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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.

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.

anti- ^{1}H NMR ($CDCl_3$): 1.27 (d, 6.3 Hz, 3H), 1.5 (s, 1H), 1.90–1.93 (m, 2H), 3.6 (s, 1H), 4.05–4.15 (m, 1H), 5.58–5.62 (m, 1H), 7.35 (dd, 8.0, 1.4 Hz, 1H), 7.57 (dd, 7, 1.2 Hz, 1H), 7.85–7.89 (m, 2H) ppm.

Enantiomeric excess: HPLC, Chiralpak IC column, Hex/*i*prOH 92/8, 1 mL/min, 254 nm, ((+)1*S*,3*S* 25.81; (−)1*R*,3*S* 17.38 min).

*2.3.3. 1-(3-Nitrophenyl)-1,3-butanediol (**3c**)*

syn- ^{1}H NMR ($CDCl_3$): 1.27 (d, 6.3 Hz, 3H), 1.6 (s, 2H), 1.79–1.84 (m, 2H), 4.17–4.24 (m, 1H), 5.04–5.09 (m, 1H), 7.54–7.64 (m, 1H), 7.71–7.74 (m,1H), 8.11–8.14 (m, 1H), 8.24–8.26 (m, 1H) ppm.

anti- ^{1}H NMR ($CDCl_3$): 1.29 (d, 6.3 Hz, 3H), 1.58 (s, 1H), 1.90–1.94 (m, 2H), 3.57 (s, 1H), 4.06–4.12 (m, 1H), 5.16–5.20 (m, 1H), 7.50–7.53 (m, 1H), 7.71–7.73 (m,1H), 8.10–8.14 (m, 1H), 8.25–8.27 (m, 1H) ppm.

Enantiomeric excess: HPLC, Chiralpak IC column, Hex/*i*prOH 95/5, 1 mL/min, 254 nm, ((−)1*S*,3*S* 60.1 min; (+)1*R*,3*S* 31.4 min).

2.3.4. 1-(4-Chlorophenyl)-1,3-butanediol (3d)[10]

syn- ^{1}H NMR ($CDCl_3$): 1.24 (d, 6.0 Hz, 3H), 1.69–1.87 (m, 2H), 2.64 (s, 1H), 3.38 (s, 1H), 4.10–4.19 (m, 1H), 4.93 (dd,9.6, 3.3 Hz,1H), 7.31 (s, 4H) ppm.

anti- ^{1}H NMR ($CDCl_3$): 1.25 (d, 6.3 Hz, 3H), 1.84–1.89 (m, 2H), 2.1 (s, 1H), 3.04 (s, 1H), 4.04–4.10 (m, 1H), 5.03–5.07 (m,1H), 7.31 (s, 4H) ppm.

Enantiomeric excess: HPLC, Chiralpak IA column, Hex/*i*prOH 97/3, 1 mL/min, 214 nm, ((+)1*R*,3*R* 29.1 min; (−)1*S*,3*S* 28.04 min; (+)1*R*,3*S* 36.8 min).

2.3.5. 1-(2-Chlorophenyl)-1,3-butanediol (3e) [10]

syn- ^{1}H NMR ($CDCl_3$): 1.19 (d, 6.0 Hz, 3H), 1.56–1.67 (m, 1H), 1.81–1.87 (m, 1H), 2.7 (s, 1H), 3.46 (s, 1H), 4.13–4.22 (m, 1H), 5.28 (dd, 9.9, 1.8 Hz; 1H), 7.13 (dd, 6.6, 1.8 Hz,1H) 7.21–7.26 (m, 2H), 7.55–7.58 (m, 1H) ppm.

anti- ^{1}H NMR ($CDCl_3$): 1.22 (d, 6.0 Hz, 3H), 1.84–1.87 (m, 2H), 2.1 (s, 1H), 3.2(s, 1H), 3.98–4.04 (m, 1H), 5.36–5.42 (m,1H), 7.13 (dd, 6.9, 1.8 Hz,1H) 7.24 (dd, 7.8, 0.9 Hz, 2H), 7.55–7.59 (m, 1H) ppm.

Enantiomeric excess: HPLC, Chiralcel ODH column, Hex/*i*prOH 98/2, 1 mL/min, 214 nm, ((+)1*R*,3*R* 24.13 min; (−)1*S*,3*S* 45 min; (+)1*R*,3*S* 32.7 min).

*2.3.6. 1-Phenyl-1,3-butanediol (**3f**)[13]*

syn- ^{1}H NMR ($CDCl_3$): 1.23 (d, 6.0 Hz, 3H), 1.73–1.93 (m, 2H), 2.89 (s, 1H), 3.09 (s, 1H), 4.13–4.18 (m, 1H), 4.95 (dd, 9.9, 3 Hz, 1H), 7.35–7.37 (m, 5H) ppm.

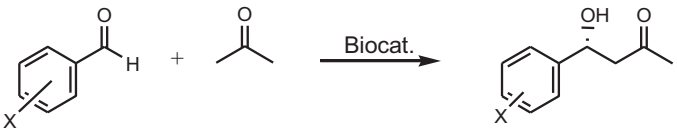
anti- ^{1}H NMR ($CDCl_3$): 1.25 (d, 6.0 Hz, 3H), 1.86–1.93 (m, 2H), 2.2 (s, 1H), 2.84 (s, 1H), 4.05–4.11 (m, 1H), 5.04–5.1 (m, 1H),7.35–7.40 (m, 5H) ppm.

Enantiomeric excess: HPLC, Chiralpak IC column, Hex/*i*prOH 96/4, 1 mL/min, 214 nm, ((+)1*R*,3*R* 35.2 min; (−)1*S*,3*S* 24.7 min; (+)1*R*,3*S* 28.46 min).

3. Results and discussion

Microorganisms used as whole cell catalysts can display different enzymatic activities depending on the medium conditions and the substrates supplied [3,4]. In a previous work, we studied the ability of several yeast strains to carry out asymmetric aldol reaction between 4-nitrobenzaldehyde and acetone to yield 4-(4-nitrophenyl)-4-hydroxybutan-2-one (**2a**). For this purpose, lyophilized cells from stationary phase cultures were employed.

In the present work, we wanted to extend the reaction described above to other biocatalysts and substrates. Scheme 1 and Table 1 show the results obtained with the microorganisms *K. marxianus* and *P. jardinii* for the reaction of different activated benzaldehydes and acetone under conditions that were previously optimized



1a (X= 4-NO_2)
1b (X= 2-NO_2)
1c (X= 3-NO_2)
1d (X= 4-Cl)
1e (X= 2-Cl)

2a (X= 4-NO_2)
2b (X= 2-NO_2)
2c (X= 3-NO_2)
2d (X= 4-Cl)
2e (X= 2-Cl)

Scheme 1. Biocatalytic aldol reaction.

(2.5% water in acetone used as a solvent) [8]. In all cases, the *R* enantiomer was obtained in moderate excess, the stereoselectivity being slightly better when *K. marxianus* was used as a catalyst.

In reactions carried out in a 1:1 mixture of acetone and water as a solvent at 30 °C, the reaction was quantitative after 72 h (as shown in Scheme 1), but no stereoselectivity was achieved with any of the catalysts under these conditions (less than 5% ee). Unfortunately, the reaction was effective only when strongly activated aldehydes were used as substrates.

It is worth mentioning that under both conditions the carbonyl reduction of the starting materials does not occur, and only the aldol product and the aldehyde substrate are recovered in all cases.

Based on these results, we decided to explore the behaviour of these microorganisms under different conditions. So, first of all, we checked their ability to reduce the racemic aldol product derived from 4-nitrobenzaldehyde and acetone (±**2a**) using fresh cells in aqueous medium (2% glucose w/v). Scheme 2 shows the complementary results obtained for both biocatalysts.

K. marxianus showed excellent stereoselectivity in the resolution of the racemic starting material and allowed the recovery of the *S* enantiopure aldol (***S*-2a**,95% ee). Moreover excellent enantioselectivity (95%) was achieved in the 1,3-diol product of the reduction reaction. Indeed, the *syn* diol with the 1*R*,3*R* configuration was almost the only product. On the other hand, *P. jardinii* was unable to resolve the racemic mixture, and both diastereomers (relation *syn:anti* 1.17) with excellent enantioselectivity were obtained (**1*S*,3*S*–3a**, 95% ee and **1*R*,3*S*–3a**, 99% ee).

Both microorganisms showed a different enantioselectivity in the generation of the new stereogenic center (*R* in the case of *K. marxianus* and *S* when *P. jardinii* was used), and three of the four possible diol stereoisomers were available. In order to obtain the 1,3-diol with 1*S*,3*R* configuration, we unsuccessfully screened seven other yeast strains [11]. *Saccharomyces cerevisiae* Lalvin T-73, *Torulospora delbrueckii*, *Saccharomyces cerevisiae* FY86, *Pichia*

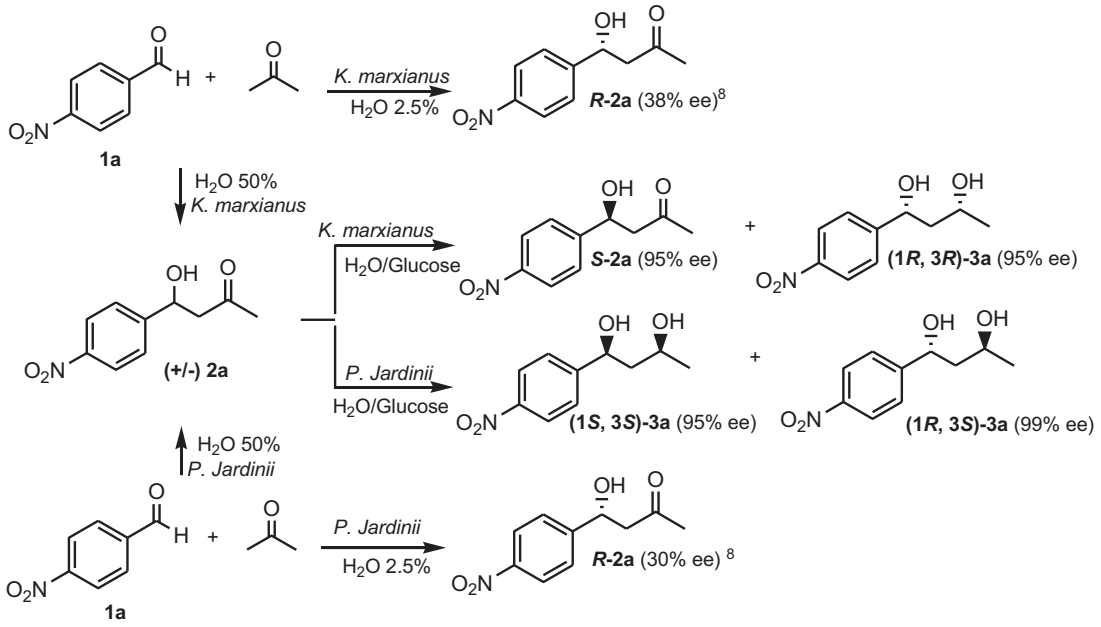
Table 1
Scope of aldehydes for the aldol reaction with acetone under optimized conditions.

Aldehyde	Biocatalyst	Conversion (%)[a]	Product/ee (%)[b]
1a[8]	*P. jardinii*	35	(*R*)–**2a**/30
1a[8]	*K. marxianus*	42	(*R*)–**2a**/38
1b	*P. jardinii*	25	(*R*)–**2b**/35
1b	*K. marxianus*	25	(*R*)–**2b**/47
1c	*P. jardinii*	30	(*R*)–**2c**/27
1c	*K. marxianus*	30	(*R*)–**2c**/44
1d	*P. jardinii*	nd	(*R*)–**2d**/33
1d	*K. marxianus*	nd	(*R*)–**2d**/50
1e	*P. jardinii*	80	(*R*)–**2e**/39
1e	*K. marxianus*	60	(*R*)–**2e**/50

Conditions [8]: lyophilized biocatalyst (40 mg), substrate (4 mg), water in acetone (2.5-vol.%, 2.5 mL), 25 °C; 96 h.

[a] Conversion: determined by ^{1}H NMR. No reaction was observed in the absence of biocatalyst.

[b] Determined by chiral HPLC. Absolute configuration according to literature data [12].



Scheme 2. Use of both yeast strains allows access to a structural and stereochemical diversity of compounds, depending on the starting reagents and conditions.

Table 2
Reduction of several racemic aldols with yeast *K. marxianus*.

Substrate/time (h)/conversion (%)[b]	Product 2/ee (%)[d]	Product 3- syn/anti[c]	Product 3-conf/ee (%)[d]
(±)**2a**/96/60	***S*–2a**/95	83/17	1*R*,3*R*-3a/95
(±)**2b**/96/10	–	–	–
(±)**2c**/96/10	–	–	–
(±)**2d**/96/50	***S*–2d**/57	83/17	1*R*,3R-**3d**/99
(±)**2e**/72/52	***S*–2e** 78	90/10	1*R*,3*R*-**3e**/85
(±)**2f**[a]/72/62	***S*–2f**/92	60/40	1*R*,3*R*-**3f**/99

Conditions: biocatalyst (500 OD_{600} unit) in 25 mL of sterile water containing 2.5% glucose, substrate (10 mg), 30 °C.
[a] **2f** (X = H).
[b] Calculated by ^{1}H NMR.
[c] Relation between diastereomeric dialcohols was determined by both ^{1}H NMR and chiral HPLC. Assignation of the ^{1}H NMR signals for each one was done in accordance with the literature data [10,13] and was confirmed by NOE experiments (Supplementary Data).
[d] Absolute configuration was established in accordance with the literature data [10,12,13]. Enantioselectivity was less than 5% for the minoritary *anti* diastereomer.

glucozyma, and *Pichia fermentants* were inactive in the reduction of the starting material. *Ogatea minuta* showed the same stereoselectivity than *P. jardinii*, but was considerably less active in the reduction reaction. On the other hand, *Debaromyces etchellsii* produced the four possible diasteroemers with some diastereoselectivity (*syn:anti* 2) but without enantioselectivity.

Then, we decided to explore the general scope of this methodology. Racemic aldols obtained by condensation of acetone and several aromatic aldehydes were employed as substrate (Tables 2 and 3). The stereoselectivity and the reactivity of the reaction were affected by the nature and position of the substituents in the aromatic ring when *K. marxianus* was the biocatalyst. Thus, 2- and 3-nitro derivatives (**2b**, ***2c***) were not transformed by this microorganism, but surprisingly 2-chloro (**2e**) was. In those cases in which reduction was possible, the transformation of the starting material fell between 50 and 60% after 72–96 h and the enantioselectivity in the resolution of the racemic aldol was between good and excellent. The stereoselectivity in *syn* diol production was

Table 3
Reduction of several racemic aldols with yeast *P. jardinii*.

Substrate/time (h)/conversion (%)[b]	Product 3 [*syn*/*anti*][c]	Product 3 *syn*/conf/ee(%)[d]	Product 3 *anti*/conf/ee (%)[d]
(±)**2a**/48/96	**3a** [54/46]	(1*S*,3*S*)/95	(1*R*,3*S*)/99
(±)**2b**/96/72	**3b** [63/37]	(1*S*,3*S*)/99	(1*R*,3*S*)/99
(±)**2c**/48/86	**3c** [53/47]	(1*S*,3*S*)/99	(1*R*,3*S*)/99
(±)**2d**/96/90	**3d** [55/45]	(1*S*,3*S*)/99	(1*R*,3*S*)/99
(±)**2e**/72/95	**3e** [51/49]	(1*S*,3*S*)/99	(1*R*,3*S*)/99
(±)**2f**[a]/72/89	**3f** [50/50]	(1*S*,3*S*)/99	(1*R*,3*S*)/99

Conditions: biocatalyst (500 OD_{600} unit) in 25 mL of sterile water containing 2.5% glucose, substrate (10 mg), 30 °C.
[a] **2f** (X = H).
[b] Calculated by ^{1}H NMR.
[c] Relation between diastereomeric dialcohols was determined by both ^{1}H NMR and chiral HPLC. Assignation of the ^{1}H NMR signals for each one was done in accordance with the literature data [10,13] and was confirmed by NOE experiments (Supplementary Data).
[d] Absolute configuration was established according to the literature data [10,12,13].

always excellent. In the case of *P. jardinii*, all the racemic aldols were substrates, affording excellent conversions and enantioselectivities in both the diastereoisomeric diols obtained, which were easily separated by column chromatography. Once again, the enantioselectivity in the generation of the new stereogenic center was complementary for both yeast strains.

4. Conclusion

In summary, this work explores the use of two yeast strains to produce the chiral products deriving from the aldol reaction between acetone and some aromatic aldehydes, and also the chiral 1,3-diols deriving from their reduction. An aldol reaction is catalyzed by both microorganisms when a large amount of acetone is present in the medium, and is moderately stereoselective for the aldol with the *R* absolute configuration under almost anhydrous conditions (2.5% water). The racemic aldol (produced by the microorganism itself when employing larger amounts of water, or obtained by conventional methods) was reduced in an aqueous medium provided with glucose (2%). Under these conditions, the resolution of the racemic starting material was possible in several cases with *K. marxianus* and the aldol product with the *S* configuration was recovered. Finally, both microorganims show complementary enantioselectivity in the generation of the new stereogenic center by the reduction of the carbonyl moiety, which provides access to 3 of the 4 possible diastereomeric diols with high enantiomerical purity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2013.03.017.

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