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Exploring the potential of some yeast strains in the stereoselective synthesis of aldol reaction products and its reduced 1,3-dialcohol derivatives

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ABSTRACT

The behavior of two yeast strains has been studied under different conditions. Both microorganims catalyzed the aldol reaction between activated aldehydes and acetone when a large amount of the latter was present in the reaction medium producing, with moderate stereoselectivity, the aldol product with the *R* configuration. No reduction of any of the products present in the medium was detected. On the other hand, the carbonyl group of the racemic aldol was reduced to produce chiral 1,3-dialcohol derivatives when water was employed as the only solvent. In this case, the resolution of the racemic starting material was also possible with one of the biocatalysts, and the aldol was recovered with the *S* configuration. A complementary enantioselectivity was shown by both microorganisms in the generation of the new stereogenic center, which allowed access to 3 of the 4 possible diastereomeric diols with high enantiomerical purity.

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1. Introduction

Biocatalysis refers to the use of natural enzymes to perform chemical transformations of organic compounds. Enzymes used as catalysts in synthesis offer considerable advantages, not the least of which include their high efficiency and their capacity to produce regio- and stereoselectively building blocks of great value and utility in the pharmaceutical and food industries [1]. If compared with conventional chemical catalysis, biocatalysis is the most convenient from the experimental and ecological points of view: its high specificity results in few side products, the reaction conditions are very mild, and the low production level of waste pollutants involves a negligible environmental impact [2]. In the biocatalysis field, not only have isolated enzymes been used, but also whole microorganisms [3]. Whole cells of different yeast species have been widely used for a number of asymmetric transformations, especially in bioreduction reactions for the preparation of chiral alcohols [4]. The use of whole cells entails simpler catalyst preparation and easy strategies for efficient cofactor recycling or multistep conversions than the utilization of isolated enzymes as catalysts [5].

Catalytic promiscuity has been recognized as enzymes' ability to catalyze different chemical transformations depending on the reaction conditions [6]. Whole cell catalysts containing multiple enzymes are also capable of catalyzing different reactions depending on the medium conditions and the substrates supplied. This potential has been exploited for synthetic purposes [4a–c].

The stereoselective aldol reaction is considered one of the most important carbon-carbon bond-forming reactions in organic synthesis, and is a way to obtain chiral β-hydroxy carbonyl compounds [7]. Recently, we described the use of different yeast strains to study the aldol reaction between acetone and *p*-nitrobenzaldehyde. We carried out the reaction using lyophilized cells in an organic medium (acetone), and studied the influence of different conditions to achieve the best results from the stereoselective viewpoint. In all cases the excess enantiomer shows *R* configuration, although moderate conversion and stereoselectivity were achieved for all the strains checked. In this study we were unable to identify the respective enzyme responsible for this catalytic activity, not discarding the possibility that some amino acid residue acts as organocatalyst in the reaction. Under the reported conditions, the carbonyl reduction of the starting materials did not occur, and only the aldol product and the aldehyde substrate were recovered in all cases [8].

Chiral 1,3-diols, with two stereogenic centers, are important building blocks in the synthesis of pharmaceutically active compounds [9]. All the possible stereoisomers of these interesting compounds have been obtained by different groups by

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combining organocatalysis, for aldol asymmetric reaction between an aldehyde and acetone, and biocatalysis, using isolated alcohol dehydrogenases, for the asymmetric carbonyl reduction of the aldol product [10].

In this work we describe results obtained with whole cells from yeasts *Pichia jardinii* (CECT 1060) [11] and *Kluyveromyces marxianus* (CECT 1018) [11] as biocatalysts under different reaction conditions. Selection of the medium, substrate and biocatalyst allowed us to prepare chiral aldols in both configurations and/or their chiral 1,3-diols derivatives.

2. Experimental

2.1. General

All the commercially available reagents were purchased from Sigma–Aldrich. Reactions were monitored by thin layer chromatography (TLC) on Merck silica plates 60 F_{254}. Flash chromatography was performed on Merck silica gel (60 particle size: 0.040–0.063 mm). The NMR spectra were recorded with Bruker DRX 300 spectrometers using deuterated chloroform as solvent. Chemical shifts are reported in ppm in relation to the residual solvent peak. Absolute configurations were determined by comparison with the optical rotations reported in the literature and these were performed on a Perkin Elmer 241 Polarimeter at $\lambda = 589$ nm. Determination of enantiomeric excess was carried out by high performance liquid chromatography (HPLC), with a Merck Hitachi Lachrom system. The specific conditions are described for each case.

Racemic mixtures of diastereomeric dialcohols were synthesized by reduction of the respective racemic aldol with $NaBH_4$ in methanol following standard methods. They were used as HPLC patterns.

2.2. Typical procedure for biocatalytic aldol condensation using dried whole cells [8]

Reactions were carried out in capped vials (15 mL), where lyophilized cells (40 mg), prepared as we described previously [8], were resuspended in 2.5 mL of the solvent mixture (acetone:water, 97.5:2.5 or 1:1). The corresponding aldehyde (4 mg) was added and the mix was shaken on an orbital shaker at 25 °C. The reaction was monitored by tlc and, finally, the mixture was centrifuged (3500 rpm, 3 min), treated with an ammonium chloride solution and acetone was evaporated in vacuum. The aqueous solution was extracted with methylene chloride and the organic phase was dried over anhydrous sodium sulfate. Crude material was employed to determine the yield by NMR and the ee by HPLC using the chiral stationary phase.

The reaction products were purified by column chromatography (hexane:ethyl acetate 4:1) and characterized by NMR and chiral HPLC. Data were consistent with those described in the literature [12].

2.2.1. 4-(4-Nitrophenyl)-4-hydroxybutan-2-one (**2a**)[12]

^{1}H NMR ($CDCl_3$): 2.21 (s, 3H), 2.84 (m, 2H), 3.60 (s, 1H), 5.26 (m, 1H), 7.53 (d, 8.8 Hz, 2H), 8.20 (d, 8.8 Hz, 2H) ppm. Enantiomeric excess: HPLC, Chiralpak IC column, Hex/*i*prOH 94/6, 1 mL/min, 254 nm (t_r = 31.5 min *S*; t_r = 33.6 min *R*).

2.2.2. 4-(2-Nitrophenyl)-4-hidroxybutan-2-one (**2b**) [12]

^{1}H NMR ($CDCl_3$): 2.16 (s, 3H), 2.63 (dd, 17.8 Hz, 9.4 Hz, 1H), 3.09 (dd, 17.8 Hz, 2.0 Hz, 1H), 3.90 (s, 1H), 5.60 (m, 1H), 7.46 (m, 1H), 7.67 (m, 1H), 7.88 (m, 1H), 7.96 (m, 1H). Enantiomeric excess: HPLC, Chiralpak IA column, Hex/*i*prOH 97/3, 0.8 mL/min, 254 nm (t_r = 47 min *R*; t_r = 49.8 min *S*); Chiralpak IC column, Hex/*i*prOH 92/8, 1 mL/min, 254 nm (t_r = 24.4 min *R*; t_r = 41.9 min *S*).

2.2.3. 4-(3-Nitrophenyl)-4-hydroxybutan-2-one (**2c**) [12]

^{1}H NMR ($CDCl_3$): 2.23 (s, 3H), 2.90 (m, 2H), 3.59 (s, 1H), 5.30 (m, 1H), 7.53 (m, 1H), 7.70 (m, 1H), 8.09 (m, 1H), 8.24 (m, 1H). Enantiomeric excess: HPLC, Chiralpak IA column, Hex/*i*prOH 97/3, 0.8 mL/min (t_r = 70 min *R*; t_r = 80 min *S*).

2.2.4. 4-(4-Chlorophenyl)-4-hydroxybutan-2-one (**2d**) [12]

^{1}H NMR ($CDCl_3$): 2.19 (s, 3H), 2.80 (m, 2H), 3.38 (s, 1H), 5.11 (dd, 8, 4 Hz, 1H), 7.30 (m, 4H). Enantiomeric excess: HPLC, Chiralpak IA column, Hex/*i*prOH 97/3, 1 mL/min, 214 nm (t_r = 25.5 min *R*; t_r = 27.3 min *S*).

2.2.5. 4-(2-Chlorophenyl)-4-hydroxybutan-2-one (**2e**) [12]

^{1}H NMR ($CDCl_3$): 2.21 (s, 3H), 2.68 (dd,18, 9 Hz, 1H), 2.98 (dd,18, 2.2 Hz, 1H), 3.65 (s, 1H), 5.50 (dd, 9, 2.1 Hz, 1H), 7.20 (m, 1H), 7.34 (m, 2H), 7.62 (m, 1H). Enantiomeric excess: HPLC, Chiralpak IA column, Hex/*i*prOH 97/3, 1 mL/min, 214 nm (t_r = 16 min *R*; t_r = 17 min *S*).

2.3. General procedure for the reduction of racemic aldols **2**

To ensure that in independent experiments the same amount of biomass was used, the OD_{600} (which measures the turbidity of the cellular suspension) was determined in overnight cultures of the microorganisms in YPD medium (1% (w/v) yeast extract, 2% (w/v) bactopeptone, 2% (w/v) glucose), and the volume corresponding to 500 OD_{600} was taken for each experiment (for example, 50 mL of a culture with OD_{600} of 10). After centrifugation, the pellet was resuspended in 25 mL of sterile water containing 2.5% glucose (to ensure the metabolic activity of the cells, and guarantee the regeneration of the cofactor NADH). The mixture was incubated in a flask at 30 °C for 30 min with orbital shaking. Then racemic aldol 2 (10 mg) was added, and the reaction was maintained under the above conditions for the time indicated in each case (previously determined by a chiral chromatography analysis of the various aliquots taken at different times). Next 1 mL of 40% glucose was added periodically every 24 h during the incubation time. Afterwards, the reaction mixture was centrifuged and the aqueous supernatant was extracted with ethyl acetate (3 × 20 mL). Organic phases were combined and dried with sodium sulfate. After solvent evaporation, the crude material was analyzed by ^{1}H NMR, to determine the percentage of transformation, and also by chiral HPLC. Afterward, it was purified by column chromatography (hexanes:ethyl acetate 5:1) to afford quantitatively the products described latter.

2.3.1. 1-(4-Nitrophenyl)-1,3-butanediol (**3a**)[10]

syn- ^{1}H NMR ($CDCl_3$): 1.27 (d, 6.2 Hz, 3H), 1.76–1.82 (m, 2H), 2.34 (s, 1H), 4.05 (s, 1H), 4.2–4.26 (m, 1H), 5.05–5.09 (m, 1H), 7.54 (d, 8.3 Hz, 2H), 8.20 (d, 8.3 Hz, 2H) ppm.

anti- ^{1}H NMR ($CDCl_3$): 1.28 (d, 6.3 Hz, 3H), 1.89–1.92 (m, 2H), 2.00 (s, 1H), 3.55 (s, 1H), 4.04–4.10 (m, 1H), 5.16–5.20 (m, 1H), 7.55 (d, 8.4 Hz, 2H), 8.21 (d, 8.4 Hz, 2H) ppm.

Enantiomeric excess: HPLC, Chiralpak IC column, Hex/*i*prOH 96/4, 1 mL/min, 254 nm, ((+)1*R*,3*R* 49.2 min; (−)1*S*,3*S* 42.0 min; (+)1*R*,3*S* 33.4 min).

2.3.2. 1-(2-Nitrophenyl)-1,3-butanediol (**3b**)

syn- ^{1}H NMR ($CDCl_3$): 1.27 (d, 6.2 Hz, 3H), 1.5 (s, 1H). 1.69–1.81 (m, 1H),1.97–2.09 (m, 1H), 4.0 (s, 1H), 4.20–4.34 (m, 1H), 5.49 (dd, 9.8, 1.8 Hz, 1H), 7.41 (dd, 8.3, 1.6 Hz, 1H), 7.66 (dd, 8, 1 Hz, 1H), 7.91–7.94 (m, 2H) ppm.

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