Journal of Molecular Catalysis B: Enzymatic 114 (2015) 19–24

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Lyophilized *Rhodotorula* yeast as all-in-one redox biocatalyst: Access to enantiopure building blocks by simple chemoenzymatic one-pot procedures

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ARTICLE INFO

Article history:
Available online 2 August 2014

Dedicated to Professor Carlos E. Tonn for his great contribution to the development of the chemistry of natural products in Argentina and on the occasion of his retirement.

Keywords:
Rhodotorula
Stereoselective reduction
(Chemo)enzymatic
One-pot procedures
Chiral alcohols

ABSTRACT

Rhodotorula sp. LSL, isolated from a local landfarming was able to catalyze the reduction of prochiral arylketones into sec-alcohols with excellent Prelog stereoselectivity (ee > 99%). The use of resting and lyophilized cells was optimized accessing to an easy-to-use whole cell biocatalyst that efficiently works even under non-sterile conditions, without the addition of external cofactors and using plain water as solvent. The catalyst resistance in alkaline media enabled (chemo)enzymatic one-pot procedures at high pH values. So a simple and efficient methodology was applied to prepare alternatively enantiopure β-halohydrins, terminal diols and epoxides from aromatic α-haloketones.

1. Introduction

The development of new eco-friendly processes for the production of chiral molecules is still an open challenge for organic chemists. Biotechnological methods have already provided with major advances to synthetic chemistry, although there are still several practical issues that may be improved [1]. Methodologies based on the use of commercially available hydrolytic enzymes which do not require the use of cofactors are widely accepted by chemists to obtain enantiopure molecules mainly by (dinamic)kinetic resolutions [2,3]. On the contrary, the industrial application of isolated redox enzymes to prepare enantioenriched chiral compounds is still less developed even though several smart strategies for cofactor recycling have been described [4]. The operational simplicity and availability of wild type whole cell systems make still worthing the screening for new selective and robust microorganisms to perform valuable transformations at low cost [5].

It is well known that enantiopure β-halohydrins are valuable precursors of bioactive products applicable in several fields, mainly agriculture and pharmaceutical industry [6,7]. They also acquire additional value since can be easily transformed to the corresponding epoxides with controlled stereochemistry [8–10]. These compounds along with non-racemic chiral vicinal diols are important intermediates in the synthesis of pharmaceuticals such as (R)-(−)-Eliprodil, a NMDA receptor antagonist, arylalkylamine calcimimetics, NK-1 receptor antagonists, antiviral nucleosides analogs and anticonvulsivant agents [11]. Traditionally, β-halohydrins were obtained by asymmetric reduction of haloketones mediated by metal-based reducing agents [12,10]. Logically, one might consider certain strategies for accessing such building blocks by biocatalytic redox methods: (i) the stereoselective bioreduction of prochiral haloketones, and (ii) the kinetic resolution of racemic *sec*-halohydrins by enantioselective oxidation. Since it has been demonstrated the *quasi*-irreversible reduction of haloketones due to thermodynamic control [13], the former would be the best approach to use ADH enzymes for this

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http://dx.doi.org/10.1016/j.molcatb.2014.07.011
1381-1177/

purpose. Consecuently, several bacteria belonging to the genera *Bacillus*, *Leisfonia*, *Rhodococcus* and fungi such as *Saccharomyces*, *Geotrichum* and *Rhodotorula* have been recognized for their ability to catalyze the reduction of haloketones into enantioenriched halohydrins [8,14–18].

Following the elegant strategy developed by Kroutil et al. [8,19–21] to convert a prochiral α-chloroketone to the enantiopure epoxide based on a one-pot, one-step procedure by lyophilized bacterial cells from *Rhodococcus ruber* at pH ~13, the aim of our work was to detect and optimize the use of new fungal whole cell for the preparation of enantiomerically enriched chiral terminal diols and epoxides. Hence, we undertook the study of "extrachemophile" microorganisms [22] to broaden the toolbox of catalysts able to tolerate high pH values necessary to lead the epoxide formation in a one-pot combination of chemo- and biocatalysis, in minimal aqueous media.

2. Experimental

2.1. Chemicals

Acetophenone derivatives 2-chloro-1-phenylethanone (**1a**), 2-bromo-1-phenylethanone (**1b**), 2-chloro-1-(4′-chlorophenyl)ethanone (**1c**), 2-chloro-1-(4′-nitrophenyl)ethanone (**1d**), 2-bromo-1-(3′-nitrophenyl)ethanone (**1e**), 2-bromo-1-(4′-methoxyphenyl)ethanone (**1f**), 1-phenylethanone (**1j**) and (*rac*)-phenyloxirane (**3a**) were purchased from Sigma–Aldrich, Argentina. The compounds 2-hydroxy-1-phenylethanone (**1g**) and 2-azido-1-phenylethanone (**1h**) were prepared from **1b** by nucleophilic substitution with sodium acetate or sodium azide, respectively. For compound **1g**, a hydrolysis step after the ester formation was carried out. The compound 2-azido-1-(4′-chlorophenyl)ethanone (**1i**) was prepared from **1c** by nucleophilic substitution with sodium azide. The characterization data is presented in the Supporting Information. The racemic *sec*-alcohol standards were obtained by reduction with $NaBH_4$ from the corresponding ketones.

2.2. Analytical methods

Analytical TLC was performed on silica gel 60 F_{254} plates (Merck), employing *n*-hexane: ethyl acetate mixtures and visualized by UV irradiation and further sprayed with acidic anisaldehyde solution. Flash chromatography was carried out on silica gel (70–230 mesh) with *n*-hexane: ethyl acetate mixtures as eluents. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 in $CDCl_3$ at 200 and 50.2 MHz, respectively. GC/LR-MS analyses were performed in a GCQ-plus Finnigan Mat instrument equipped with a Restex-5-MS column (30 m, 0.25 mm ID, 0.25 mm df). Temperature setting: 70 °C (10 min), 20 °C/min, 110 °C (6 min), 10 °C/min, 130 °C (15 min). GC-FID analyses were performed in a Perkin Elmer Clarus 500 instrument equipped with a Restek RT-BetaDEXse column (30 m, 0.25 mm ID, 0.25 mm df). Temperature settings: Program A: 70 °C (4 min), 20 °C/min, 110 °C (10 min), 10 °C/min, 130 °C (15 min), 20 °C/min, 200 °C (10 min) for determination of haloketones **1a–g**, **1j**, the alcohols **2a–g**, **2j** and the epoxides **3a–f**. Program B: 130 °C (10 min), 1 °C/min, 140 °C, 2 °C/min, 150 °C (10 min) for determination of **1h**. Program C: 120 °C (3 min), 5 °C/min, 150 °C (5 min), 2 °C/min, 190 °C for determination of **1i**. Optical rotations were determined on a Perkin Elmer-341 polarimeter.

2.3. Microorganisms

Fungal strains of *Rhodotorula* sp. UBA 236 and *Penicillium chrysogenum* UBA 1179 were obtained from the culture collection of Facultad de Ciencias Exactas – Universidad de Buenos Aires, Argentina. Stock cultures were stored in Petri dishes on solid PDA or Czapek media at 4 °C.

Rhodotorula sp. LSL and *Penicillium* sp. LSL strains were isolated from a soil sample of a landfarming belonging to a local chemical industry dedicated to the synthesis of saturated and unsaturated polyester resins. The procedure is described as follows: initially soil samples (5 g) were inoculated in 100 mL of liquid YGM (yeast extract 5 g/L and glucose 10 g/L) medium in 250 mL Erlenmeyer flasks and incubated for 48 h at 28 °C with orbital shaking (160 rpm). Subsequently, 1 mL of this culture was transferred to fresh YGM medium and incubated under the same conditions for 24 h. Finally, 0.2 mL of the culture were planted onto agar plates with basal medium supplemented with chloramphenicol (0.05 g/L) and incubated for 48 h at 28 °C. When single colonies were observed, they were individually transferred to fresh agar plates and incubated at 28 °C. Isolated strains were stored at 4 °C.

Penicillium sp. LSL strain was identified by classic biochemical tests [23], while *Rhodotorula* sp. LSL was identified by both biochemical tests and molecular studies as internal transcribed spacer (ITS) sequence analysis. For this, isolation of genomic DNA was performed by standard procedures [24] and a fragment containing the yeast ITS1, 5.8S rDNA and ITS2 were amplified using the specific pair of primers ITS1–ITS4 [25]. The full-length PCR fragment was analyzed by BLAST, indicating 100% identity with Rhodotorula genus. The nucleotide sequence retrieved in this study was deposited in the GenBank database under the accession number.

2.4. General biotransformation procedures

2.4.1. Preparation of growing and resting cell biocatalysts

Rhodotorula and *Penicillium* strains were inoculated in 30 mL of YM and Czapek media in 250 mL Erlenmeyer flasks, respectively. Incubation was carried out during 48 h at 28 °C with orbital shaking (160 rpm). After incubation cells were harvested by centrifugation (5000 rpm, 10 min) and directly used as biocatalysts.

2.4.2. Preparation of lyophilized biocatalysts

After cultivation, *Rhodotorula* sp. LSL cells were harvested by centrifugation (5000 rpm, 10 min) without washing, and further resuspended in 1 mL of potassium phosphate buffer (pH 6.5). Aliquots of 100 mL were frozen in liquid nitrogen and freeze dried under vacuum for 24 h. The tubes were airtight sealed and stored at 4 °C and −20 °C.

2.4.3. Biocatalytic experiments with growing cells

Cell cultures obtained as described in Section 2.4.1 were transferred to fresh media (30 mL) and 5 mg of substrate **1a** (0.032 mmol) dissolved in 50 μL of DMSO were added to biotransformation batches. The bioreaction progress was monitored every 24 h. The withdrawn samples were extracted with ethyl acetate and analyzed by GC-FID.

2.4.4. Biocatalytic experiments with resting and lyophilized cells

To a 100 mL Erlenmeyer flask containing 30 mL of potassium phosphate buffer (0.1 M, pH 6.5), 1.2 g of sucrose, 1.5 g of freshly harvested cells or 200 mg of lyophilized cells rehydrated in the same buffer (see Supporting Information), and substrates **1a–j** dissolved in DMSO (1 mg/10 μL) were added. The bioreaction mixture was shaken at 28 °C (160 rpm) and its progress was monitored by GC-FID. Extraction was carried out by adding ethyl acetate (10 mL ×3) and the organic layer dried over anhydrous Na_2SO_4 and analyzed by GC for determining yields and enantiomeric excesses of **2a–k**.

For isolated yields, 10 batches of the bioreactions were run in parallel using substrates **1a** and **1b** (10 mg/batch) by using 200 mg of lyophilized *Rhodotorula* sp. LSL in 30 mL of water incubating for

6 h at room temperature. After purification, isolated yields of **(*R*)-2a** and **(*R*)-2b** were 71% and 69%, respectively (ee > 99%).

2.5. One-pot procedures

2.5.1. One-pot, two-step procedure to obtain (R)-phenyloxirane

Non-rehydrated lyophilized cells of *Rhodotorula* sp. LSL (200 mg) and the substrate 1-bromo-2-phenylethanone, **1b** (6.4 mg, 0.032 mmol) dissolved in DMSO (50 μL) were added to 30 mL of potassium phosphate buffer (0.1 M, pH 4.3) under non-sterile conditions. The reaction mixture was stirred in orbital shaker (160 rpm) for 30 min at room temperature. Afterwards, NaOH pellets (300 mg, pH ~10) were added and the reaction was shaken for additional 30 min.

2.5.2. One-pot, one-step procedure to obtain (R)-phenyloxirane

Non-rehydrated lyophilized cells of *Rhodotorula* sp. LSL (200 mg), **1a** (5 mg 0.032 mmol) in DMSO (50 μL) and NaOH pellets (300 mg, pH ~10) were added to 30 mL of potassium phosphate buffer (0.1 M, pH 6.5) in non-sterile conditions. The reaction mixture was maintained for 60 min in orbital shaker (160 rpm) at room temperature.

2.5.3. One-pot, one-step procedure to obtain (R)-diols

Non-rehydrated lyophilized cells of *Rhodotorula* sp. LSL (200 mg), **1b** (6.4 mg, 0.032 mmol) in DMSO (50 μL) were added to 30 mL of potassium phosphate buffer (0.1 M, pH 6.5) in non-sterile conditions. Immediately, the pH was adjusted to 12.0 by adding NaOH pellets (600 mg). Reaction mixture was agitated (160 rpm) at room temperature for 60 min.

After neutralization with HCl (2.0 M), extraction was carried out with ethyl acetate and the organic layer was dried with anhydrous Na_2SO_4.

In all the biotranformation procedures above described, blank assays without substrates and without fungi were performed in parallel, and all the experiments were carried out in triplicate. In every case, samples were analyzed by chiral GC-FID according to Section 2.2.

2.6. Alternative preparation of (R)-phenyloxirane

(*R*)-1-Chloro-2-phenylethanol, **(*R*)-2a** (68.7 mg, 0.44 mmol) obtained by bioreduction with *Rhodotorula* sp. LSL according to Section 2.4.4. was purified, dissolved in 3.0 mL of diethyl ether and NaOH (172 mg, 4.4 mmol) was added. The mixture was stirred at 30 °C. After 1 h the solvent was evaporated and the reaction mixture was extracted with ethyl acetate (30 mL ×2). The combined organic layers were concentrated under vacuum to give **(*R*)-3a** as a colorless liquid in 70% isolated yield (37.0 mg, 0.30 mmol, ee > 99%).

2.7. NMR and MS data of isolated compounds

(R)-2-Chloro-1-phenylethanol (**2a**): colorless liquid. $[\alpha]_D^{25} = -54.06$ (*c* 1.00, $CHCl_3$) {lit. [26] $[\alpha]_D^{25} = -50.0$ (*c* 0.87, CH_2Cl_2) for 99% ee, (*R*)}. ^{1}H NMR: (200 MHz, $CDCl_3$) δ: 2.79 (bs, 1H), 3.65 (dd, *J* = 11.2, 8.7 Hz, 1H), 3.73 (dd, *J* = 11.2, 3.5 Hz, 1H), 4.87 (dd, *J* = 8.7, 3.5 Hz, 1H), 7.36 (bs, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.9, 74.1, 126.1, 128.5, 128.7, 140.0 EM: (EI) m/z 158–156 (M^+), 108, 107, 88, 86, 84, 79, 78, 77, 51, 50. ee > 99%, determined by chiral GC, r_t 26.7 min (program A).

(R)-2-Bromo-1-phenylethanol (**2b**): yellow liquid. $[\alpha]_D^{25} = -40.1$ (*c* 0.60, $CHCl_3$) {lit. [17] $[\alpha]_D^{25} = -53.06$ (*c* 0.55, $CHCl_3$) for 99% ee, (*R*)}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.65 (d, *J* = 2.6 Hz, 1H), 3.66 (dd, *J* = 10.4, 8.7 Hz, 1H), 3.75 (dd, *J* = 10.4, 3.5 Hz, 1H), 4.93 (dt, *J* = 5.4, 2.7 Hz, 1H), 7.25–7.42 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 40.2, 73.8, 126.0, 128.5, 128.8, 140.3. EM: m/z (EI, 70 eV): 200–202 (M^+). ee > 99%, determined by chiral GC, r_t 28.0 min (program A).

(R)-2-Chloro-1-(4′-chlorophenyl) ethanol (**2c**): yellow oil. $[\alpha]_D^{25} = -42.8$ (*c* 0.55, $CHCl_3$) {lit. [17] $[\alpha]_D^{25} = -43.06$ (*c* 0.49, $CHCl_3$) for 99% ee, (*R*)}. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 2.63 (s, 1H), 3.60 (dd, *J* = 11.2, 8.4 Hz, 1H), 3.70 (dd, *J* = 11.2, 3.6 Hz, 1H), 4.88 (dd, *J* = 8.4, 3.6 Hz, 1H), 7.30–7.40 (m, 4H). ee > 99%, determined by chiral GC, r_t 30.4 min.

(R)-2-Chloro-1-(4′-nitrophenyl) ethanol (**2d**): white solid, mp: 87–88 °C. $[\alpha]_D^{25} = -40.0$ (*c* 1.00, $CHCl_3$) {lit. [27] $[\alpha]_D^{25} = -32.60$ (*c* 1.00, $CHCl_3$) for 99% ee, (*R*)}. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 2.63 (s, 1H), 3.64 (dd, *J* = 11.3, 8.2 Hz, 1H), 3.80 (dd, *J* = 11.3, 3.6 Hz, 1H), 5.05 (dd, *J* = 8.2, 3.6 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 8.24 (d, *J* = 8.8 Hz, 2H). ee > 99%, determined by chiral GC, r_t 28.2 min (program A).

(R)-2-Azido-1-phenylethanol (**2h**): yellow oil. $[\alpha]_D^{25} = -80.1$ (*c* 1.00, $CHCl_3$) {lit. [28] $[\alpha]_D^{25} = -89.03$ (*c* 1.00, CH_3OH) for 99% ee, (*R*)}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.38 (bs, 1H), 3.44 (dd, *J* = 12.6, 4.7 Hz, 1H), 3.52 (dd, *J* = 12.6, 7.4 Hz, 1H), 4.90 (dd, *J* = 7.4, 4.7 Hz, 1H), 7.30–7.40 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 57.9, 73.3, 125.8, 128.2, 128.6, 140.5. EM: m/z (EI, 70 eV): 163 (M^+), 145, 106, 105, 85, 77, 71, 57. ee > 99%, determined by chiral GC, r_t 29.2 min (program B).

(R)-2-Azido-1-(4′-chlorophenyl) ethanol (**2i**): colorless oil. $[\alpha]_D^{25} = -79.1$ (*c*. 1.25, $CHCl_3$) {lit. [27] $[\alpha]_D^{25} = -103.0$ (*c* 1.00, CH_3OH) for 99% ee, (*R*)}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.82 (bs, 1H), 3.40 (d, *J* = 6.0 Hz, 2H), 4.80 (t, *J* = 6.0 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 7.32 (d, *J* = 8.7 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 57.3, 72.4, 127.2, 128.8, 133.9, 138.9. EM: m/z (EI, 70 eV): 197–199 (M^+), 171, 170, 146, 145, 117, 116, 105, 77. ee > 99%, determined by chiral GC, r_t 31.6 min (program C).

(R)-1-Phenyl-1,2-diol (**2g**): white solid, mp: 62–65. $[\alpha]_D^{25} = -43.49$ (*c* 1.00, $CHCl_3$) {lit. [29] $[\alpha]_D^{25} = +68.23$ (*c* 0.58, $CHCl_3$) for 95% ee, (*S*)-enantiomer}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.45 (bs, 1H), 2.85 (bs, 1H), 3.64 (dd, *J* = 11.2, 8.2 Hz, 1H), 3.73 (dd, *J* = 11.2, 3.6 Hz,1H), 4.79 (dd, *J* = 8.2, 3.6 Hz, 1H), 7.28–7.38 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 68.0, 74.7, 126.1, 127.9, 128.5, 140.5. EM: m/z (EI, 70 eV): 138 (M^+), 121, 107, 79, 77. ee > 99%, determined by chiral GC, r_t 19.0 min (program A).

(R)-Phenyloxirane (**3a**): colorless liquid. $[\alpha]_D^{25} = -22.3$ (*c* 1.00, $CHCl_3$) {lit. [30] $[\alpha]_D^{25} = -+25.10$ (*c* 1.00, $CHCl_3$) for 99% ee, (*S*)-enantiomer}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.80 (dd, *J* = 5.5, 2.6 Hz, 1H), 3.14 (dd, *J* = 5.5, 4.1 Hz, 1H), 3.85 (dd, *J* = 4.1, 2.6 Hz, 1H), 7.29–7.39 (5H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 51.2, 52.4, 125.5, 128.2, 128.5, 137.6. EM: m/z (EI, 70 eV): 120 (M^+), 119, 104, 91, 77, 65. ee > 99%, determined by chiral GC, r_t 11.8 min (program A).

3. Results and discussion

3.1. Screening for robust microorganisms able to perform selective ketoreductions

2-Chloro-1-phenylethanone (**1a**) was chosen as model substrate to screen the ketoreductase activity of microbial strains isolated from a landfarming where a local company that synthesizes polyester resins dumps the liquid effluents from polymerization processes. Soil samples were taken 15 days after the effluent discharge in order to increase the chances of finding viable microorganisms from soils with pH values ranged between 7.3 and 10.0 (see Supporting Information). Five strains, including filamentous fungi and yeasts, were isolated and their ability to reduce **1a** was tested by employing a classical growing cell biotransformation procedure toward 1 mM of the substrate in liquid Czapek and YM media for molds and yeasts, respectively. Only two of the isolated strains, a filamentous fungus and a pink yeast, were able to reduce the model substrate into 2-chloro-1-phenylethanol (**2a**). The mold reduced

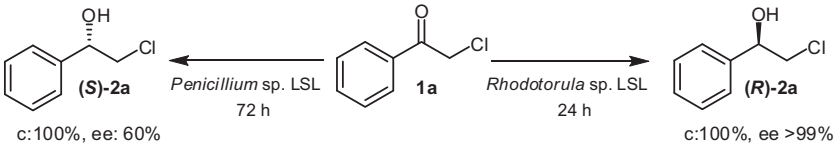


Fig. 1. Stereodivergent bioreduction of 2-chloro-1-phenylethanone, **1a**, catalyzed by isolated strains from a landfarming.

the substrate completely after 72 h with preference to the **(*S*)-2a** enantiomer (ee: 60%), meanwhile the yeast completed the reaction at 24 h with total selectivity to the **(*R*)-2a** isomer (*ee* > 99%) (Fig. 1). The importance of having stereo-complementary whole cell catalysts that provide both enantiomers of the halohydrins has already been standed out by other authors, for example, *R. ruber* and *Pseudomona flourescens* to prepare the Prelog and anti-Prelog isomers, respectively [8] and *Rhodotorula glutinis* and *Geotrichum candidum* in a more recent report [27].

Our selected strains were identified to the genus level by morphological and biochemical methods as a *Penicillium sp*. and *Rhodotorula sp*. Due to its remarkable biocatalytic potential, the yeast was further identified by phylogenetic analysis. The gene 5.8S of the ribosomal large subunit presents high homology with several *Rhodotorula* species, so it was named as *Rhodotorula* sp. LSL.

After that, we compared the biocatalytic performance of the two new isolates with the collection strains *Rhodotorula* sp. UBA 236 and *P. chrysogenum* UBA1179. Again growing cell systems in standard conditions toward 1 mM of substrate were used. Table 1 shows that after 72 h the mold *P. chrysogenum* reduced **1a** with almost the same selectivity to the *S* isomer (65% ee) as the *Penicillium sp*. isolated from the landfarming. On the contrary, our extrachemophile yeast, *Rhodotorula* sp. LSL, proved to be more selective than *Rhodotorula* sp. UBA 236 for the preparation of **(*R*)-2a** in both growing and resting cell procedures (Table 1). Consequently, further developments were carried out using *Rhodotorula* sp. LSL as biocatalyst.

3.2. Optimization of 1a bioreduction by Rhodotorula sp. LSL

The catalytic potential of *Rhodotorula* sp. LSL in a resting cell system at standard conditions (28 °C and pH 7.0) was demonstrated by the above depicted time course experiment, standing out that a complete substrate conversion is achieved at only 30 min of bioreaction working with a substrate concentration of 1 mM (Table 1). Keeping in mind the idea of using the whole cell biocatalysts as an easy-to-use stereoselective tool, we performed time course experiments with *Rhodotorula* sp. LSL at room temperature and at different pH values ranged from 4.0 to 8.0. No differences neither in conversion nor in stereoselection were observed. Then, we tested the ability of the biocatalyst to reduce higher concentrations of **1a** at two different bioreaction times 1 h and 24 h (Table 2). In this experiment, we also compared the performances of *Rhodotorula* sp. LSL with *Rhodotorula* sp. UBA 236. Again it was demonstrated the better abilities of the new isolate in both substrate conversion and stereoselectivity. It is noteworthy that the optical purity of the β-halohydrins **2a** did not vary regardless the increment of the substrate concentration (Table 2). This is particulary interesting since whole cells can have several ADHs with different stereopreference. Moreover, the conversion at 24 h was very good (85%) even at 15 mM of substrate concentration.

Since the separation of the biomass for up-scale processes might be an important factor requiring extra time and/or space, lyophilization of cells and its applicability as biocatalysts were studied. Then, freeze dried *Rhodotorula* sp. LSL were tested. Hence, cultures in their stationary phase of growth were lyophilized without the use of crioprotectors. Preliminary assays were performed just adding the lyophilized cells (100 mg equivalent to 1.5 g/fresh weight) into a buffer solution containing 1 mM of **1a** at pH 6.5. Despite the conversion decreased by half at 1 h compared to restings cells, the stereoselection was not affected at all. When the biocatalyst concentration was doubled (200 mg/batch), conversion was complete (Table 3). It is important to remark that this procedure with lyophilized cells did not require sterile conditions.

The rehydration time and media were also considered as parameters in the recovery of the ADH activity. By soaking the cells for 24 h in a rehydration medium, 100% conversion of **1a** (10 mM) was reached. Although the use of water without any additive was effective for the revival of cells, the optimal solution was based on buffer phosphate at pH 6.5 supplemented with sucrose (4%) (for optimization details, see Supporting Information).

Although a rehydration step with frozen dried cells is required to reach complete conversion for higher substrate concentrations,

Table 1
Biocatalytic conversion of **1a** (1 mM) into **2a** by isolated and collection strains.

Strains	Reaction time (h)	Conversion [%][a]	ee [%][a]	Config.[a]
Rhodotorula sp. UBA 236[b]	0.15	80	90	*R*
	0.30	85	90	*R*
	3	90	90	*R*
	6	98	90	*R*
	24	100	90	*R*
Rhodotorula sp. LSL[c]	0.15	97	>99	*R*
	0.30	100	>99	*R*
Penicillium sp. LSL[c]	72	100	60	*S*
P. chrysogenum UBA 1179[b]	72	100	65	*S*

[a] Determined by chiral GC analyses.
[b] Collection strains.
[c] Isolated strains.
0.032 mmol (5 mg)/50 μL of DMSO were added to 1.5 g FW/30 mL of Czapek for *Penicillium* or YM for *Rhodotorula*.

Table 2
Influence of the substrate concentration in the bioreduction of **1a** to **(*R*)-2a** by *Rhodotorula* sp. strains.

Biocatalyst[b]	**1a** conc. (mM)	Reaction time (h)	Conversion [%][a]	ee [%][a]
Rhodotorula sp. UBA 236	1	24	100	90
	2	24	85	90
	3	24	75	90
Rhodotorula sp. LSL	2	1	100	>99
		24	100	>99
	4	1	62	>99
		24	100	>99
	8	1	26	>99
		24	100	>99
	10	1	20	>99
		24	100	>99
	15	24	85	>99
	30	24	50	>99

[a] Determined by chiral GC analyses.
[b] Resting cells, 1.5 g FW (~100 mg DW), in phosphate buffer (30 mL, 0.1 M, pH 6.5).

Table 3
Influence of the lyophilized *Rhodotorula* sp. LSL cell amount toward the bioreduction of **1a** (2 mM).

Lyophilized biocatalyst[b]	Reaction time (h)	Conversion [%][a]	ee [%][a]	Config.[a]
100 mg	1	50	>99	*R*
	24	100	>99	*R*
200 mg	1	100	>99	*R*
	24	100	>99	*R*

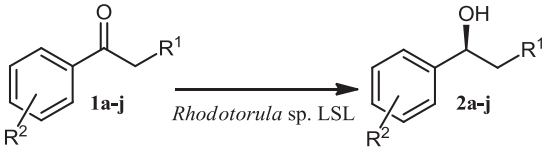
[a] Determined by chiral GC analyses.
[b] In phosphate buffer (30 mL, 0.1 M, pH 6.5) without rehydration.

they still show some additional advantages by making the separation of the biomass easier in time and space, and providing an easy-to-store-and transport whole cell biocatalyst [28].

3.3. Performance of the biocatalytic systems toward a series of α-substituted arylketones (*1a–j*)

A series of α-chloro and α-bromo arylketones with different substituents in the aromatic ring, as well as a series of non-halogen α-substituted acetophenones were tested as depicted in Table 4. Particularly, the α-hydroxy (**1g**) and α-azido (**1h** and **1i**) derivatives were selected since their derived non-racemic *sec*-alcohols are important chiral precursors for the preparation of pharmaceutically interesting compounds [11,29,31–35]. The experiments were performed with rehydrated lyophilized cells at substrate concentration of 2 mM for 1 h. Under these conditions, full conversions and excellent stereoselectivity (ee > 99%) were achieved for all the ketones tested furnishing as expected the *R* isomers of the corresponding *sec*-alcohols according to Prelog's rule [(*S*)-enantiomer for alcohol **2j** due to the switch in Cahn–Ingold–Prelog priority of the substituents] [36].

Table 4
Asymmetric reductions of aryl ketones (**1a–j**) by lyophilized *Rhodotorula* sp. LSL[b].



a- R^1=Cl, R^2= H
b- R^1=Br, R^2= H
c- R^1=Cl, R^2= *p*-Cl
d- R^1=Cl, R^2= *p*-NO_2
e- R^1=Br, R^2= *m*-NO_2
f- R^1=Br, R^2= *p*-OCH_3
g- R^1=OH, R^2= H
h- R^1=N_3, R^2= H
i- R^1=N_3, R^2= *p*-Cl
j- R^1=H, R^2= H

Substrate	Product	Conversion [%][a]	ee [%][a]
1a	**(*R*)-2a**	100	>99
1b	**(*R*)-2b**	100	>99
1c	**(*R*)-2c**	100	>99
1d	**(*R*)-2d**	100	>99
1e	**(*R*)-2e**	100	>99
1f	**(*R*)-2f**	100	>99
1g	**(*R*)-2g**	100	>99
1h	**(*R*)-2h**	100	>99
1i	**(*R*)-2i**	100	>99
1j	**(*S*)-2j**	100	>99

[a] Determined by chiral GC analyses.
[b] 2 mM of ketone/50 μL of DMSO was added to 200 mg of lyophilized yeast in 30 mL of phosphate buffer (0.1 M) at pH 6.5 for α-chloroketone and 4.3 for α-bromoketone.

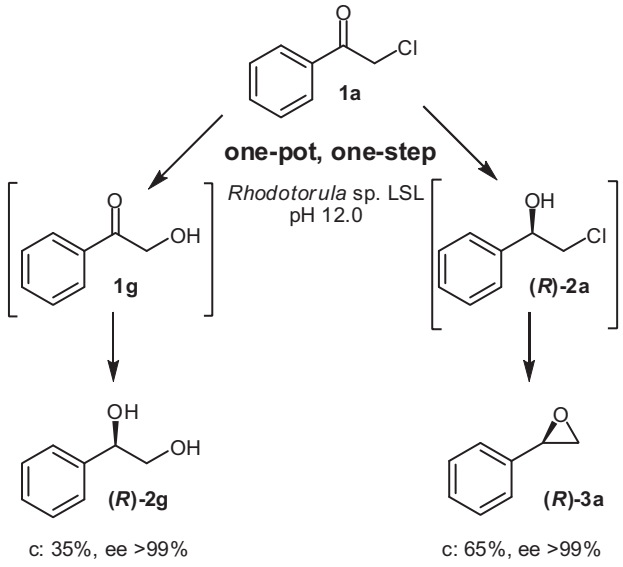


Fig. 2. Chemodivergent one-pot, one-step procedure achieved at pH 12.0 in 1 h with *Rhodotorula* sp. LSL from the α-chloroketone **1a**.

3.4. Chemoselective preparation of halohydrins, diols and epoxides from α-haloketones by a one-pot strategy

Firstly, we worked on the biotransformation of the model substrate **1a**, in a one-pot, one-step procedure at pH 12.0, by using either resting or lyophilized cells of *Rhodotorula* sp. LSL (Fig. 2). Interestingly, the substrate was totally consumed in only 1 h, to give two optically pure molecules, the expected (*R*)-phenyloxirane, **(*R*)-3a** (65%) and (*R*)-1-phenylethane-1,2-diol, **(*R*)-2g** (35%). We ruled out the possibility that the occurrence of the **(*R*)-2g** was due to the base-promoted epoxide ring opening, since **(*R*)-3a** remained unchanged under the same conditions in a blank assay. Moreover, when 2-hydroxy-1-phenylethanone, **1g**, was used as substrate, the complete reduction to the *R* enantiomer was evident (Table 4). Besides, in our blank assays without biocatalyst, it was possible to detect the presence of small amounts of **1g** in the medium, even in very early bioreaction times. Thus, we assume that the presence of the enantiopure diol is owing to a rapid, but partial, halogen substitution from the starting substrate caused by the alkaline medium (pH 12.0) and the further stereoselective bioreduction of **1g**.

On the other hand, when the one-pot, one-step experiment was carried out at pH 10.0, it was possible to obtain the epoxide **(*R*)-3a** as the only product starting from **1a** (*c*: 100%, ee > 99%, isolated yield: 35%). We extended this method to prepare the enantiopure oxiranes **(*R*)-3c–f** in 100% conversion.

Given that optically pure α-arylic vicinal diols are very interesting building blocks [11], we focused in the optimization of a simple biocatalytic process for their preparation. Taking into account that bromine is a better leaving group than chlorine in S_N2 reactions, we applied the above described one-pot, one-step procedure for the biotransformation of 2-bromo-1-phenylethanone, **1b**. At pH 12.0, either with resting or lyophilized cells, the diol **(*R*)-2g** was achieved not only with complete conversion of the substrate, but also with excellent chemo- and stereo-selectivity (ee > 99%, 60% isolated yield) in less than 1 h (Fig. 3). Afterward, we optimized a one-pot, two-step procedure to obtain **(*R*)-3a** from **1b**, just lowering the pH to 4.3 in the first biocatalytic step to avoid the bromine substitution. These experiments were also carried out using the brominated ketones **1e** and **1f** as starting materials.

Although the concept of cascade reactions by one-pot, one-step strategies to access to enantiopure epoxides from prochiral α-haloketones has been vastly explored in the last decade [8,19–21],

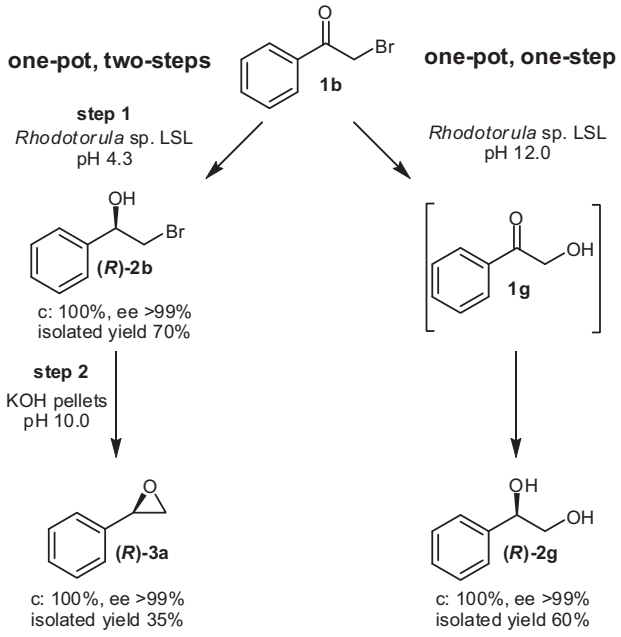


Fig. 3. One-pot, one-step and one-pot, two-step procedures for the preparation of enantiopure terminal vicinal diol (***R***)-**2g**, bromohydrin (***R***)-**2b** and epoxide (***R***)-**3a** from **1b** by *Rhodotorula* sp. LSL.

our systems based on *Rhodotorula* sp. LSL lyophilized cells present practical advantages. Among them, it was possible to work in plain water and under non-sterile conditions. Besides with substrate concentration up to 10 mM, it was not necessary the addition of external expensive cofactors [NAD(P)H] or hydrogen donors. It is likely that at higher substrate concentrations (see conversion data at 15 and 30 mM, Table 2) the hydride source is limited and, therefore the conversion can not be complete.

In order to improve the epoxide isolated yields, we also tested the efficiency in the independent processes by purifying the halohydrin intermediates (**2a** and **2b**) before performing the alkali-induced oxirane formation. Likewise, we could obtain the epoxide **3a** in 70% isolated yield starting from both chloro- and bromoketones. The lower yields of the one-pot procedures could be attributed to the instability of the epoxide in the chromatographic purification through silica gel.

As a summary, we could produce (*R*)-β-halohydrins, terminal (*R*)-epoxides and terminal vicinal (*R*)-diols with high stereo- and chemoselectivity by means of *Rhodotorula* sp. LSL resting or lyophilized cells, just choosing the suitable substrate and tuning the pH of the reaction. Thus, by following one of the three one-pot, one-step procedures, we could obtain as sole products: (i) the β-halohydrins from α-chloroketones at pH 6.5 and α-bromoketones at pH 4.3; (ii) the oxirane **3a** from the α-chloroketone **1a** at pH 10.0; and (iii) the vicinal diol **2g** from the α-bromoketone **1b** at pH 12.0.

4. Conclusions

We isolated *Rhodotorula* sp. LSL, a wild robust extrachemophile yeast capable of reducing a significant number of arylketones with excellent stereoselectivity. After optimization, proper conditions for using the microorganism as resting and lyophilized cells were settled. The main advantages are the possibility of working under non-sterile conditions, extreme alkaline pH values and, remarkably, the use of water or a minimal buffer solution as reaction media. With this biocatalyst, we developed one-pot procedures to alternatively access to valuable enantiopure building blocks from α-chloro or α-bromoacetophenone derivatives.

Acknowledgments

This work was supported by grants from Universidad Nacional de San Luis PROICO 2-1412, PIP 00360 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and PICT 2011-1416 Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT). C.A.P. is a doctoral CONICET fellow. F.R.B, A.A.O and M.K.S are members of the Research Career of CONICET. We especially acknowledge Dr. Delia Benuzzi for her helpful contributions in the isolation and biochemical identification of the microorganisms and Lic. Mónica M. Ferrari for technical assistance.

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.2014.07.011.

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