Journal of Molecular Catalysis B: Enzymatic 100 (2014) 78–83

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

# Lipase activity and enantioselectivity of whole cells from a wild-type *Aspergillus flavus* strain

Carmen Solarte[a,1], Edinson Yara-Varón[a,1], Jordi Eras[a], Mercè Torres[b], Mercè Balcells[a], Ramon Canela-Garayoa[a,*]

[a] Department of Chemistry, University of Lleida, Rovira Roure 191, Lleida 25198, Spain
[b] Department of Food Science and Technology, University of Lleida, Rovira Roure 191, Lleida 25198, Spain

ARTICLE INFO

*Article history:*
Received 23 July 2013
Received in revised form
18 November 2013
Accepted 9 December 2013
Available online 17 December 2013

*Keywords:*
*Aspergillus flavus*
(*R*)-1-Phenylethyl acetate
Ethyl laurate
2-Chloro-1-(chloromethyl)ethyl acrylate
2,3-Dichloro-1-propyl acrylate

ABSTRACT

This study reports the high enantiomeric preference of whole cell lipase from *Aspergillus flavus* wild-type that allows the preparation of a chiral secondary alcohol. Whole cells prepared from a wild-type *Aspergillus flavus* strain were used as biocatalysts to prepare (*R*)-1-phenylethyl acetate. (*R*)-1-Phenylethanol was esterified into (*R*)-1-phenylethyl acetate with a 94.6% enantiomeric excess (*ee*) within 24 h at 40 °C and (*S*)-1-phenylethanol remained in the reaction medium with a >99%*ee*. Besides, this biocatalyst allows the preparation of ethyl laurate and a mixture of 2-chloro-1-(chloromethyl)ethyl acrylate and 2,3-dichloro-1-propyl acrylate. The ethyl laurate yield was 96%, whereas the synthesis of a mixture of the acrylate regioisomers, 2-chloro-1-(chloromethyl)ethyl acrylate and 2,3-dichloro-1-propyl acrylate gave similar yields to those obtained using commercial lipases.



## 1. Introduction

Recent years have witnessed the rapid development of lipase applications for the preparation of esters and chiral alcohols. This growth, in part, is driven by EU and US legislation stating that natural flavouring and fragrance products can be prepared from natural sources only by physical processes (extraction from natural sources) or by biotechnological transformations that involve precursors isolated from nature [1]. Indeed, several biotransformation processes using either enzymes or whole cells have been implemented to conduct the kinetic resolution of 1-phenylethanol a compound used in various pharmaceutical and personal care products [2–5].

Biocatalysts have also been reported for the preparation of ethyl laurate, which has a coconut aroma [6]. A regioisomeric mixture of 2-chloro-1-(chloromethyl)ethyl acrylate and 2,3-dichloro-1-propyl acrylate (DCPA), have been used as monomers to prepare poly(dichloropropyl acrylates), which have applications in many fields [7].

Lipase-catalyzed biotransformation is one of the most popular and practical enzymatic technologies. Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are enzymes that catalyze a broad spectrum of reactions, such as the hydrolysis of ester bonds and transesterification and ester synthesis, at the interface between substrate and water or in non-aqueous organic solvents [8]. Consequently, lipases are used in a wide range of industrial processes, including food, chemical, pharmaceutical and detergent production. In many cases, however, these enzymes have been used in an immobilized form, which is costly and time-consuming, thus hindering the widespread use of enzymatic processes. Lipases from various microorganisms have been reported. There are two types of lipase preparation: (i) extracellular lipase, which is secreted into culture broth and (ii) intracellular or whole cell lipase, which remains either inside the cell or in the cell-wall [9]. Many of these have been purified and their properties are described. To date, several lipases are commercially available, and their applications have been extensively described [10,11]. However, while most of these are extracellular enzymes, little research has been devoted to intracellular or cell-bound lipases.

Cell-bound lipases are economically attractive because they can be produced at low cost. The biomass can be used directly, thus avoiding isolation, purification and immobilization procedures. Moreover, the biocatalyst can be easily recovered by filtration. Also,

*Abbreviations:* $a_w$, water activity; PDA, potato/dextrose/agar; *r*, specific activity; *R*-1-PEA, (*R*)-1-phenylethyl acetate; $ee_s$, enantiomeric excess substrate; $ee_p$, enantiomeric excess product; DCPA, mixture of regioisomers (2-chloro-1-(chloromethyl)ethyl acrylate and 2,3-dichloro-1-propyl acrylate); CALB, lipase B from *Candida antarctica*.

* Corresponding author. Tel.: +34 973 702843; fax: +34 973238264.
*E-mail address:* canela@quimica.udl.cat (R. Canela-Garayoa).
[1] These authors contributed equally to this paper.

1381-1177/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molcatb.2013.12.005

naturally immobilized lipases show potential for applications. Nagy et al. [3], studied thirty-eight filamentous fungi cultivated under solid state fermentation (SSF). The majority of these preparations proved to be effective as enantiomer selective biocatalyst and some were successfully applied in preparative scale kinetic resolution of secondary alcohols.

The use of naturally immobilized lipases has been proposed in the oil and fat industry [12,13], in the preparation of flavours [1,14] and, recently, in the synthesis of biodiesel [15,16]. Recombinant microbial whole-cell biocatalysts expressing lipases have also been proposed for enantioselective transesterification in non-aqueous medium. Nevertheless, the increasing concern regarding non-natural approaches for the preparation of additives and fragrances has prompted us to look for wild microorganisms that could be used to prepare aroma and fragrance compounds.

In our attempt to obtain a new fungal cell biocatalyst from our environment, we isolated a lipolytic *Aspergillus flavus* strain from sunflower seeds collected in a sunflower mill. Several studies under submerged fermentation and solid-state fermentation has been performed to produce whole cell lipase from *A. flavus*. Its stability and activity has been reported [17–21]. Here we describe several characteristics of the whole cell lipase prepared from the isolated wild-type *A. flavus* strain. These whole cells were used as biocatalysts to prepare (*R*)-1-phenylethyl acetate, ethyl laurate and DCPA. In this study we describe the high enantiomeric preference of a wild-type whole cell lipase from *A. flavus* that allows the preparation of a chiral secondary alcohol.

## 2. Materials and methods

A strain of *Aspergillus flavus* was isolated from sunflower seeds and was maintained on potato/dextrose/agar (PDA) at 4 °C. The microorganism was deposited in the culture collection "Colección Española de Cultivos Tipo" (Burjassot, Valencia-Spain), reference number CECT 20475.

### 2.1. Reagents and solvents

Asparagine, $K_2HPO_4$, $MgSO_4$, glucose, thiamine hydrochloride, $Fe(NO_3)_3.9H_2O$, $ZnSO_4{\cdot}7H_2O$, isopropenyl acetate, *rac*-1-phenylethanol, (*S*)-1-phenylethanol, lauric acid, and toluene were purchased from Sigma–Aldrich (Sigma–Aldrich Quimica, S.A., Madrid, Spain). $MnSO_4{\cdot}H_2O$ was supplied by Fisher Scientific (Madrid, Spain). Oleic acid was acquired from Merck (Barcelona, Spain). Ethyl laurate, ethyl acrylate, methyl palmitate and methyl oleate were from Fluka (Sigma–Aldrich, Madrid, Spain). Ethanol and hexane were supplied by J.T. Baker (Quimega, Lleida, Spain). Isooctane and sodium carbonate were purchased from Panreac (Barcelona, Spain). 1-Propanol was from Acros Organics (Barcelona, Spain).

### 2.2. Microorganism, growth media, and culture conditions

A non-aflatoxigenic strain of *A. flavus*, isolated from sunflower seeds, was cultivated in a synthetic liquid medium that contained 2.0 g asparagine, 1.0 g $K_2HPO_4$, 0.5 g $MgSO_4$, 5.0 mg thiamine hydrochloride, 1.45 mg $Fe(NO_3)_3{\cdot}9H_2O$, 0.88 mg $ZnSO_4{\cdot}7H_2O$ and 0.31 mg $MnSO_4{\cdot}H_2O$ per litre of distilled water. The pH was adjusted to 6.0 using 1 M HCl. Two hundred and fifty millilitres of the liquid medium were sterilized in a 1 L Erlenmeyer flask at 121 °C for 15 min, and 2% of refined sunflower oil was added. The medium was inoculated with 2.5 mL of a spore suspension ($5 \times 10^6$ spores/mL) of *A. flavus* grown on PDA. The medium was then incubated at 28 °C for 5 days on an orbital shaker at 200 rpm.

### 2.3. Preparation of whole cell lipase

Mycelium obtained from the culture medium was harvested using a Buchner funnel, washed with distilled water followed by acetone, and freeze-dried for 18 h. It was then ground to powder consistency.

### 2.4. Equilibration of water activity ($a_w$)

The $a_w$ in the experiments was set by independently equilibrating reagents, solvent and biocatalyst with aqueous saturated solutions of LiCl ($a_w = 0.12$), $MgCl_2$ ($a_w = 0.33$), $K_2CO_3$ ($a_w = 0.42$), $Mg(NO_3)_2$ ($a_w = 0.54$) and NaCl ($a_w = 0.75$). Separate closed containers were used for each reactant and biocatalyst [22]. Equilibration was performed at room temperature for at least 48 h. The $a_w$ of the biocatalyst was measured using an Aqua Lab series 3TE from Decagon Devices Inc. (Pullman, WA, USA).

### 2.5. Biocatalyst activity

Two mL of a 0.09 M isooctane solution of oleic acid (0.05 g) containing 0.08 g of 1-propanol was stirred for 1 h at 28 °C in the presence of various amounts of whole cell lipase ($a_w = 0.12$). Samples were collected every 15 min. These samples were diluted at *ca.* 1 mg $mL^{-1}$ using methyl palmitate as internal standard. Resulting samples were analyzed by gas-chromatography (GC-FID) as described below. All experiments were performed in triplicate.

### 2.6. Evaluation of substrate adsorption by the whole cell lipase biomass

Eighty mL of a 0.022 M isooctane solution of methyl oleate (500 mg) containing 3 g of whole cell lipase ($a_w = 0.12$) were stirred for 24 h at 28 °C. Samples were collected at 0, 2, 4 and 24 h. These samples were diluted at *ca.* 1 mg $mL^{-1}$ using methyl palmitate as internal standard and analyzed by gas chromatography (GC-FID) as described below. All experiments were performed in triplicate.

### 2.7. Kinetic resolution of rac-1-phenylethanol

Kinetic resolution reactions were carried out using dry conditions in flame-dried glassware following a previously described method [23]. *rac*-1-Phenylethanol was dried over a molecular sieve (4 Å) before use. Isopropenyl acetate was dried over $CaCl_2$ and distilled before use. Dry toluene was dried by refluxing under nitrogen in the presence of sodium wire and benzophenone.

Whole cell lipase (25 mg) and sodium carbonate (53 mg, 0.5 mmol) were added to a vial. The corresponding *rac*-1-phenylethanol or (*S*)-1-phenylethanol (55 mg, 0.5 mmol) dissolved in dry toluene (1 mL) was added to the vial, and the mixture was stirred for 6 min. Next, isopropenyl acetate (110 μL, 1.0 mmol) was added to the reaction. Experiments were carried out at 25, 40 and 60 °C. Samples were collected between 30 min and 44 h depending on the temperature and reagent used, and then analyzed by chiral GC as described below. A blank study was conducted without using isopropenyl acetate.

### 2.8. Preparation of ethyl laurate

Half a mL of a solution containing 25 mg $mL^{-1}$ (0.125 mmol $mL^{-1}$) of lauric acid and ethanol 11.52 mg $mL^{-1}$ (0.250 mmol $mL^{-1}$) in hexane was added to a reaction vial (1.5 mL) fitted with a PTFE-lined cap. Then 20 mg of biocatalyst ($a_w = 0.54$) was added to the vial, and the mixture was stirred and heated to 40 °C for 24 h. The resulting solution was analyzed using GC-FID.

Product quantification was achieved using a standard curve obtained from ethyl laurate.

### 2.9. Procedure for the preparation of 2,3-dichloro-1-propyl laurate

2-Chloro-1-(chloromethyl)ethyl laurate was prepared from glycerol and lauric acid following a previously described method [24]. The isomerization of 2-chloro-1-(chloromethyl)ethyl laurate to 2,3-dichloro-1-propyl laurate was achieved by microwave irradiation (300 W max, 17 atm max, 243–247 °C) for 1 h in a solvent-free system. The isomerization process used was previously evaluated [25].

### 2.10. Enzymatic transesterification

A 1:1 mixture of ethyl acrylate (1 mmol, 100 mg) and dichloropropyl laurates (32:68 isomeric mixtures, 1 mmol, 311 mg) was continuously reciprocally shaken (Eppendorf® Thermomixer Comfort) at 1200 rpm at atmospheric pressure in a reaction vial (1.5 mL) fitted with a PTFE-lined cap. Reaction vials were used as received. A set of experiments were carried out in a solvent-free system at 40 °C using 15% (47 mg) of biocatalyst on the basis of the weight of dichloropropyl laurates. Samples were collected at 24 h. Once the experiment had ended, an aliquot of 10 mg of the crude product was dissolved in hexane containing butyl acrylate as internal standard. The resulting solution was analyzed by GC-FID. Each compound was quantified using the internal standard. Experiments were performed in triplicate.

### 2.11. GC analysis

The progress of each reaction was determined by GC using an Agilent (Barcelona, Spain) HP6890 series gas chromatograph coupled to an FID detector. A 30 m × 0.25 mm fused silica capillary coated with a 0.20-μm film of poly(80% biscyanopropyl-20% cyanopropylphenyl siloxane) (SP-2330; Supelco, Madrid, Spain) was used for fatty acid analysis.

The temperature programme used was 40 °C for 5 min, followed by an increase of 20 °C/min until reaching the final temperature of 225 °C, which was then held for 3 min. A 1:20 split injection ratio was applied. Hydrogen was used as the carrier gas at a constant pressure of 620 kPa. The injection volume was 1 μL. The injection system was held at 250 °C and the FID system at 280 °C. Quantification was carried out by a conventional internal standard calibration method using the corresponding ester standard.

Chiral analysis was performed using a 25 m × 0.250 mm fused silica capillary coated with a 0.25-μm film of modified β-cyclodextrins bonded to dimethylpolysiloxane (CP-Chirasil-Dex CB Varian, Madrid, Spain). The oven temperature was held constant at 120 °C for 10 min. A 100:1 split injection ratio was applied. Hydrogen was used as the carrier gas at flow rate of 2.0 mL/min. The injection volume was 1 μL. The injection system was held at 250 °C and the FID system at 250 °C.

## 3. Results and discussion

Several studies have been carried out to describe the optimal conditions for the production of lipase by *A. flavus* [17–19]. In our research group we have used an optimal method to cultivate the fungal mycelium [26]. In this method, refined sunflower oil was added to induce the lipase activity. Mycelium was harvested from the culture medium, washed with water and acetone, freeze-dried and ground to a powder. The yield was 10 g $L^{-1}$. Finally, the mycelium was equilibrated at various $a_w$. An initial study was carried out using pure oleic acid to investigate the specific activity ($r$) of the whole cell lipase. This specific activity was determined at five substrate:whole cell lipase ratios (Fig. 1). The activity was between 65 and 91 μmol $min^{-1}$ $g^{-1}$, a range that could be a consequence of the inherent heterogeneity of the cells. No apparent ratio trends were observed in the total number of reactions performed. Nevertheless, the standard deviation for a given ratio did not exceed 7% (ratio 2.50).

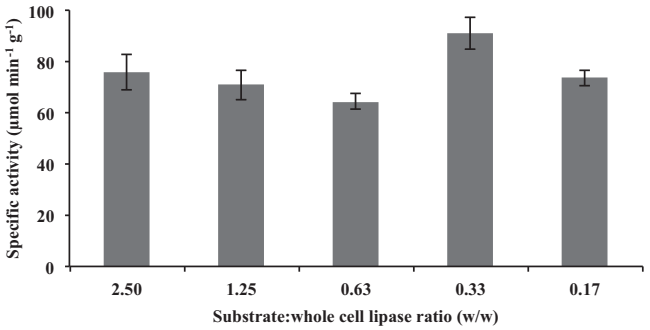


**Fig. 1.** Effect of the substrate:whole cell lipase ratio on the specific activity of *A. flavus*, calculated on the basis of the synthesis of propyl oleate from oleic acid and 1-propanol.

Considering that the fungal biomass used as biocatalyst could partially retain the product and the reagent used, a new set of experiments was conducted to preclude this possibility. Fig. 2 shows that the average percentage of methyl oleate absorbed by 1 g of whole cell lipase after 24 h using a substrate:whole cell lipase ratio of 0.12 was 5%. Given these results obtained with the lowest substrate:whole cell lipase ratio, we performed all the subsequent experiments using substrate:whole cell lipase ratios higher than 1.7 to assure that the biocatalyst could not retain any chemical compound.

### 3.1. Kinetic resolution of rac-1-phenylethanol using fungal whole cell lipase

Table 1 shows the progress of the kinetic resolution of *rac*-1-phenylethanol (**1**) at three temperatures. Whole cell lipase from *A. flavus* were used at a substrate:whole cell lipase ratio of 2.0 and $a_w$ = 0.12 (Fig. 3A). The conversion to (*R*)-1-phenylethyl acetate (**3**) (R-1-PEA) calculated as $ee_s/(ee_s + ee_p)$ was 48% with 96%*ee* ($E$ = 150) within 44 h of reaction at 25 °C with an apparent 87% $ee_s$. Nevertheless, the conversion reached 51% with 94.6%*ee* ($E$ > 200) within 24 h of reaction at 40 °C. In this case, the remaining substrate showed >99% $ee_s$. The increase in the reaction temperature (60 °C) did not lead to an improvement of these results. The conversion rate was 49.5% for 12 h of reaction at this temperature and R-1-PEA dropped to 92.2%*ee* ($E$ = 73). An experiment was carried out using (*S*)-1-phenylethanol. The formation of 1.56% of (*S*)-1-phenylethanol acetate (**4**) was observed after 24 h of reaction at

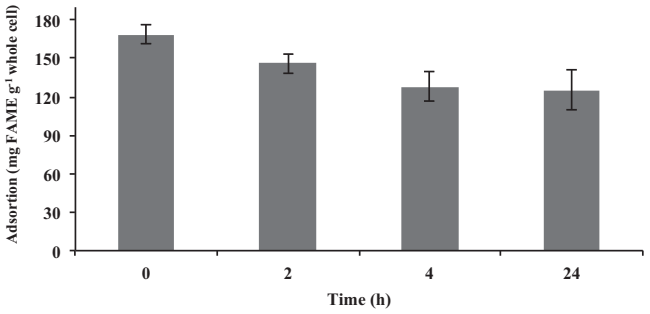


**Fig. 2.** Effect of contact time on the absorption of methyl oleate (FAME) by the whole cell lipase from *A. flavus*. The substrate:whole cell lipase ratio used was 0.12.

**Table 1**
Time course of (*R*)-1-phenylethyl acetate (R-1-PEA) yield in enantioselective transesterification at various temperatures using whole cell lipase from a wild-type *A. flavus* strain.

| *t* (h) | *T* = 25 °C | | | | *T* = 40 °C | | | | *T* = 60 °C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $ee_s$ (%)[a] | $ee_p$ (%)[a] | *c* (%)[b] | *E*[c] | $ee_s$ (%)[a] | $ee_p$ (%)[a] | *c* (%)[b] | *E*[c] | $ee_s$ (%)[a] | $ee_p$ (%)[a] | *c* (%)[b] | *E*[c] |
| 0.5 | 0 | 0 | 0 | – | <1 | >99 | <1 | >200 | 1.6 | >99 | 1.5 | >200 |
| 1 | 0 | 0 | 0 | – | 1.2 | >99 | 1.2 | >200 | 3.7 | >99 | 3.9 | >200 |
| 2 | 2 | >99 | 2.2 | >200 | 2.7 | >99 | 2.7 | >200 | 10.2 | >99 | 9.2 | >200 |
| 3 | – | – | – | – | 4.8 | >99 | 5.0 | >200 | 17.9 | >99 | 15.2 | >200 |
| 5 | – | – | – | – | 10.7 | >99 | 9.7 | >200 | 19.5 | >99 | 16.7 | >200 |
| 7 | – | – | – | – | 28.2 | 98.4 | 22.3 | 166 | 36.9 | >99 | 27.0 | >200 |
| 9 | – | – | – | – | 42.7 | 97.0 | 30.6 | 101 | 73.7 | 94.2 | 43.9 | 71 |
| 12 | – | – | – | – | 55.6 | 95.0 | 36.9 | 69 | 90.3 | 92.2 | 49.5 | 73 |
| 24 | 46 | >99 | 31.7 | >200 | >99 | 94.6 | 51.4 | >200 | – | – | – | – |
| 44 | 87.1 | 96.2 | 47.5 | 150 | – | – | – | – | – | – | – | – |

[a] Determined by chiral GC.
[b] $c = ee_s/(ee_s + ee_p)$.
[c] $E = \ln[(1-c)\times(1+ee_p)]/\ln[(1-c)\times(1-ee_p)]$.

40 °C, thereby confirming the high enantiopreference of whole cell lipase from *A. flavus* for (*R*)-1-phenylethanol.

Several studies have addressed the enantioselective activity of intracellular enzymes present in fungal cells. Thus, a recombinant strain overexpressing *Aspergillus oryzae* lipase derived from the wild-type strain RIB40 was immobilized in biomass-support particles and used as a whole-cell biocatalyst. The immobilized lipase-overexpressing strain showed high activity and was used to selectively synthesize R-1-PEA from rac-1-phenylethanol and vinyl acetate. The yield and enantiomeric excess (%*ee*) of R-1-PEA after 48 h of reaction at 30 °C using a substrate:resting cell ratio of 0.4 was 90% and 95%*ee*, respectively [27]. The same *A. oryzae* whole-cell biocatalyst expressing the lipase-encoding gene from *C. antarctica* shows a higher performance in the enantiomeric transesterification of rac-1-phenylethanol, yielding 88.1% of R-1-PEA with a 99.9%*ee* after 3.5 h of reaction [19,28]. Although this performance

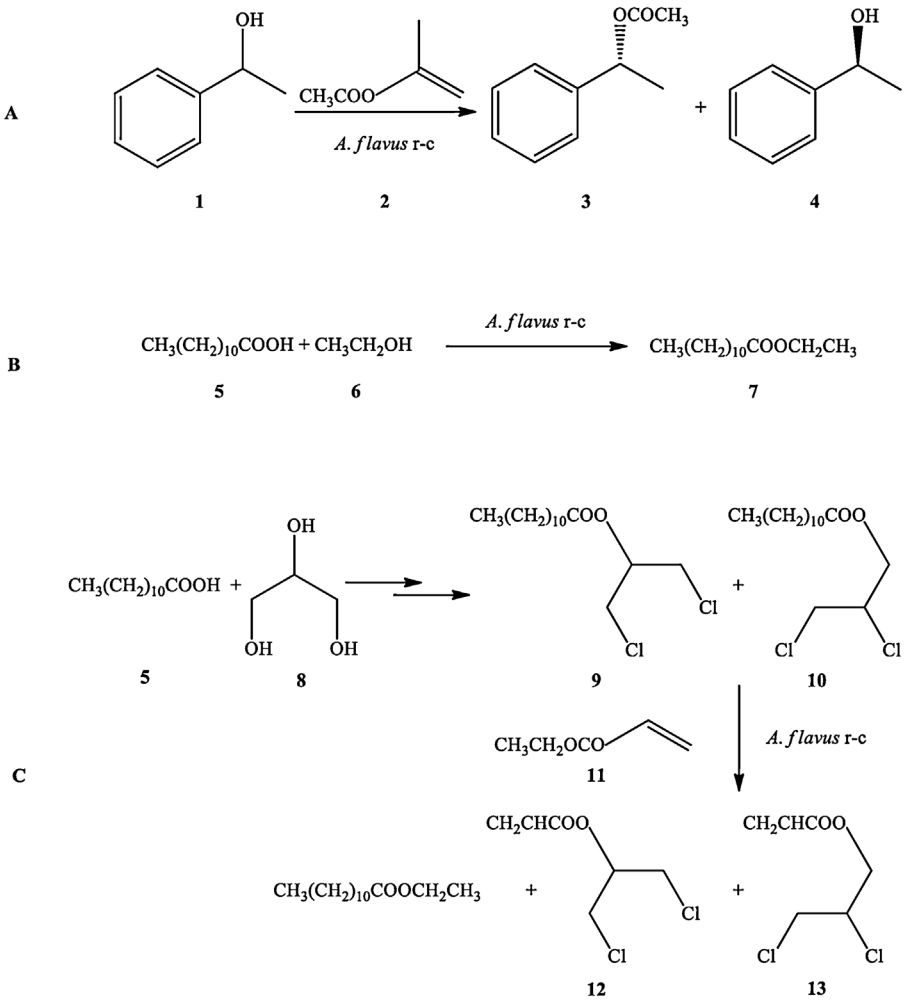


**Fig. 3.** Biocatalytic reactions using whole cell lipase from an *A. flavus* strain. (A) Kinetic resolution of *rac*-1-phenylethanol. (B) Synthesis of ethyl laurate. (C) Synthesis of mixture of regioisomers (DCPA).

is superior to that of the *A. flavus* resting cell, it should be considered that it was reached by expressing *C. antarctica* lipase, the enzyme shown to provide the highest yield and *ee* [29], and using a substrate:whole-cell ratio of 0.6. This ratio implies that those authors used approximately three-fold more biocatalyst than that used in the present study in the same reaction with wild resting cells. Nagy et al. [3] studied 38 filamentous fungi cultivated under SSF conditions for kinetic resolutions of racemic secondary alcohols. The freeze-dried solid prepared form two *Aspergillus* species (*Aspergillus terreus* and *Aspergillus niger*) were tested. The reaction was conducted for 120 h at room temperature. The acetylation of racemic 1-phenylethanol showed a conversion of 45.6% and 87.8%*ee* of R-1-PEA by *A. terreus*. *A. niger* showed a conversion of 24% and 56.9%*ee* of R-1-PEA. Both biocatalysts showed lower enantiomeric excess of R-1-PEA than that showed by our strain of *A. flavus*. The best biocatalyst in terms of *ee* (98.4%) described by these authors was the solid material prepared from a *Gliocladium catenulatum* strain. Nevertheless, they used a substrate:biocatalyst ratio of 1 in all the studies, twice than that used in our study.

### 3.2. Biocatalytic synthesis of ethyl laurate

The biocatalytic esterification of lauric acid (**5**) was carried out using ethanol (**6**) and the whole cell lipase from *A. flavus* (Fig. 3B). Ethyl laurate (**7**) was synthesized with a 95% yield after 24 h of reaction. This result was similar to that described by [6], who reported a 92% yield of ethyl laurate using Lipolase®, a commercial preparation containing the lipase from *Thermomyces lanuginosa*. Mycelium-bound lipase of a locally isolated *A. flavus* have been used previously to catalyze the acidolysis of several vegetables oils [21] and the transesterification of acylglycerides to the production of FAME [30]. The works mentioned above demonstrate that *A. flavus* have been identified as robust whole-cell biocatalysts. The use of lipases as catalysts allows the preparation of high purity esters. Moreover, the products prepared using these enzymes can be labelled “natural”, provided that natural raw materials are also used.

### 3.3. Biocatalytic preparation of regioisomeric mixture of DCPA

Initially, 2-chloro-1-(chloromethyl)ethyl laurate (**9**) was prepared from lauric acid (**5**) and glycerol (**8**). Subsequently, an isomerization reaction of **9** to 2,3-dichloro-1-propyl laurate (**10**) was achieved by microwave irradiation, and a mixture of regioisomers **9**:**10** (32:68%, respectively) was obtained (Fig. 3C).

In a first experiment, the transesterification reaction between **9** and ethyl acrylate (**11**) was carried out using a 1:1 mole ratio of reagents **9** and **11** and a solvent-free system (Fig. 3C). The results showed a 14% yield of ester **12** for 24 h of reaction at $a_w = 0.33$. Given this result and considering that the biocatalysts might react with the primary ester bond much more readily than with the secondary ester bond, we performed the isomerization reaction mentioned above. When this regioisomer mixture **9**:**10** was used for 24 h, the yield of esters (**12**, **13**) increased to 21% ($a_w = 0.33$). In an attempt to improve the activity of the resting cells, we tested four different water activities. The results of ester yields obtained for the transesterification for 24 h of reaction were 9, 23, 41 and 0%, respectively for $a_w = 0.12$; 0.42, 0.54 and 0.75. Yoshida et al. [31] found that lipases from *Rhizopus* species work efficiently at low $a_w$, although their catalytic performance changes slightly depending on the reaction. The behaviour of *Rhizopus oryzae* lipases was similar to that the found with *A. flavus*, with the higher yield at $a_w = 0.54$. Similar behaviour was reported using CALB for the same reaction, with 50% yields at $a_w = 0.54$ [7]. These results confirm that optimum water content is required to maintain sufficient conformational flexibility and activity of the lipase. These dichloropropyl esters can be readily transformed into polymeric compounds [7].

## 4. Conclusions

The kinetic resolution of racemic 1-phenylethanol was successfully achieved using whole cell lipase from a wild-type *A. flavus* strain. The (*R*)-1-phenylethanol was transesterified into (*R*)-1-phenylethyl acetate, leaving the (*S*)-1-phenylethanol in the reaction medium with >99%*ee*. Our results show that the biocatalyst proved highly selective, yielding (*R*)-1-phenylethyl acetate in 24 h at 40 °C with 94.6%*ee*. Moreover, we have shown that this biocatalyst shows acceptable performance in esterification and transesterification reactions. Ethyl laurate can be prepared at very high yield while the regioisomeric mixture of DCPA can be synthesized at similar yields to those obtained using a commercial lipase.

## Acknowledgements

This work was supported in part by a Grant-in-Aid for the Secretaría de Estado de Política Científica y Tecnológica of the Spanish Ministry of Education and Culture (contract grant number: CTQ2009-14699-C02-01). The authors are grateful to the Comissionat per a Universitats i Recerca del Departament d’Innovació, Universitats i Empresa de la Generalitat de Catalunya and to the European social Fund (EFS) for the FI grant of Carmen Solarte Orozco and to the Vicerrectorat de Recerca de l’Universitat de Lleida for the UdL grant of Edinson Yara Varón. We also thank Tania Yates for the proofreading of the manuscript.

## References

[1] S. Serra, C. Fuganti, E. Brenna, Trends Biotechnol. 23 (2005) 193–198.
[2] S.H. Schöfer, N. Kaftzik, U. Kragl, P. Wasserscheid, Chem. Commun. (2001) 425–426.
[3] V. Nagy, E.R. Tőke, L.C. Keong, G. Szatzker, D. Ibrahim, I.C. Omar, G. Szakács, L. Poppe, J. Mol. Catal. B: Enzym 39 (2006) 141–148.
[4] B.M. Nestl, C.V. Voss, A. Bodlenner, U. Ellmer-Schaumberger, W. Kroutil, K. Faber, Appl. Microbiol. Biotechnol. 76 (2007) 1001.
[5] T. Matsumoto, M. Ito, H. Fukuda, A. Kondo, Appl. Microbiol. Biotechnol. 64 (2004) 481–485.
[6] M.L.M. Fernandes, N. Krieger, A.M. Baron, P.P. Zamora, L.P. Ramos, D.A. Mitchell, J. Mol. Catal. B: Enzym. 30 (2004) 43–49.
[7] E. Yara-Varón, J. Eras Joli, M. Torres, M. Balcells, G. Villorbina, R. Canela-Garayoa, J. Mol. Catal. B: Enzym. 92 (2013) 7–13.
[8] E. Busto, V. Gotor-Fernandez, V. Gotor, Chem. Soc. Rev. 39 (2010) 4504–4523.
[9] M.M.R. Talukder, H.Z.S. Lee, R.F. Low, L.C. Pei-Lyn, D. Warzecha, J. Wu, J. Mol. Catal. B: Enzym. 89 (2013) 108–113.
[10] F. Hasan, A.A. Shah, A. Hameed, Enzyme Microb. Technol. 39 (2006) 235–251.
[11] H. Zhao, Z. Song, Biochem. Eng. J. 49 (2010) 113–118.
[12] A. Lopez-Hernandez, C. Otero, E. Hernandez-Martin, H.S. Garcia, C.G. Hill, Eur. J. Lipid Sci. Technol. 109 (2007) 1147–1159.
[13] P. Srimhan, K. Kongnum, S. Taweerodjanakarn, T. Hongpattarakere, Enzyme Microb. Technol. 48 (2011) 293–298.
[14] S. Tamalampudi, S. Hama, T. Tanino, M.R. Talukder, A. Kondo, H. Fukuda, J. Mol. Catal. B: Enzym. 48 (2007) 33–37.
[15] S. Hama, H. Yamaji, T. Fukumizu, T. Numata, S. Tamalampudi, A. Kondo, H. Noda, H. Fukuda, Biochem. Eng. J. 34 (2007) 273–278.
[16] T. Sun, W. Du, D. Liu, Process Biochem. 46 (2011) 661–664.
[17] K. Long, H.M. Ghazali, A. Ariff, K. Ampon, C. Bucke, J. Chem. Technol. Biotechnol. 67 (1996) 157–163.
[18] H.S. Pau, I.C. Omar, Pak. J. Biol. Sci. 7 (2004) 1249–1256.
[19] B.T.N. Parveen, S. Manikandaselvi, Int. J. Pharm. Technol. Res. 3 (2013) 1299–1302.
[20] J. Yu, S.M. Mohawed, D. Bhatnagar, T.E. Cleveland, J. Appl. Microbiol. 95 (2003) 1334–1342.
[21] K. Long, H.M. Ghazali, A. Ariff, C. Bucke, J. Am. Oil Chem. Soc. 74 (1997) 1121–1128.
[22] M. Nordblad, P. Adlercreutz, Biotechnol. Bioeng. 99 (2008) 1518–1524.
[23] A. Träff, K. Bogár, M. Warner, J.E. Bäckvall, Org. Lett. 10 (2008) 4807–4810.
[24] J. Eras, M. Escriba, G. Villorbina, M. Oromi-Farrus, M. Balcells, R. Canela, Tetrahedron 65 (2009) 4866–4870.
[25] M. Escriba, J. Eras, M. Duran, S. Simon, C. Butchosa, G. Villorbina, M. Balcells, R. Canela, Tetrahedron 65 (2009) 10370–10376.

[26] M. Torres, E. Barbosa, V. Loscos, R. Canela, Biotechnol. Lett. 22 (2000) 1265–1268.
[27] M. Kaieda, M. Nagayoshi, S. Hama, A. Kondo, H. Fukuda, Appl. Microbiol. Biotechnol. 65 (2004) 301–305.
[28] S. Tamalampudi, M.M.R. Talukder, S. Hama, T. Tanino, Y. Suzuki, A. Kondo, H. Fukuda, Appl. Microbiol. Biotechnol. 75 (2007) 387–395.
[29] Z. Maugeri, W. Leitner, P.D. de María, Tetrahedron Lett. 53 (2012) 6968–6971.
[30] M. Torres, V. Loscos, V. Sanahuja, R. Canela, J. Am. Oil Chem. Soc. 80 (2003) 347–351.
[31] A. Yoshida, S. Hama, K. Nakashima, A. Kondo, Enzyme Microb. Technol. 48 (2011) 334–338.