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Pseudomonas sp. lipase immobilized in polymers versus the use of free enzyme in the resolution of (*R*,*S*)-methyl mandelate

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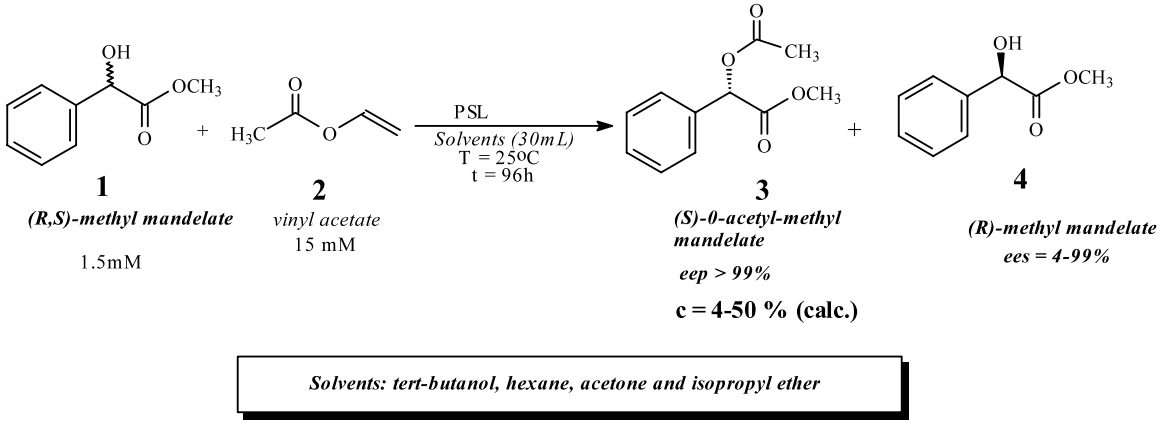
Abstract—In the present report, we describe the preparative-scale resolution of (*R*,*S*)-methyl mandelate by acylation with vinyl acetate catalyzed by free *Pseudomonas* sp. lipase or immobilized in poly(ethylene) oxide (PEO) and agar gel, in organic media. Under experimental conditions the method was very effective using the enzyme immobilized in PEO. The degree of conversion was near 50% and yielded both enantiomers with practically 100% of optical purity. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Mandelic acid and its derivatives are useful chiral reagents widely employed both for synthetic purposes and for stereochemical investigations. In fact, the enantiomers of mandelic acid can be used for the resolution of alcohols, amines, etc., thus permitting the determination of enantiomeric excess by HPLC or NMR.[1–3] Lipases are versatile catalysts, as they catalyze a plethora of reactions such as esterification, amidation, and transesterification of esters in addition to the natural reaction of fatty ester hydrolysis. Applications of lipases include production of food additives, chiral intermediates, and pharmaceutical products.[4] Many of the characteristics which make their use difficult in organic media may be circumvented by immobilization in a solid support. In general, immobilized enzymes are more stable in relation to changes in pH and temperature when compared with soluble forms. Moreover, they remain stable for months and the catalyst can be used repetitively. Thus, they are easy to handle and can be recuperated from the solution, which makes the process practical and economically viable. Enzymes can be immobilized in organic and/or inorganic materials to be used for synthetic purposes. These materials include, glass, silica, alumina, biopolymers, organogels and chrysotille.[5–8]

In this work, *Pseudomonas* sp. lipase (PSL) was immobilized in two polymers, poly(ethylene oxide) (PEO)



Scheme 1. Acylation reaction of *R*,*S*-methyl mandelate.

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and agar of which the latter is a complex mixture of polysaccharides including agarose as the principal gelling component. These systems were used in the acylation reaction of *R*,*S*-methyl mandelate with vinyl acetate in several organic solvents (Scheme 1). The enzyme immobilization in PEO was performed by dissolving 500 mg of the polymer and 100 mg of PSL in 20 mL of water, with further solvent evaporation forming a film, which was then cut into several regular sections. The enzyme immobilization in agar was performed by dissolving 0.4 g of agar into 9.0 mL of water at 100°C. After the system reached ~50°C, 100 mg PSL was added and it was shaken until it became homogeneous. Regular sections of agar gel containing enzyme were obtained by passing it through a wire sieve at room temperature. The reaction's progress and enantiomeric excess values were measured by gas chromatography (GC) equipped with a chiral column (CP-chirasil-Dex CB) using H_2 as the carrier gas, with a detector, an injector set at 275°C, and a column set to a temperatures of 80–140°C (2°C/min). The enantiomeric ratio (*E*) values were calculated from the degree of conversion and the e.e. of the product, according to Sih, Sharpless and Fajans' equation.[9]

2. Results and discussion

Many devices are commonly suggested to improve the performance of the catalytic system. Thus, for comparative purposes, PSL was immobilized in agar gel and in PEO film. As reported by Wang et al., the lipase immobilization can increase the reaction rate.[10] Our results showed the dependence of the enzymatic activity according to the support employed. No product was detected using PSL immobilized in agar gel. However, immobilization of PSL in PEO increased its activity. One explanation for this result was related to diffusion process of the reagents and products from the support to the reaction media. Using PEO, the diffusion was faster since it formed the products in a shorter time than when agar gel was used. All reactions were performed under the same experimental conditions. Table 1 shows the effects of organic solvents on the enantioselectivity of PSL free and immobilized in PEO in the resolution of (*R*,*S*)-methyl mandelate with vinyl acetate as the acylating agent. The results showed that PSL had the same stereochemical preference for the *S* enantiomer, regardless of the system used. It was verified that the solvent had a profound effect on the % conversion of the product, but had no effect on the enzyme enantioselectivity. In all solvents, ***R*-4** and ***S*-3** were obtained, confirmed by GC comparison with an authentic sample. Kinetic resolution of **1** can be better achieved by using PSL immobilized in PEO. ***S*-3** was obtained with e.e. >99% at 50% conversion with 96 h reaction, while only 13% conversion was obtained with the free form. Considering the nature of the organic solvents and support, isopropyl ether as solvent and PEO as support were the most appropriate for the biocatalytic conversion of **1** (Fig. 1). The influence of temperature was also evaluated in the acylation of **1** using PSL immobilized in PEO and isopropyl ether as solvent. No macroscopic changes in the physical integrity of the system PSL/PEO were observed after 72 h at 35°C. The variation of the conversion percentage with temperature was evaluated by measuring the

Table 1. Effects of solvents on the immobilized and free *Pseudomonas* sp.[a] lipase-catalyzed acylation of *R*,*S*-methyl mandelate **1** with vinyl acetate

Solvents	*t* (h)	E.e.s[b] (%)		E.e.p[c] (%)		Conversion (%)		*E*[d]	
		PSL/free	PSL/PEO	PSL/free	PSL/PEO	PSL/free	PSL/PEO	PSL/free	PSL/PEO
Isopropyl ether	24	2	33	99	99	1	25	200	274
	48	3	47	99	99	3	32	205	315
	72	9	69	99	99	8	41	216	413
	96	15	99	99	99	13	50	230	1057
Hexane	24	0	17	0	99	0	17	0	242
	48	1	39	0	99	1	28	200	290
	72	6	56	99	99	6	36	211	349
	96	7	72	99	99	7	42	214	429
tert-Butanol	24	0	20	0	99	0	15	0	242
	48	1	33	99	99	1	25	200	274
	72	3	47	99	99	3	32	205	315
	96	3	61	99	99	3	38	205	370
Acetone	24	0	5	0	99	0	5	0	209
	48	0	7	0	99	0	7	0	214
	72	2	13	99	99	2	12	203	227
	96	2	14	99	99	2	15	203	236

Reactions were conducted in each solvent (30 mL) containing *R*,*S*-methyl mandelate (0.0015 mol), lipase PS (100 mg) and vinyl acetate (0.015 mol) at 25°C.

[a] Amano 30, 30000 U/g.

[b] Enantiomeric excess of the recovered product **4**.

[c] Enantiomeric excess of compound **3**.

[d] Enantiomeric ratio, parameter describes the selectivity of enzyme between two enantiomers.

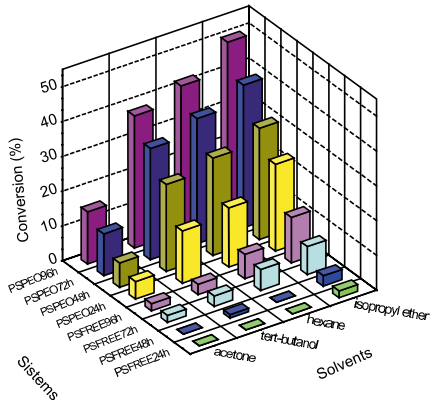

Figure 1. Immobilized *Pseudomonas* sp. lipase in PEO versus free form in the resolution of (*R*,*S*)-methyl mandelate at 25°C.

formed products at definite times (24, 48 and 72 h). In the case of 48 h at 35°C, the (*S*)-(+)-**3** was obtained with 50% conversion, e.e. >99% and *E* >200, the yield being 18% higher than at 25°C. However, 96 h at 25°C was necessary to reach 50% conversion. This indicates that the enzyme had higher catalytic activity at 35°C. Therefore, it is clear that poly (ethylene oxide) when used as a polymeric support to increase lipase reactivity, is far more efficient than the corresponding free form and agar gel under the same conditions.

In conclusion, immobilization of PSL in PEO proves to be an excellent methodology for the production of (*S*)-(+)-**3** on a laboratorial scale. In addition, the support increases the PSL reactivity, another further advantage of this system is that it can be re-used three times without losing its reactivity and enantioselectivity.

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