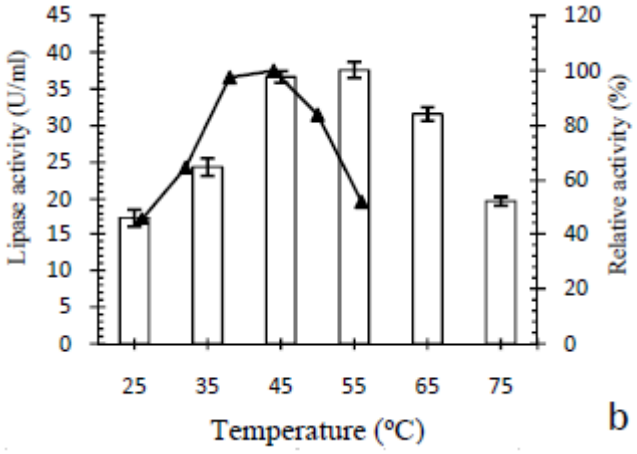


Figure 4. Effect of pH of the broth (a) and incubation temperature (b) on the production of lipase by *Streptomyces* sp. STL-D8

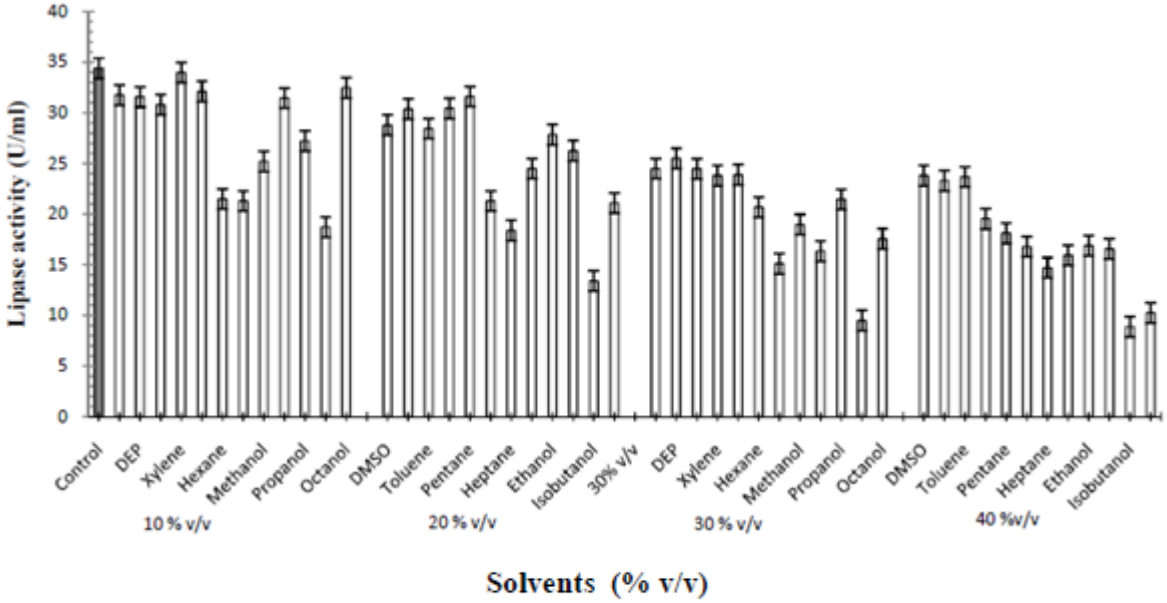


Figure 5. Effect of addition of different organic solvents to the broth on the production of lipase by *Streptomyces* sp. STL-D8

tional decrease in the lipase activity. This might be on the account of precipitation or aggregation of proteins that prompted a decline in enzyme activity.

Influence of DMSO on the growth and lipase production by *Streptomyces* sp.

It has been previously reported that the lipase producing *Streptomyces* strain is more stable in DMSO at higher concentration, thus DMSO is widely used as a solvent for lipase- catalyzed esterification reactions [4,5]. The solvent tolerance of *Streptomyces* sp. was determined by adding defined amount of DMSO in the production broth at regular interval of time and it was observed that the *Streptomyces* sp. produced lipase in the broth at all the tested concentrations (10-40 % v/v) of DMSO at 55°C after 48 h (Figure 6).

Discussion

The selection of a proper habitat for sample collection is utmost necessary during the isolation and screening of a particular microbial strain capable of producing desired enzyme activity. Soil con-

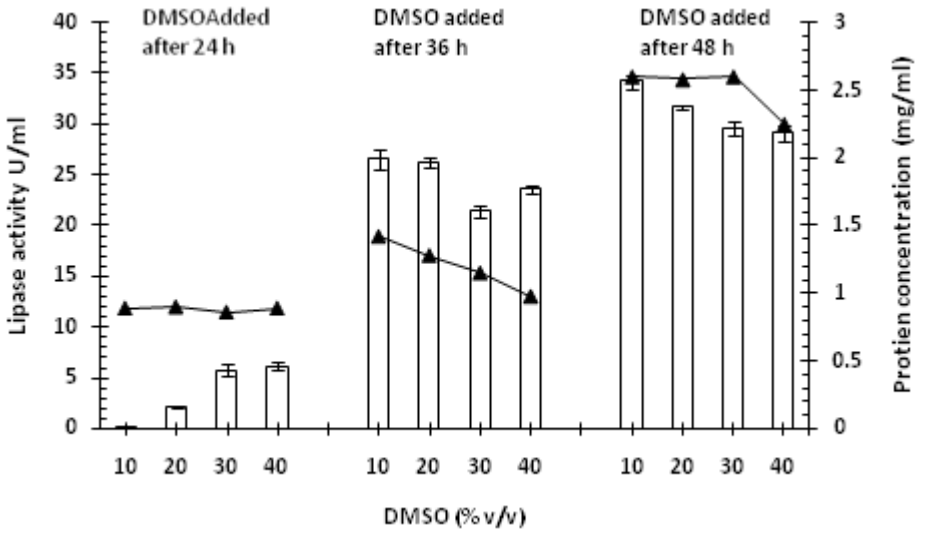


Figure 6. Effect of DMSO addition on the cell growth and production of lipase added after 24, 36 and 48 h in production

taining lipid, oil and fat containing material are most suited for the isolation of lipase producing bacteria. Depending upon the need of physical conditions like temperature, stability of microorganism at wide range of pH and in the presence of various metabolizable or non-metabolizable chemicals, adequate care should be taken during the collection of water/ and or soil sample(s) for isolation of microbial culture harboring desired enzymatic activity. Majority of studies reported the lipase production from mesophilic solvent-tolerant bacterial strains. Recently, a mesophilic lipase (39.8 kDa) produced by *Streptomyces* sp. CS133 has been reported as a solvent-stable enzyme [23]. Lipase is produced by *Streptomyces lividans*, *Streptomyces clavuligerus*, *Streptomyces coelicolor, Streptomyces rimosus* and a wild-type besides a recombinant strain of *Saccharopolyspora-erythraea* [24]. In our work, a lipase producing *Streptomyces* sp.was obtained by direct isolation on tributyrin plates after 72 h of incubation at 55°C. This *Streptomyces* sp. designated STL-D8 showed filamentous growth and developed rapidly on agar plate at 55°C in 48 h. As reported previously that in *S. clavuligerus* the lipase activity was detected only in the presence of an oil substrate, and lipase activity in *S. coelicolor* was detected only if oil was present at high concentration in the culture medium.

The optimization of physic-chemical parameters for the extracellular lipase production by *Streptomyces* sp. as well as assay conditions were approached systematically. In a recent study, the maximum activity (21.9 U/ml) of lipase was reported with 50 % cottonseed oil by *Aspergillus* sp. RDB-01 [25], and in the present work only 2 % (v/v) cottonseed oil added to the production broth provided a lipase activity of 17.8 U/ml as compared to control (*i.e*. 11.4 U/ml). Further, the addition of an inducer like glucose 0.4 % (v/v) also increased the lipase activity to 18.8 U/ml. In a previous study, hexadecane was used as a sole carbon source for the production of extracellular lipase by *Pseudomonas pseudomellai* 12sM [26]. Aulakh and Prakash [25] used peptone (0.5 % w/v) as a nitrogen source. However, in our study both organic as well as inorganic nitrogen sources were added and presence of both yeast extract and sodium nitrate (0.4 % w/v each) in the production broth caused a rapid increase in the production of lipase up to 24.4 U/ml by *Streptomyces* sp. Cumulative effect of optimized nitrogen and carbon sources was studied as a function of pH and temperature which yielded approximately 2-fold increase in the lipase activity (37.6 U/ml) in comparison toinitial activity (16.1U/ml). Recently, in a similar study an organic solvent tolerant lipase was isolated and purified from *Streptomyces* sp. CS133 which was stable in pH range 5.0-9.0 and at temperature lower than 50°C [23]. Two lipases from *S.*

*coelicolor*were characterized fully and both enzymes catalyzed the hydrolysis of mid (C10) to long-chain fatty acids up to C18, and both enzymes were active at alkaline pH, a characteristic that is typical of true lipases [27].

When the solvent-tolerance ability of *Streptomyces* sp. STL-D8 to produce lipase in the production brothwas studied in presence of different organic solvents, it was observed that the *Streptomyces* sp. strongly tolerated most of the commonly used organic solvent at considerable higher concentration. *Streptomyces* sp. STL-D8 showed good cell growth as well as the production of lipase even in the presence of 40 % (v/v) *n*-pentane, *n*-hexane, DMSO and DEP. In a previous study *Bacillus sphaericis*, an organic solvent tolerant lipase producing bacterial isolate showed high solvent tolerance of up to 75 % (v/v) BTEX (*n*-benzene, toluene, ethyl benzene and *p*-xylene) [28] at 30°C.

Our study is the first report of a thermophilic and solvent-tolerant *Streptomyces* sp. that produced good lipase activity in a wide range of organic solvents. A benzene tolerant lipase by *Acinetobacter baylyi* from marine sludge in Angsila, Thailand displayed maximum activity for extracellular lipase at 60°C and pH 8 [29].

Conclusion

The present study was carried out to determine the culture conditions for optimalextracellular lipase production by a newly isolated thermophilic *Streptomyces* sp. STL-D8 from a hot-spring. Medium composition (carbon and nitrogen sources), initial pH and incubation temperature were examined for optimization of extracellular lipase production by *Streptomyces* sp. STL-D8. Cottonseed oil and yeast extract were observed to be most effective carbon and nitrogen sources for lipase production by this bacterium. A maximum lipase activity of 37.6 U/ml was obtained when the initial pH of the broth was 8.5 at 55°C by using 2 % (v/v) cottonseed oil as the main carbon source and 0.4 % w/v yeast extract. The study presented a significant observation on the use of cottonseed oil as a main carbon source to enhance the synthesis of lipase even in the presence of 10-40 % (v/v) of organic solvents but DMSO being the best solvent. Thus solvent-tolerant thermophilic lipases are the promising enzymes to replace the conventional enzyme processes of biotechnological industries. However, a more extensive effort is needed required to overcome several bottlenecks like high enzyme cost, low activity and/or stability under environmental conditions, low reaction yields and low biodiversity of thermostable microbes is yet to be explored.

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