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Preparation of key intermediates of adrenergic receptor agonists: Highly enantioselective production of (*R*)-α-halohydrins with *Saccharomyces cerevisiae* **CGMCC** 2.396

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ABSTRACT

In our effort to screen for strains producing carbonyl reductases with high activity and enantioselectivity, *Saccharomyces cerevisiae* **CGMCC** 2.396 was found to be able to catalyze the biotransformation of a series of α-haloacetophenones to Prelog's configurated alcohols in excellent optical purity (>99% ee). The optimal reaction condition was obtained after the investigation of various crucial factors. Under the optimal condition, the product was obtained with high yield (97%) and excellent enantioselectivity (>99% ee). The usefulness of this strain has been further demonstrated by the synthesis of several (*R*)-α-halohydrins (>99% ee) of pharmaceutical importance.

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1. Introduction

Optically pure α-halohydrins or styrene oxides are important synthetic building blocks for some optically active molecules. In this regard, they play an important role in the synthesis of various pharmaceuticals such as α1-, β2-, β3-adrenergic receptor agonists [1–5]. Recently, a number of chemical [6] and biological methods have been developed to obtain these optically pure molecules. The biological methods include resolution of racemic styrene oxides [7–13] or α-halohydrins [14] by lipases and bioreduction of α-haloacetophenones [15–26]. A notable method among the bioapproaches developed for the preparation of chiral α-halohydrin is the enantioselective bioreduction of α-haloacetophenone.

Saccharomyces cerevisiae is by far the most widely used microorganism for the asymmetric reduction of ketones. It was used in the reduction of α-haloacetophenones as well, while the results of these reports were unsatisfactory with low enantiomeric excesses of the products [15,16]. Other strains, including *Geotrichum* sp. [17], *Rhodotorula* sp. [18–21], *M. subtilissimus* [22] or recombinant ketoreductase enzymes [23–27], were also used for the preparation of α-halohydrins. However, most of these reductions failed to yield optically pure products (>99% ee), and few results about substituted α-haloacetophenone has been reported [17–19,26]. Problems such as incomplete transformation, moderate enantiomeric purity and narrow substrate spectrum remain to be solved.

In most cases, biotransformations would give products with high enantioselectivities under mild and environmentally compatible reaction conditions [18–23]. Bioreductions can be carried out with whole microbial cells or isolated enzymes. In the case of isolated dehydrogenases, the external addition of expensive coenzymes (NADPH/NADH) and a recycling system is required [23–26]. In contrast, the use of microbial cells is particularly advantageous since it is unnecessary to add the coenzymes.

In this paper, we screened out a novel strain, *S. cerevisiae* **CGMCC** 2.396, from 52 strains. The whole cells of *S. cerevisiae* **CGMCC** 2.396 showed high reaction activity and enantioselectivity for the reduction of 2-chloro-1-(3-chlorophenyl)ethanone to (*R*)-2-chloro-1-(3-chlorophenyl)ethanol. We also optimized the reaction conditions for high reaction activity. The substrate spectrum was tested by subjecting the strain to the synthesis of some optically active alcohols from the corresponding acetophenones.

2. Experimental

2.1. General

S. cerevisiae **CGMCC** 2.396 was obtained from our laboratory and registered at the China General Microbiological Culture Collection

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Center (**CGMCC**). Acetophenone derivatives (**2a**–**6a**, **16a**–**18a**) were prepared by Friedel–Crafts reaction. Monobromination of acetophenone derivatives with bromine yielded **7a**–**12a**, and the other acetophenone derivatives were obtained from commercial suppliers.

^{1}H NMR spectra were recorded on a Brucker-300 (300/75 MHz) spectrometer in $CDCl_3$. Gas chromatographic analyses were performed using a Fuli GC9790 with a chiral column (CP-Chirasil-DEX CB, Varian, USA) and using a flame ionization detector, nitrogen was used as the carrier gas at 1.5 mL/min, split ratio was 1:50 (v/v), the injector and the detector temperatures were both set at 250 °C, the column temperature was programmed as being kept at 80 °C for 3 min and then upgraded to 220 °C at a rate of 3 °C/min.

2.2. Cultivation of *S. cerevisiae* **CGMCC** 2.396

S. cerevisiae **CGMCC** 2.396 was inoculated into 250 mL Erlenmeyer flasks containing 100 mL medium (peptone 1% (w/v), yeast extract 0.5% (w/v), glucose 0.5% (w/v), malt extract 0.5% (w/v), pH 6.8–7.0) and was incubated 48 h at 27 °C with reciprocal shaking. Cells were harvested by centrifugation and washed twice with a physiological saline (0.85% NaCl).

2.3. Screening method

To a 50 mL Erlenmeyer shaking-flask was added 10 mL potassium phosphate buffer (0.01 M, pH 7.5), 1.0 g freshly harvested cells, 0.1 mL ethanol, 0.1 g glucose and 25 mg **1a**, and the mixture was shaken for 24 h at 30 °C. After the reaction was completed, ether (30 mL) was added to the reaction mixture, and the organic layer was analyzed by GC to determine the yield and enantiomeric excesses of **1b**.

2.3.1. (R)-1-(3-Chlorophenyl)-2-chloroethanol (**1b**)

Yellow oil; $[\alpha]_D^{22} = -31.33$ (c 0.9972, $CHCl_3$) {lit. [19] $[\alpha]_D^{20} = -33.62$ (c 1.0, CH_3OH) for 99% ee, (*R*)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70 (s, 1H), 3.61 (dd, 1H, *J* = 11.3 Hz and 8.6 Hz), 3.74 (dd, 1H, *J* = 11.3 Hz and 3.5 Hz), 4.90 (dd, 1H, *J* = 8.6 Hz and 3.5 Hz), 7.24–7.32 (m, 3H), 7.40 (s, 1H). Enantiomeric excess: 99%, determined by GC, t_R 32.3 min.

2.4. General bioreduction of **2a**–**18a**

To a 50 mL Erlenmeyer shaking-flask was added a suspension of *S. cerevisiae* **CGMCC** 2.396 cells (2.0 g, wet weight) in 10 mL of potassium phosphate buffer (0.01 M, pH 7.5), one of the ketones (100 mg), glucose (1.0 g) and *tert*-butyl alcohol (0.5 mL), and the mixtures were shaken for 48 h at 35 °C. After the reaction completed, the mixture was centrifugated at 10,000 rpm for 10 min, the supernatant was saturated with sodium chloride and then extracted with ether (3 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The chemical yield and ee of the product were determined by GC analysis. The product was purified by silica gel column chromatography, and was identified by ^{1}H NMR analysis. The absolute configuration of the product was determined by the optical rotation and comparison with the literature data, or the product was transformed into the corresponding styrene oxide to determine the optical rotation and then established by comparison of the optical rotation reported in the literature.

2.4.1. (R)-1-Phenyl-2-chloroethanol (**2b**)

Yellow oil; $[\alpha]_D^{22} = -45.1$ (c 1.0013, $CHCl_3$) {lit. [28] $[\alpha]_D^{25} = +52.0$ (c 1.0, cyclohexane) for 99% ee, (*S*)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.55 (s, 1H), 3.64 (dd, 1H, *J* = 11.2 Hz and 8.7 Hz), 3.74 (dd, 1H, *J* = 11.2 Hz and 3.5 Hz), 4.90 (dd, 1H, *J* = 8.7 Hz and 3.5 Hz), 7.39 (m, 5H). Enantiomeric excess: 99%, determined by GC, t_R 25.8 min.

2.4.2. (R)-1-(4-Fluorophenyl)-2-chloroethanol (**3b**)

Light yellow oil; $[\alpha]_D^{22} = -51.1$ (c 0.9953, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 1H), 3.61 (dd, 1H, *J* = 11.2 Hz and 8.7 Hz), 3.71 (dd, 1H, *J* = 11.2 Hz and 3.5 Hz), 4.88 (dd, 1H, *J* = 8.7 Hz and 3.5 Hz), 7.03–7.09 (m, 2H), 7.33–7.38 (m, 2H). Enantiomeric excess: 99%, determined by GC, t_R 25.5 min.

2.4.3. (R)-1-(4-Chlorophenyl)-2-chloroethanol (**4b**)

Yellow solid; $[\alpha]_D^{22} = -43.4$ (c 1.0351, $CHCl_3$) {lit. [29] $[\alpha]_D^{22} = 40.2$ (c 2.1, $CHCl_3$) for 90.5% ee (*S*)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.56 (s, 1H), 3.60 (dd, 1H, *J* = 11.3 Hz and 8.6 Hz), 3.71 (dd, 1H, *J* = 11.3 Hz and 3.5 Hz), 4.88 (dd, 1H, *J* = 8.6 Hz and 3.5 Hz), 7.26–7.36 (m, 4H). Enantiomeric excess: 99%, determined by GC, t_R 33.7 min.

2.4.4. (R)-1-(4-Bromophenyl)-2-chloroethanol (**5b**)

Yellow solid; $[\alpha]_D^{22} = -35.87$ (c 1.1072, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.39 (s, 1H), 3.60 (dd, 1H, *J* = 11.3 Hz and 8.7 Hz), 3.71 (dd, 1H, *J* = 11.3 Hz and 3.4 Hz), 4.87 (dd, 1H, *J* = 8.7 Hz and 3.4 Hz), 7.27 (AB, 2H, *J* = 8.4), 7.51 (AB, 2H, *J* = 8.4). Enantiomeric excess: 99%, determined by GC, t_R 36.6 min.

2.4.5. (R)-1-(4-Methylphenyl)-2-chloroethanol (**6b**)

Yellow oil; $[\alpha]_D^{22} = -44.9$ (c 0.9852, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H), δ 2.54 (s, 1H), 3.63 (dd, 1H, *J* = 11.2 Hz and 8.6 Hz), 3.72 (dd, 1H, *J* = 11.2 Hz and 3.7 Hz), 4.86 (dd, 1H, *J* = 8.6 Hz and 3.7 Hz), 7.19 (AB, 2H, *J* = 8.1), 7.27 (AB, 2H, *J* = 8.1). Enantiomeric excess: 99%, determined by GC, t_R 28.4 min.

2.4.6. (R)-1-Phenyl-2-bromoethanol (**7b**)

Yellow oil; $[\alpha]_D^{22} = -53.7$ (c 0.5517, $CHCl_3$) {lit. [28] $[\alpha]_D^{25} = -44.4$ (c 1.0, cyclohexane) for 99% ee, (*R*)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 1H), 3.55 (dd, 1H, *J* = 10.4 Hz and 8.9 Hz), 3.64 (dd, 1H, *J* = 10.4 Hz and 3.4 Hz), 4.93 (dd, 1H, *J* = 8.9 Hz and 3.4 Hz), 7.33–7.38 (m, 5H). Enantiomeric excess: 99%, determined by GC, t_R 29.6 min.

2.4.7. (R)-1-(3-Nitrophenyl)-2-bromoethanol (**8b**)

Light yellow solid; $[\alpha]_D^{22} = -37.1$ (c 0.4993, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.54 (s, 1H), 3.54 (dd, 1H, *J* = 10.6 Hz and 8.3 Hz), 3.68 (dd, 1H, *J* = 10.6 Hz and 3.5 Hz), 5.04 (dd, 1H, *J* = 8.3 Hz and 3.5 Hz), 7.25–8.28 (m, 3H), 8.29 (s, 1H). Enantiomeric excess: 99%, determined by GC, t_R 36.5 min.

2.4.8. (R)-1-(3-Chlorophenyl)-2-bromoethanol (**9b**)

Light yellow oil; $[\alpha]_D^{22} = -22.1$ (c 0.9671, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.74 (s, 1H), 3.65 (dd, 1H, *J* = 11.5 Hz and 8.5 Hz), 3.68 (dd, 1H, *J* = 11.5 Hz and 3.5 Hz), 5.04 (dd, 1H, *J* = 8.5 Hz and 3.5 Hz), 7.25–7.38 (m, 3H), 7.39 (s, 1H). Enantiomeric excess: 99%, determined by GC, t_R 33.8 min.

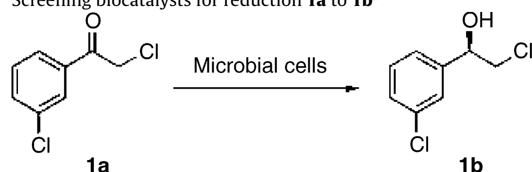
2.4.9. (R)-1-(4-Nitrophenyl)-2-bromoethanol (**10b**)

Light yellow solid; $[\alpha]_D^{22} = -60.8$ (c 0.4732, $CHCl_3$) {lit. [30] $[\alpha]_D^{23} = 29.6$ (c 1.03, $CHCl_3$) for 84.2% ee, (*S*)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.79 (s, 1H), 3.53 (dd, 1H, *J* = 10.6 Hz and 8.3 Hz), 3.69 (dd, 1H, *J* = 10.6 Hz and 3.5 Hz), 5.04 (dd, 1H, *J* = 8.3 Hz and 3.5 Hz), 7.59 (AB, 2H, *J* = 8.7), 8.25 (AB, 2H, *J* = 8.7). Enantiomeric excess: 99%, determined by GC, t_R 49.2 min.

2.4.10. (R)-1-(4-Chlorophenyl)-2-bromoethanol (**11b**)

White solid; $[\alpha]_D^{22} = -59.5$ (c 0.9966, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 1H), 3.50 (dd, 1H, *J* = 10.5 Hz and 8.7 Hz),

Table 1
Screening biocatalysts for reduction **1a** to **1b**[a]



Entry	Microorganism	Yield (%)[b]	ee (%)[b]
1	*Saccharomyces cerevisiae* **CGMCC** 2.396	90	>99
2	*Saccharomyces cerevisiae* **CGMCC** 2.346	87	>99
3	*Trichosporon cutaneum* **CGMCC** 2.571	87	>99
4	*Alcaligenes faecalis* **CGMCC** 1.1799	9	>99
5	*Trichosporon cutaneum* **CGMCC** 2.570	82	98.3
6	*Arthrobacter* sp. **CGMCC** 1.0008	9	97.8
7	*Trichosporon cutaneum* **CGMCC** 2.1795	6	50.6
8	*Alcaligenes faecalis* **CGMCC** 1.924	9	25.4
9	*Trichosporon cutaneum* **CGMCC** 2.1570	71	9.9
10	*Saccharomyces cerevisiae* **CGMCC** 2.1396	26	7.1

[a] The reaction was performed by suspending 1.0 g freshly harvested cells in 10 mL potassium phosphate buffer (0.01 M, pH 7.5), glucose (1.0 g), **1a** 50 mg and ethanol 0.5 mL in a 50 mL Erlenmeyer shaking-flask, was shaken for 24 h at 30 °C and 160 rpm.

[b] Determined by GC analyses.

3.61 (dd, 1H, J = 10.5 Hz and 3.4 Hz), 4.90 (dd, 1H, J = 8.7 Hz and 3.4 Hz), 7.25–7.37 (m, 4H). Enantiomeric excess: 99%, determined by GC, t_R 37.7 min.

*2.4.11. (R)-1-(4-Bromophenyl)-2-bromoethanol (**12b**)*

White solid; $[\alpha]_D^{25} = -42.7$ (c 0.5102, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 1H), 3.50 (dd, 1H, J = 10.5 Hz and 8.7 Hz), 3.61 (dd, 1H, J = 10.5 Hz and 3.4 Hz), 4.90 (dd, 1H, J = 8.7 Hz and 3.4 Hz), 7.27–7.51 (m, 4H). Enantiomeric excess: 99%, determined by GC, t_R 40.9 min.

*2.4.12. (S)-1-(3-Nitrophenyl)ethanol (**13b**)*

Light yellow solid; $[\alpha]_D^{22} = -30.2$ (c 0.9893, $CHCl_3$) {lit. [30] $[\alpha]_D^{20} = 33.2$ (c 0.86, $CHCl_3$) for 83.3% ee, (R)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.51 (d, 3H, J = 6.5 Hz), 2.12 (s, 1H), 5.02 (q, 1H, J = 6.5 Hz), 7.48–8.12 (m, 3H), 8.23 (s, 1H). Enantiomeric excess: 99%, determined by GC, t_S 36.6 min.

*2.4.13. (S)-1-(3-Chlorophenyl)ethanol (**14b**)*

Colorless oil; $[\alpha]_D^{22} = -12.2$ (c 1.0052, $CHCl_3$) {lit. [31] $[\alpha]_D^{20} = -38.6$ (c 1.0, $CHCl_3$) for 100% ee, (S)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (d, 3H, J = 6.5 Hz), 2.04 (s, 1H), 4.81 (q, 1H, J = 6.5 Hz), 7.22–7.25 (m, 3H), 7.35 (s, 1H). Enantiomeric excess: 99%, determined by GC, t_S 25.5 min.

*2.4.14. (S)-1-(4-Nitrophenyl)ethanol (**15b**)*

Yellow oil; $[\alpha]_D^{22} = -34.5$ (c 0.9967, $CHCl_3$) {lit. [25] $[\alpha]_D^{25} = -30.5$ (c 1.083, EtOH) for 99% ee, (S)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (d, 3H, J = 6.5 Hz), 2.09 (s, 1H), 4.99 (q, 1H, J = 6.5 Hz), 7.52–7.55 (m, 2H), 8.15–8.20 (m, 2H). Enantiomeric excess: 99%, determined by GC, t_S 38.6 min.

*2.4.15. (S)-1-(4-Chlorophenyl)ethanol (**16b**)*

Colorless oil $[\alpha]_D^{22} = -41.0$ (c 1.0073, $CHCl_3$) {lit. [25] $[\alpha]_D^{27} = -45.0$ (c 0.9, $CHCl_3$) for 94%, ee (S)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (d, 3H, J = 6.5 Hz), 1.82 (s, 1H), 4.88 (q, 1H, J = 6.5 Hz), 7.26–7.34 (m, 4H). Enantiomeric excess: 99%, determined by GC, t_S 25.0 min.

*2.4.16. (S)-1-(4-Bromophenyl)ethanol (**17b**)*

Colorless oil; $[\alpha]_D^{22} = -33.5$ (c 0.4981, $CHCl_3$) {lit. [25] $[\alpha]_D^{27} = -37.3$ (c 1.1, $CHCl_3$) for 98% ee, (S)}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (d, 3H, J = 6.4 Hz), 1.71 (s, 1H), 4.88 (q, 1H, J = 6.4 Hz), 6.99–7.04(m, 2H), 7.30–7.35 (m, 2H). Enantiomeric excess: 99%, determined by GC, t_S 29.0 min.

*2.4.17. (S)-1-(4-Methoxyphenyl)ethanol (**18b**)*

Light yellow oil; $[\alpha]_D^{25} = -31.5$ (c 0.5002, $CHCl_3$) {lit. [32] $[\alpha]_D^{27} = -4.2$ (c 0.9, $CHCl_3$) for 6% ee, (S)}.^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (d, 3H, J = 6.4 Hz), 1.83 (s, 1H), 3.80 (s, 3H), 4.84 (q, 1H, J = 6.4 Hz), 6.87–6.90 (m, 2H), 7.26–7.31 (m, 2H). Enantiomeric excess: 99%, determined by GC, t_S 25.5 min.

3. Results and discussion

Herein we explored the potential usefulness of *S. cerevisiae* **CGMCC** 2.396 in the reduction of a wide range of acetophenone derivatives to the corresponding alcohols, which have great biological significance. The biotransformation using *S. cerevisiae* **CGMCC** 2.396 followed Prelog's rule [33] and the products were obtained from the biotransformation with high yields and excellent enantioselectivities.

Table 2
Optimize the biotransformation conditions for *S. cerevisiae* **CGMCC** 2.396[a]

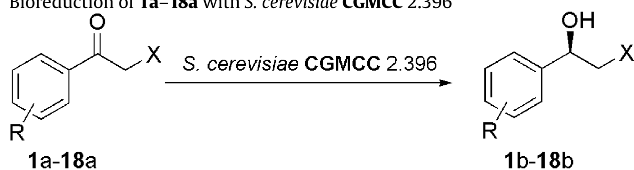
Entry	Time	pH	Solvent 5% (v/v)	Temperature (°C)	Substrate (mg)	Yield (%)[b]	ee (%)[b]
1	24	7.5	Ethanol	30	50	90	>99
2	48	7.5	Ethanol	30	50	98	>99
3	48	6.5	Ethanol	30	50	100	>99
4	48	8.5	Ethanol	30	50	82	>99
5[c]	48	6.5	Ethanol	30	50	1	>99
6	15	6.5	Ethanol	30	50	58	>99
7	15	6.5	*tert*-Butyl alcohol	30	50	63	>99
8	15	6.5	*iso*-Propanol	30	50	55	>99
9	15	6.5	–	30	50	<1	>99
10	48	6.5	*tert*-Butyl alcohol	30	100	83	>99
11	48	6.5	*tert*-Butyl alcohol	35	100	100	>99
12	48	6.5	*tert*-Butyl alcohol	40	100	93	>99
13	48	6.5	*tert*-Butyl alcohol	35	120	100	>99
14	48	6.5	*tert*-Butyl alcohol	35	140	99	>99
15	48	6.5	*tert*-Butyl alcohol	35	280	97	>99
16	48	6.5	*tert*-Butyl alcohol	35	560	82	>99

[a] The reaction was performed by suspending 1.0 g *S. cerevisiae* **CGMCC** 2.396 wet cells in 10 mL potassium phosphate buffer (0.01 M), glucose (1.0 g), **1a** and alcohol in a 50 mL Erlenmeyer shaking-flask.

[b] Determined by GC analyses.

[c] The reaction without added glucose.

Table 3
Bioreduction of **1a**–**18a** with *S. cerevisiae* **CGMCC** 2.396



Entry	Substrate	R	X	Yield[a] (isolated yield) (%)	ee (%)[a]	Configuration[b]
1	**1a**	*m*-Cl	Cl	100 (92)	>99	*R*
2	**2a**	H	Cl	100 (93)	>99	*R*
3	**3a**	*p*-F	Cl	100 (95)	>99	*R*
4	**4a**	*p*-Cl	Cl	92 (85)	>99	*R*
5	**5a**	*p*-Br	Cl	85 (81)	>99	*R*
6	**6a**	*p*-CH_3	Cl	71 (64)	>99	*R*
7	**7a**	H	Br	26 (11)	>99	*R*
8	**8a**	*m*-NO_2	Br	78 (62)	>99	*R*
9	**9a**	*m*-Cl	Br	59 (52)	>99	*R*
10	**10a**	*p*-NO_2	Br	30 (27	>99	*R*
11	**11a**	*p*-Cl	Br	38 (29)	>99	*R*
12	**12a**	*p*-Br	Br	13 (8)	>99	*R*
13	**13a**	*m*-NO_2	H	40 (32)	>99	*S*
14	**14a**	*m*-Cl	H	40 (34)	>99	*S*
15	**15a**	*p*-NO_2	H	4 (3)	>99	*S*
16	**16a**	*p*-Cl	H	14 (11)	>99	*S*
17	**17a**	*p*-Br	H	15 (9)	>99	*S*
18	**18a**	*p*-OCH_3	H	8 (6)	>99	*S*

[a] Determined by GC analyses.
[b] The absolute configurations of **1b**, **2b**, **4b**, **7b**, **10b**, **13b–16b** were established by comparisons with the optical rotation reported in the literatures [19,25,28–32]. The absolute configurations of **3b**, **5b**, **6b**, **8b**, **9b**, **11b**, **12b** were obtained by checking the optical rotations of styrene oxides which were prepared from the corresponding optical halohydrins with a chemical correlation method [9].

3.1. Screening of phenacyl halide reducing microorganisms

Fifty two strains were screened for the enantioselective reduction of 2-chloro-1-(3-chlorophenyl)ethanone **1a** to (*R*)-2-chloro-1-(3-chlorophenyl)ethanol **1b**. The reaction progress was monitored by GC analysis. Results (yields and ee of (*R*)-**1b**) are shown in Table 1. *S. cerevisiae* **CGMCC** 2.396, *S. cerevisiae* **CGMCC** 2.346 and *Trichosporon cutaneum* **CGMCC** 2.571 gave especially high yields (87–90%) and excellent enantioselectivity (>99%) (entries 1–3). The best result for the bioreduction was obtained when *S. cerevisiae* **CGMCC** 2.396 was used, the yield and ee were 90% and >99%, respectively (entry 1). On the contrary, the reduction with commercially available dry yeast (YSC-2, sigma) gave (*R*)-**1b** in less than 1% yield. Other strains, such as *Alcaligenes xylosoxidans, Alcaligenes faecalis*, *Candida magnoliae*, *Stenotrophomonas maltophilia*, *T. cutaneum*, *Trichosporon variabilis*, *Variovorax paradoxus*, *Geotrichum candidum*, *S. cerevisiae*, *Candida boidinii*, *Yarrowia lipolytica*, *Pichia farinose*, *Arthrobacter* sp., *Arthrobacter sulfurous*, *Microbacterium terregens* or *Acinetobacter calcoaceticus*, showed poor bioreduction activities (**1b** yield <1%). Since the highest activity was observed by using *S. cerevisiae* **CGMCC** 2.396 as biocatalyst, it was selected for further studies.

3.2. Optimal biotransformation conditions

Reaction conditions such as reaction time, pH value, glucose, colsovents and reaction temperature were investigated for the biotransformation of **1a** by *S. cerevisiae* **CGMCC** 2.396 (Table 2). The best result was observed after 48 h (98% yield and >99% ee) (entry 2). The results, shown in Table 2, indicate that the optimum pH for the bioreduction activity was found to be 6.5. When pH 6.5 was used, the yield and ee of **1b** were 100% and >99% ee, respectively (entry 3). Under the optimum conditions (pH 6.5, 48 h), the effect of glucose was investigated in order to determine whether it could affect the yield of **1b** or not. The bioreduction gave a very low yield (about 1%) without glucose (entry 5). Cosolvents also play an important role in this process. The best result (63% yield and >99% ee) was obtained by adding 5% (V/V) tert-butyl alcohol in the reaction mixture (entry 7), while poor yield (<1%) was observed when alcohol was absent from the reaction mixture (entry 9). Under the optimum conditions (pH 6.5, 48 h, 10% (W/V) glucose, 5% (V/V) tert-butyl alcohol), the effect of different reaction temperatures was investigated in order to determine whether it could increase the bioreduction activity. The results show that the temperature clearly had a significant effect on the yield of **1b**. The highest yield (100%) was obtained at 35 °C. Another reaction parameter, initial substrate concentration, was studied under optimum conditions (pH 6.5, 48 h, 10% (W/V) glucose, 5% (V/V) tert-butyl alcohol, 35 °C). The results shown that 280 mg **1a** can be biotransformed into **1b** by 1 g *S. cerevisiae* **CGMCC** 2.396 wet cells with high yield and ee, the yield and ee were 97% yield (88% isolated yield) and >99% ee, respectively(entries 13–16).

3.3. Bioreduction of acetophenones

Acetophenone derivatives with different substituents in the alpha position and in the benzene ring were chosen to test the efficiency and stereoselectivity of ketone group bioreduction by *S. cerevisiae* **CGMCC** 2.396, all α-haloacetophenones **2a**–**12a** were transformed into the corresponding (*R*)-aclohols in high enantiomeric excess (more then 99% ee) (Table 3, entries 1–12). Selectivities of previously reported reduction systems were relatively high, while enantiomerically pure compounds (>99% ee) could be obtained in a few cases.

As can be seen in Table 3, *S. cerevisiae* **CGMCC** 2.396 perform the bioreduction of the α-chloroacetophenones **1a–6a** better than α-bromoacetophenones **7a–12a**. The bioreduction of **1a–6a** performed with *S. cerevisiae* **CGMCC** 2.396 gave excellent results (71%–100% yield) in the exploratory experiments. In contrast, bioreduction of α-bromoacetophenones was observed in low yields (13–59%) except for **8a** (78% yield).

In all cases, the bioreduction of the *meta*-substituted ketones performed better than the *para*-substituted ketones (**1a** *vs* **4a**, **8a** *vs* **10a, 9a** *vs* **11a**). This fact can be explained by the electronic and hindrance effects caused by the substituents. The rates of the bioreduction also depended on the size of the substituent on the phenyl ring at *para*-position, the smallest substituent was favor for the *S. cerevisiae* **CGMCC** 2.396 mediated reduction (entries 2–6).

Alpha-haloacetophenones have been used as mechanistic probes for identification and differentiation between reduction processes which occur via hydride transfer (H^-) or by a multi-step electron transfer ($e^\cdot$, H^+). Acetophenones are the reduction products obtained by electron transfer while optically active α-halohydrins are obtained by hydride transfer mechanism [34–40]. No free radical reduction has been observed which did not yielded the dehalogenation product [41]. The results (Table 3, entries 1–12) show that the biotransformation by the *S. cerevisiae* **CGMCC** 2.396 adopted a hydride transfer way.

A series of acetophenone derivatives (**13a–18a**) without halogen substituent on the alpha position were also investigated for the biotransformation with *S. cerevisiae* **CGMCC** 2.396. Poor reduction activities (4–40% yield) were observed for **13a–18a** and the corresponding (*S*)-alcohols were obtained with excellent enantioselectivities (all >99% ee) in all cases (Table 2, entries 13–18). The results obtained for the bioreduction of **13a**–**18a** provides additional evidence towards the preferential reduction of *meta*-substituted ketones by *S. cerevisiae* **CGMCC** 2.396.

4. Conclusion

In summary, *S. cerevisiae* **CGMCC** 2.396 was selected among 52 strains having the ability to reduce acetophenone derivatives. It provides an important approach for the enantioselectively reduction of a wide range of α-haloacetophenones to (*R*)-α-halohydrins with whole microorganism cells. The reduction of α-haloacetophenones gives the corresponding (*R*)-α-halohydrins with high chemical yield (up to 100% yield) and excellent enantioselectivites (>99% ee), and the biocatalyst presented the highest activity with α-chloroacetophenones. This biocatalyst provides a significant method for the preparation of the (*R*)-α-halohydrins which are important chiral building blocks of various pharmaceuticals.

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