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Highly enantioselective bioreduction of ethyl 3-oxohexanoate

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ABSTRACT

Seven wild-type microorganism strains were used to reduce ethyl 3-oxohexanoate to ethyl (*R*)-3-hydroxyhexanoate. Free cells of *Kluyveromyces marxianus* and *Aspergillus niger* led to higher than 99% of conversion with higher than 99% ee. After immobilization in calcium alginate spheres, cells of *K. marxianus* exhibited high enantioselectivity (>99% ee) and conversion level (99%) within 24 h even if substrate was added at concentration of 10 g/L (62 mM).

Chiral β-hydroxyesters are widely used as building blocks for the synthesis of fine chemicals, pharmaceuticals, and natural products.[1,2] Ethyl 3-hydroxyhexanoate is a key intermediate in the synthesis of (+)-neopeltolide, a bioactive marine macrolide with potent antiproliferative activity against cancer cell lines. The cellular target for this class of substance is the inhibition of cytochrome *bc*1 complex.[3]

Many efforts have been made in the search for highly selective methods to obtain chiral β-hydroxyesters, as ethyl 3-hydroxyhexanoate. Asymmetric reduction of prochiral ketoesters is an alternative route. Bioreductions are attractive methods, mainly due to high enantioselectivity, mild and safe reaction conditions, and lower environmental impact compared to conventional reactions in organic chemistry.[4–7]

Isolated enzymes and whole cells can be used as biocatalysts in enantioselective reductions. Reductase enzymes require reduced nicotinamide cofactors and when isolated enzymes are used, a second catalytic cycle is necessary to regenerate cofactors and sustain catalytic activity.[4–7] So, whole cells are frequently preferred because of their own cofactor regeneration system even if competing enzymes within cells could decrease stereoselectivity. Reactions conditions and suitable microorganisms should be investigated to improve yields.[4–9]

In recent years we have been investigating the microbial reduction of ketoesters.[2,8–11] In the present work, seven wild-type microorganism strains were employed in the asymmetric reduction of ethyl 3-oxohexanoate to ethyl (*R*)-3-hydroxyhexanoate (Fig. 1). Some yeasts were also tested after immobilization in calcium alginate spheres.

In the first step of this work, free cells of five yeasts (*Saccharomyces cerevisiae* 40, *Hansenula* sp., *Geotrichum candidum*, *Kluyveromyces marxianus*, and *Rhodorotula rubra*) and two filamentous fungi (*Trichoderma harzianum* and *Aspergillus niger*) were tested (Table 1).[12] All the strains were able to catalyze the reaction with excess of the (*R*)-hydroxyester (determined by the measurement of optical rotation)[13] with an excellent conversion level (>99%) within 24 h. The enantiomeric excess varied from 9% (with *T. harzianum*) to higher than 99% (with *K. marxianus* and *A. niger*). Examples of chromatograms obtained are shown in Figure 2.

In previous studies, these microorganisms were successfully used in the reduction of methyl acetoacetate,[10] ethyl acetoacetate,[2,10] ethyl 2-methylacetoacetate,[10] ethyl benzoylacetate,[8,11] and ethyl 4-chloroacetoacetate.[9] However, excess of the (*S*)-hydroxyester was obtained in almost all of the cases. Only *K. marxianus*, *T. harzianum* and *A. niger* led to the excess of the (*R*)-hydroxyester in the reduction of ethyl acetoacetate (67% ee, 51% ee, and 19% ee, respectively) and *A. niger* maintained the

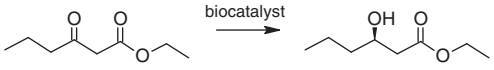

Figure 1. Bioreduction of ethyl 3-oxohexanoate to ethyl 3-(*R*)-hydroxyhexanoate.

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Table 1
Bioreduction of ethyl 3-oxohexanoate (5 g/L) to ethyl (*R*)-3-hydroxyhexanoate by free cells. Incubation: 30 °C, 150 rpm, 24 h

Microorganism	Conversion[a] (%)	ee (%)
Saccharomyces cerevisiae 40	>99	97.5
Hansenula sp.	>99	87.2
Geotrichum candidum	>99	85.7
Kluyveromyces marxianus	>99	>99
Rhodotorula rubra	>99	90
Trichoderma harzianum	>99	9
Aspergillus niger	>99	>99

[a] Determined by GC analysis.

(*R*)-enantioselectivity in the reduction of methyl acetoacetate (45% ee). So, enantioselectivity depends critically on the length of the carbon chain, as also observed by other researchers.[4,14]

Some authors described such enantioselectivity and conversion level employing recombinant microorganisms[15] and isolated enzymes.[6,16,17] Rodríguez et al.[15] showed that it was possible to obtain both (*S*)- and (*R*)-hydroxyesters with higher than 98% ee by using genetic engineering of baker's yeast, although lower substrate concentration (10 mM) had been used in comparison with the present work (31 mM). Kaluzna et al.[16] also achieved high (*S*)-enantioselectivity (>99% ee) by using isolated *S. cerevisiae* reductases overproduced in *Escherichia coli*, while Zhu et al.[17] used recombinant ketoreductases to reach both isomers with higher than 99% ee. However, wild-type microorganism strains are generally preferred than recombinant strains because of their robustness.[2] Moreover, whole cells do not require the addition of expensive cofactors, which is an advantage over the use of isolated enzymes, besides the lower cost.[18]

Among the wild-type microorganisms, *S. cerevisiae* was most frequently assayed in the reduction of ethyl 3-oxohexanoate. North[19] obtained higher than 98% ee of the (*S*)-hydroxyester and conversion of 55% with *S. cerevisiae* in petrol; Rotthaus et al.[20] employed *S. cerevisiae* in the reduction using different solvents and obtained 100% conversion and 86% ee of the (*R*)-isomer when the reaction was conducted in water. Rodriguez et al.[15] achieved 98% ee of the same isomer when *S. cerevisiae* was used as the biocatalyst, substrate at 12.6 mM, and glucose or galactose as the carbon source. Dahl et al.[21] achieved 99% ee of the (*R*)-hydroxyester using baker's yeast but they added allyl alcohol to the reaction medium, which is a carcinogenic and volatile enzymatic inhibitor not recommended for industrial processes.[22] Buisson et al.[22] described that the yeast *G. candidum* afforded the same isomer with 98% ee and 90% of conversion within 24 h. In the present work, as can be seen in Table 1, free cells of *K. marxianus* and *A. niger* carried out in the (*R*)-hydroxyester production with more than 99% ee and more than 99% conversion in medium containing substrate at 5 g/L or 31 mM.

In order to improve the method, some yeasts (*Hansenula* sp., *K. marxianus,* and *R. rubra*) were immobilized in calcium alginate spheres and tested for their ethyl (*R*)-3-oxohexanoate reduction ability in two cycles (Table 2).[23] This entrapment technique makes the product recovery much easier and the biocatalyst can be readily reused. However, the immobilization can influence enantiomeric excess and conversion level.[2,8,9,11,24,25]

As shown in Table 2, the immobilized cells of *K. marxianus* exhibited the best results because they led to the high enantioselectivity (>99% ee) and conversion level (>99%) after immobilization in the two cycles, with storage of 12 days between the cycles. The yeast *Hansenula* sp. gave high conversion level in the two cycles, but with decrease in enantioselectivity. In the first use of immobilized cells of *R. rubra,* a little decrease was observed in conversion level and, in the second cycle, still lower conversion was obtained.

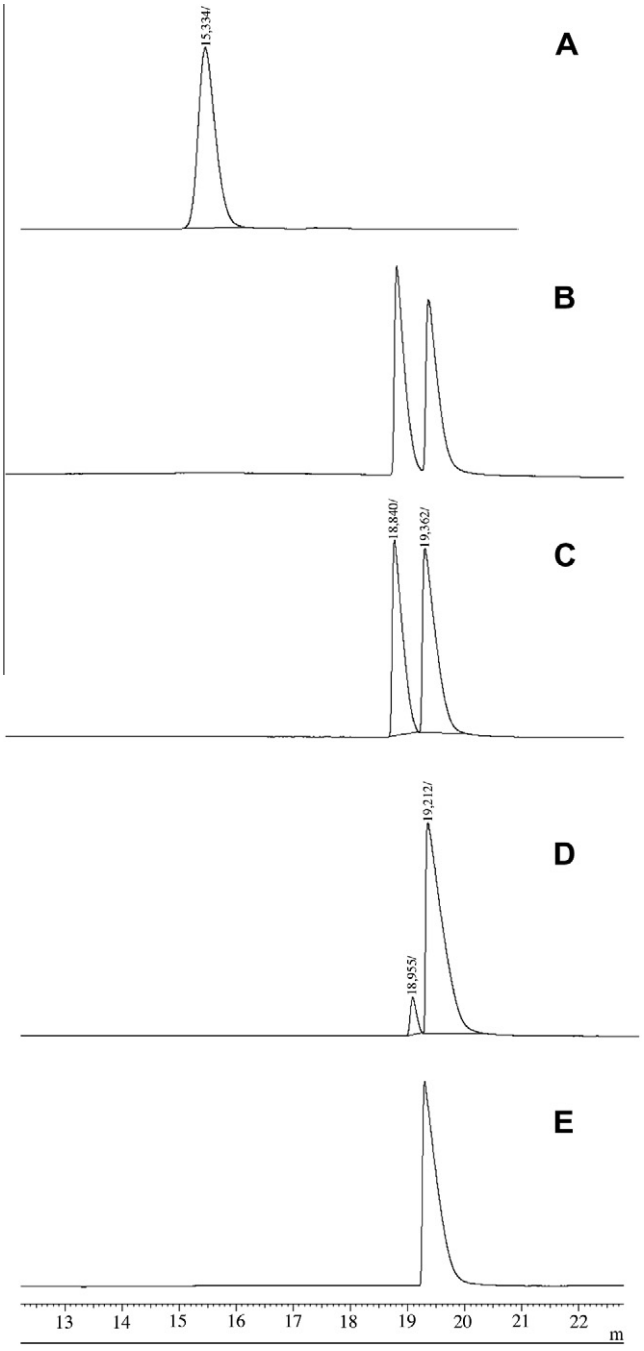

Figure 2. Typical chromatograms showing the selectivities achieved. Chiral GC analysis on column Beta DEX325 (30 m × 0.25 mm × 0.25 μm), at 90 °C (23 min): (A) ethyl 3-oxohexanoate (substrate); (B) ethyl 3-hydroxybutanoate (racemate obtained via NaBH4 reduction); (C) *Trichoderma harzianum* reduction; (D) *Hansenula* sp. reduction; (E) *Kluyveromyces marxianus* reduction. Retention times (t_R): t_R (substrate) = 15.3 min, $t_{R\ ((S)\text{-enantiomer})}$ = 18.8 min, $t_{R\ ((R)\text{-enantiomer})}$ = 19.3 min.

High substrate concentration is important for an industrial biotransformation process[24,26] but it can decrease conversion level and enantioselectivity.[27] This effect was previously observed in the reduction of ethyl 4-chloroacetoacetate to ethyl (*S*)-4-chloro-3-hydroxybutanoate by the immobilized cells of *K. marxianus*.[9] In the reactions catalyzed by whole cells, substrate toxicity is an aggravating factor.[28] Immobilization could reduce toxic effects by a diffusion barrier that protects cells and it could allow the addition of substrate at higher concentrations.[24] To evaluate

Table 2
Bioreduction of ethyl 3-oxohexanoate (5 g/L) to ethyl (*R*)-3-hydroxyhexanoate by immobilized cells. Incubation: 30° C, 150 rpm, 24 h

Microorganism	First cycle		Second cycle (after 12 d)	
	Conversion[a] (%)	ee (%)	Conversion[a] (%)	ee (%)
K. marxianus	>99	>99	>99	>99
Hansenula sp.	>99	75.1	>99	60.8
R. rubra	96.5	80.6	61.1	80.5

[a] Determined by GC analysis.

whether substrate concentration could influence catalytic activity, the cells of *K. marxianus* were immobilized again and used in the reduction of ethyl 3-oxohexanoate at substrate concentrations of 7.5 g/L and 10 g/L. This biocatalyst also showed the same conversion level (>99%) and enantioselectivity (>99% ee) observed before.

In conclusion, the filamentous fungus *A. niger* and the yeast *K. marxianus* led to the high conversion of ethyl 3-oxohexanoate to ethyl (*R*)-3-hydroxyhexanoate (>99%) with extremely high enantioselectivity (>99% ee). To our knowledge, this is the first Letter on the use of these two microorganisms in the reduction of ethyl 3-oxohexanoate. The high conversion level, high enantiomeric excess, tolerance to substrate and the possibility of immobilization, and reuse make *K. marxianus* a promising biocatalyst for industrial applications.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.09.023.

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13. $[\alpha]_D^{25}$ −30.8° (*c* 1 g/100 mL, $CHCl_3$); literature: $[\alpha]_D^{25}$ −26.9° (*c* 1.14 g/100 mL, $CHCl_3$). Optical rotations were measured from $CHCl_3$ solutions using a JASCO DIP-370 polarimeter at the sodium D line (589 nm) operating at room temperature and compared to literature: Roche, C.; Desroy, N.; Haddad, M.; Phansavath, P.; Genet, J. P. *Org. Lett.* **2008**, *10*, 3911–3914.
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