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Investigation of steapsin lipase for kinetic resolution of secondary alcohols and synthesis of valuable acetates in non-aqueous reaction medium

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ABSTRACT

In present study, the application of steapsin lipase (as a biocatalyst) was investigated for kinetic resolution of secondary alcohols (1-phenyl ethanol and their derivatives) using vinyl acetate as an activated acyl donor. The enzymatic protocol was optimized for various reaction parameters such as effect of the molar ratio, solvent, temperature, time and biocatalyst loading to obtain best reaction conditions. On optimization, developed enzymatic methodology provided considerable enantiomeric excess of the product (up to 92% ee) at 55 °C in *n*-hexane as a solvent. Furthermore using the developed protocol, synthesis of several industrially important acetates was successfully achieved with excellent yield (up to 99%). During acetate synthesis, the biocatalyst was remarkably reused for eight consecutive recycles without any significant loss in its catalytic activity. This revealed the good potential of steapsin lipase for application in organic solvents.

1. Introduction

Biocatalysis has gained a special attention for the synthesis of several important biomolecules possessing a great application in pharmaceuticals, foods, textiles, flavour and fragrance industries [1,2]. Enzymes being highly specific in nature with regard to remarkable stereo-, regio- and chemo-selectivities, the enzymes from oxido-reductase and hydrolase family have achieved a special importance in the synthesis of chiral molecules [3,4]. Enantiomerically pure alcohols are one of the most important building blocks and chiral auxiliaries for the synthesis of bioactive drug molecules. In this regard, lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) have received an outstanding credit for kinetic resolution of secondary alcohols and chiral synthesis of several esters and amides mainly used in flavour and pharmaceutical preparations [1–4]. Among lipases, *Candida antartica* lipase B has been widely explored for chiral resolution of alcohols and amines in organic solvents as well in neoteric solvents like ionic liquids, supercritical fluids, polyethylene glycol or their combinations [5–7]. Several other lipases like *Candida rugosa* lipase, *Pseudomonas* sps. lipase, *Mucor miehei* lipase, Amano PS, Amano AK, *Bacillus subtilis* lipase, porcine pancreatic lipase (PPL) and many more are reported for kinetic resolution of racemic 1-phenyl ethanol in various non-aqueous reaction medium [8–11]. However, use of enzymes like lipases has always been a high cost issue and hence studies in recyclability of lipase for several recycles would make the enzymatic processes economically viable. Apart from lipase, esterase is also reported for kinetic resolution of racemic glycerol ester derivatives via selective hydrolysis [12].

On the other side, increasing demand of flavour and fragrance compounds in modern society surges the development in chemical and biocatalytic procedures rather than natural extraction method. Since the compound of interest (ester) isolated using extraction procedure is comparatively low and thus makes the process economically unviable. Moreover, increasing health consciousness encourages the development in enzymatic protocols as synthesis of these esters via biocatalysis endows them a green label [13]. Among various esters, acetates find a special interest as they are widely used in food and flavour industries. Recently, we reported the novel immobilization support as a film prepared using blend of hydroxypropyl methyl cellulose (HPMC) and polyvinyl alcohol (PVA) for *R. oryzae* lipase and was studied for the synthesis of several industrially important esters [14,15].

Steapsin lipase, a very well known digestive enzyme [16] has not yet been well studied for kinetic resolution of secondary alcohols. In past decades, Francalanci and co-workers [17] reported the kinetic resolution and chiral synthesis of amino alcohol via

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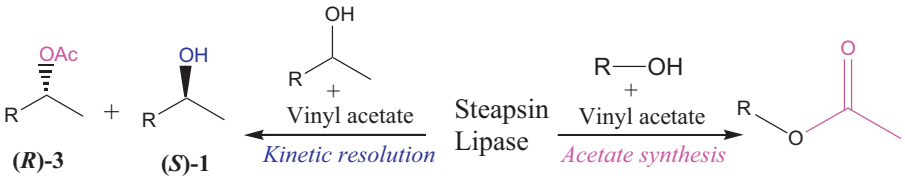

Fig. 1. Steapsin lipase catalyzed kinetic resolution of sec-alcohol and acetate synthesis.

transesterification followed by alkaline hydrolysis using steapsin lipase immobilized on celite. Growing awareness in lipase catalyzed reactions and remarkable development in enzyme immobilization procedures has made the steapsin lipase to be available as small globules like preparation insoluble in non-polar solvents (commercially made available by Sisco Research Laboratory). Recently Kumar et al., immobilized steapsin lipase on celite 545 and studied for ethyl ferulate synthesis using DMSO as an organic solvent [18]. However, from the literature we acquainted that steapsin lipase does not find a special attention for kinetic resolution application, considering which we investigated the fact.

In continuation to our interest on exploration of lipase for organic reactions [19,20], in present work we describe the first effort for investigation of steapsin lipase for kinetic resolution of 1-phenyl ethanol and their derivatives in organic solvent (Fig. 1). The reaction was optimized to obtain best reaction conditions for the resolution of secondary alcohols. Further, we employed steapsin lipase for the synthesis of various industrially important acetates.

2. Experimental

2.1. Enzymes and chemicals

The steapsin lipase (immobilized form, readily disperse in water/polar solvents, small globules with activity ≥20 U/mg) was purchased from Sisco Research Laboratories, Mumbai, India whereas *Candida antartica* lipase B immobilized on acrylic resin (with ≥10,000 U/g, recombinant, and expressed in *Aspergillus oryzae*) was purchased from Sigma–Aldrich, India. The (*R*)-menthol (ee 99%) was purchased from Sigma–Aldrich, India. The various substrates, chemicals and solvents used were purchased from firms of repute with their highest purity available and were used without any further treatment.

2.2. Synthesis of racemic phenyl ethanol derivatives

The substituted racemic phenyl ethanol derivatives were synthesized from corresponding acetophenone derivatives namely *p*-chloro acetophenone, *p*-methyl acetophenone using sodium borohydride ($NaBH_4$) as a reducing reagent with reported method in literature [21]. The authentic standards of (*S*)-(−)-1-phenyl ethanol and (*R*)-(+)-1-phenyl ethyl acetate were synthesized using CaL B lipase. The enantiomeric compounds were then purified using column chromatography and the purity was then examined by chiral gas chromatography. The sign of optical rotation were recorded using the digital polarimeter (JASCO, DIP-370) and were compared with reported literature values. The similar protocol was followed for preparation of authentic samples of other racemic secondary alcohols.

2.3. General procedure for steapsin lipase catalyzed kinetic resolution of secondary alcohols

In typical experimental procedure, 0.5 mmol of secondary alcohol and 3 mL of *n*-hexane were added to 10 mL glass stoppered tube. After gentle stirring, 2 mmol of vinyl acetate was added and to this 50 mg of steapsin lipase was added to initiate the reaction. The reaction was placed at 55 ± 1 °C in an orbital shaker at 160 rpm speed for a period of 24–48 h. All the experiments were carried out at least in duplicate and the mean values are herein reported. Progress of reaction was monitored by TLC/GC analysis and was then quantitatively analyzed using gas chromatography technique (Perkin-Elmer, Clarus 400) equipped with flame ionization detector (FID) and chiral capillary column CP-Chirasil-Dex CB Column (Varian capillary column, 25 m × 0.25 mm × 0.25 μm). The column temperature was kept at 80 °C for 3 min and then raised to 250 °C for 30 min with a rise of 1 °C/min. The temperature of the injector and detector was maintained at 220 °C and 240 °C, respectively. On completion of reaction, the reaction mixture was filtered and biocatalyst was thoroughly washed 2–3 times with *n*-hexane to remove any traces of reactant/product if remained adhered. The reaction mixture was then evaporated under vacuum at very low pressure. The oily residue obtained was then subjected for column chromatography (silica gel, mesh size 60–120) using pet ether:ethyl acetate (99:1) as eluent to afford pure products. All the products are well known in literature [22] and were compared with authentic samples and further characterized using FT-IR (Perkin-Elmer, Spectrum 100), GC–MS (Shimadzu QP 2010) and ^1H and ^{13}C NMR spectra recorded on NMR spectrometer (Varian-300) using TMS as internal standard. The sign of optical rotation was compared with the results obtained for authentic samples prepared using CaL B lipase and as well as from the values reported in literature. The % enantiomeric excess (ee%) was calculated as the ratio of $\{[S]-[R]/[S]+[R]\} \times 100$, where [*S*] and [*R*] are the concentration of (*S*) and (*R*) enantiomers respectively. The (%) conversion was calculated as $[ee_s/(ee_s + ee_p)] \times 100$. The enantiomeric ratio (*E*) was calculated as $\ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.

2.4. General procedure for steapsin lipase catalyzed acetate synthesis

The 1 mmol of primary alcohol was added to 3 mL of *n*-hexane in 10 mL glass stoppered tube. On gentle stirring, 5 mmol of vinyl acetate and 70 mg of steapsin lipase were subsequently added. The reaction tubes were then placed at 55 ± 1 °C in an orbital shaker at 160 rpm speed for a period of 24–72 h. All the experiments were carried out at least in duplicate and the mean values are herein reported. The progress of reaction was monitored by TLC/GC analysis and was quantitatively analyzed using gas chromatography technique (Perkin-Elmer, Clarus 400) equipped with flame ionization detector (FID) and a capillary column (Elite-1, 30 m × 0.32 mm × 0.25 μm). The column temperature was kept at 80 °C for 3 min and then raised to 250 °C for 30 min with a rise of 10 °C/min. Temperature of the injector and detector was maintained at 220 °C and 240 °C, respectively. After completion of reaction, the reaction mixture was filtered and the biocatalyst was thoroughly washed 2–3 times with *n*-hexane to remove traces of reactant/product if remained adhered. The reaction mixture was then evaporated under vacuum at very low pressure. The oily residue obtained was then subjected for column chromatography

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2.5. Recyclability study of steapsin lipase

The steapsin lipase was recycled for four consecutive recycles for kinetic resolution of 1-phenyl ethanol whereas eight consecutive recycles were studied for the synthesis of isoamyl acetate following the experimental procedure discussed in Sections 2.3 and 2.4 respectively. On completion of reaction, the reaction mixture was filtered and the biocatalyst was thoroughly washed 2–3 times with *n*-hexane to remove traces of reactant/product if remained adhered. The biocatalyst was dried at 42–45 °C for 10–12 h and then used for next recycle. All the experiments were carried out at least in duplicate and the mean values are reported.

2.6. Morphological analysis of the steapsin lipase biocatalyst

The photographic image of steapsin lipase (before use and after eight recycle) versus black background was captured using SONY Camera (12.1 megapixels, 4× zoom). To further elucidate the detailed physical appearance of beads, the SEM analysis (FEI, Quanta 200) of steapsin lipase before use and at the end of eight recycle was studied. This study was performed so as to investigate any breakage or major damage if occurred to the steapsin lipase globules due to more number of recycles.

3. Results and discussion

3.1. Application of steapsin lipase for kinetic resolution

Lipase catalyzed kinetic resolution of 1-phenyl ethanol is the most widely studied model substrate in literature [6–11], and thus was used to investigate the catalytic behaviour of steapsin lipase. The effect of various optimization parameters like molar ratio, solvent, temperature, biocatalyst loading and time was studied. The molar ratio has always played a vital role in transesterification reaction and hence was firstly considered [14]. The different molar ratio was studied ranging from 1:1 to 1:6 of alcohol to vinyl acetate. During the study, we observed that for molar ratio of 1:4 (alcohol: vinyl acetate) the ee_p (enantiomeric excess of product (*R*)-3) was optimum whereas further increase in molar ratio decreases ee_p (Fig. 2a). Also we observed that as the molar ratio increases from 1:1 to 1:4, the *E* value has also increased (from 13 to 66) while further increase in the molar ratio led to noticeable decrease in the *E* value (from 66 to 21) and hence molar ratio of 1:4 was selected for further study. Similar results were obtained by de Souza et al. for kinetic resolution of 1-phenyl ethanol using lipase Amano PS, where increase in certain molar ratio increased the conversion while further increased in molar ratio decreased the conversion [24]. During the study, we observed that the ee_p was optimum at 24 h of reaction time. The maximum *E* value was obtained at this time period while at lower time no considerable *E* value was observed, may be due to lower conversion (data not shown). From our previous studies [14,20], we observed that use of polar solvents has adverse effect on lipase activity as they have tendency to strip out the essential water from the surface of catalytic site while such was not the case with non-polar solvents. The non-polar solvent like *n*-hexane, toluene and cyclohexane were screened for the present study. The *n*-hexane ($\log P$ value = 3.5) [25] was found to be the best solvent for present reaction, whereas other solvents like toluene and cyclohexane hampered the ee_p to a certain extent (Fig. 2b). With regards to *E* value we found the optimum *E* value for *n*-hexane while for other solvents it decreased from 66 to 20, and hence *n*-hexane was the best suitable solvent for the present system. The similar observation was made for kinetic resolution of secondary alcohols with other sources of lipase by several research groups [10,24].

Enzymes being protein in nature are sensitive to higher temperature and thus effect of temperature was deliberated. We found that as temperature of the reaction was increased from 35 °C to 55 °C, ee_p also increased where the optimum ee_p was observed at 55 °C while further increase or decrease in temperature lowers the ee_p (Fig. 2c). Also, we recognized that the *E* value was optimum for 55 °C. The observed results are in acceptance to the literature data, where Xia et al. reported that increase in temperature increases the conversion of the reaction while further increase in temperature above a certain level decreases the ee_p of the reaction [10]. However, the optimum temperature for every different lipase catalyzed reaction is variable and thus optimization of reaction parameters is crucial to obtain the best result for the respective enzymatic protocol.

In order to obtain the optimum yield of desired (*R*)-ester, the biocatalyst loading was studied ranging from 30 mg to 90 mg (Fig. 2d). It was found that 50–70 mg of lipase concentration was sufficient, however 70 mg was considered for further study as the other substituted secondary alcohol would rather require higher biocatalyst loading. The more interesting results were obtained with 90 mg of biocatalyst loading where ee_s was increased than ee_p, this strategy could be applied for one's interest in isolation of either (*S*) or (*R*) enantiomer. As the amount of biocatalyst loading increases it tends to lose its selectivity for (*R*)-ester and hence the *E* value declines. Thus, amount of biocatalyst loading is quite important parameter to be considered during the optimization study. A control reaction in absence of enzyme was also performed, however we observed no product formation thus elucidating that steapsin lipase was essential to catalyze the reaction. Most of the time, the optimal performance of biocatalyst depends on the water activity of enzyme [26]. The solvents (i.e. *n*-hexane, toluene and cyclohexane) used for the present study were of hydrous grade, thus contained trace % of water which we believed was quite sufficient to maintain the essential water content for lipase activity. This assumption was further confirmed by controlling the water content of the reaction medium via drying agent like molecular sieves. Molecular sieves (4 Å) are most widely reported for such experiments [27]. The study was performed by addition of the molecular sieves (4 Å) ranging from 50 mg to 200 mg to make the reaction system anhydrous (Fig. 2e). During the study, we observed that as the concentration of the molecular sieves increases (above 50 mg) there is marginal decrease in the ee_p while the *E* value had drastically decreased (up to 27). This signifies that as the medium is dehydrated the steapsin lipase loses its selectivity, might be due to the removal of the essential water from the active site of enzyme. The water molecules present near the active site of enzyme are vital to maintain the three-dimensional structure of the protein, while loss of this essential water may lead to distortion in the protein conformation and thus finally loss in its catalytic activity [27].

Furthermore to investigate the general applicability of developed methodology, the kinetic resolution of substituted racemic 1-phenyl ethanol derivatives was studied (Table 1, entries 1–3). The (%) conversion was good for 1-phenyl ethanol (in 24 h) and *para*-methyl-1-phenyl ethanol (in 36 h) whereas for *para*-chloro-1-phenyl ethanol (in 48 h) the conversion has decreased with marginal low enantioselectivity. The presence of halogen like chlorine, an electron withdrawing group might have hindering effect on lipase activity and thus have led to lower conversion in case of

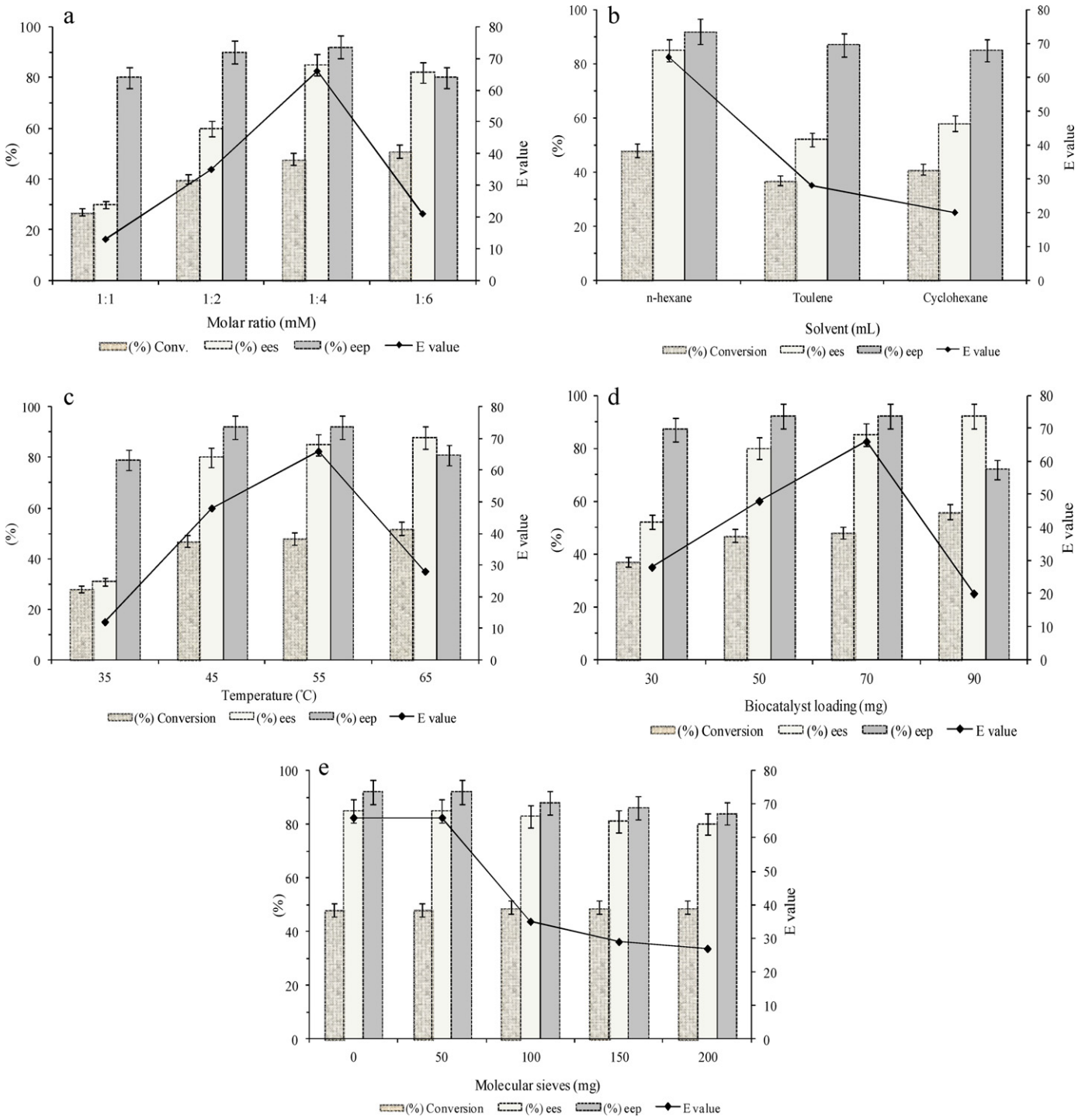


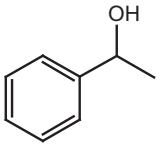
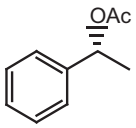
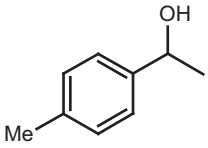
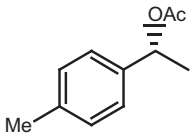
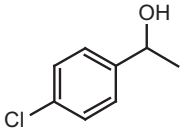
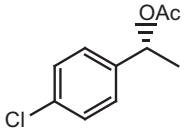
Fig. 2. Effect of various reaction parameters on steapsin lipase catalyzed kinetic resolution of secondary alcohol. Reaction conditions: *rac*-secondary alcohol (0.5 mmol), agitation speed (160 rpm), time (24 h). Conversion (%) = $[ee_s/(ee_s + ee_p)] \times 100$. ee (%) = $[\%(S) - \%(R)]/[\%(S) + \%(R)] \times 100$. $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.

para-chloro-1-phenyl ethanol. The similar effect was observed by Xia et al. for the wheat germ lipase catalyzed kinetic resolution of *para*-chloro-1-phenyl ethanol where the conversion was too low, however the selectivity reported was against the Kazlauskas route [10]. Moreover, to make the biocatalytic protocol more economical the recyclability of steapsin lipase was studied for four consecutive recycles (Fig. 3). We observed that as recycle runs are increased the enantiomeric excess of product ee_p has decreased although conversion (%) remained the same. Also, the decline in *E* value for the third and fourth recycle is observed.

3.2. Application of steapsin lipase for acetate synthesis

Apart from its kinetic resolution behaviour study, we employed the steapsin lipase for the synthesis of industrially important acetates using primary alcohols and vinyl acetate as an activated acyl donor. In order to obtain the best optimized reaction conditions, the transesterification reaction of isoamyl alcohol with vinyl acetate was selected as a model reaction. During optimization study, we observed that the optimized reaction conditions were very similar to the protocol developed for kinetic resolution

Table 1
Steapsin lipase catalyzed kinetic resolution of *rac*-secondary alcohols.[a]

Entry	Substrate *rac*-alcohol	Product (***R***)-3	Time (h)	Conv. (%)[b]	ee_s (%)[c] (***S***)-1	ee_p (%)[c] (***R***)-3	*E*[d]
1			24	48 ± 0.5	85 ± 1.1 (95 ± 1.2)	92 ± 1.4 (91 ± 1.4)	66
2			36	52 ± 0.5	92 ± 0.7 (92 ± 0.8)	86 ± 1.6 (95 ± 1.5)	40
3			48	26 ± 0.3	25 ± 0.9 (94 ± 1.8)	71 ± 1.5 (30 ± 1.6)	08

[a] Reaction conditions: *rac*-secondary alcohol (0.5 mmol), vinyl acetate (2 mmol), *n*-hexane (3 mL), steapsin lipase (70 mg), temperature (55 °C), agitation speed (160 rpm).
[b] Conversion (%) = $[ee_s/(ee_s + ee_p)] \times 100$.
[c] ee (%) = $[\%(S) - \%(R)]/[\%(S) + \%(R)] \times 100$. The GC yields are mentioned in parentheses.
[d] $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.

of secondary alcohols (data not shown). The time course of acetate synthesis was then studied for model reaction with defined interval period (Fig. 4). It was observed that the yield of isoamyl acetate increases as reaction time increased where maximum yield (99%) was obtained within 24 h. In present reaction system, the formation of acetate mainly depends on the three types of reaction going on i.e. firstly hydrolysis of vinyl acetate, then formation of desired product (i.e. iso amyl acetate) and thirdly hydrolysis of the product formed [28]. However, even on prolonged period (of 36 h), the yield of desired product was not altered.

Further to explore the potential of steapsin lipase as biocatalyst, the obtained optimized conditions were employed for transesterification of several aliphatic, alicyclic, allylic and aromatic alcohols using vinyl acetate as an acyl donor (Table 2). Contentedly, steapsin lipase was found to be a suitable biocatalyst for transesterification reaction providing various valuable acetates [29,30] with remarkable yields within 24–72 h. The straight chain aliphatic acetates like *n*-butyl acetate (found in fruits like red delicious apple), *n*-octyl acetate (fruity orange) were synthesized with remarkable yield (99%) within 24–48 h (Table 2, entries 1–2). While the branched aliphatic acetates such as isoamyl acetate (pear, banana flavour) one of the most widely used flavour compound in food industries and 2-ethyl-hexan-1-ol acetate were synthesized with excellent yield within 24–30 h (Table 2, entries 3–4). The 1, 4 butane diol was also subjected for the developed methodology and was found to provide di-acetate as a product in 24 h (Table 2, entry 5). One of the industrially valuable acetate i.e. citronellol acetate was synthesized with 94% yield using the developed enzymatic protocol (Table 2, entry 6). The substrates like 2-ethyl-hexan-1-ol and citronellol used in the present study were of racemic type and we didn't observe any significant chiral resolution of these compounds during the study, whereas the chiral (*R*)-menthol (ee 99%) was used for synthesis of its acetate derivative. The alicyclic alcohols like cyclopentanol, cyclohexanol, (*R*)-menthol were also studied and found to provide appreciable yield (57–77%) of desired product (Table 2, entries 7–9). The *R*-menthol is a naturally occurring enantiomercially pure compound, and its acetyl derivative finds a great application in food and flavour industries. The kinetic resolution of (R/S)-menthol was studied by Zhou et al., using Novozyme 435 in

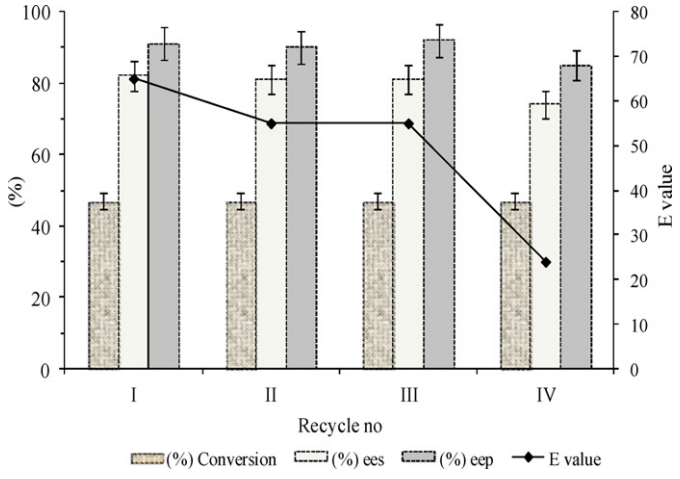


Fig. 3. Recyclability study of steapsin lipase for kinetic resolution of 1-phenyl ethanol. Reaction conditions: *rac*-secondary alcohol (0.5 mmol), vinyl acetate (2 mmol), *n*-hexane (3 mL), steapsin lipase (70 mg), temperature (55 °C), agitation speed (160 rpm), time (24 h). Conversion (%) = $[ee_s/(ee_s + ee_p)] \times 100$. ee (%) = $[\%(S) - \%(R)]/[\%(S) + \%(R)] \times 100$. $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.

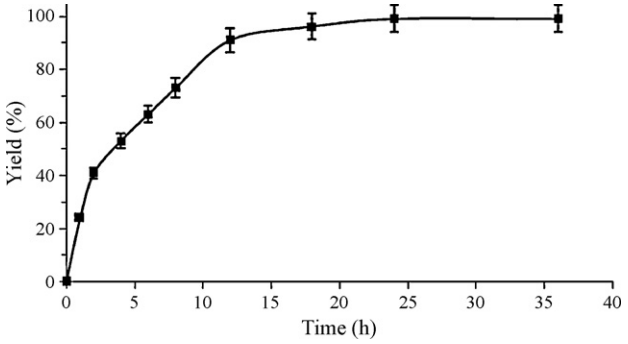


Fig. 4. Time course for lipase catalyzed isoamyl acetate synthesis. Reaction conditions: isoamyl alcohol (1 mmol), vinyl acetate (5 mmol), *n*-hexane (3 mL), steapsin lipase (70 mg), temperature (55 °C), agitation speed (160 rpm). Yields based on GC analysis.

Table 2
Steapsin lipase catalyzed acetate synthesis.[a]

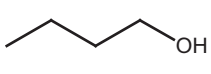
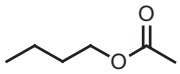
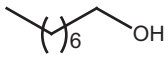
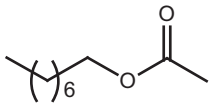
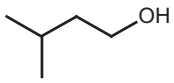
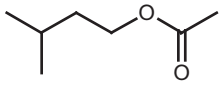
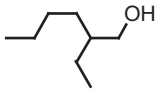
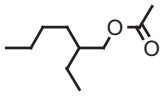
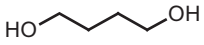
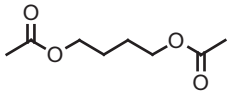
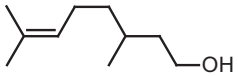
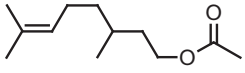
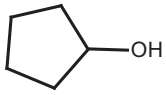
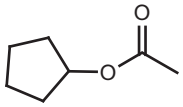
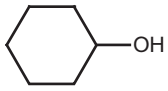
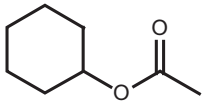
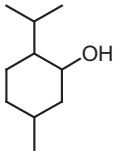
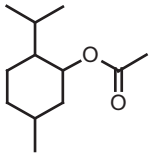
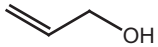
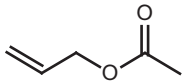
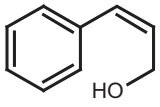
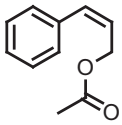
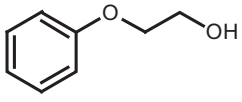
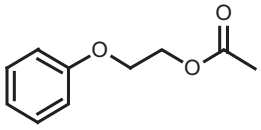
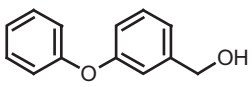
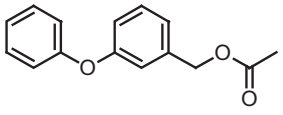
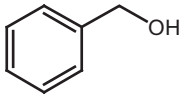
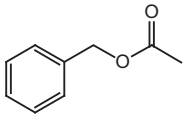
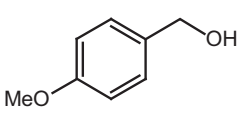
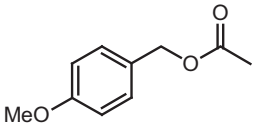
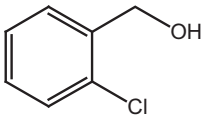
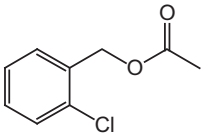
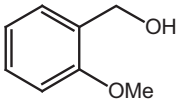
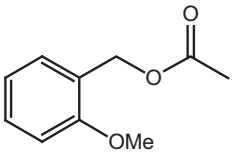
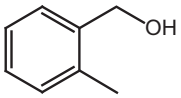
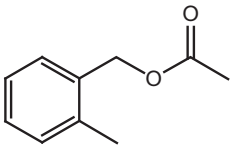
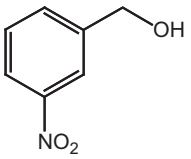
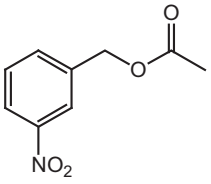
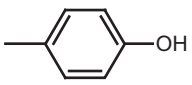
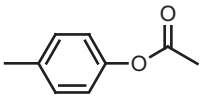
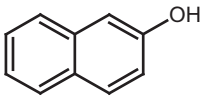
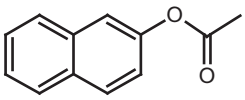
Entry	Substrate (alcohol)	Product (acetate)	Time (h)	Yield (%)[b]
1			24	99 ± 0.3
2			48	99 ± 0.2
3			24	99 ± 0.3
4			30	99 ± 0.4
5			24	99 ± 0.2
6			24	94 ± 1.1
7			48	74 ± 1.7
8			48	77 ± 1.4
9			72	57 ± 1.9
10			24	99 ± 0.3
11			24	99 ± 0.3
12			24	99 ± 0.4
13			24	99 ± 0.3
14			24	99 ± 0.5

Table 2 (*Continued*)

Entry	Substrate (alcohol)	Product (acetate)	Time (h)	Yield (%)[b]
15			48	99 ± 0.3
16			48	99 ± 0.2
17			48	99 ± 0.3
18			48	99 ± 0.3
19			48	99 ± 0.6
20			60	81 ± 1.3
21			60	24 ± 1.8

[a] Reaction conditions: alcohol (1 mmol), vinyl acetate (5 mmol), steapsin lipase (70 mg), temperature (55 °C), agitation speed (160 rpm).
[b] Yields based on GC analysis.

ionic liquids, however 15% of conversion with 86 ee_p was obtained after 24 h [11]. The allyl alcohol also reacted well providing excellent yield of 99% while industrially important cinnamyl acetate was synthesized efficiently with appreciable yield in 24 h (Table 2, entries 10–11). The 2-phenoxy derivatives like 2-phenoxy ethanol and 2-phenoxy benzyl alcohol were compatible for the developed protocol furnishing excellent yield (Table 2, entries 12–13). Benzyl alcohol and its several derivatives like *p*-OMe, *o*-Cl, *o*-OMe, *o*-Me, *m*-NO_2 were investigated for the developed protocol and found to furnished the corresponding substituted benzyl acetate with excellent yields (Table 2, entries 14–19). The reaction of phenol derivative like *p*-cresol was found to be sluggish as good yield of the desired product was obtained after a long period of 60 h (Table 2, entry 20), similar was the case with alicyclic alcohols where good yields were obtained for (*R*)-menthol after 72 h. The 2-napthol was not so compatible substrate as poor yield of desired product was obtained even after a prolonged reaction time (Table 2, entry 21). The lower yields obtain for *p*-cresol, (*R*)-menthol and 2-napthol may be due to the steric hindrance effect.

The feasibility of biocatalytic methodology sounds superior only when they are economical for application. In contribution to this, recyclability of enzyme is one of the vital parameter for large scale application of enzymes. Hence, the recyclability of steapsin lipase was studied for isoamyl acetate synthesis under the optimized reaction conditions (Fig. 5). We observed that the biocatalyst was capable to furnish appreciable yield of isoamyl acetate even at the

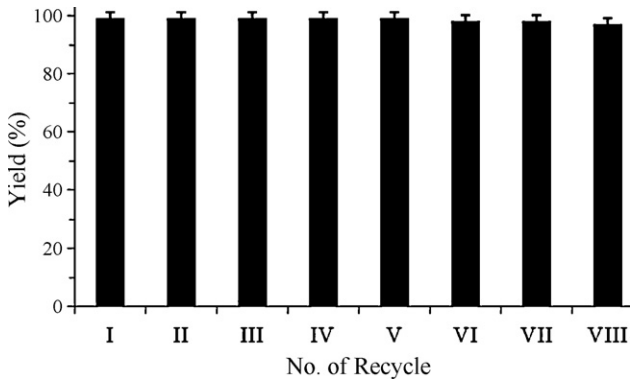


Fig. 5. Recyclability study of steapsin lipase for acetate synthesis. Reaction conditions: isoamyl alcohol (1 mmol), vinyl acetate (5 mmol), *n*-hexane (3 mL), steapsin lipase (70 mg), temperature (55 °C), agitation speed (160 rpm), time (24 h). Yields based on GC analysis.

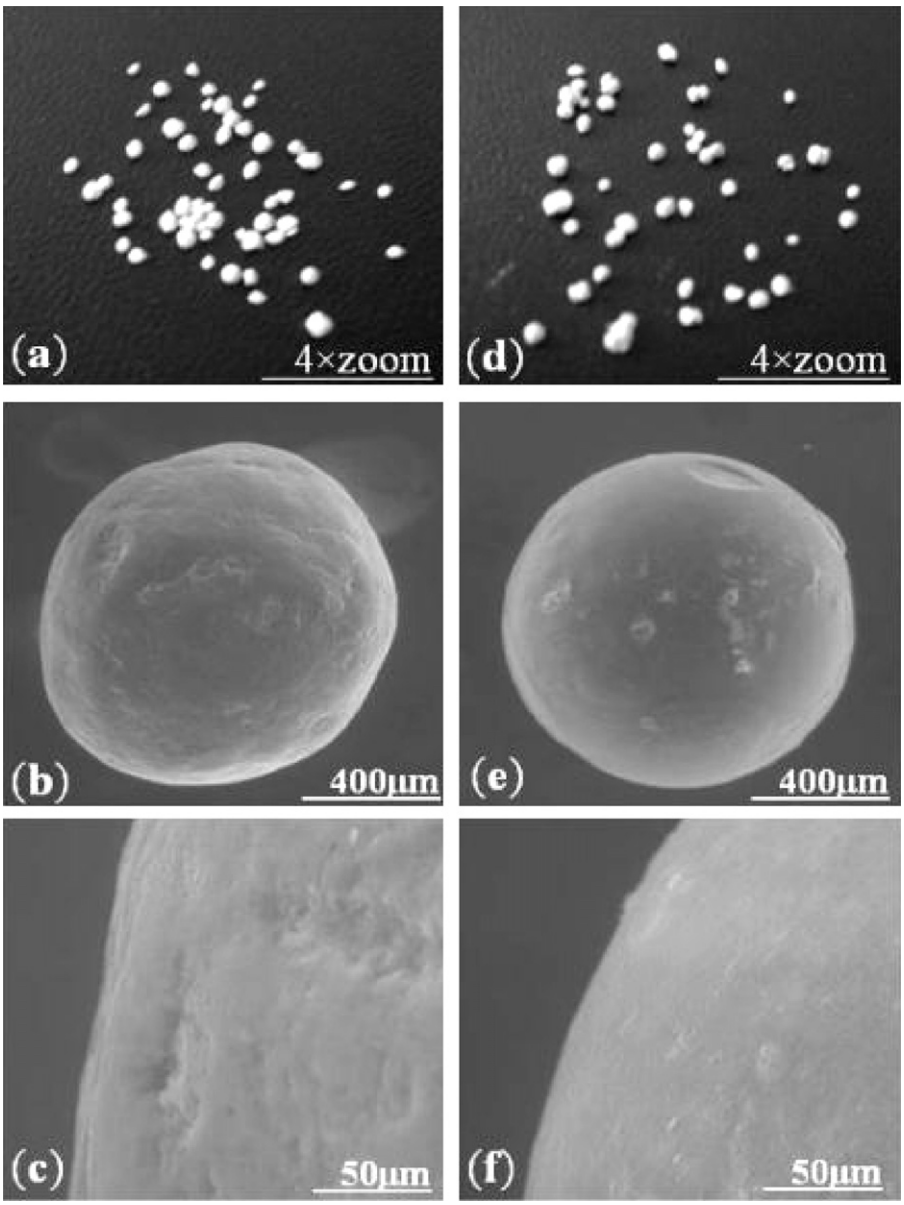


Fig. 6. Images of steapsin lipase beads. Photographic image captured from SONY Camera (12.2 megapixel, 4× zoom) before use [a] and after eight recycle [d]. SEM images of biocatalyst before use [b, c] and after eight recycle [e, f].

end of eight consecutive recycles without any significant loss in its catalytic activity demonstrating that the biocatalyst was catalytically and thermally stable.

3.3. Morphological study of steapsin lipase

To investigate the occurrence of possible distortion in the physical appearance of steapsin lipase, the photographic image and SEM analysis of the biocatalyst before use and after eight recycle was examined (Fig. 6). The SEM analysis of steapsin lipase revealed no major structural changes in appearance as the surface of steapsin lipase globules was observed to remain intact even after eight recycle.

4. Conclusions

In conclusion, for the first time steapsin lipase as a biocatalyst was investigated for its kinetic resolution ability. The biocatalyst has satisfactorily resolved the racemic mixture of secondary alcohols (up to 92% ee). In addition, several valuable acetates were successfully synthesized with good to excellent yield (up to 99%) using the developed methodology. The recyclability of steapsin lipase was appreciable for eight consecutive cycles. The present investigation hence appeals for exhaustive research with respect to immobilization strategies, genetic engineering protocols to modify the catalytic properties of the steapsin lipase thus endowing it as a robust biocatalyst with excellent stereo-selectivity and higher catalytic activity.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2012.01.009.

References

[1] V. Gotor-Fernandez, F. Rebolledo, V. Gotor, in: R.N. Patel (Ed.), Biocatalysis in the Pharmaceutical and Biotechnology Industry, CRC Press, Boca Raton, FL, 2007, pp. 203–248.
[2] J.M. Woodley, Trends Biotechnol. 26 (2008) 321–327.
[3] F. Hasan, A. Alishah, A. Hameed, Enzyme Microb. Technol. 39 (2006) 235–251.
[4] D. Acetti, E. Brenna, C. Fuganti, F.G. Gatti, S. Serra, Eur. J. Org. Chem. (2010) 142–151.
[5] P. Lozano, Green Chem. 12 (2010) 555–569.
[6] P. Lozano, T.D. Diego, C. Mira, K. Montague, M. Vaultier, J. Iborra, Green Chem. 11 (2009) 538–542.
[7] A. Paiva, P. Vidinha, M. Angelova, S. Rebocho, S. Barreiros, G. Brunner, J. Supercrit. Fluid 55 (2011) 963–970.
[8] M. Singh, R.S. Singh, U.C. Banerjee, Process Biochem. 45 (2010) 25–29.
[9] P. Hara, J.P. Mikkola, D.Y. Murzin, L.T. Kanerva, J. Mol. Catal. B: Enzym. 67 (2010) 129–134.
[10] X. Xia, Y.H. Wang, B. Yang, X. Wang, Biotechnol. Lett. 31 (2009) 83–87.
[11] H. Zhou, J. Chen, L. Ye, H. Lin, Y. Yuan, Bioresour. Technol. 102 (2011) 5562–5566.
[12] S. Hamaguchi, T. Ohashi, K. Watanabe. Eur. Pat. Appl. EP 189878 A2 (1986).
[13] V. Athawale, N. Manrekar, M. Athawale, Biotechnol. Prog. 19 (2003) 298–302.
[14] K.P. Dhake, P.J. Tambade, Z.S. Qureshi, R.S. Singhal, B.M. Bhanage, ACS Catal. 1 (2011) 316–322.
[15] K.P. Dhake, K.M. Deshmukh, Y.P. Patil, R.S. Singhal, B.M. Bhanage, J. Biotechnol. 156 (2011) 46–51.
[16] R.J. Jandacek, J.A. Whiteside, B.N. Hokombe, R.A. Volpenhein, J.D. Taulbee, Am. J. Clin. Nutr. 45 (1987) 940–945.
[17] F. Francalanci, P. Cesti, W. Cabri, D. Bianchi, T. Martinengo, M. Foa, J. Org. Chem. 52 (1987) 5079–5082.
[18] A. Kumar, S.S. Kanwar, Bioresour. Technol. 102 (2011) 2162–2167.
[19] K.P. Dhake, Z.S. Qureshi, R.S. Singhal, B.M. Bhanage, Tetrahedron Lett. 50 (2009) 2811–2814.
[20] K.P. Dhake, P.J. Tambade, R.S. Singhal, B.M. Bhanage, Tetrahedron Lett. 51 (2010) 4455–4458.
[21] M. Vallin, P.O. Syren, K. Hult, ChemBioChem 11 (2010) 411–416.
[22] M. Paivio, D. Mavrynsky, R. Leino, L.T. Kanerva, Eur. J. Org. Chem. (2011) 1452–1457.
[23] T. Mino, T. Hasegawa, Y. Shirae, M. Sakamoto, T. Fujita, J. Organomet. Chem. 692 (2007) 4389–4396.
[24] R.O.M.A. de Souza, O.A.C. Antunes, W. Kroutil, C.O. Kappe, J. Org. Chem. 74 (2009) 6157–6162.
[25] C. Laane, S. Boeren, K. Vos, C. Veeger, Biotechnol. Bioeng. (1987) 81–87.
[26] H. Hirata, K. Higuchi, T. Yamashina, J. Biotechnol. 14 (1990) 157–167.
[27] S. Gayot, X. Santarelli, D. Coulon, J. Biotechnol. 101 (2003) 29–36.
[28] V. Kasche, Enzyme Microb. Technol. 8 (1986) 4–16.
[29] P. Kraft, K.A.D. Swift, Perspectives in Flavor and Fragrance Research, VHCA, Verlag Helvetica Chimica Acta AG/WILEY–VCH, GmbH & Co. KGaA, Zurich/Weinheim, Germany, 2005.
[30] D.J. Rowe, Chemistry and Technology of Flavors and Fragrances, first ed., Blackwell Publishing/CRC Press, Boca Raton, FL, USA, 2005.