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Screening of bacterial strains for lipase production and its application in biodiesel synthesis

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Abstract
The qualitative screening of 46 bacterial isolates/strains for lipase production was carried out using Rhodamine olive oil agar assay. On this basis, four bacterial strains, *viz. Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Bacillus aryabhattai* were identified to be positive for lipase production. The lipase activity index (LAI) of the selected bacterial strains ranged from 1.57 to 2.90 with highest LAI observed for *P. aeruginosa* (2.90). *P.aeruginosa* exhibited significantly high lipase activity of 726.35, 505.33 and 355.98 pkat ml^{-1} in olive, karanj and jatropha oil liquid cultures, respectively. Among four bacterial strains, *P. aeruginosa* possessed significantly high lipase activity in all three oil liquid cultures. Olive oil served as most suitable carbon source for production of bacterial lipases followed by karanj and jatropha oil. The conversion of olive, karanj, jatropha and soybean oil into biodiesel (methyl ester) by using *P. aeruginosa* lipase as biocatalyst was 64.2, 59.7, 57.4 and 57.9 percent, respectively.

Keywords: Lipase, *P. aeruginosa*, olive oil, biodiesel

Introduction
The increasing severity of the global crisis, shortage of fossil fuels, increase in the crude oil prices, an increasing number of environmental problems and concerns to reduce pollution have resulted in rapid growth of research into alternative energy sources, as well as the use of such sources [1, 2]. Among potential alternate energy sources, biodiesel is an important substitute for petroleum-based diesel. The attractive features of biodiesel are that it is plant–derived and its combustion does not increase current net atmospheric levels of CO_2, a "greenhouse" gas [3, 4]. The vegetable or plant oils and animal fats cannot be used directly in diesel engine due to its high viscosity. These oils can be converted into biodiesel by various methods such as transesterification, blending, micro-emulsions and pyrolysis among which, transesterification is the most commonly used method [5, 6]. Chemically, biodiesel is produced by transforming triglycerides into fatty acid alkyl esters in the presence of alcohol such as methanol or ethanol and an acid or alkali catalyst, generating glycerol as a by-product [7, 8]. This process suffers from several inherent drawbacks such as being energy-intensive and involving environment unfriendly processing steps including catalyst and product recovery and waste water treatment [9].

Enzymes such as lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) are water- soluble enzymes having the ability to hydrolyse triacylglycerols to release free fatty acids and glycerol. Lipases represent an environment friendly alternative to chemical catalysts for biodiesel synthesis. The important characteristics of lipase catalyzed synthesis of biodiesel include its reusability, specificity, ability to accept new substrates, thermo-stability, mild reaction conditions and environmental friendliness [10, 11, 12].

Lipases have been isolated and purified from fungi, yeast, bacteria, plant and animal sources [13]. Many lipases are limited in use because they are substrate specific and regioselective. However, majority of the lipases are capable of converting triglycerides, diglycerides, monoglycerides and free fatty acids to fatty acid ethyl esters in addition to fat hydrolysis [1, 13]. Microbial lipases have gained special industrial attention due to their stability, selectivity and broad substrate specificity [14]. The demand for microbial lipases as biocatalyst with novel and specific properties is increasing day by day.

In the view of above facts, there is a need for extensive and rigorous screening of new microorganisms and their lipolytic enzymes that can provide novel alternatives for synthetic processes and consequently, open novel possibilities to solve environmental problems [15]. The present study was thus carried out to screen bacterial isolates/strains for novel lipases that can efficiently catalyze esterification/ transesterification reaction for biodiesel production from plant oils.

Material and Methods

Procurement of culture

Forty six (46) bacterial isolates/strains were procured from the Pulses Microbiology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Standard culture of *Pseudomonas fluorescens* MTCC 103 was procured from Institute of Microbial Technology, Chandigarh.

Qualitative screening of bacterial isolates/ strains for lipase production

The qualitative screening of forty six (46) bacterial isolates/strains and standard culture of *P. fluorescens* MTCC 103 for lipase production was carried out using Rhodamine-B olive oil agar (ROA) plate assay [16]. The growth medium contained (g/l): peptone, 5; beef extract, 1.5; yeast extract, 1.5; NaCl, 5 and agar, 20. The medium was adjusted to pH 7.0, autoclaved and cooled. Then, 31.25 ml of olive oil (sterile filtrated) and 10 ml of rhodamine B solution (1.0 mg/ml distilled water and sterilized by filtration) was added, followed by vigorous stirring. After this, 20 ml of media was poured into each Petri plate under aseptic conditions and allowed to solidify. The prepared plates were spot inoculated with different bacterial isolates and incubated at ±28˚C for 48 h. The plates were observed for the formation of orange fluorescent halos around bacterial colonies visible upon UV irradiation. The enzymatic activity was measured using lipase activity index (LAI), which is the ratio of total diameter of the orange halo to the diameter of the bacterial colony [17].

Determination of lipase activity of bacterial strains

The standard bacterial culture *P. fluorescens* (MTCC 103) and bacterial strains (4) identified after qualitative screening for lipase production were repeatedly sub cultured and maintained on nutrient agar slants. The extracellular lipase activity of selected bacterial strains was studied in minimal salt media using different oils as carbon source.

Lipase production

Lipase production in liquid culture was carried out by the method of Gokbulut and Arslanoglu [18] (2013) with slight modifications. The minimal medium used for lipase production consisted of 200 ml of 5X minimal salt solution ($Na_2HPO_4.7H_2O$, 64 g/l; KH_2PO_4, 15 g/l; NaCl, 2.5 g/l and NH_4Cl, 5 g/l), 800 ml of water and 0.02 percent gum arabic. The medium was adjusted to pH 7.0, autoclaved and cooled. Then 2 ml 1M $MgSO_4$ (sterile filtrated) and 0.1ml 1M $CaCl_2$ (sterile filtrated) was added to medium. Lipase production was carried out by transferring 10 ml of standard inoculum into 250 ml triplicate flasks containing 100 ml of minimal media broth supplemented with different oils, *viz*. olive, karanj and jatropha (1% w/v) as carbon source. The inoculated flasks were incubated at ±28˚C with constant shaking at 150 rpm in an orbital shaking incubator. The aliquots of culture broth were withdrawn at an interval of 24, 36, 48, 60, 72, 96 and 120 h and bacterial cells were harvested from broth by centrifugation at 10,000 rpm for 15 min at 4˚C. The cell free supernatant, thus, collected was used for assay of lipase activity.

Assay of lipase activity

Lipase activity assay in liquid cultures was performed spectro photometrically using p-nitrophenyl palmitate (p-NPP) as substrate by the method of Winkler and Stuckmann [19](1979) with slight modifications. The substrate solution consisted of solution A and solution B. Solution A was prepared by adding 40mg of p-NPP in 12 ml of isopropanol. Solution B was prepared by adding 0.1 g of gum arabic and 0.4 ml Triton X-100 in 90 ml of distilled water. Finally, 1 ml of solution A was added to 19 ml of solution B drop wise with continuous stirring to obtain a substrate emulsion that remained stable for 2 h. The reaction mixture for lipase assay contained 1 ml of substrate emulsion; 0.5 ml of buffer (50 mM Tris HCl, pH-8.0), 0.1 ml of crude enzyme and volume was made up to 3 ml with distilled water. The reaction mixture was incubated at 40˚C for 45 min in a temperature-controlled water bath. The reaction was stopped by adding 0.5 ml of isopropanol and the absorbance was measured at 410 nm against blank. The standard curve was prepared by using p-nitrophenol (0.01 to 0.2 µ moles). The amount of enzyme activity was expressed as pkat of p-nitrophenol (p-NP) produced per ml under standard assay conditions. The protein content of enzyme extracts was estimated by method of Lowry *et al.* [20] (1951). The specific activity was expressed as pkat of p-nitrophenol (p-NP) produced per mg protein.

Lipase catalysed biodiesel production from plant oils

Lipase catalysed transesterification of plant oils for biodiesel (methyl esters) production was carried out by the method of Aranisola [21] (2013) with some modifications. The reaction was performed in screw-capped bottles (50 ml) containing 10 ml of different oils, *viz*. olive, karanj, jatropha and soybean oil. In respective bottles, 10 ml of crude lipase was added. The bottles were kept in an incubator shaker at ±40˚C with constant shaking at 200 rpm. Then, 0.5 ml of methanol was added stepwise at an interval of 12, 24 and 36 h of reaction. After this, the reaction was stopped after 48 h by removing the respective bottles from incubator shaker. The product was centrifuged to separate the upper layer of biodiesel (methyl ester).

The estimation of percent conversion of oil into biodiesel was carried out by determining the total glycerides in the oil and unconverted glycerides in the ester (biodiesel) in terms of glycerol, which was obtained after saponification of the test sample.

Saponification was carried out by adding 0.8 ml of 33 percent aqueous potassium hydroxide and 20 ml of 95 percent ethyl alcohol to 0.5 ml of oil/ester sample in a round-bottomed flask. The mixture was refluxed for 90 minutes on boiling water bath. Immediately after refluxing, 20 ml of 2N HCl was added. Thereafter, the mixture was cooled and 40 ml of petroleum ether (40-60˚C) was added. The solution was thoroughly mixed again and allowed to settle down for about 20 min. The upper layer of the petroleum ether containing free fatty acid was discarded. The lower layer of ethanol water containing glycerol was evaporated to half the volume in order to remove the excess alcohol. Then, the volume of the solution containing glycerol was made up to 50 ml with distilled water.

Glycerol in the above solution was estimated by the method of Kates [22] (1972). To 0.2 ml of the sample, 1.8 ml of

distilled water and 0.1 ml of 10N H_2SO_4 was added, followed by the addition of 0.5 ml of 0.1M sodium periodate ($NaIO_4$). This mixture was mixed thoroughly and left at room temperature for 5 minutes. Thereafter, 0.5 ml of 10 percent sodium bisulfite ($NaHSO_3$) was added, mixed and placed a 0.5 ml aliquot in a glass-stoppered tube, followed by addition of 5 ml of chromotropic acid reagent (0.18%, 100mg dissolved in 10 ml distilled water and added to it 45 ml of 24N H_2SO_4). The contents were mixed thoroughly and the tubes were placed in the boiling water bath for approximately 30 min. The tubes were cooled and the absorbance of solution was recorded at 570 nm. Standard glycerol (0.1 to 0.6 µmole) and blank containing distilled water was also run simultaneously. Total glycerides in oil and unconverted glycerides left in the ester were estimated and the percent conversion of oil into biodiesel (methyl ester) was calculated by subtracting unconverted glycerides from the total glycerides.

Statistical Analysis

ANOVA was used to test the significance of difference among lipase activities of different bacterial strains by split plot design using CPCS1 software. The correlation coefficient between mean lipase activity and bacterial growth was also worked out.

Results and Discussion

Qualitative screening of lipase activity

The lipase activity of 46 bacterial isolates/strains and standard culture of *Pseudomonas fluorescens* MTCC 103 was assessed qualitatively using Rhodamine olive oil agar (ROA) plate assay after 48 h of incubation (Table 1). The 4 bacterial strains produced orange fluorescent halos, indicating the release of free fatty acid during growth on ROA plates (Plate 1). The four bacterial strains exhibiting lipase activity were identified as *Stenotrophomonas maltophilia*, *P. aeruginosa*, *P. fluorescens* and *B. aryabhattai*. Similarly, Kouker and Jaeger [16] (1987) screened lipase producing bacterial strains in a medium containing trioleoylglycerol and fluorescent dye Rhodamine B. The orange fluorescent halos were formed around the lipase producing bacterial strains, *viz. P. aeruginosa* PAC 1R, *P. aeruginosa* ATCC 9027, *P. aeruginosa* PAO, *Serratia marcescens*, *Staphylococcus aureus* and *B. subtilis* at 48 h of incubation. The bacterial strains such as *E. coli* showed no orange fluorescence upon UV irradiations. Stathopoulou *et al.* [23] (2013) screened 101 thermophilic bacterial strains for lipase activity using ROA plate assay and only 17 out of 101 (16.8%) strains showed positive test for lipase activity. Among them, nine bacterial strains, *viz.* SP14, SP22, SP29, SP75, SP76, SP79, SP83, SP93, and SP73 produced intense fluorescent halo in Rhodamine media and exhibited high extracellular lipolytic activity in liquid culture (> 0.75 nkat ml^{-1}).

Table 1: Qualitative screening of bacterial isolates/strains for lipase activity on Rhodamine olive oil agar (ROA) medium

Bacterial isolates/strains	Reaction to ROA plate assay	% of ROA positive
Pseudomonas aeruginosa (KF 853103), *Pseudomonas fluorescens* (KR08703.1), *Bacillus aryabhattai* (KF 853102), *Stenotrophomonas maltophilia* (KR080703.1) and *Pseudomonas fluorescens* MTCC 103 (standard)	+ ve (5 strains)	10.6
1P, 2P, 3P, 4P, 5P, 6P, 7P, 8P, 9P, 10P, 11P, 12P, 13P, 14P, 15P, 16P, 17P, 18P, 19P, 20P, 1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B, 9B, 10B, 11B, 12B, 14B, 15B, 16B, 17B, 18B, 19B, 20B, *Pseudomonas argentinensis* (JX 239745), *Klebsiella* sp. (KF 424316), *Staphylococcus aureus*	- ve (42 isolates/strains)	89.3

In ROA assay, qualitative evaluation for lipase activity was performed based on fluorescent halo production (+ ve: halo formation; - ve: no halo formation; P: *Pseudomonas*; B: *Bacillus*)

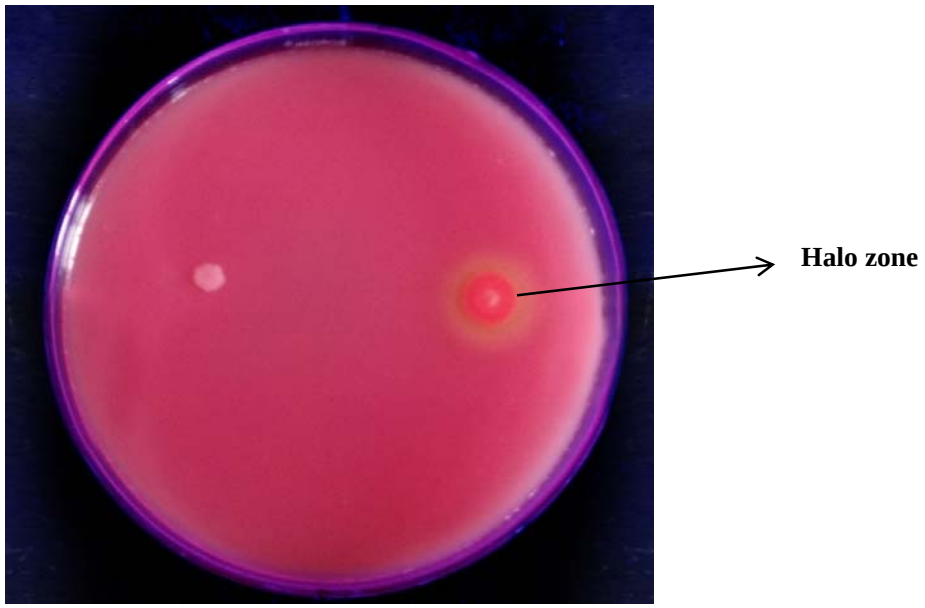

Plate 1: Lipase production on Rhodamine olive oil agar plate by bacterial strains

Lipase activity index (LAI) of four bacterial strains and standard bacterial culture which showed positive reaction to ROA plate assay was determined. The enzyme indices of these bacterial strains ranged from 1.57 to 2.90 (Table 2).The highest LAI of 2.90 was reported for *P. aeruginosa,*as compared to standard bacterial culture (2.33). *B. aryabhattai* was reported to have lowest enzyme index (1.57). Similarly, Willerding *et al*. [17] (2011) reported that LAI of selected bacterial strains on olive oil culture media ranged from 1.60 to 2.41. Nine bacterial strains, *viz.* R-024, R-016, R-048, U-027, E-170, E-005, E-100, E-139 and U-068 exhibited a higher or similar LAI to the reference strain *B. subtilis* ATCC 6633 with LAI of 1.80.

Table 2: Lipase activity index (LAI) of selected bacterial strains

Bacterial strain	LAI
P. aeruginosa	2.90
P. fluorescens	1.84
S. maltophilia	1.65
B. aryabhattai	1.57
P. fluorescens MTCC 103 (standard)	2.33

Lipase production by bacterial strains in liquid cultures

The lipase production by the four selected bacterial strains was studied in liquid cultures using the minimal salt medium supplemented with different oils, *viz*. olive, karanj and jatropha oil as carbon source. The lipase production by bacterial strains was expressed in terms of lipase activity (pkat of p-nitrophenol produced ml^{-1}) at different hours after incubation.

Lipase activity in olive oil liquid culture

The mean lipase activity of different bacterial strains in olive oil liquid culture ranged from 18.97 to 553.20 pkat of p-nitrophenol produced ml^{-1} at different time intervals (Table 3). Among the different bacterial strains, *P. aeruginosa* recorded significantly high mean lipase activity (553.20 pkat ml^{-1}), followed by *P. fluorescens* (394.92 pkat ml^{-1}) as compared to standard culture of *P. fluorescens* MTCC 103 (296.19 pkat ml^{-1}). However, the mean lipase activities of *S. maltophilia* and *B. aryabhattai* were reported to be significantly low (18.97 and 36.43 pkat ml^{-1}, respectively) as compared to the other bacterial strains. The mean lipase activity of bacterial strains at different hours after incubation (24 to 120 h) ranged from 50.14 to 348.79 pkat ml^{-1}. The mean lipase activity was significantly high (348.79 pkat ml^{-1}) at 72 h after incubation, followed by that at 96 and 120 h (335.94 and 317.29 pkat ml^{-1}) after incubation. The decrease in lipase activity at later stages could be due to changes in pH as well as proteolytic degradation of enzyme by proteases released into the culture medium at the end of exponential phase of growth [24].

The interaction of bacterial strains and time intervals revealed that there were significant differences in the lipase activities of individual bacterial strains at different hours after incubation. The bacterial strain, *P. aeruginosa* recorded significantly high lipase activity of 726.35 pkat ml^{-1} at 72 h after incubation, followed by *P. fluorescens*, with highest lipase activity 536.97 pkat ml^{-1} at 96 h after incubation. The standard bacterial culture of *P. fluorescens* MTCC 103 and *S. maltophilia* recorded their highest lipase activities of 438.93 and 29.48 pkat ml^{-1} at 72 h after incubation, respectively. However, *B. aryabhattai* exhibited its highest lipase activity of 48.90 pkat ml^{-1} at 96 h after incubation. Similar trend was observed for specific activity of lipase of different bacterial strains in olive oil liquid culture at different hours after incubation. Stathopoulou *et al.* [23] (2013) studied the lipase production pattern of nine thermophillic bacterial strains in minimal media supplemented with various carbon sources. Among different carbon source, olive oil induced significant lipase production in all the nine strains with maximum lipase production (4.61 nkat ml^{-1}) in bacterial strain SP75.

Table 3: Lipase activity (pkat p-nitrophenol ml^{-1}) of bacterial strains in olive oil liquid culture at different hours after incubation

Bacterial strain	Incubation time (h)							Mean
	24	36	48	60	72	96	120	
Pseudomonas aeruginosa	88.46 (1010.28)	451.63 (1251.59)	583.48 (1316.87)	595.22 (1345.90)	726.35 (1444.58)	717.24 (1404.03)	710.05 (1379.12)	553.20
Pseudomonas fluorescens	68.32 (994.50)	259.86 (1018.53)	407.28 (1234.16)	470.09 (1282.57)	502.45 (1335.32)	536.97 (1372.90)	519.47 (1343.83)	394.92
Stenotrophomonas maltophilia	8.39 (199.51)	12.22 (239.81)	17.98 (414.35)	20.62 (431.32)	29.48 (435.29)	22.53 (343.18)	21.57 (324.24)	18.97
Bacillus aryabhattai	20.85 (497.34)	30.44 (735.22)	37.16 (828.57)	39.31 (834.28)	46.74 (935.34)	48.90 (966.84)	31.64 (839.02)	36.43
Pseudomonas fluorescens MTCC 103 (standard)	64.72 (878.75)	234.68 (1009.06)	267.52 (1079.96)	409.68 (1162.30)	438.93 (1170.10)	354.07 (1054.63)	303.72 (990.69)	296.19
Mean	50.14	197.76	262.68	306.98	348.79	335.94	317.29	
CD 5 (p=0.05)	Strain (S)	2.29						
	Time interval (T)	3.32						
	S x T	7.42						

Each value is mean of triplicates

Values in parenthesis represent specific activity of enzyme

Lipase activity in karanj oil liquid culture

The mean lipase activity of different bacterial strains in karanj oil liquid culture ranged from 40.06 to 383.58 pkat ml^{-1} at different time intervals (Table 4). Among the different bacterial strains, *P. aeruginosa* exhibited significantly high mean lipase activity (383.58 pkat ml^{-1}), followed by standard culture of *P. fluorescens* MTCC 103 (301.91 pkat ml^{-1}) and *P. fluorescens* (261.46 pkat ml^{-1}). However, the mean lipase activity of *B. aryabhattai* and *S. maltophilia* was observed to be significantly low (40.06 and 44.51 pkat ml^{-1}, respectively) as compared to the other bacterial strains.

The mean lipase activity of bacterial strains at different hours after incubation (24 to 120 h) ranged from 66.06 to 274.48 pkat ml^{-1}. The mean lipase activity was significantly high

(274.48 pkat ml^{-1}) at 72 h after incubation, followed by that at 96 h after incubation (261.39 pkat ml^{-1}).

The interaction of bacterial strains and time intervals revealed that there were significant differences in the lipase activities of individual bacterial strains at different hours after incubation. The bacterial strain, *P. aeruginosa* was found to have significantly high lipase activity of 505.33 pkat ml^{-1} at 96 h after incubation, followed by standard bacterial culture of *P. fluorescens* MTCC 103, with lipase activity of 414.24 pkat ml^{-1} at 72 h after incubation. The bacterial strains of *P. fluorescens* and *S. maltophilia* recorded their highest lipase activities of 374.92 and 52.02 pkat ml^{-1} at 72 and 96 h after incubation, respectively. However, *B. aryabhattai* exhibited its highest lipase activity of 49.86 pkat ml^{-1} at 72 h after incubation. Similar trend was observed for specific activity of lipase of different bacterial strains in karanj oil at different hours after incubation. Kalyani and Saraswathy [25] (2014) reported that the lipase produced by isolate LB5 cleaved all the tested non-edible oils with highest affinity to punnakka oil (7.36 U/ml) followed by karanja oil. The lipases produced by the bacterial strains are generally induced in the medium that contained adequate amount of all fatty acids and oils. The carbon sources i.e. lipidic or non-lipidic substrates trigger the associated genes responsible for lipase proteins [26].

Table 4: Lipase activity (pkat p-nitrophenol ml^{-1}) of bacterial strains in karanj oil liquid culture at different hours after incubation

Bacterial strain	Incubation time (h)							Mean
	24	36	48	60	72	96	120	
Pseudomonas aeruginosa	104.03 (1026.26)	302.76 (1104.64)	444.68 (1181.02)	446.12 (1200.63)	486.39 (1258.36)	505.33 (1265.14)	395.78 (1095.70)	383.58
Pseudomonas fluorescens	66.88 (866.99)	210.47 (973.16)	230.13 (1023.50)	277.59 (1104.14)	374.92 (1156.17)	349.03 (1031.76)	321.23 (1012.88)	261.46
Stenotrophomonas maltophilia	36.67 (827.73)	36.91 (822.93)	43.86 (881.77)	46.02 (880.72)	46.98 (898.51)	52.02 (929.81)	49.14 (869.64)	44.51
Bacillus aryabhattai	34.76 (745.59)	39.79 (834.03)	40.75 (834.51)	45.06 (839.02)	49.86 (890.41)	36.19 (703.85)	34.04 (679.26)	40.06
Pseudomonas fluorescens MTCC 103 (standard)	87.98 (952.43)	229.17 (989.03)	312.12 (1085.90)	357.42 (1143.50)	414.24 (1169.37)	364.38 (1068.11)	348.08 (1017.77)	301.91
Mean	66.06	163.82	214.31	234.44	274.48	261.39	229.65	
CD 5 (p=0.05)	Strain (S)	2.13						
	Time interval (T)	2.44						
	S x T	5.47						

Each value is mean of triplicates

Values in parenthesis represent specific activity of enzyme

Lipase activity in jatropha oil liquid culture

The mean lipase activity of different bacterial strains in jatropha oil liquid culture ranged from 18.69 to 244.84 pkat ml^{-1} at different time intervals (Table 5). Among the different bacterial strains, *P. aeruginosa* recorded significantly high mean lipase activity (244.84 pkat ml^{-1}), followed by standard culture of *P. fluorescens* MTCC 103 (215.54 pkat ml^{-1}) and *P. fluorescens* (186.09 pkat ml^{-1}). However, the mean lipase activity of *S. maltophilia* and *B. aryabhattai* were observed to be significantly low (18.69 and 28.73 pkat ml^{-1}, respectively) as compared to the other bacterial strains.

Table 5: Lipase activity (pkat p-nitrophenol ml^{-1}) of bacterial strains in jatropha oil liquid culture at different hours after incubation

Bacterial strain	Incubation time (h)							Mean
	24	36	48	60	72	96	120	
Pseudomonas aeruginosa	86.54 (993.09)	260.57 (1070.62)	307.08 (1138.58)	316.67 (1161.73)	355.98 (1205.29)	260.51 (1140.28)	126.57 (1040.03)	244.84
Pseudomonas fluorescens	66.88 (837.37)	196.33 (936.19)	200.65 (1009.00)	257.70 (1090.67)	287.18 (1123.15)	187.70 (848.78)	106.19 (802.76)	186.09
Stenotrophomonas maltophilia	7.43 (168.84)	11.98 (233.06)	17.98 (405.90)	20.38 (419.51)	27.09 (427.07)	23.49 (344.01)	22.53 (334.17)	18.69
Bacillus aryabhattai	12.94 (272.73)	21.09 (439.49)	26.13 (444.01)	32.12 (682.17)	44.11 (867.33)	35.96 (708.69)	28.77 (671.40)	28.73
Pseudomonas fluorescens MTCC 103 (standard)	77.90 (940.24)	230.85 (1004.95)	280.71 (1038.59)	287.90 (1137.23)	301.57 (1179.33)	212.63 (976.68)	117.22 (837.28)	215.54
Mean	50.34	144.16	166.51	182.95	203.18	144.05	80.25	
CD 5 (p=0.05)	Strain (S)	1.85						
	Time interval (T)	1.84						
	S x T	4.12						

Each value is mean of triplicates

Values in parenthesis represent specific activity of enzyme

The mean lipase activity of bacterial strains at different hours after incubation (24 to 120 h) ranged from 50.34 to 203.18 pkat ml^{-1}. The mean lipase activity was significantly high (203.18 pkat ml^{-1}) at 72 h after incubation and declined thereafter. The interaction of bacterial strains and time intervals revealed that there were significant differences in the lipase activities of individual bacterial strains at different hours after incubation. The bacterial strain, *P. aeruginosa*

recorded its significantly high lipase activity of 355.98 pkat ml^{-1} at 72 h after incubation, followed by standard bacterial culture of *P. fluorescens* MTCC 103, which recorded its highest lipase activity (301.57 pkat ml^{-1}) at 72 h after incubation. The other bacterial strains *viz., P. fluorescens*, *B. aryabhattai* and *S. maltophilia* were found to have high lipase activities of 287.18, 44.11 and 27.09 pkat ml^{-1}, respectively at 72 after incubation. A similar trend was observed for specific activity of lipase of different bacterial strains in jatropha oil liquid culture at different hours after incubation. Thakur *et al*. [27] (2014) used different lipidic (0.5 v/v) and non-lipidic (0.5 w/v) substrates to enhance lipase production by *P. stutzeri* MTCC 5618 in jatropha oil production medium. The bacterial strain showed maximum biomass formation of 8.8 log cells ml^{-1} and intracellular enzyme production of 68 U mg^{-1} in jatropha oil containing production medium at 72 h of fermentation.

Comparison of different oils as carbon sources for lipase production

The comparison of different oils as carbon source for lipase production by bacterial strains has been depicted in Fig.1. On the basis of mean lipase activities of different bacterial strains evaluated, olive oil served as the most suitable carbon source for the production of bacterial lipases, followed by karanj and jatropha oil. This may probably be due to higher content of oleic acid in olive oil [28] as compared to jatropha and karanj oil, thus indicating the preferential use of this fatty acid (oleic acid) by the bacterial strains. Among the different bacterial strains, *P. aeruginosa* was found to possess significantly high mean lipase activity in all three liquid oil cultures.

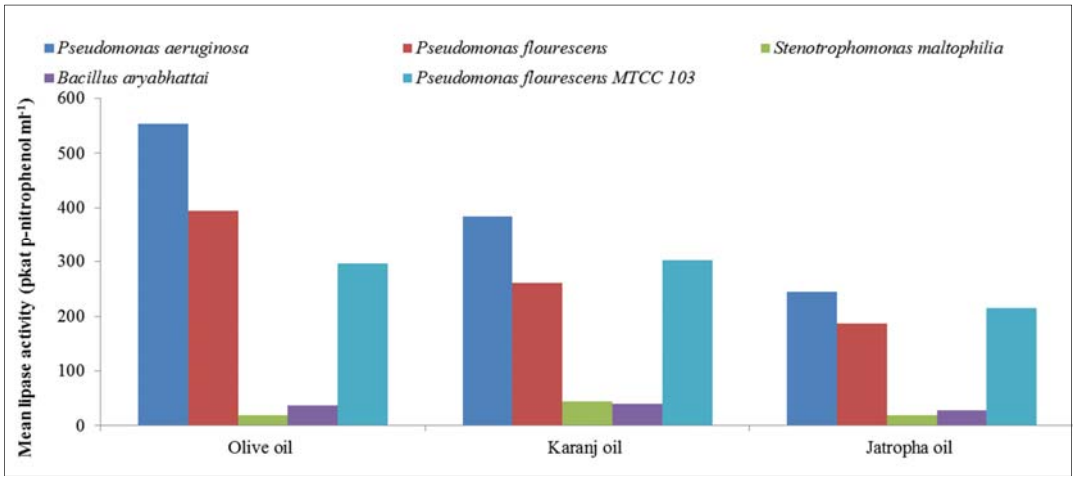

Fig 1: Comparison of different oils as carbon source for lipase production

Percent conversion of oil into biodiesel (methyl ester)

The crude lipase produced from *P. aeruginosa* was used as biocatalyst for transesterification of plant oils, *viz*. olive, karanj, jatropha and soybean oil. With crude *P. aeruginosa* lipase, the percent conversion of olive, karanj, jatropha and soybean oil into methyl ester was observed to be 64.2, 59.7, 57.4 and 57.9 after 48 h of transesterification reaction (Fig.2). The three different lipases, *viz. Chromobacterium viscosum, C. rugosa* and *Porcine pancreas* were used as biocatalyst for transesterification of jatropha oil to produce biodiesel by Shah *et al.* [29] (2004). The maximum yield of 62 percent of ethyl ester was obtained with *Chromobacterium viscosum* lipase after 8 h of reaction.

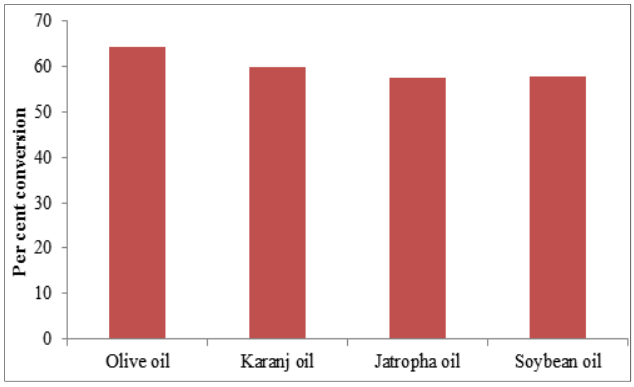

Fig 2: Percent conversion of plant oils into biodiesel (methyl ester) by lipase from *P. aeruginosa*

Conclusion

The present study revealed that among different bacterial strains, *P. aeruginosa* was an efficient producer of lipase in all three oil liquid cultures and thus, can be used as a novel biocatalyst for biodiesel synthesis.

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