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Enantioselective transesterification of (*RS*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol using *Pseudomonas aeruginosa* lipases

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Abstract—Lipases from the bacterial strain, *Pseudomonas aeruginosa*, isolated from the soil by enrichment techniques, are assessed for the enantioselective transesterification of (*RS*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol (*rac*-CDPP) to (*R*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol, a key intermediate in the synthesis of the chiral drug (*S*)-Lubeluzole. The lipases produced by the organism yielded the (*S*)-ester and the (*R*)-alcohol as the remaining substrate with an excellent yield (>49.9%) and almost complete enantioselectivity (ee >99.9%) in the presence of vinyl butyrate as an acyl donor in an organic medium. In contrast, purified and expensive commercially available lipases of *Candida rugosa* and porcine pancreas achieved much lower conversion with enantioselectivities of 15% and 5%, respectively. A well-mixed (~1000 rev min^{-1}) batch reactor having the aqueous phase finally dispersed in hexane was used in these studies. The parameters of the transesterification reaction were optimized and the optimal concentrations of *rac*-CDPP and vinyl butyrate were found to be 5 and 150 mM at 30 °C. A preparative-scale reaction yielded the (*S*)-ester at 42% conversion and ee >99%.

1. Introduction

Biocatalysts are increasingly being used to synthesize complex molecules of industrial interest.[1,2] The biggest role of biocatalysis still remains in the pharmaceutical sector where the exquisite regioselective and stereoselective properties of biocatalysts enable the difficult synthesis.[3] In the manufacturing of modern pharmaceutical drugs and in the drug discovery process, chiral reagents and chiral building blocks play a pivotal role. For the synthesis of various drugs and drug intermediates, among the various chiral compounds, chiral alcohols represent a highly versatile and attractive group of chiral building blocks. However, the problem lies with the solubility of these chiral intermediates, most of the time they are not miscible in the aqueous phase. Organic synthesis employing the enzymes as a catalyst for *stereo*-controlled reactions has grown significantly as these are inexpensive and easy to operate under mild conditions of temperatures and pH.

Production of enantiomerically pure intermediates by the enzymatic processes requires large-scale screening programs that reveal a number of enzymes (hydrolases, oxidoreductases, aminotransferases, aldolases, monooxygenases and dioxygenases) with the ability to catalyze a large number of enantioselective reactions.[4] Among the various biocatalysts, lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) have provided chemists with the opportunity for the development of novel chemical synthesis.[5] The screening of microbial strains found in soil samples is the best way to obtain microorganisms producing enzymes capable of carrying out a specific chemical reaction. To obtain the specific microorganism, soil samples are enriched with the substrate itself or its easily available derivative as a sole carbon source.[6]

Lubeluzole [(*S*)-4-(2-benzothiazolylmethylamino)-α-((3,4-difluorophenoxy)methyl)-1-piperidineethanol] is a novel benzothiazole compound that has shown neuroprotective activity in preclinical models of ischemic stroke.[7] Lubeluzole contains one stereocenter, and a retrosynthetic strategy reveals that enantiopure (*R*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol and *N*-methyl-*N*-(4-piperidinyl)-2-benzothiazolamine should be suitable building blocks for its synthesis.[8,9]

The synthesis of (*R*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol using commercially available (*R*)-epichlorohydrin and its reaction with 3,4-difluorophenol is

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reported.[8] The reaction is complete in 96 h with an average conversion of 48%. The chemically synthesized enantiomerically pure alcohol was then used for the convergent synthesis of (*S*)-Lubeluzole. Overall yields ranged from 20% to 35% with a moderate enantiomeric excess of 94%, which needs to be increased. There is precedent for synthesis of the enantiomerically pure alcohol through lipase catalyzed transesterification reaction, but on a very small scale[8] (<5 mM).

So far, there has been no report regarding the optimization of reaction conditions for the enzymatic resolution of *rac*-CDPP and its preparative-scale synthesis. Herein we report for the first time the transesterification of *rac*-CDPP to enantiopure (*R*)-alcohol using crude lipases from *Pseudomonas aeruginosa* MTCC 5113. The various parameters for the transesterification reaction have been optimized with the preparative-scale synthesis of (*R*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol, for its subsequent use in the production of anti-ischemic drug, Lubeluzole.

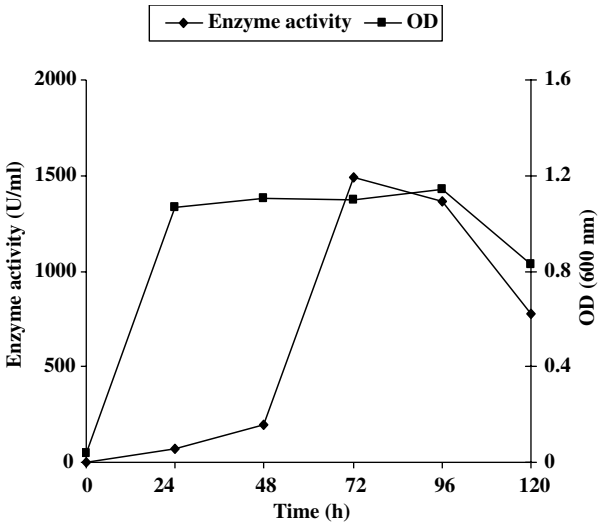


Figure 1. Time-course of growth and enzyme production by lipases from *P. aeruginosa*.

2. Results and discussion

2.1. Screening of lipase producer

The screening was carried out as described in Section 4, using 3,4-difluorophenol as the sole carbon source. We successfully isolated 20 lipase-producing microorganisms. All of these were tested for their enantioselective transesterification capabilities on *rac*-CDPP. Among them, only one strain gave moderate conversion (15%) with excellent (*S*)-enantioselectivity (ee >99%). Morphological, biochemical and physiological tests for the identification of the organism isolated were carried out in the Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology, Chandigarh. It was found that the isolated organism was a bacterial strain, *P. aeruginosa*. All the taxonomic studies of the organism indicated that it is the same organism (99% similarity), which was earlier isolated in our laboratory and deposited in MTCC at the Institute of Microbial Technology, Chandigarh, under the accession number 5113.[10] Previously, the same organism *P. aeruginosa* was used for the enantiospecific hydrolysis of methoxyphenyl glycidic acid methyl ester (±)-MPGM.[11] To further characterize the new isolate, the time-course of the enzyme production and growth was monitored by cultivating the organism in the production medium and performing the enzyme assay as described in Section 4. As seen in Figure 1, lipase activity started increasing after 24 h of incubation and continued up to 72 h, thereafter, there was a fall in the enzyme activity.

2.2. Time-course for transesterification

The time-course of the transesterification of *rac*-CDPP for the production of (*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-butanoate is shown in Figure 2. The reaction was allowed to proceed for 50 h and the conversion and ee were calculated. The conversion of alcohol to ester increased with time, while the ee decreased as commonly observed in such

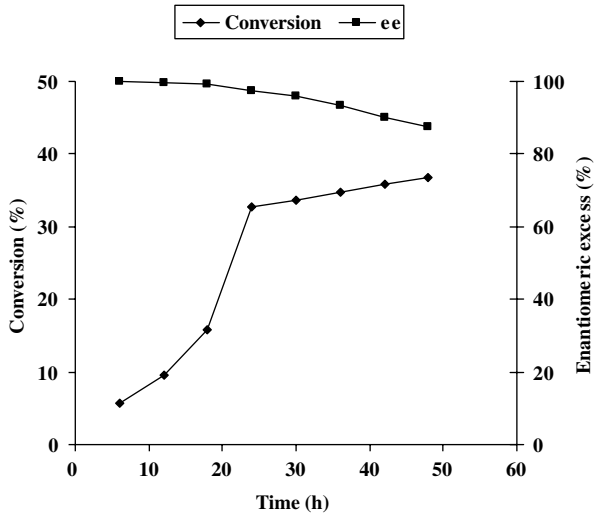


Figure 2. Time-course of conversion and enantioselectivity of (*RS*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol by crude lipase of *P. aeruginosa* at 30 °C.

biotransformations. Therefore, all the subsequent reactions were carried out up to 24 h.

2.3. Effect of temperature

The transesterification of *rac*-CDPP by lipase from *P. aeruginosa* was examined at different temperatures ranging from 20 to 60 °C. Both above and below 30 °C, a decrease in conversion was observed. All the subsequent experiments were carried out at 30 °C (Table 1).

2.4. Effect of solvents

Keeping in mind the effect of organic solvents on lipase activity as well as the specificity and insolubility of the substrates in aqueous phase, various solvents with different

Table 1. Effect of temperature on the conversion of *rac*-CDPP

Temperature (°C)	Conversion (%)
20	28.57
30	40.05
40	39.45
50	37.01
60	35.02

log *P* (*P* is octanol–water partition coefficient for the solvent) values were selected. Since solvents with high log *P* values stabilize lipases compared to the solvents with low log *P* values, six different types of solvents, that is, hexane, heptane, toluene, benzene, tetrahydrofuran (THF), and water-miscible solvent like acetonitrile (ACN), were assessed for the lipase catalyzed transesterification reaction (Fig. 3). Hexane was found to offer the maximum conversion rate when compared to the others.

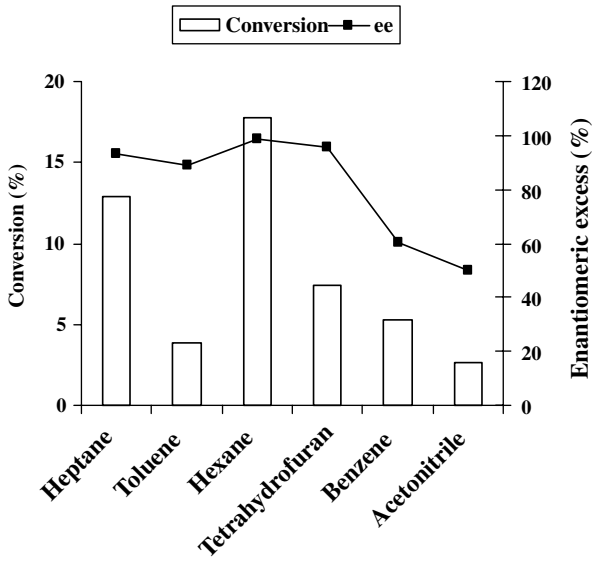


Figure 3. Effect of solvent type on the transesterification reaction of *rac*-CDPP by *P. aeruginosa* lipase at 30 °C. [All reaction mixtures had 50% (v/v) of organic solvent and the initial substrate concentrations were 5 mM (*rac*-CDPP) and 150 mM (vinyl butyrate).]

As reported by Goswami and Goswami in 2005, using dimethyl sulfoxide (DMSO) as a co-solvent enhances the enantioselectivity as well as the catalytic activity of lipase-mediated transesterification of chiral alcohols.[12] In view of this effect, the reactions were also evaluated using DMSO (5%) as a co-solvent. This did not have any significant effect on the conversion rate when hexane, heptane, benzene, and ACN were individually used as solvents. It was indeed observed that when 5% DMSO was supplemented into THF, the conversion rate as well as the ee increased (Fig. 4); however, the conversion rate was still lower than that in hexane. Consequently, hexane was used in all the subsequent experiments, in view of its superior performance.

2.5. Effect of *rac*-CDPP concentration on transesterification

The substrate concentration in the reaction medium influences sufficient expression of the enzyme activity, or may

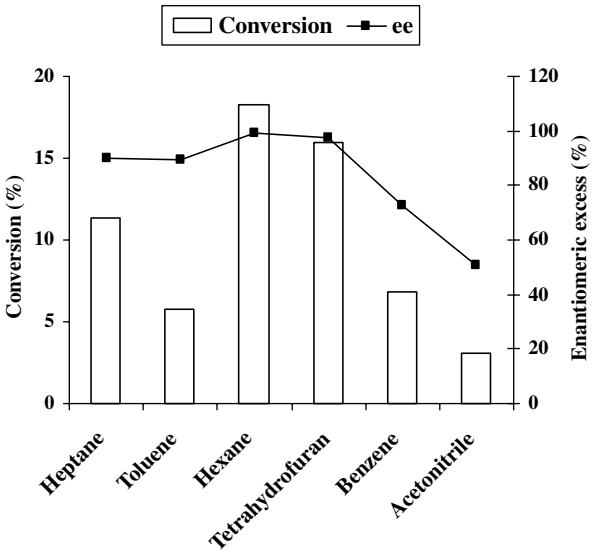


Figure 4. Effect of addition of DMSO (5%) in different organic solvents.

result in substrate inhibition. Therefore, the effect of substrate concentration on the conversion rate is a very important parameter, worthy of careful investigation. Several concentrations of *rac*-CDPP (2–8 mM) were used for the transesterification reaction at 30 °C while keeping constant amount of the following components in the reaction mixture: vinyl butyrate (150 mM), hexane (25 ml), enzyme (25 ml). The experiments were carried out in triplicate and the samples were withdrawn after every 6 h up to 24 h. It is clear from Figure 5 that the final conversion was highest when the initial concentration of *rac*-CDPP was 5 mM. However, there was a decrease in conversion rate beyond 5 mM, suggesting the toxicity of *rac*-CDPP in higher concentration. Also, with the increase of *rac*-CDPP concentration in the reaction mixture, there was a decrease in the enantiomeric excess, indicating changing

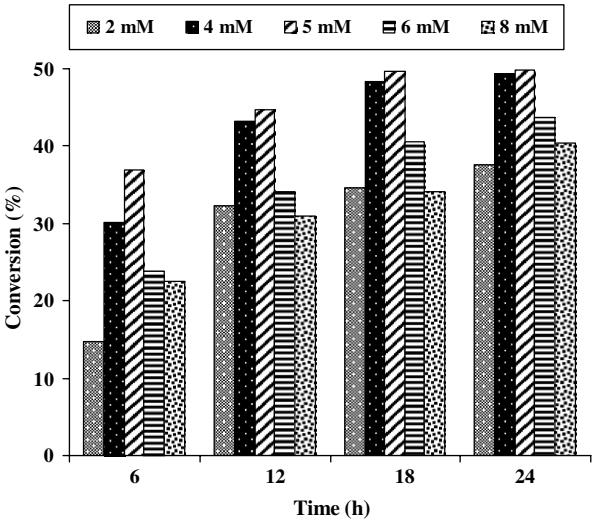


Figure 5. Effect of substrate concentration on the synthesis of (*S*)-butyric acid 1-chloromethyl-2-(3,4-difluorophenoxy)-ethyl ester by lipases from *P. aeruginosa* at 30 °C.

of the enzyme affinity for the (*S*)-enantiomer as compared to its counterpart, that is, the (*R*)-enantiomer (Fig. 6).

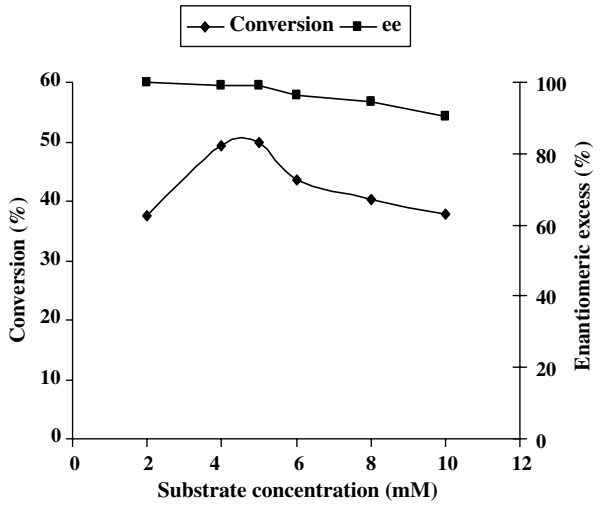


Figure 6. Effect of substrate concentration on the conversion and enantioselectivity of (*R*,*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol by lipases from *P. aeruginosa*.

2.6. Effect of vinyl butyrate concentration

Figure 7 shows the effect of vinyl butyrate concentration on the conversion of *rac*-CDPP. The concentration of vinyl butyrate was varied from 100 to 200 mM while keeping the constant concentrations of *rac*-CDPP (5 mM), hexane (25 ml), and lipase solution (25 ml) in the reaction mixture. It can be seen from Figure 7 that there was an increase in the conversion rate with an increase in vinyl butyrate concentration up to 150 mM and it decreased with a further increase of its concentration. This may be due to the decrease in surface area for *rac*-CDPP molecules at the interface of the reaction mixture at a higher concentration of vinyl

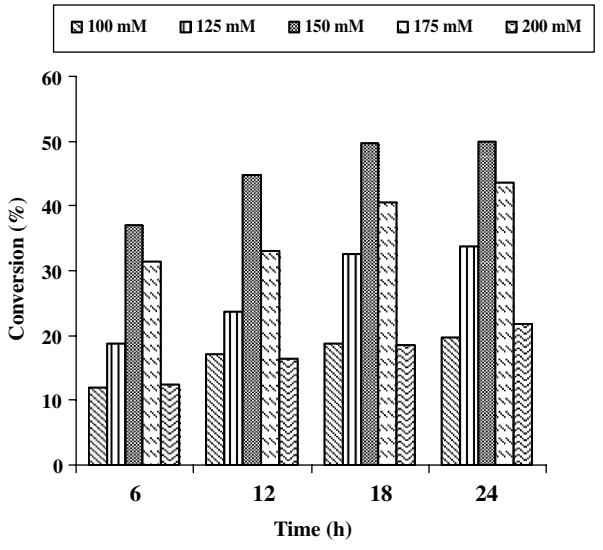


Figure 7. Effect of vinyl butyrate concentration on the transesterification reaction of *rac*-CDPP by lipases from *P. aeruginosa* at 30 °C.

butyrate. This is in contradiction with an earlier report,[13] where the conversion rate was insensitive to vinyl ester concentration.

2.7. Effect of various biocatalysts

Purified lipases from *C. antarctica* and porcine pancreatic are known to be excellent catalysts for transesterification reactions (Table 2); however, in the current study, *C. antarctica* lipase showed very low conversion (12%) as well as low enantioselectivity when compared to *P. aeruginosa* lipase. Pancreatic lipase also showed almost no conversion over 24 h. The higher activity of *P. aeruginosa* lipase is probably due to its stability in the presence of acetaldehyde. It has been reported that acetaldehyde liberated by the vinyl group of esters deactivates few enzymes through the formation of a Schiff base with lysine residue.[14–16] This may be the reason for the poor conversion rate and enantioselectivity of *C. antarctica* and porcine pancreatic lipase in the reaction mixture containing vinyl butyrate as acyl donor.

Table 2. Effect of lipases from different sources on the conversion of *rac*-CDPP

Enzyme source	Conversion (%)
Candida rugosa	15
Porcine pancreatic	5
Pseudomonas aeruginosa MTCC 5113	>49

3. Conclusion

The biocatalytic transesterification of racemic alcohols has been extensively explored and is now one of the best methods for the preparation of enantiomerically pure alcohols. However, the application of biocatalysts in such a process has been hampered by the unavailability of suitable biocatalysts. In our study, after a thorough screening of 20 lipase producers, we used the best one for the transesterification of *rac*-CDPP. This led us to a bacterial strain (*P. aeruginosa*) that can catalyze the stereoselective transesterification of *rac*-CDPP with almost absolute enantioselectivity to (*R*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol, a key intermediate of (*S*)-Lubeluzole.

The crude lipases from *P. aeruginosa* efficiently catalyzed the enantioselective transesterification of *rac*-CDPP. Studies illustrated that a solvent, such as ACN, having a low log*P* value decreases the reaction rate because such types of solvents are hydrophilic in nature and tend to rip off the water available for the enzyme activity. Also, DMSO (mild base) when added in minute quantities into the reaction mixture enhanced the conversion and enantioselectivity to a certain extent. Optimization of the substrate concentration in the reaction revealed that with an increase in substrate concentration, there is a substantial decrease in both the conversion and the ee. This could be due to the dead-end inhibition complex formation between lipases and *rac*-CDPP or might be due to the presence of different

lipases in the crude mixture possessing opposite stereochemical specificities and different affinities for *rac*-CDPP. The reaction conditions were optimized so that *rac*-CDPP was transesterified quantitatively and both the enantiomers could be separated with ee >99% on a preparative scale (conversion 42% and ee >99%). Thus, the lipases from *P. aeruginosa* were used here for the first time on a preparative scale and an efficient enzymatic resolution of *rac*-CDPP was developed.

In conclusion, it can be said that the stereoselective transesterification of *rac*-CDPP has been successfully carried out with the lipase from a new bacterial strain, *P. aeruginosa*. The result described here, in combination with the existing knowledge, makes the extracellular lipases from *P. aeruginosa* very interesting biocatalysts for further investigation, as well as for a variety of biotechnological applications. Purification of lipases and cloning of the lipase gene involved in the process and its application for the synthesis of certain other chiral compounds are currently in progress in our laboratory.

4. Experimental

4.1. Chemicals and medium

3,4-Difluorophenol, epichlorohydrine, Triton X-100, Tween-80, *p*-nitrophenyl palmitate and *p*-nitrophenol were procured from Sigma–Aldrich Corporation (St. Louis, MO, USA). Vinyl butyrate used in biological reactions was purchased from Fluka. *rac*-CDPP was synthesized in the laboratory and the structure was confirmed by ^{1}H and ^{13}C NMR.[17] Various components of growth media were obtained from Himedia (Mumbai, India). Cultures were grown in a medium containing peptone (0.5%), beef extract (0.5%), yeast extract (0.1%), NaCl (0.5%) and agar (1.5%). A minimal salt medium (MSM) used for the screening and growth of organisms under selection pressure includes dihydrogen potassium phosphate (0.1%), disodium orthophosphate (0.2%), ammonium chloride (0.04%), magnesium chloride (0.04%) with 3,4-difluorophenol as the sole carbon source. Agar plates were prepared by supplementing MSM with 2 mM 3,4-difluorophenol. Soil samples were collected from the different sites of an oil industry in Punjab, India. Various solvents were of HPLC grade and were obtained from Ranbaxy Fine Chemicals Limited (New Delhi, India). *Candida rugosa* lipase was obtained from Amano Pharmaceuticals Co. (Japan) and porcine pancreas lipase was purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA).

4.2. Chemical synthesis of (*RS*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol

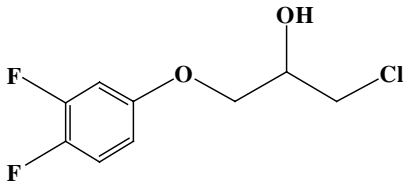
4.2.1. Synthesis of 2-(3,4-difluoro-phenoxymethyl)-oxirane. 3,4-Difluorophenol (1.0 g, 3.8 mM), epichlorohydrin (0.52 g, 5.7 mM) and potassium carbonate (1.05 g, 76 mM) or cesium carbonate were dissolved in acetonitrile (10 ml). The reaction was stirred at reflux for 16 h, extracted, dried over Na_2SO_4, and concentrated in vacuo. The reaction was carried out in acetonitrile to avoid the use of water in workup. The yield obtained was 95%. Low melting white solid, Yield: 95%, ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.00$–7.09 (m, 1H), 6.72–6.77 (m, 1H), 6.60–6.62 (d, $J = 6.96$ Hz, 1H), 4.19–4.23 (d, $J = 11.04$ Hz, 1H), 3.81–3.85 (d, $J = 10.98$ Hz, 1H), 3.31 (s, 1H), 2.89 (s, 1H), 2.73 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 155.34$, 166.52, 149.24, 147.33, 144.14, 177.83, 110.57, 104.89, 70.13, 50.42, 44.85. MS (ESI) $m/z = 186$ (M^+), 57 (100). On each occasion, the spectral data (IR, NMR, and MS) of the prepared known compounds were found to be identical with those reported in the literature.

4.2.2. Synthesis of *rac*-CDPP from 2-(3,4-difluoro-phenoxymethyl)-oxirane. *rac*-CDPP was synthesized by dissolving 3,4-difluorophenoxy methyl oxirane (1.0 g, 5.3 mM), lithium chloride (0.4 g, 9.5 mM) in acetic acid (1 ml) and THF (10 ml) at room temperature. The reaction was carried out for 6 h and 90% yield of the product was obtained. The reaction mixture was diluted with ethyl acetate (15 ml), washed with water (5 ml), dried over Na_2SO_4, and was concentrated under vacuum to afford (*RS*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol.

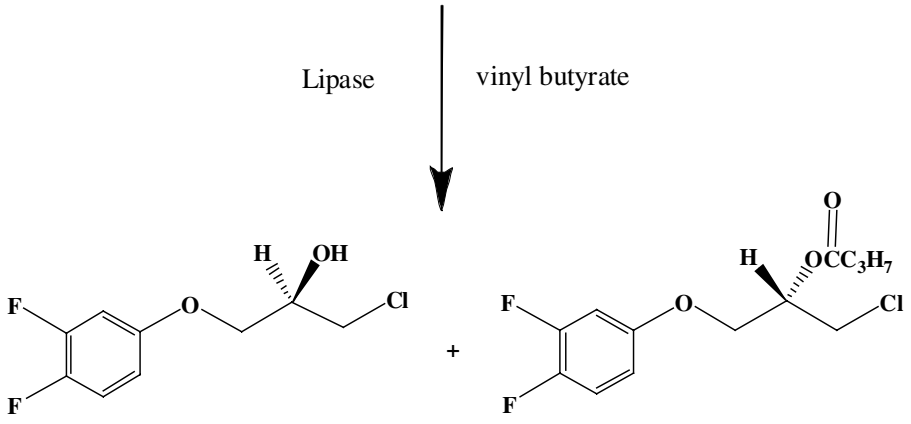
^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.02$–7.11 (q, 1H), 6.70–6.63 (m, 2H), 4.12–4.24 (m, 1H), 3.98–4.03 (m, 1H), 3.98–4.03 (d, $J = 5.106$ Hz, 1H), 3.68–3.79 (m, 1H), 2.92 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 155.09$, 152.620, 149.42, 147.52, 144.33. 118.37, 110.39, 104.92, 70.10, 46.33. MS (ESI) $m/z = 222$ (M^+), 130 (100); spectroscopic data were identical with an authentic sample.[8]

4.3. Isolation of microorganism

Soil samples were collected from various places in and around the oil industries in Punjab, India. A soil suspension was prepared by adding 10 ml of tap water to 1 g of the soil sample. This was vortexed and 1 ml of its supernatant used as an inoculum in 100 ml MSM containing 1 mM 3,4-difluorophenol or 1 mM *rac*-CDPP as a sole carbon source and incubated at 30 °C in an orbital shaker (200 rpm) for 4–5 days. A loop full of the broth was plated onto selective 3,4-difluorophenol and *rac*-CDPP plates. All positive isolates were transferred to nutrient agar plates (0.5% peptone, 0.1% (v/v) yeast extract, 0.5% beef extract, 0.5% sodium chloride, pH 8). The enzyme production was carried out using 1% (v/v) preculture (12 h) in 500 ml flask containing 100 ml production medium with 2 mM 3,4-difluorophenol or *rac*-CDPP as inducer. The production was carried out at 30 °C (200 rpm) for 24 h. Extracellular broth containing lipase was harvested by centrifugation (10,000*g*, 10 min) and enzyme activity and the enantioselectivity for *rac*-CDPP transesterification were determined. The reaction mixture was incubated at 30 °C (~1000 rpm). Samples (1 ml) containing solvent layer were withdrawn after every 3 h up to 48 h. The organic phase was dried and removed under reduced pressure. Finally, the conversion and ee of the (*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-butanoate formed were determined using chiral HPLC. The transesterification of *rac*-CDPP by *P. aeruginosa* is shown in Scheme 1.



1-Chloro-3-(3,4-difluoro-phenoxy)-propan-2-ol



(*R*)-1-Chloro-3-(3,4-difluoro-phenoxy)-propan-2-ol (*S*)-Butyric acid 1-chloromethyl-2-(3,4-difluoro-phenoxy)-ethyl ester

Scheme 1. Transesterification of (*RS*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol to (*S*)-butyric acid 1-chloromethyl-2-(3,4-difluorophenoxy)-ethyl ester by crude lipase from *P. aeruginosa*.

4.4. Enzyme production

A loop full of microorganisms maintained on nutrient agar plates (0.5%, w/v peptone; 0.15%, w/v yeast extract; 0.5%, w/v beef extract; 0.5%, w/v sodium chloride; 1.5%, w/v agar; pH 8) was transferred to 20 ml sterilized (121 °C, 20 min) nutrient broth (as above, without agar) to produce the seed culture. A 1% (v/v) seed inoculum was used to inoculate 100 ml nutrient broth in 500 ml shake flask and incubated at 30 °C (200 rpm, 96 h). Crude supernatant of the fermentation broth was separated from the cells by centrifugation (10,000*g*, 10 min). The supernatant was used as crude lipase preparation.

4.5. Enzyme assay

A modified form of the Winkler and Stuckmann method was applied to the quantification of lipase activity in the crude mixture of *P. aeruginosa*.[18] The substrate (*p*-nitrophenyl palmitate) was dissolved in 2-propanol (3 mg/ml) and an aqueous solution (9 ml) of gum arabic (0.11% w/v) and Triton X-100 (0.44% w/v) was added into the substrate solution. Intense agitation (vortex mixture) was used to emulsify the mixture. This emulsion (0.9 ml) was mixed with 1.5 ml tris–HCl buffer (50 mM, pH 8) and 0.5 ml $CaCl_2$ (75 mM). After 5 min of incubation, 100 μl of appropriately diluted (in 50 mM tris–HCl buffer, pH 8.0) enzyme solution was added. Incubation was continued for a further 10 min. The reaction was stopped by putting the test tubes in ice and the optical density was measured spectrophotometrically at 410 nm against substrate blank. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μmol *p*-nitrophenol per minute under the above specified assay condition.

4.6. Enantioselective transesterification of *rac*-CDPP

The transesterification reaction was carried out by extracellular crude lipase in 100 ml capacity stoppered flask containing substrate *rac*-CDPP and vinyl butyrate, that was magnetically agitated (~1000 rpm) and held at the desired constant temperature (30–60 °C) in an incubator for 24 h (Scheme 1). Samples (1 ml) were collected and processed, as described above, to monitor the conversion and enantioselectivity.

4.7. Optimization of transesterification reaction

The effect of various solvents (hexane, heptane, toluene, benzene, tetrahydrofuran, and water-miscible solvents such as acetonitrile) on the transesterification of *rac*-CDPP was evaluated. Unless otherwise specified, the total volume of the solvent was always 50% of the total volume of the reaction mixture. It has been reported that the addition of a trace amount of mild base enhances the enantioselectivity and catalytic activity in *Pseudomonas* lipase catalyzed resolution of racemic alcohols.[12] To evaluate the effect of the co-solvent on the transesterification reaction, a catalytic amount of denaturing organic DMSO was added into each solvent in separate experiments.

The optimum temperature was determined by incubating the lipase with the substrate at different temperatures in the range 20–60 °C. To find out the effect of substrate concentration, varying amounts of *rac*-CDPP (1–10 mM) were subjected to transesterification by *P. aeruginosa*. Finally, in order to optimize the concentration of the acyl donor, reactions were carried out by varying the concentrations of vinyl butyrate (100–200 mM) and keeping the other parameters constant. The samples were taken and analyzed for transesterification efficiency and enantioselectivity.

4.8. Preparative-scale transesterification

In order to scale-up the transesterification of *rac*-CDPP, when the reaction was carried out at a preparative scale with 3 g *rac*-CDPP, (*S*)-1-chloro-3-(3,4-difluorophenoxy)-2-butanoate was obtained at a conversion of about 42% with an ee >99%. This suggests that the crude lipase mixture of *P. aeruginosa* is a versatile biocatalyst, able to transesterify (*RS*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol with excellent enantioselectivity. Also, the results demonstrated promising prospects for the practical application of *P. aeruginosa* in the production of (*R*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol, a key intermediate in the synthesis of anti-ischemic drug (*S*)-Lubeluzole.

For the (*S*)-ester, $[\alpha]_D^{22} = +18.9$ (*c* 1, $CHCl_3$).Yellowish liquid, IR (Neat): 2965, 2934, 1743, 1609, 1518, 1462, 1435, 1327, 1262, 1216, 1204, 1162, 1101, 1043, 983, 963, 851, 786, 704 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.02–7.11$ (m, 1H), 6.71–6.77 (m, 1H), 6.60–6.63 (m, 1H), 5.31–5.34 (m, 1H), 4.13 (s, 1H), 4.11 (s, 1H), 3.81–3.85 (m, 1H), 3.73–3.78 (m, 1H), 2.33–2.38 (t, $J = 7.18$ Hz, 2H), 1.62–1.73 (m, $J = 7.16$ Hz, 2H), 0.83–0.87 (t, $J = 7.07$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 171.78$, 153.53, 150.99, 146.08, 116.42, 108.86, 103.61, 69.50, 65.81, 41.3, 34.99, 17.37, 13.09. MS (MALDI-TOF) $m/z = 315.50$ (M^+), 242 (100).

For the (*R*)-alcohol, $[\alpha]_D^{22} = -1.9$ (*c* 1.14, $CHCl_3$). IR (KBr): 3399, 2937, 2873, 1611, 1517, 1464, 1437, 1260, 1211, 1162, 1116, 1037, 957, 840, 783 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.01–7.11$ (m, 1H), 6.70–6.77 (m, 1H), 6.59–6.63 (m, 1H), 4.17–4.24 (m, 1H), 4.01–4.03 (m, 2H), 3.68–3.79 (m, 1H), 3.13 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 155.1$, 155.0, 152.6, 152.5, 149.4, 149.2, 147.5, 147.4, 144.4, 144.2, 117.9, 117.7, 110.4, 105.0, 104.7. The above spectroscopic data were in agreement with the literature.[8]

4.9. Effect of various biocatalysts

Purified lipases from different sources (*C. rugosa* and Porcine pancreatic) were evaluated for the transesterification of *rac*-CDPP with vinyl butyrate at 30 °C. The results for conversion and enantioselectivity were compared with the crude lipases from *P. aeruginosa*.

4.10. Analytical methods

Conversion and the enantiomeric excess of the transesterification reaction were monitored by HPLC performed on a Shimadzu 10AVP Instrument equipped with a UV detector using a Chiracel ODH column (0.46 mm diam., 250 mm long, 5 μm, Chiralcel). The mobile phase was hexane–isopropyl alcohol at 95:05 (v/v) with a flow rate of 0.5 ml/min and detected at 220 nm. The retention times of (*S*)- and (*R*)-1-chloro-3-(3,4-difluorophenoxy)-2-propanol were 23 and 24.7 min, respectively, and the retention time for the (*S*)-ester was 12.2 min in the Chiracel ODH column. The ee was defined as the ratio of $[R]-[S]/[R]+[S]\times 100\%$, where $[R]$ and $[S]$ are the concentrations of the (*R*)- and (*S*)-enantiomers, respectively. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance DPX 300 NMR spectrometer and optical rotations were measured on a Rudolph polarimeter (Rudolph Research Autopol IV).

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