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Short communication

Microbiological enantioselective reduction of ethyl acetoacetate

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Abstract

Different microorganisms (MOs) were used to carry out the enantioselective reduction of ethyl acetoacetate to (*S*)-(+)-3-hydroxybutanoate or (*R*)-(−)-3-hydroxybutanoate. Surprisingly, the commercially available *Saccaromyces cerevisiae* led to only 58% ee of (*S*)-(+)-3-hydroxybutanoate. Other MOs such as *Hansenula* sp. and *Dekera* sp. furnished the same enantiomer with greater ees, 81 and 73%, respectively. *Aspergilus niger* and *Kluyveromyces marxianus* although leading to lower ees, 30 and 18%, yielded the opposite enantiomer. All reactions proceeded to greater than 85% conversion. This is the first report on the use of *Hansenula* sp. and *Dekera* sp. in reductions of β-ketoesters.

Keywords: *Hansenula* sp.; *Dekera* sp.; Enantioselective; Reduction; Ketoesters

1. Introduction

Asymmetric reduction of prochiral ketones is one of the most investigated methods to produce chiral compounds [1–6]. Many successful approaches have been published so far, including the catalytic use of chiral oxazaborolidines [7–9] for hydride transfer and hydrogenation via the use of homogeneous chiral metal complexes [10–12] and heterogeneous chiral modified surfaces [13–24]. However, microbiological reduction of activated carbonyls is still an interesting method and although vastly investigated, the search for alternative microorganisms to *Sacharomyces cerevisae* to effect the desired reductions is the aim of several groups [25–30], including our own.

The need for environmentally friendly biotechnological methods that could generate either enantiomer upon reduction of prochiral ketones is still a challenge. The purpose of the present paper is to describe briefly our findings with different microorganisms (MOs) [31–35] in the reduction of ethyl acetoacetate.

Since the original work of Prelog and coworkers [34,35] and Seebach et al. [36], many other groups contributed significantly in this field. However, the insistence in working with Baker's yeast, due to its low cost, facility of use and good stereochemical product prediction has masked the fact that many other MOs could give similar results and perhaps provide either enantiomer [31–35,37,38].

It is worth noting that enzymatic or microbiological processes have many advantages compared to conventional ones [39–53], despite the fact that many of the conventional chemical processes, as described above, lead to very good yields and ees. The main points in favor of the biotechnological process are its green and

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"natural" appeal and the potential scope for changing the MO and/or the feedstock.

The whole cells can be used as "formal" reagents mainly in three different stages of growth: (a) the feedstock is added immediately upon starting the cultivation, after choosing the exact conditions to achieve the desired MO growth; (b) the MO is collected after the initial stages, via centrifugation or filtration and resuspended in the reaction medium within the substrate; and/or (c) dried cells are prepared, stored and used whenever necessary. While the first method is simple and useful for those not very experienced in this kind of process, the second presents the advantage of possible reuse of the MO and the third offers the possibility of storing the MO as a "formal" reagent.

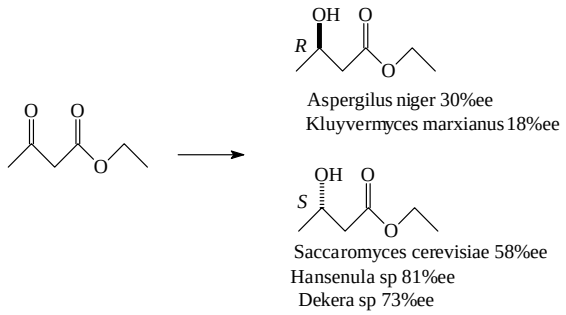
The low cost of the process using whole cells compared to the use of isolated enzymes is quite often affected by the fact that side products are frequently obtained, low enantiomeric excesses or diastereoisomeric excesses are achieved and the permeability of the cell does not allow the entrance of the feedstock. Additionally, substrate solubility, which can be a drawback when using pure enzymes as reagents, can become critical when using whole cells. However, the plethora of MOs available in nature [51–53] encouraged us into the empirical [54] search of MOs capable of conducting the desired transformations.

Prelog and coworkers [36–42] have successfully rationalized the outcome of these reactions and presently the role of the coenzymes is understood and the stereochemistry of the products is anticipated easily using Baker's yeast (or the original *Curvularia falcata*) as the reduction reagent.

2. Results and discussion

In order to perform the present screening *Kluyveromyces marxianus*, *Dekera* sp. and *Hansenula* sp. were selected in addition to the well-known commercial Baker's yeast (*Sacharomyces cerevisae*) and *Aspergilus niger*.

The reactions (Scheme 1) were followed by IR and/or HRGC. Conversions were always above 85%. The racemate standard was obtained quantitatively via $NaBH_4$ reduction of the feedstock, ethyl acetoacetate. All the results showed in the present paper were reproduced at least three times and analyzed (for ee determination) several times without any noticeable bias (Table 1).



Scheme 1.

Table 1
Reduction of ethyl acetoacetate with different MOs

Microorganism	ee
Saccaromyces cerevisiae	58% *S*
Aspergilus niger	30% *R*
Hansenula sp.	81% *S*
Dekera sp.	73% *S*
Kluyveromyces marxianus	18% *R*

To our surprise, the commercial *Saccaromyces cerevisiae* used in the present work furnished the reaction product in moderate ee. Two other MOs, *Hansenula* sp. and *Dekera* sp. were able to reach higher ees, 81 and 73%, respectively. Both *A. niger* and *K. marxianus* showed the reversed enantioselectivity, i.e. they afforded the (*R*)-enantiomer, although in low ee, so opening a new perspective in microbiological access to chiral building blocks.

With the aim to improve the ees, some initial studies were conducted on immobilization of *K. marxianus* in calcium alginate [55]. Before their use, the immobilized cells were activated during 4 h in the growth medium. However, although leading to a very high conversion (99%), the enantiomeric excesses was lower, 14%, when compared with free cells. Other reaction conditions are under investigation with this and with the other microorganisms used in the present work.

3. Experimental

The ee and absolute configuration of the reaction products were determined by chiral high-resolution

chromatography, performed on a commercially available BDB-176 capillary column (25 m × 0.25 mm, i.d.), isothermally at 343 K. Ethyl (*R*)-(−)-hydroxybutanoate, (*S*)-(+)-hydroxybutanoate and *rac*-3-hydroxybutanoate, were used as chromatographic standards. The elution order is *S* (t_r = 8.8 min) followed by *R* (t_r = 9.3 min). Ethyl acetoacetate eluted at t_r = 8.6 min. The reaction products were isolated and characterized by ^{1}H NMR and IR. The reaction mixture was submitted to HRGC-MS analysis. All spectra data support the present findings.

All microorganisms used in the present work were collected from soil (*A. niger*) or from different fruits (*Hansenula* sp., *Dekera* sp. and *K. marxianus*); they belong to the collection of the "Departamento de Bioquímica, Escola de Química, UFRJ" and are freely available upon request.

The cells were allowed to grow during 48 h in a medium containing 1% glucose, 0.5% of yeast extract, 0.5% peptone, 0.1% NH_4OH and 0.1% $MgSO_4$. After that period, they were harvested by centrifugation, resuspended in water and added to the reaction medium which contained 20% glucose, 0.1% $MgCl_2$ and 0.5% of the substrate. The quantity of cells was not determined; instead the volume of *inocula* was always 10% of the medium volume. Reactions were carried out under stirring (150 rpm) at 303 K during 24 h. Work up was undertaken by centrifugation, decantation and extraction with EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum.

Some initial studies were conducted on immobilization of *K. marxianus*. Therefore, after 48 h of cell growth, cells were harvested by centrifugation and resuspended in water. To this suspension, under stirring, was added 1.5% (w/v) aqueous solution of sodium alginate. This solution was then added to a 0.1 mol l^{-1} aqueous solution of $CaCl_2$ and stocked at 10 °C until use. Spheres obtained by decantation were activated by allowing them to rest in the growth medium during 4 h and decanted before their addition to the reaction medium.

4. Conclusions

To conclude, this is the first report on the use of *Hansenula* sp. and *Dekera* sp. for enantioselective reduction of β-ketoesters, which furnished higher ees than the known *S. cerevisiae* and *A. niger*. In addition, the ee obtained with *K. marxianus*, although lower, deserves attention since this MO afforded the (*R*)-enantiomer. Preliminary immobilization studies with this microorganism led to higher conversion and lower ee. A complete study is in progress.

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