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Optimization of Lipase Production by Thermo-Alkalophilic *Bacillus* sp. 8C

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ABSTRACT

Microbial lipases catalyzing the hydrolysis and synthesis of esters from glycerol and fatty acids are commercially important in food, dairy, detergent and leather industries. In the present study, 15 thermo-alkalophilic bacterial strains isolated from a compost soil sample were screened for lipase production in beef extract nitrate medium containing 1% tributyrin. The strain 8C, giving maximum lipolytic activity was morphologically and biochemically identified as *Bacillus* sp. Lipase production from strain 8C was optimized by studying effects of incubation period, initial pH of medium, carbon and nitrogen sources, metals, substrates and thiols under static conditions. Lipase production was maximum after 7 days of incubation at 50°C and pH 9.0 and when sesame oil was used as a substrate. Supplementation of ribose or raffinose and ammonium sulphate (1%) as sole sources of carbon and nitrogen also stimulated lipase production over control. Of the different thiols tested, methionine greatly increased lipase production (175 Eu mL⁻¹) whereas, FeCl₃ (50 mM) found to be most suitable metal salt for optimal lipase production (300 Eu mL⁻¹). Under optimized conditions, lipase production by thermo-alkalophilic *Bacillus* sp. 8C was increased by 6.36 fold compared to unoptimized conditions.

Key words: Thermophilic, alkaline, ribose, FeCl₃, methionine, sesame oil

INTRODUCTION

Lipases (triglycerol hydrolases (EC 3.1.1.3) are the hydrolytic enzymes belonging to serine hydrolase family and catalyze conversion and synthesis of esters from glycerol and fatty acids. Lipolytic reactions are highly chemo-, regio and or enantioselective and operate at the interface of oil and water (Nguyen *et al.*, 2010). High quality and low energy consumption of lipase catalyzed reactions make them superior over conventional steam splitting methods operating at high temperature and pressure (Treichel *et al.*, 2010). These characteristics of lipases increased their demand in different industries including food and dairy, detergent, cosmetics, leather, paper and pulp, biodiesel and pharmaceutical (Hasan *et al.*, 2007).

Thermophilic lipases are of special interest for industrial applications due to their stability and high activity at higher temperatures and being stable in presence of chemicals (Messias *et al.*, 2009; Uttatree *et al.*, 2010). Lipases being third largest selling group of enzymes with more than 1000 t of annual production and approaching to a billion dollar business. Lipases active at alkaline pH are of great importance in detergent industry where these enzymes are used for removal of fat stains and dirt (Bora and Bora, 2012). The use of alkaline lipases in detergent formulation substantially

acts as a substitute for the phosphate builders in chemical detergent which are considered as major pollutant from detergent industry (Jellouli *et al.*, 2011). The extremozymes working at high temperatures and alkaline pH decrease the need of added steps in the process, reduce the cost and increase the process efficiency (Myers *et al.*, 2000). Lipases from different thermo-alkalophilic *Bacillus* sp. including *Bacillus stearothermophilus* (Kim *et al.*, 2000), *Bacillus licheniformis* (Sangeetha *et al.*, 2010) and *Bacillus thermocatenuatus* BTL-2 (Schmidt-Dannert *et al.*, 1994) were reported earlier. The research on identification and isolation of new lipase produced in microorganisms has been intensified in recent years (Hasan *et al.*, 2006; Shu *et al.*, 2010; Treichel *et al.*, 2010).

Enzyme production process is closely controlled in microorganisms and for improving its productivity. It is necessary to mitigate these controlling factors of production medium. This can be achieved by manipulating growth conditions to optimal level that generally improves the lipase productivity as evidenced in earlier studies (Gupta *et al.*, 2007; Treichel *et al.*, 2010; Rabbani *et al.*, 2013). The most important parameters that critically affect the lipase production are carbon and nitrogen sources (Kumari *et al.*, 2009; Zhao *et al.*, 2008). However, optimization of other nutritional and growth parameters affecting lipase production will be the added advantage for improvement studies. Knowing suitable conditions required for higher lipase production will reduce the production cost, minimize production time and other critical conditions required for production process. The present research work deals with the isolation of lipase producing thermo-alkalophilic *Bacillus* sp. from compost soil sample and optimization of conditions required for lipase production.

MATERIALS AND METHODS

Sample collection and isolation of lipolytic bacteria: A compost soil sample was collected from Trikut, Nanded, Maharashtra, India. One gram of air dried soil was suspended in 100 mL beef extract-nitrate broth (peptone 3%, beef extract 5% and KNO₃ 0.1%) supplemented with 1% tween-80 for enrichment of lipolytic bacteria. The pH of medium was adjusted to 9.0 by using 0.1 N NaOH. The flask was kept for incubation at 50°C under static conditions for 7 days. After incubation, 0.1 mL of enriched broth sample was spread on the surface of sterile beef extract-nitrate agar plates containing 1% Tween-80 in triplicates. The plates were incubated at 50°C for 7 days and observed for colonies showing zone of lipolysis. The lipolytic activity of selected isolates were further confirmed on tributyrin agar plates (peptone 3%, beef extract 5%, KNO₃ 0.1% and tributyrin 1%) by spot inoculation and incubating the plates under previously mentioned conditions.

Quantitative determination of enzyme activity: The selected isolates were grown in tributyrin broth (peptone 3%, beef extract 5%, KNO₃ 0.1% and tributyrin 1%) with pH 9.0 as mentioned before. After incubation, the culture broths were centrifuged at 10,000 rpm for 20 min at 4°C and supernatant was used as a source of crude enzyme and used for estimating lipase activity.

Lipase assay: The assay was performed by using the method described by Selvam *et al.* (2010), on the basis of hydrolysis of olive oil. To the reaction mixture containing 1 mL of Tris- HCl buffer of pH 9.0, 2.5 mL of deionized water and 3 mL of olive oil emulsion (10% gum arabic and 5% olive oil in deionized water), 1 mL of culture supernatant for test and 1 mL of deionized water for blank were added. The reaction mixture was mixed thoroughly by swirling and incubated at 50°C for 30 min. After incubation, enzyme substrate reaction was terminated by addition of 3 mL of 95% ethanol and mixed by swirling. The amount of fatty acid liberated due to lipase activity was

estimated by titrating the contents of assay mixture against 0.05 M NaOH using thymolphthalein as pH indicator. The end point observed was from colorless to light blue.

One unit of lipase was defined as the amount of enzyme required to release 1 μmol of fatty acid per milliliter under assay conditions. The enzyme activity is expressed as Eu mL^{-1} .

Identification of lipase producing bacterial isolate: The potential lipase producer strain 8C was selected for further studies and identified on the basis of morphological traits and biochemical pattern as per the standard criteria given in Bergy's Manual of Systematic Bacteriology (Holt *et al.*, 1994).

Optimization of process variables

Effect of incubation period: The 8C was inoculated in beef extract-nitrate broth of pH 9.0 containing 1% tributyrin as a substrate and incubated at 50°C for 11 days. Five milliliter culture media was removed periodically at an interval of 48 h and used for lipase assay.

Effect of initial pH of medium: Active culture of 8C was inoculated in beef extract-nitrate media containing 1% tributyrin. pH of media were adjusted to different pH (7, 8, 9, 10, 11 and 12) with the help of 0.1 N NaOH on a pH meter. The media were incubated at 50°C for 7 days. Enzyme units were calculated by performing the lipase assay.

Effect of carbon sources: To study the effect of carbon sources on lipase production by 8C, 11 different carbon sources such as xylose, fructose, dextrin, mannitol, galactose, sucrose, maltose, raffinose, ribose, lactose and gum acacia at 1% concentration were added individually to the beef extract-nitrate media. The broths were incubated at 50°C for 7 days at static condition. After incubation, broth was centrifuged and used as crude enzyme for lipase assay as mentioned earlier.

Effect of nitrogen sources: For the selection of suitable nitrogen source for lipase production by 8C different organic and inorganic nitrogen sources such as yeast extract, pyruvate, bile salts, glycerol, ammonium sulphate and arginine were screened. By supplementing those at 1% concentration in beef extract-nitrate media of pH 9.0, the active culture was inoculated and incubated at 50°C for 7 days and assayed to determine the enzyme units.

Effect of metal salts: The effect of different metal salts (NaCl, KCl, CaCl_2 , MgCl_2 , ZnCl_2 , NH_4Cl and FeCl_3 1%) on lipase production was assessed by inoculating active culture of 8C in beef extract-nitrate broth containing ribose and ammonium sulphate. The flasks were incubated at 50°C for 7 days at static condition and used for lipase assay as mentioned before.

Effect of substrates: Lipase production was accelerated by incorporation of suitable lipidic substrate. Therefore, effect of different lipidic substrates on lipase production by 8C was screened. Different substrates such as castor oil, mustard oil, eucalyptus oil, soya bean oil, coconut oil, sesame oil and clove oil at 1% concentration were supplemented individually in beef extract-nitrate media of pH 9.0. The flasks were incubated at 50°C for 7 days at static conditions. After incubation enzyme activity was determined as mentioned above.

Effect of thiols: To determine the effect of thiols on lipase production, different thiol containing compounds (methionine, dithiothreitol, β -mercaptoethanol, cysteine, sodium sulphite and thiourea)

were added individually to beef extract-nitrate medium at 50 mM concentration. After 7 days of incubation at 50°C under static conditions, the enzyme activity was determined as stated earlier.

RESULTS AND DISCUSSION

Isolation and identification of lipolytic bacteria: In the present study, 15 bacterial isolates obtained from compost soil were tested for lipase production potential. Out of the 15 isolates, 5 (1C, 2C, 3C, 8C and 15C) showed good lipolytic activity on tributyrin agar and broth (Table 1). The isolate 8C showing higher activities (28 mm, 55 Eu mL⁻¹), was selected as a potent lipase producer strain for further studies.

The isolate 8C was characterized morphologically and biochemically as per the criteria given in Bergy's Manual of Systematic Bacteriology. The isolate was gram positive, non-motile, catalase positive rod, with ability to ferment lactose, mannose, maltose, fructose and ribose while not utilizing salicin, mannitol and arabinose. The isolate was positive for vogues-proskeur test, hydrolyzed starch and showed citrate utilization capacity. Based on these characteristics the isolate 8C was identified as *Bacillus* sp. and used further for optimization studies. Thermophilic *Bacillus* sp. have previously shown the lipase production at 50-60°C (Kim *et al.*, 2000; Bora and Bora, 2012). However, the reports on lipase production by *Bacillus* sp. under thermo-alkalophilic conditions are scanty.

Optimization of process variables

Effect of incubation period: Lipase production by *Bacillus* sp. started after 72 h at 50°C and increased thereafter with the increase in incubation time. The maximum enzyme activity (125 Eu mL⁻¹) was obtained by 7 days of incubation and then decreased gradually from 8th day to 11th day of incubation (Fig. 1). The high incubation time required for enzyme production might be due to the growth of microorganism at high temperature (50°C) under alkaline (pH-9.0) and static conditions. Under these conditions the organism may have high lag period before its exponential growth. The gradual decrease in enzyme production after 7 days might be due to the accumulation of toxic end products of metabolism or exhaustion of available nutrients in medium. Kumar *et al.*

Table 1: Qualitative and quantitative screening of isolates showing lipolytic activity

Isolates	Lipase activity (Eu mL ⁻¹)	Zone of lipolysis (mm)
1C	11.80	28
2C	7.80	15
3C	6.49	12
8C	4.13	10
15C	2.95	11

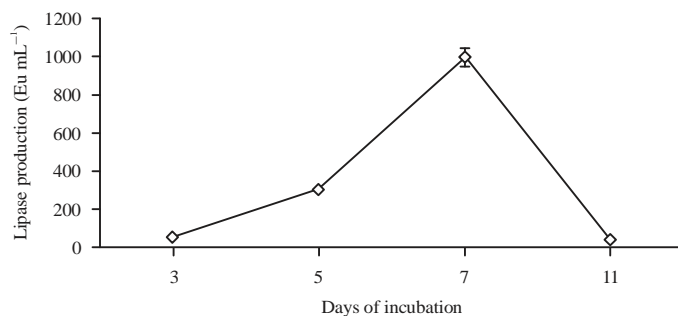


Fig. 1: Effect of incubation period on lipase production by *Bacillus* sp. 8C. Each value represents Mean±percentage error

(2012) have found that *Bacillus* sp gave the maximum yield of (1.2 Eu mL⁻¹) lipase after 72 h of incubation whereas Sharma *et al.* (2002) showed the lipase production was highest after 12 h of incubation.

Effect of initial pH of medium: Changes in the initial pH of production medium may induce the production of new metabolites that affect the biosynthesis of desired products. Hence, we examined the effect of pH on lipase production by *Bacillus* sp. and the results are summarized in Fig. 2. pH 9.0 was found to be the optimum for enzyme production (125 Eu mL⁻¹). Most of the published literature responds pH 8.0 as optimum pH for lipase production in thermophilic *Bacillus* sp. (Hasan and Hameed, 2001; Kim *et al.*, 1994; Duza and Mastan, 2014). Increased cultivation of the organism at an unfavorable pH, reduce the growth rate of organism and also has marked effects on the level of enzyme production (Bhosale *et al.*, 2011) as evidenced by drastically reduced enzyme production at all other pH.

Effect of carbon sources: Lipase production by thermo-alkalophilic *Bacillus* sp. was determined by measuring the enzyme activity when the strain was growing on different carbon sources. The highest lipase production was obtained with 1% ribose and raffinose (165 Eu mL⁻¹) followed by gum acacia (140 Eu mL⁻¹) and lactose (115 Eu mL⁻¹). Dextrin, sucrose, maltose and most of the carbon sources except xylose could stimulate lipase activity as they supported the growth of thermo-alkalophilic *Bacillus* sp. (Fig. 3). These results are in agreement with those reported by Sztajer and

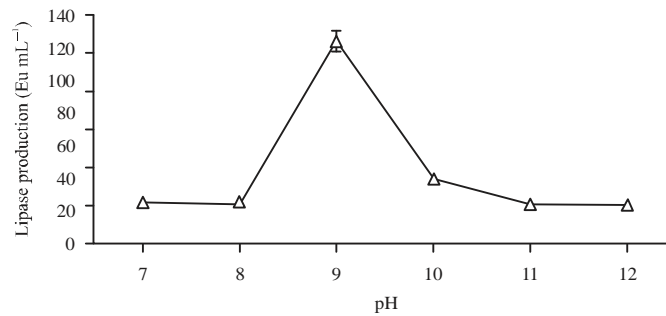


Fig. 2: Effect of pH on lipase production by *Bacillus* sp. 8C. Each value represents Mean±percentage error

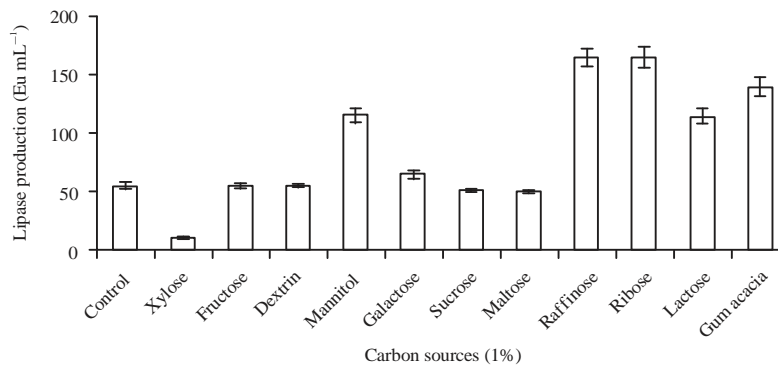


Fig. 3: Effect of different carbon sources on lipase production by *Bacillus* sp. 8C. Each value represents Mean±percentage error

Maliszewska (1989). Ability of an organism to drive a metabolic reaction and grow in presence of specific carbon source is dependent on typical enzymatic machinery present in the cell. In the present study, *Bacillus* grew well in presence of ribose and raffinose. Gum acacia, lactose and mannitol also supported for maximum enzyme production. However, lowest enzyme units recorded in the presence of xylose indicating that xylose is not suitable for lipase production.

Effect of nitrogen sources: The addition effect of different nitrogen sources on enzyme production is shown in Fig. 4. The maximum production of lipase was observed with 1% ammonium sulphate (140 Eu mL^{-1}) compared to glycerol (35 Eu mL^{-1}) and pyruvate (50 Eu mL^{-1}). Lipase production was also noticeable in presence of peptone, arginine and bile salts. Ammonium sulphate is highly soluble in water. Hence, readily available nitrogen source for microbial growth. Further, its high ionic strength releases ammonium ions in aqueous media that can be easily incorporated as part of different amino acids and hence plays important role in protein synthesis process.

Effect of metal salts: The effect of different metal salts added to the production medium on lipase production is presented in Fig. 5. Salts of ferric ions (FeCl_3) highly stimulated (300 Eu mL^{-1}) the lipase production by *Bacillus* sp. whereas, enzyme production was considerably high when ZnCl_2 and MgCl_2 were supplemented in the medium. Presence of KCl did not affected enzyme production and the production increase was marginal in presence of CaCl_2 , NaCl and MnCl_2 .

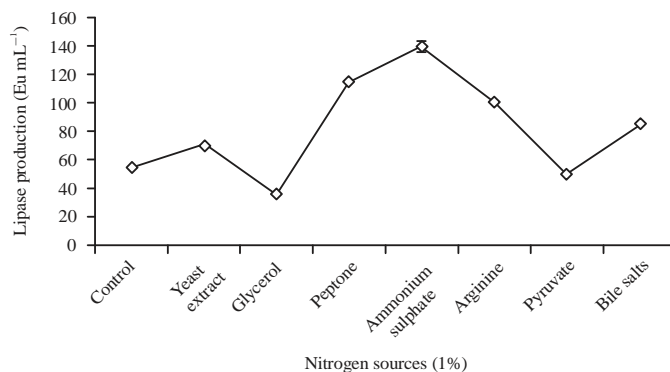


Fig. 4: Effect of different nitrogen sources on lipase production by *Bacillus* sp. 8C. Each value represents Mean±percentage error

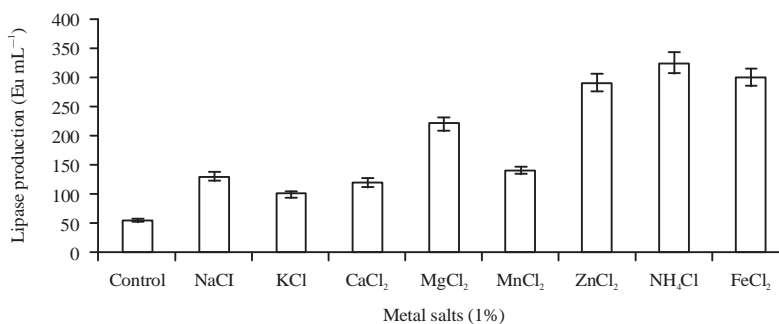


Fig. 5: Effect of metal salts on lipase production by *Bacillus* sp. 8C. Each value represents Mean±percentage error

Effect of different substrates: Supplementation of different lipidic substrate in production medium greatly affected the lipase production capacity of *Bacillus* sp. The enzyme production was unchanged when tributyrin in production media was replaced with castor oil, eucalyptus oil, soya bean oil and coconut oil as indicated in Fig. 6. However, production was highly stimulated when sesame oil was used as a substrate (165 Eu mL^{-1}) and was increased considerably in presence of mustard oil (110 Eu mL^{-1}) and clove oil (77 Eu mL^{-1}). Lipase is an extra-cellular inducible enzyme whose production is only detectable in presence of suitable substrate. Further, the growth and enzyme production capacity of an organism while growing on different substrate depend on its substrate utilization efficiency that ultimately depends on the presence of specific metabolic route required for metabolism of substrate.

Effect of thiols: In present study, we also examined the effects of different thiol compounds on lipase production by *Bacillus* sp. Interestingly, supplementation of different thiols in production medium either did not affected or stimulated the lipase production (Fig. 7). The effect was not detectable in the presence of dithiothreitol, β -mercaptoethanol and thiourea. However, addition of sodium sulphite, cysteine, sodium thiosulphate and methionine gradually increased the lipase

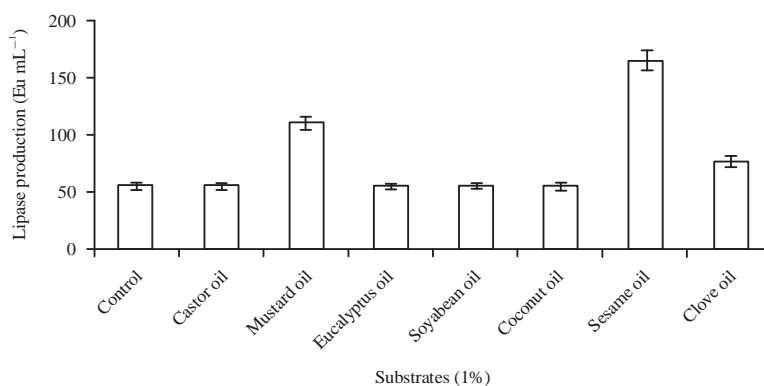


Fig. 6: Effect of different substrates on lipase production by *Bacillus* sp. 8C. Each value represents Mean \pm percentage error

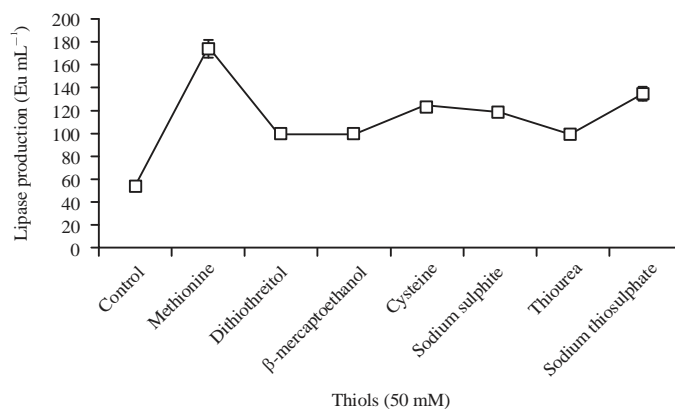


Fig. 7: Effect of different thiols on lipase production by *Bacillus* sp. 8C. Each value represents Mean \pm percentage error

production. Methionine and cysteine are sulphur containing amino acids that play an important role in stabilizing tertiary structure of protein molecules as well as they act as good nitrogen source for microbial growth. Enhanced lipase production in presence of methionine and cysteine indicated good growth of *Bacillus* sp. that might have supported higher production of enzyme.

The enzyme activity determined in tributyrin beef extract-nitrate broth before optimization was 55 Eu mL⁻¹ and the production found in optimized medium (peptone 3%, beef extract 5% , KNO₃ 0.1%, ribose 1%, ammonium sulphate 1%, sesame oil 1%, FeCl₃.7H₂O 1% and methionine 50 mM) was 350 Eu mL⁻¹ indicating 6.36 fold increase in lipase production by thermo-alkalophilic *Bacillus* sp. 8C.

CONCLUSION

In the present study, a thermo-alkalophilic lipase producer strain of *Bacillus* sp. 8C has been optimized for improved lipase production. The parameters and conditions used for lipase optimization studies are simple and easy to manage. This study first time demonstrated the role of raffinose and ribose sugars and thiols like methionine in improvement of thermo-alkalophilic *Bacillus* produced lipase yield.

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REFERENCES

- Bhosale, H.J., S.R. Sukalkar, Z.U. Shaheen and T.A. Kadam, 2011. Production of xylanase by *Streptomyces rameus* grown on agricultural wastes. *Biotechnol. Bioinform. Bioeng.*, 1: 505-512.
- Bora, L. and M. Bora, 2012. Optimization of extracellular thermophilic highly alkaline lipase from thermophilic *Bacillus* sp. isolated from hot spring of Arunachal Pradesh, India. *Braz. J. Microbiol.*, 43: 30-42.
- Duza, M.B. and S. Mastan, 2014. Optimization of lipase production from *Bacillus thuringiensis* (TS11BP), *Achromobacter xylosoxidans* J2 (TS2MCN)-isolated from soil sediments near oilseed farm. *IOSR J. Pharm. Biol. Sci.*, 9: 66-76.
- Gupta, N., V. Sahai and R. Gupta, 2007. Alkaline lipase from a novel strain *Burkholderia multivorans*: Statistical medium optimization and production in a bioreactor. *Process Biochem.*, 42: 518-526.
- Hasan, F. and A. Hameed, 2001. Optimization of lipase production from *Bacillus* sp. *Pak. J. Bot.*, 33: 789-796.
- Hasan, F., A.A. Shah and A. Hameed, 2006. Industrial applications of microbial lipases. *Enzyme Microb. Technol.*, 39: 235-251.
- Hasan, F., A.A. Shah and A. Hameed, 2007. Purification and characterization of a mesophilic lipase from *Bacillus subtilis* FH5 stable at high temperature and pH. *Acta Biologica Hungarica*, 58: 115-132.
- Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. *Bergey's Manual of Determinative Bacteriology*. 9th Edn., Lippincott Williams and Wilkins, Baltimore, USA., ISBN-13: 9780683006032, Pages: 787.

- Jellouli, K., O. Ghorbel-Bellaaj, H.B. Ayed, L. Manni, R. Agrebi and M. Nasri, 2011. Alkaline-protease from *Bacillus licheniformis* MP1: Purification, characterization and potential application as a detergent additive and for shrimp waste deproteinization. *Process Biochem.*, 46: 1248-1256.
- Kim, H.K., M.H. Sung, H.M. Kim and T.K. Oh, 1994. Occurrence of thermostable lipase in thermophilic *Bacillus* sp. strain 398. *Biosci. Biotechnol. Biochem.*, 58: 961-962.
- Kim, M.H., H.K. Kim, J.K. Lee, S.Y. Park and T.K. Oh, 2000. Thermostable lipase of *Bacillus stearotherophilus*: High-level production, purification and calcium-dependent thermostability. *Biosci. Biotechnol. Biochem.*, 64: 280-286.
- Kumar, D.J.M., R. Rejitha, S. Devika, M.D. Balakumaran, A.I.N. Rebecca and P.T. Kalaichelvan, 2012. Production, optimization and purification of lipase from *Bacillus* sp. MPTK 912 isolated from oil mill effluent. *Adv. Applied Sci. Res.*, 3: 930-938.
- Kumari, A., P. Mahapatra and R. Banerjee, 2009. Statistical optimization of culture conditions by response surface methodology for synthesis of lipase with *Enterobacter aerogenes*. *Brazil. Arch. Biol. Technol.*, 52: 1349-1356.
- Messias, J.M., B.Z. da Costa, V.M.G. de Lima, R.F.H. Dekker, M.I. Rezende, N. Krieger and A.M. Barbosa, 2009. Screening *Botryosphaeria* species for lipases: Production of lipase by *Botryosphaeria ribis* EC-01 grown on soybean oil and other carbon sources. *Enzyme Microb. Technol.*, 45: 426-431.
- Myers, N., R.A. Mittermeier, C.G. Mittermeier, G.A.B. da Fonseca and J. Kent, 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403: 853-858.
- Nguyen, L.N., T.T. Dao, T. Zivkovic, M. Fehrholz, W. Schafer and S. Salomon, 2010. Enzymatic properties and expression patterns of five extracellular lipases of *Fusarium graminearum* *in vitro*. *Enzyme Microb. Technol.*, 46: 479-486.
- Rabbani, M., M.R. Bagherinejad, H.M. Sadeghi, Z.S. Shariat and Z. Etemadifar *et al.*, 2013. Isolation and characterization of novel thermophilic lipase-secreting bacteria. *Braz. J. Microbiol.*, 44: 1113-1119.
- Sangeetha, R., A. Geetha and I. Arulpandi, 2010. Concomitant production of protease and lipase by *Bacillus licheniformis* VSG1: Production, purification and characterization. *Braz. J. Microbiol.*, 41: 179-185.
- Schmidt-Dannert, C., H. Sztajer, W. Stocklein, U. Menge and R.D. Schmid, 1994. Screening, purification and properties of a thermophilic lipase from *Bacillus thermocatenuatus*. *Biochimica Biophysica Acta (BBA)-Lipids Lipid Metabol.*, 1214: 43-53.
- Selvam, K., B. Vishnupriya and V.S.C. Bose, 2010. Screening and quantification of marine actinomycetes producing industrial enzymes amylase, cellulase and lipase from South Coast of India. *Int. J. Pharmaceut. Biol. Arch.*, 2: 1481-1487.
- Sharma, R., S.K. Soni, R.M. Vohra, L.K. Gupta and J.K. Gupta, 2002. Purification and characterisation of a thermostable alkaline lipase from a new thermophilic *Bacillus* sp. RSJ-1. *Process Biochem.*, 37: 1075-1084.
- Shu, Z.Y., H. Jiang, R.F. Lin, Y.M. Jiang, L. Lin and J.Z. Huang, 2010. Technical methods to improve yield, activity and stability in the development of microbial lipases. *J. Mol. Catal. B: Enzymatic*, 62: 1-8.
- Sztajer, H. and I. Maliszewska, 1989. The effect of culture conditions on lipolytic productivity of *Penicillium citrinum*. *Biotechnol. Lett.*, 11: 895-898.

- Treichel, H., D. de Oliveira, M.A. Mazutti, M. di Luccio and J.V. Oliveira, 2010. A review on microbial lipases production. *Food Bioprocess Technol.*, 3: 182-196.
- Uttatree, S., P. Winayanuwattikun and J. Charoenpanich, 2010. Isolation and characterization of a novel thermophilic-organic solvent stable lipase from *Acinetobacter baylyi*. *Applied Biochem. Biotechnol.*, 162: 1362-1376.
- Zhao, L.L., J.H. Xu, J. Zhao, J. Pan and Z.L. Wang, 2008. Biochemical properties and potential applications of an organic solvent-tolerant lipase isolated from *Serratia marcescens* ECU1010. *Process. Biochem.*, 43: 626-633.