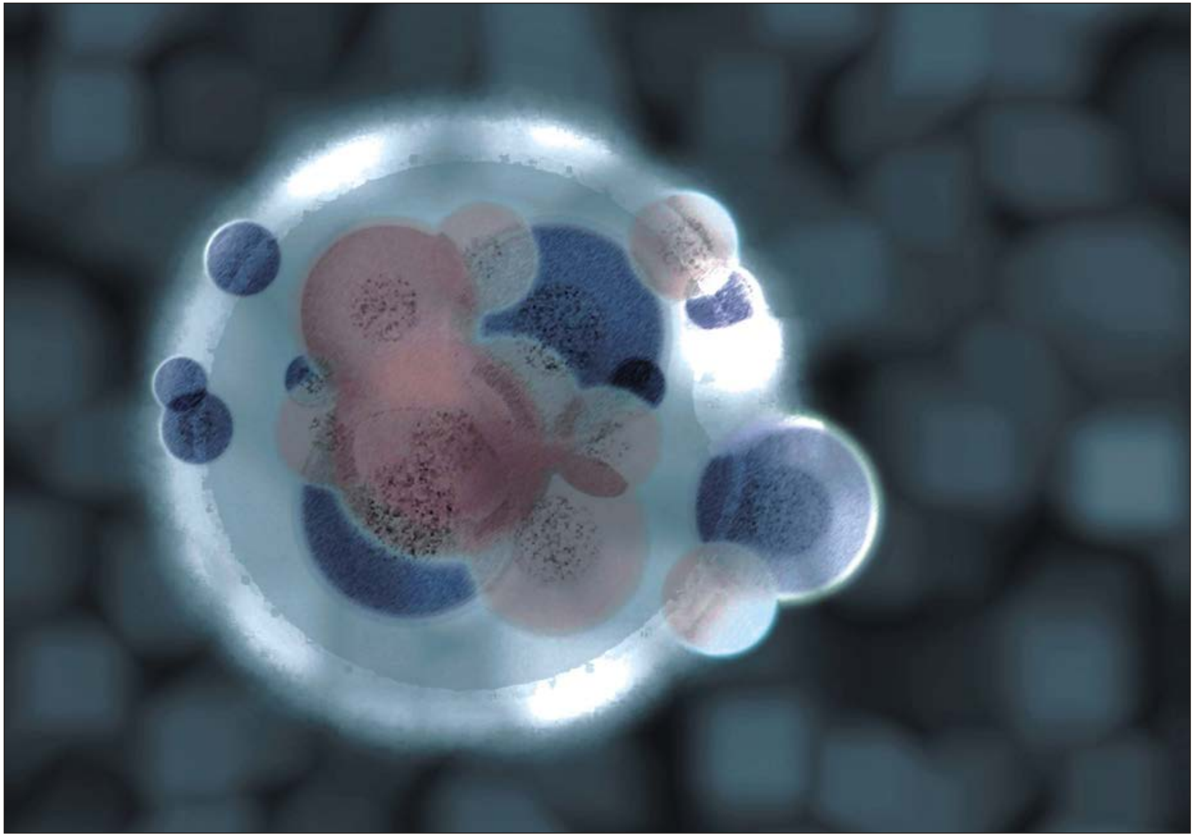

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Isolation, Screening and Production of Extracellular Alkaline Lipase from a Newly Isolated *Bacillus* sp. PD-12

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Abstract: There is limited available information on the characterization of alkali-stable *Bacillus* sp. producing lipase and its commercial production. Therefore, thirty bacterial strains were isolated from oil industry soil samples and were screened for lipolytic activity. *Bacillus* sp. PD-12 was selected for lipase production because of its high lipolytic activity (4.2 IU mL^{-1}). *Bacillus* sp. PD-12 lipase was optimally active at pH 8.0 and at 40°C temperature. The lipase is capable of hydrolyzing vegetable oils and synthetic triglycerides. Maximum lipase production by *Bacillus* sp. PD-12 was obtained when grown under shaking conditions (250 rpm) at 30°C for 24 h. In order to increase lipase production, optimization of carbon and nitrogen sources was studied. Maximum lipolytic activity (4.25±0.020 IU mL^{-1}) was obtained with olive oil as a carbon source followed by coconut oil (2.5±0.030 IU mL^{-1}). Among nitrogen sources, ammonium nitrate resulted in maximum lipolytic activity (15.6±0.036 IU mL^{-1}). Lipase production by *Bacillus* sp. PD-12 was studied in a 3 liter fermentor with a working volume of 1.8 liter under optimized conditions resulted in lipolytic activity of 22 IU mL^{-1} after 21 h. Thus, short fermentation time (21 h) makes this fermentation system a promising one in terms of lipase productivity and alkali-stable *Bacillus* sp. can be used in detergent industry.

Key words: *Bacillus* sp. PD-12, extracellular lipase, production, screening, tributyrin agar assay, olive oil PVA emulsion, enzyme characterization

INTRODUCTION

Enzymes are nature's catalyst, possessing unique properties that make them highly desirable for their application in processing industry. It is because of their bio-degradability, high specificity and high catalytic efficiency; lipases find prominent position as industrial biocatalysts (Sangeetha *et al*., 2011). Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are a class of hydrolases that catalyze the hydrolysis of triacylglycerols to diacylglycerols, monoacylglycerols, fatty acids and glycerol at oil-water interface (Gupta *et al*., 2011). Under experimental conditions, lipases can catalyze reverse reactions including synthesis of esters by esterification, transesterification and interesterification (Franken *et al*., 2011). Today, nearly 4000 enzymes are known and of these, about 200 are in commercial use (Sharma *et al*., 2011). The world market of industrial enzymes was estimated to be US $ 2 billion in 2004 which is expected to reach nearly $ 2.4 billion by 2008. On the criteria of total sales volume, lipases include the third largest group after proteases and carbohydrases among industrial enzymes (Hasan *et al*., 2006). Lipase is an important enzyme having tremendous potential in areas such as food industry, fine chemicals, biodiesel production and pharmaceutical industry (Lara and Park, 2004; Won *et al*., 2006; Larios *et al*., 2004).

Lipases are ubiquitous in nature and are widely distributed among plants, animals and microorganisms. Microbial enzymes are more useful than enzymes derived from plants or animals due to the better stability, high yields, ease of genetic manipulation, regular supply and rapid growth of microorganisms in inexpensive media and their lipase can catalyze a wide variety of hydrolytic and synthetic reactions (Hasan *et al*., 2006). Microorganisms producing lipase have been found in varied habitats including industrial wastes, vegetable oil processing units, soil contaminated with oil, dairies, oilseeds, decaying foods, compost heaps, coal tips and hot springs (Wang *et al*., 1995). Various reports on the different aspects of lipase are available beginning with isolation and screening of lipolytic organisms and optimization of production conditions (Jaeger *et al*., 1994; Abdel-Fattah, 2002; Castro-Ochoa *et al*., 2005). Further screening may leads to isolation of novel lipases which possesses the desired properties. Thus, the study was aimed at isolation, screening and production studies of an extracellular alkaline lipase from *Bacillus* sp. PD-12.

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MATERIALS AND METHODS

Study period: The present study was carried out from March, 2010 to August, 2011 at the departmental Enzymology laboratory.

Isolation and screening of lipase producing bacteria: Enrichment cultures of the 10 soil samples collected from oil industry, Hisar, Haryana were performed using enrichment medium with the following composition (%): Olive oil 2%, $(NH_4)_2SO_4$ 0.5%, K_2HPO_4 0.5%, $MgSO_4.7H_2O$ 0.1%, $CaCO_3$ 0.5% (pH 7.0). The mixture was incubated at 30°C on a rotary shaker at 200 rpm for 48 h. Repeated streaking of single colony was performed on nutrient agar medium till pure cultures were obtained. These were maintained by regular transfers on nutrient agar slants at 4°C in a refrigerator. The isolated lipase producing strains were screened both qualitatively by tributyrin agar assay and quantitatively by titrimetry.

Crude enzyme was prepared by inoculating one loop full of fresh culture from nutrient agar slant in 10 mL nutrient broth and incubated at 30°C (250 rpm). After 20 h, 10% level of inoculum was transferred to nutrient broth medium supplemented with 2% olive oil. The mixture was incubated at 30°C on a rotary shaker for 24 h. The culture supernatant obtained was centrifuged at 3000 rpm for 20 min at 4°C. The supernatant obtained was collected and residual oil was removed by filtration through Whatman filter paper No. 1.

Tributyrin agar assay (qualitative): Lipolytic activity was determined by the diffusion plate test according to the method of Lawrence *et al.* (1967). The isolates were streaked on plates containing tributyrin agar medium with the following composition (g L^{-1}): Peptone 5.0; beef extract 3.0; tributyrin 15.0; agar 20.0 (pH 7.0), incubated at 30°C for 24 h and zone of tributyrin hydrolysis was observed and measured.

Olive oil PVA emulsion method (quantitative): Lipase activity was determined titrimetrically using olive PVA method according to the procedure of Yamada *et al.* (1977). One unit of lipase was defined as the amount which liberated 1 µmole of fatty acids per minute at 30°C at pH 7.

Characterization of lipase from potential isolates: Two strains (PD-12 and PD-20) that possessed highest activity on olive oil were selected for subsequent studies. Lipase activity of both the isolates PD-12 and PD-20 was determined by carrying out the reactions at different pH ranging from pH 4.0-10.0 using various activity buffers. Different activity buffers employed were citrate-phosphate buffer (pH 4.0-6.0), phosphate buffer (pH 7.0), Tris-HCl (pH 8.0-9.0), glycine-NaOH (pH 10.0). Lipase activity of both the isolates was determined by carrying out the reaction at different temperatures viz., 30-70°C. Substrate specificity of both PD-12 and PD-20 was determined using different triacylglycerides and vegetable oils as substrates by titrimetry. Activity was expressed as % relative activity in comparison to the activity on olive oil considered as 100%.

Optimization of media for maximum lipase production: In order to select the best lipase production medium, four different media were tested. The compositions of the media were (g L^{-1}): Medium 1: peptone (5), yeast extract (1), NaCl (5), olive oil (2% v/v); Medium 2: M1+beef extract (1)+olive oil (2% v/v); Medium 3: M2+glucose (5)+olive oil (1% v/v); Medium 4: M3+ glucose (10) at pH 7.0. The inoculum (10 mL) was transferred into 100 mL of production medium and incubated for 24 h at 30°C on a shaker at 250 rpm. The fermented broth obtained was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant (crude enzyme) obtained was collected and residual oil was removed by filtration through Whatman filter paper No. 1. The supernatant was analyzed with respect to lipase activity, biomass and final pH.

Effect of nutritional factors on lipase production: For the effect of the carbon sources, the following were added to the medium 3 at 1% (w/v): olive oil, groundnut oil, canola, coconut oil, fructose, sucrose and maltose. All carbon sources were separately sterilized by milipore filter of pore size of 0.45 µm. Nitrogen sources were added to the media at 1% (w/v): The nitrogen sources used includes urea, soyabean meal, ammonium nitrate, ammonium chloride, ammonium sulfate and sodium nitrate. Nitrogen sources were sterilized by milipore filter of pore size of 0.45 µm wherever required. All the experiments were performed in triplicate and the data obtained were expressed as Mean±SD.

Fermentation profile of *Bacillus* sp. PD-12: Lipase production by *Bacillus* sp. PD-12 was studied in a 3.0 L fermentor with a working volume of 1.8 L. The media was sterilized *in-situ* at 121°C for 15 min and inoculum was prepared in nutrient broth by transferring a loop full of culture in to 150 mL of Medium-3, incubated at 30°C for 20 h on a shaker at 250 rpm. The inoculum prepared was transferred to the fermentor containing sterilized optimized medium (Medium-3) was run for 28 h. Standard operation conditions used were: stirring rate: 250 rpm, temperature: 30°C, pH: uncontrolled and air flow: 1 vvm. Aliquots

(10-15 mL) of the fermentor broth were withdrawn periodically till 28 h and centrifuged at 8000 rpm for 15 min. The supernatant was collected and residual oil was removed by filtration through Whatman filter paper No.1 (Diameter: 12.5) and analyzed for lipase activity, biomass and final pH of the medium. Fermentation parameters, such as temperature, pH, dissolved oxygen and airflow were continuously monitored using microprocessor controlled probes.

Statistical analysis: All the experiments were performed in triplicate and the data obtained were expressed as averages of triplicate tests and Mean±SD, unless specified otherwise.

RESULTS AND DISCUSSION

Thirty strains were primarily isolated from 10 soil samples using enrichment cultures containing olive oil (2% v/v) at 30°C. All the isolates were screened both qualitatively by tributyrin agar assay and quantitatively by olive PVA emulsion method. Isolates which show zone of hydrolysis on tributyrin agar, but no activity was obtained titrimetrically may be due to esterase activity. Varied lipase activity was observed titrimetrically for different isolates ranging from 0.3 IU mL^{-1} for PD-11, 0.5 IU mL^{-1} for PD-14 and PD-28 to 4.2 IU mL^{-1} for PD-12 (Table 1). Among them, the isolates PD-12 and PD-20 were chosen for subsequent experiments due to their high lipolytic activity viz., 4.2 and 3.6 IU mL^{-1}, respectively. Different isolates possess varied zones of hydrolysis on tributyrin. 1.0 mm of halozone being the lowest was observed for three isolates (PD-1, PD-8 and PD-17) and a maximum zone of hydrolysis of 8.5 and 11.0 mm was observed for isolates PD-23 and PD-3. Halozone of 7 mm for PD-12 and 6 mm for PD-20 diameter on tributyrin agar plate indicated the production of lipase (Table 1). Based on morphological and physiological characteristics isolates PD-12 and PD-20 were tentatively identified to be *Bacillus* sp.

Characterization of lipase from two potential isolates: Lipase from the two potential lipolytic *Bacillus* sp. isolates (PD-12 and PD-20) was characterized with respect to various physico-chemical properties. Optimal lipase activity of both the isolates (PD-12 and PD-20) was evaluated at different pH using various activity buffers. The lipase from PD-12 was found to be active in the alkaline pH range with maximum activity at pH 8.0 while, the lipase from PD-20 was active at pH 7.0 (Fig. 1). The activity of both the enzymes declined on either side of peak. Many lipases from *Bacillus* sp. are reported to be alkali tolerant and show maximum activity at alkaline pH (Padmapriya *et al*., 2011; Bora and Kalita, 2007), while lipases from some *Bacillus* sp. are optimal at neutral pH (Sebdani *et al*., 2011; Lee *et al*., 1999).

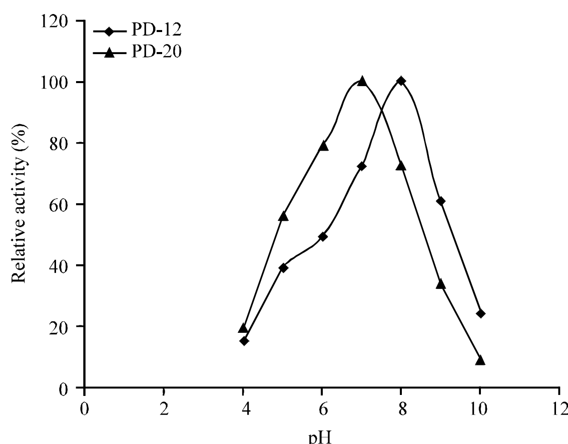

Fig. 1: pH optima of lipase from PD-12 and PD-20 using olive oil as substrate

Table 1: Screening (qualitative and quantitative analysis) of various bacterial isolates for lipase activity

Strain No.	Morphological characteristics	Qualitative analysis Zone on TB agar (mm)	Quantitative analysis enzyme activity (IU mL^{-1})
PD-1	Gram+ve rods	1.0	0
PD-2	Gram+ve cocci (chained).	5.5	0
PD-3	Gram-ve rods	11.0	0
PD-4	Gram-ve cocci	6.0	1.8
PD-5	Gram+ve cocci	3.0	0
PD-6	Gram+ve cocci	6.0	0
PD-7	Gram+ve cocci (in pairs)	5.2	0
PD-8	Gram+ve rods	1.0	0
PD-9	Gram+ve small rods	7.0	0
PD-10	Gram-ve rods in clusters	4.0	0
PD-11	Gram-ve rods	0	0.3
PD-12	Gram+ve rods	7	4.2
PD-13	Gram-ve cocci	0	0
PD-14	Gram+ve large cocci in tetrads	4.0	0.5
PD-15	Gram+ve rods	7.0	1.2
PD-16	Gram-ve rods	2.5	1.1
PD-17	Gram-ve rods	1.0	0
PD-18	Gram-ve rods (dividing)	6.0	0
PD-19	Gram-ve cocci chained	2.5	1.0
PD-20	Gram+ve rods	6	3.6
PD-21	Gram-ve rods	6.0	0
PD-22	Gram-ve rods (dividing)	7.0	0
PD-23	Gram+ve cocci(chained).	8.5	0
PD-24	Gram+ve cocci(in pairs)	5.0	0
PD-25	Gram-ve cocci in clusters	8.0	0
PD-26	Gram-ve cocci	0	0.7
PD-27	Gram+ve large cocci in tetrads	5.5	0
PD-28	Gram+ve rods in clusters	6.0	0.5
PD-29	Gram+ve rods	0	0.6
PD-30	Gram+ve coccobacilli	0	1.2

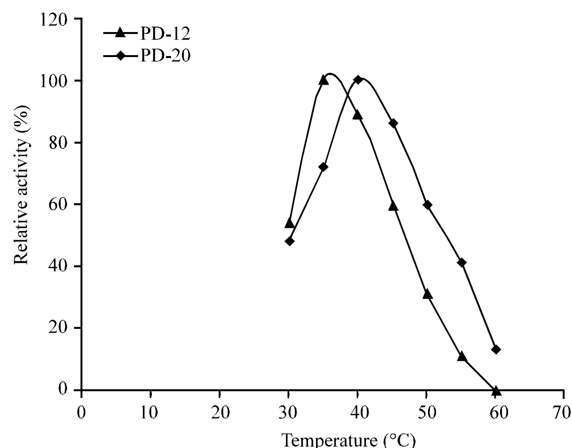

Fig. 2: Temperature optima of lipase from PD-12 and PD-20 using olive oil as substrate

Table 2: Relative hydrolysis rates of PD-12 and PD-20 lipase towards various substrates

	Relative activity (%)	
Substrate	PD-12	PD-20
Synthetic triglycerides		
Tributyrin	110	102
Triacetin	98.4	90
Vegetable oils		
Olive oil	100	100
Groundnut oil	95.6	32
Mustard oil	84	21

The lipase from PD-12 was found to have temperature optima of 40°C while PD-20 showed pH optima at 35°C as shown in Fig. 2. Also, it was found that strain PD-12 lipase possess moderate lipase activity (more than 50% relative activity) between temperature range of 35- 50°C. *Bacillus* sp. lipase possessing optimum temperature around 40°C has been reported by many investigators (Sebdani *et al*., 2011; Padmapriya *et al*., 2011) and few lipases with higher temperature optima above 60°C have also been reported from *Bacillus* sp. LBN 4 (Bora and Kalita, 2007) and *Bacillus* thermoleovorans ID-1 (Lee *et al*., 1999).

The data in Table 2 shows that lipase from PD-12 showed maximum relative activity in the presence of tributyrin (110%) followed by olive oil (100%) and triacetin (98.4%) among the synthetic triglycerides and vegetable oils tested. In contrast PD-20 lipase displayed maximum relative activity against tributyrin (102%) followed by olive oil (100%) and triacetin (90%) and while, PD-20 lipase was less active towards mustard oil and groundnut oil. Similarly, maximum hydrolytic activity in substrate tributyrin was reported Rua *et al*. (1997), Gaoa *et al*. (2000), Kojima and Shimizu (2003) and Dahiya *et al*. (2010). The substrate specificity of lipases is important for their application for analytical and industrial purposes.

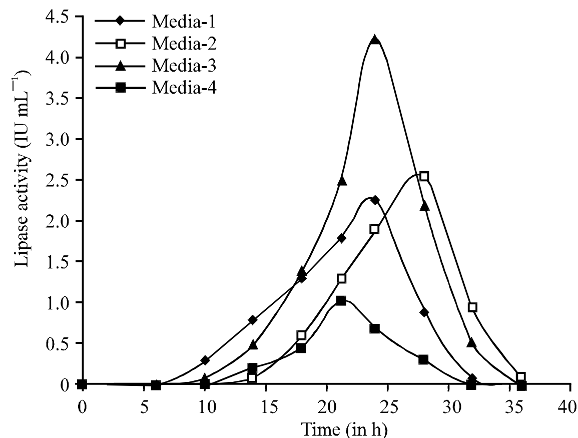

Fig. 3: Effect of different medium on lipase production by *Bacillus* sp. PD- 12

Optimization of culture parameters for maximum lipase production: Based on the results of screening and selection of potential lipolytic bacterium, strain PD-12 was selected, as it produces an alkaline lipase exhibiting wide substrate specificity as compared to PD-20. Among the desirable characteristics that commercially important lipases should exhibit, alkali tolerance and thermostability are considered to be the most important (Kulkarni and Gadre, 1999).

Influence of nutrient media on lipase production: The composition of nutrient medium influences the lipase production. In order to select the best media, four different production media were examined for their ability to support maximum lipase production by *Bacillus* sp. PD-12. It was observed that among the different media used, Medium 3 (medium 2+glucose supplemented with 2% olive oil) supported maximum lipase activity of 4.3 IU mL^{-1} after 24 h of incubation followed by medium 2 and medium 1 (Fig. 3). Medium 3 supporting maximum lipase productions was found to be best amongst all the production media tested and was used in further experiments. Generally, lipases are secreted maximally in the late or post exponential growth phase as reported by Tamerler and Keshavarz (2000) and Castro-Ochoa *et al*. (2005). *Bacillus* sp. PD-12 released lipase into culture broth when cultivated in a medium supplemented with 2% olive oil. Without the addition of olive oil, however, no lipase activity was detected in the broth even after cell lysis took place during prolonged cultivation. Thus, the addition of triglyceride like olive oil to the medium was indispensable for the production of the enzyme, as was reported by Heravi *et al*. (2008) and Wang *et al*. (1995). However, Chaturvedi *et al*. (2010) reported groundnut oil cake as a substrate for *Bacillus subtilis* MTCC 6808.

Table 3: Effect of varying carbon sources and nitrogen sources on lipase production by *Bacillus* sp. PD-12 at 30°C for 24 h

Carbon source 1%, (w/v)	Lipase activity* (U mL^{-1})	Nitrogen source 1%, (w/v)	Lipase activity (U mL^{-1})
Medium 3+Olive oil	4.32±0.020	Medium 3+Urea	0.25±0.020
M 3+ Groundnut oil	2.00±0.020	M 3+ Soybean meal	4.20±0.020
M 3+ Canola oil	1.50±0.036	M 3+ Ammonium nitrate	15.60±0.036
M 3+ Coconut oil	2.50±0.030	M 3+ Ammonium chloride	8.30±0.030
M 3+ Sucrose	1.20±0.064	M 3+ Ammonium sulfate	5.20±0.064
M 3+ Fructose	1.50±0.020	M 3+ Sodium nitrate	2.50±0.020
M 3+ Maltose	1.80±0.064		

*:Values are expressed in standard deviation

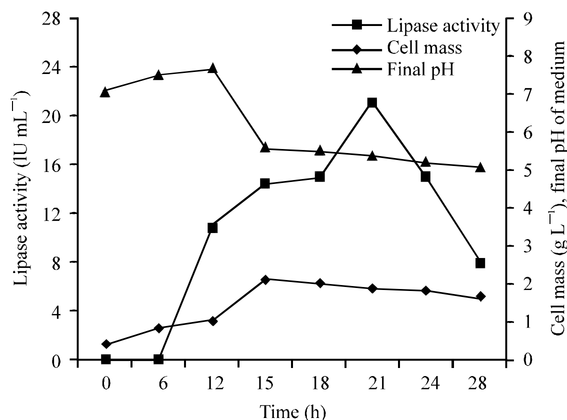

Fig. 4: Fermentation profile of *Bacillus* sp. PD-12 under optimized conditions for maximum enzyme production in a fermentor

Optimization of carbon and nitrogen sources for maximum lipase production: Effect of different carbon sources (carbohydrates and lipid sources) on *Bacillus* sp. PD-12 for lipase production was studied. Good amount of lipase was produced in all the oils tested. Maximum lipolytic activity (4.32±0.020 IU mL^{-1}) was obtained with olive oil as a carbon source, followed by coconut (2.5±0.030 IU mL^{-1}). These results are in agreement with the earlier reports of use of olive oil as lipid sources (Ellaiah *et al*., 2004; Heravi *et al*., 2008) as shown in Table 3. In order to study the effect of nitrogen sources some organic and inorganic nitrogen sources were used. Addition of ammonium nitrate resulted in maximum lipolytic activity (15.6±0.036 IU mL^{-1}) followed by ammonium chloride (8.3± 0.030 IU mL^{-1}) as shown in Table 3. Similar results on good growth and high lipase activity in the presence of inorganic nitrogen sources were reported in *Bacillus* sp. with di-ammonium hydrogen orthophosphate by Rathi *et al*. (2001) and *Pseudomonas* sp. G6 with sodium nitrate by Kanwar *et al*. (2002). Whereas, Eltaweel *et al*. (2005) reported drastically reduced lipase production in the presence of inorganic nitrogen sources in *Bacillus* sp. strain 42 and Babu and Rao (2007) reported maximum lipase production in the presence of urea in case of *Y. lipolytica* NCIM 3589.

Lipase production in batch fermentation: Lipase production by *Bacillus* sp. PD-12 was studied in a 3 L fermentor with a working volume of 1.8 L using optimized conditions. Standard operation conditions used were: stirring rate 250 rpm, temperature 30°C, uncontrolled pH, air flow 1 vvm and was run for 28 h. Aliquots were withdrawn periodically till 28 h and supernatant obtained after centrifugation and filtration was analyzed for lipase activity, biomass and final pH of the medium. The maximum lipolytic activity obtained was 22 IU mL^{-1} after 21 h, respectively (Fig. 4). The incubation period required for maximum lipase production was reduced in fermentor and maximum lipase production was obtained after 21 h. When compared with shake flasks, a good increase in the lipase production was observed. Most importantly, the time period for the production process was reduced in a fermentor. Similar to our results, lipase from most of the strains showed reduction in fermentation time as reported in *Bacillus* sp. by Heravi *et al*. (2008) and in *Candida rugosa* by Puthli *et al*. (2006).

CONCLUSION

A strain of *Bacillus* sp. PD-12 producing extracellular lipase was isolated from soil. The bacterium accumulates lipase in culture fluid when grown aerobically at 30°C for 24 h in nutrient medium supplemented with 2% olive oil as substrate. The enzyme exhibited wide substrate specificity in the presence of various synthetic triglycerides and vegetable oils. The lipase production by PD-12 was optimized which resulted in 5.2-fold increase in yield. The lipase from PD-12 isolate was found to exhibit optimum activity at 40°C and pH 8.0. The alkali stable property and wide substrate specificity of *Bacillus* sp. PD-12 lipase suggests its application for analytical and industrial purposes. The biocatalysis of *Bacillus* sp. PD-12 will be determined in organic media for the production of novel compounds for industrial applications.

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REFERENCES

Abdel-Fattah, Y.R., 2002. Optimization of thermostable lipase production from a thermophilic *Geobacillus* sp. Using Box-Behnken experimental design. Biotechnol. Lett., 24: 1217-1222.

Babu, I.S. and G.H. Rao, 2007. Lipase production by *Yarrowia lipolytica* NCIM 3589 in solid state fermentation using mixed substrate. Res. J. Microbiol., 2: 469-474.

Bora, L. and M.C. Kalita, 2007. Production and optimization of thermostable lipase from a thermophilic *Bacillus* sp. LBN4. The Int. J. Microbiol., 4: 172-179.

Castro-Ochoa, L.D., C. Rodriguez-Gomez, G. Valerio-Alfaro and R.O. Ros, 2005. Screening, purification and characterization of the thermophilic lipase produced by *Bacillus thermoleovorans* CCR11. Enzyme Microbial. Technol., 37: 648-654.

Chaturvedi, M., M. Singh, Chugh R. Man and S. Pandey, 2010. Lipase production from *Bacillus subtilis* MTCC 6808 by solid state fermentation using ground nut oil cakes as substrate. Res. J. Microbiol., 5: 725-730.

Dahiya, P., P. Arora, S. Chand, A. Chaudhury and N. Dilbaghi, 2010. Characterization of an extracellular alkaline lipase from *Pseudomonas mendocina* M-37. J. Basic Microbiol., 50: 420-426.

Ellaiah, P., T. Prabhakar, B. Ramakrishna, A.T. Taleb and K. Adinarayana, 2004. Production of lipase by immobilized cells of *Aspergillus niger*. Process. Biochem., 39: 525-528.

Eltaweel, M.A., A.B. Salleh and M. Basri, 2005. An organic solvent tolerant lipase from *Bacillus* sp. strain 42. Ann. Microbiol., 55: 187-192.

Franken, B., T. Eggert, K.E. Jaeger and M. Pohl, 2011. Mechanism of acetaldehyde-induced deactivation of microbial lipases. BMC Biochem., 12: 10-14.

Gaoa, X., S. Cao and K. Zhang, 2000. Production, properties and application to non-aqueous enzymatic catalysis of lipase from newly isolated *Pseudomonas* strain. Enz. Microb. Techol., 27: 74-82.

Gupta, P., L.S.B. Upadhyay and R. Shrivastava, 2011. Lipase catalyzed-transesterification of vegetable oils by lipolytic bacteria. Res. J. Microbiol., 6: 281-288.

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

Heravi, K.M., F. Eftekhar, B. Yakhchali and F. Tabandeh, 2008. Isolation and identification of a lipase producing *Bacillus* sp. from soil. Pak. J. Biol. Sci., 11: 740-745.

Jaeger, K.E.H., S. Ransac, B.W. Dijkstr, C. Colson, M. van Heuvel and O. Misset, 1994. Bacterial lipases. FEMS Microbiol. Rev., 15: 29-63.

Kanwar, L., B.K. Gogoi and P. Goswami, 2002. Production of a *Pseudomonas lipase* in n-alkane substrate and its isolation using an improved ammonium sulfate precipitation technique. Bioresour. Technol., 84: 207-211.

Kojima, Y. and S. Shimizu, 2003. Purification and characterization of the lipase from *Pseudomonas fluorescens* HU380. J. Biosci. Bioengin., 96: 219-226.

Kulkarni, N. and R.V. Gadre, 1999. A novel alkaline, thermostable, protease free lipase from *Pseudomonas* sp. Biotechnol. Lett., 21: 897-899.

Lara, P. and E.Y. Park, 2004. Potential applications of waste activated bleaching earth on the production of fatty acid esters using *Candida cylindraceae* lipase in organic solvent system. Enzyme Microbial. Technol., 34: 270-277.

Larios, A., H.S. Garcia, R.M. Oliart and G. Valerio-Alfaro, 2004. Synthesis of flavor and fragrance esters using *Candida antarctica* lipase. Applied Microbiol. Biotechnol., 65: 373-376.

Lawrence, R.C., T.F. Fryer and B. Reiter, 1967. Rapid method for the quantitative estimation of microbial lipase. Nature, 25: 1264-1265.

Lee, D., Y. Koh, K. Kim, B. Kim and H. Choi *et al*., 1999. Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. FEMS Microbiol. Lett., 179: 393-400.

Padmapriya, B., T. Rajeswari, E. Noushida, D.G. Sethupalan and C.K. Venil, 2011. Production of lipase enzyme from *Lactobacillus* sp. and its application in the degradation of meat. World Applied Sci. J., 12: 1798-1802.

Puthli, M.S., V.K. Rathod and A.B. Pandit, 2006. Optimization of lipase production in a triple impeller bioreactor. Biochem. Engin. J., 27: 287-294.

Rathi, P., R.K. Saxena and R. Gupta, 2001. A novel alkaline lipase from *Burkholderia cepacia* for detergent Formulation. Process Biochem., 37: 187-192.

Rua, M.L., C. Schmidt-Dannert, S. Wahl, A. Sprauer and R.D. Schimd, 1997. Thermoalkalophilic lipase of *Bacillus thermocatenulatus* large-scale production and properties: Aggregation behaviour and its effect on activity. J. Biotechnol., 56: 89-102.

Sangeetha, R., I. Arulpandi and A. Geetha, 2011. Bacterial lipases as potential industrial biocatalysts: An overview. Res. J. Microbiol., 6: 1-24.

Sebdani, R.M., M.R. Ardakani, G.R. Ghezelbash and M. Sadrinasab, 2011. Phylogenic characterization of lipase producing *Bacillus strains* isolated from Persian gulf sediments. Aust. J. Basic Applied Sci., 5: 121-126.

Sharma, D., B. Sharma and A.K. Shukla, 2011. Biotechnological approach of microbial lipase: A review. Biotechnology, 10: 23-40.

Tamerler, C. and T. Keshavarz, 2000. Lipolytic enzyme production in batch and fed-batch cultures of *Ophiostoma piceae* and *Fusarium oxysporum*. J. Chem. Technol. Biotechnol., 75: 785-790.

Wang, Y., K.C. Srivastava, G.J. Shen and H.J. Wang, 1995. Thermostable alkaline lipase from a newly isolated thermophilic *Bacillus* s train A30-1 (ATCC 53841). J. Ferment. Bioeng., 79: 433-438.

Won, K., J.K. Hong, K.J. Kim and S.J. Moon, 2006. Lipase-catalyzed enantioselective esterification of racemic ibuprofen coupled with pervaporation. Process Biochem., 41: 264-269.

Yamada, K., N. Watanabe, Y. Ota and Y. Minoda, 1977. Isolation and identification of alkaline lipase producing microorgamisms, cultural conditions and some properties of crude enzymes. Agric. Biol. Chem., 41: 1353-1358.