# Bioreduction of α-haloacetophenones by *Rhodotorula glutinis* and *Geotrichum candidum*

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**Dedicated to Prof. E. A. Ruveda on his 70th birthday**

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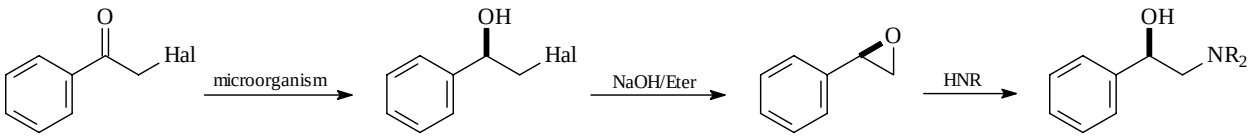
**Abstract**

Enantioselective reductions with enantiocomplementarity of α-haloacetophenones by *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 afforded the corresponding (*R*)- and (*S*)-halohydrins (halo = Cl, Br and I), respectively, in high chemical yields (89-99%) and enantiomeric excesses (92-99%). These halohydrins are potential chiral building blocks for the stereoselective syntheses of valuable compounds.

**Keywords:** Bioreduction, α-haloacetophenones, *Rhodotorula glutinis*, *Geotrichum candidum*

## Introduction

The reduction of α-haloketones mediated by whole cell of microorganisms has been frequently used to obtain chiral halohydrins,[1] that are potential chiral building blocks for use in stereoselective synthesis of valuable compounds such as enantiomerically pure 1,2-aminoalcohols *via* epoxides (Scheme 1).[1-2]




**1** (*R*)-**2** (*R*)-**3** (*R*)-**4**

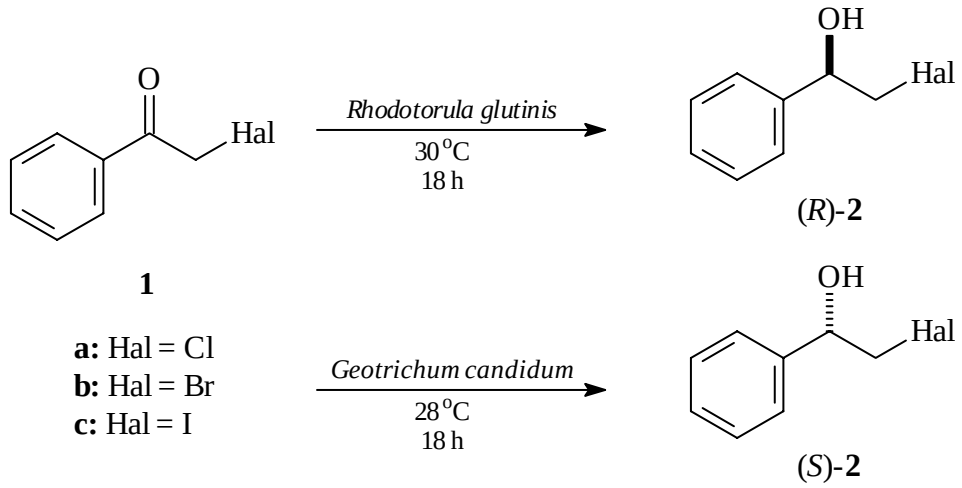
**Scheme 1**

The microorganism frequently used for reduction of α-haloacetophenones is baker´s yeast (*Saccharomyces cerevisiae*). However, the results are only fair with fluoro- and chloroacetophenone and inferior with α-bromoacetophenone giving poor yields of the corresponding bromohydrin. Also, a dehalogenation product, *i.e.* acetophenone, was obtained when α-iodoacetophenone was used as substrate.[1a,3] Recently, other microorganisms have been used with success for reduction of a large number of 4-substituted acetophenones.[1f,4] A previous report on the microbial reduction of α-haloacetophenones gave α-bromo- and α-iodo-1-phenylethanols in poor yields and low enantioselectivites.[1e] In this work, we present results of the asymmetric bioreduction of α-haloacetophenones mediated by *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 which gave halohydrins with opposite enantioselectivity and without any dehalogenated by-products.

## Results and Discussion

The bioreductions of α-haloacetophenones **1a-c** mediated by the yeast *Rhodotorula glutinis* CCT 2182 afforded the corresponding halohydrins **2a-c** with the *R* configuration, while the halohydrins **2a-c** with the *S* configuration were obtained when *Geotrichum candidum* CCT 1205 mediated the bioreductions (Scheme 2). Excellent yields and ee were obtained in both cases as shown in Table 1. These results are superior to those reported elsewhere[1a,1e] and therefore, the microorganisms used in this work should be chosen to mediate these biotransformations to obtain halohydrins **2a-c** with desired configuration *R* or *S* in excellent yields and ee. To our knowledge, this is the first time that (*S*)-(+)- and (*R*)-(-)-2-bromo-1-phenylethanol **2b** and (*S*)-(+)- and (*R*)-(-)-2-iodo-1-phenylethanol **2c** are obtained in high yields and enantioselectivity.



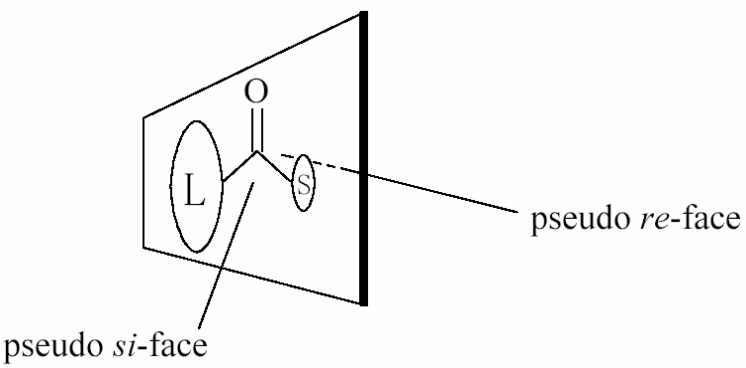

**Scheme 2**

**Table 1.** Bioreduction of aromatic ketones **1a-c** by *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205[a]

| Ketone | Yeast | T ($^oC$) | Alcohol | Yield (%) | $[\alpha]_D^{25}$ | E.e.(%)[d] |
|---|---|---|---|---|---|---|
| **1a** | *Rhodotorula glutinis* | 30 | (*R*)-**2a** | 98 | - 48.0[b] | 92 |
| **1a** | *Geotrichum candidum* | 28 | (*S*)-**2a** | 89 | + 52.0[b] | > 99 |
| **1b** | *Rhodotorula glutinis* | 30 | (*R*)-**2b** | 97 | - 44.4[c] | > 99 |
| **1b** | *Geotrichum candidum* | 28 | (*S*)-**2b** | 99 | + 40.0[c] | 90 |
| **1c** | *Rhodotorula glutinis* | 30 | (*R*)-**2c** | 98 | - 35.0[c] | 94 |
| **1c** | *Geotrichum candidum* | 28 | (*S*)-**2c** | 96 | + 37.1[c] | > 99 |

[a] Reaction time: 18 h; 2 mmol of ketone/1.5 mL of EtOH was added to 15 g of *R. glutinis* CCT 2182 or *G. candidum* CCT 1205 (wet weight) / 400 mL of YM (yeast-malt extract) or ME (malt-extract) nutrient broth respectively; [b] *c* 1, cyclohexane; [c] *c* 1, $CHCl_3$; [d] determined by GC-MS analysis (chiral capillary column CHIRASIL-DEX).

Both *Rhodotorula glutinis* CCT 2182 and *Saccharomyces cerevisiae* give products following the Prelog rule,[5] which predicts that in general, hydrogen transfer to the prochiral ketone occurs from the pseudo *re*-face (Scheme 3) were L represent a large and S a small substituent group.[6] On the contrary, the *Geotrichum candidum* CCT 1205 gives anti-Prelog products. This ability of *Geotrichum candidum* to furnish anti-Prelog reduction products has been observed elsewhere.[4a,7]




**Scheme 3**

The α-haloacetophenones have been used as mechanistic probe in the reduction reactions of NADH-dependent horse liver alcohol dehydrogenase,[8] for identification of reductants in sediments[9] and even in the whole cells.[3] This probe enables differentiation between reduction processes which proceed via hydride transfer ($H^-$) or by a multistep electron transfer ($e^-$, $H^\bullet$ or $e^-$,

$H^+$, $e^-$ as has been suggested).[10] Acetophenone is the reduction product obtained by electron transfer while optically active halohydrin is obtained when an enzyme mediates a hydride transfer process.

In this work, the yields of optically active halohydrins were high and no dehalogenation product was obtained when iodoacetophenone was used as substrate; therefore, the hydride transfer is the unique mechanism observed in the reduction of α-haloacetophenones mediated by both *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205.

## Conclusions

In conclusion, *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 should be used to mediate reduction of α-haloacetophenone to obtain halohydrins **2a-c** with the desired configuration *R* or *S* in excellent yields and ee's. Achieving these asymmetric bioreductions with enantiocomplementarity is remarkable and highlights the potential of such an approach.

## Experimental Section

**General Procedures.** IR spectra ware recorded on a Bomem MB Series spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$. The gas chromatographic analyses were performed using a Shimadzu GC/MS Class 5000 and with helium as carrier gas, with a chiral GC-column CHIRASIL-DEX (30 m x 0.25 mm x 0.25 μm). Optical rotation was measured with a J-720, VRDM306 JASCO, 589.3 nm spectropolarimeter.
Ketones **1a-b** were acquired from Aldrich Co. Ketone **1c** was obtained by reacting **1a** with NaI in acetone at rt. The racemic **2a-c** were obtained by reacting the corresponding **1a-c** with $NaBH_4$ in water/methanol at rt. All other reagents and solvents were reagent grade.

### Growth conditions for yeast culture

The yeasts *Rhodotorula glutinis* CCT 2182 and Geotrichum candidum CCT 1205 were stored at Fundação André Tosello Pesquisa e Tecnologia.[11] *Rhodotorula glutinis* was cultivated in YM (yeast-malt extract) nutrient broth (400 mL) at 30°C and *Geotricum candidum* was cultivated in ME (malt extract) nutrient broth (400 mL) at 28°C. Both yeasts were incubation for 2 days on an orbital shaker (200 rpm) before use. Sterile material was used to perform the experiments and the yeasts were manipulated in a laminar flow cabinet.

### General procedure for bioreduction of α-haloacetophenones

The compound **1** (2 mmol), dissolved in 1.5 mL of ethanol, was added to slurry of growing yeast (400 mL). The resulting suspension was stirred in an orbital shaker (200 rpm) at 28°C for *Geotricum candidum* and at 30°C for *Rhodotorula glutinis* until full conversion of **1** (18 h). The

product was extracted with $CH_2Cl_2$ and was purified by column chromatography using hexane/ethyl acetate (7:3).

**(*R*)-2-Chloro-1-phenyl-1-ethanol (2a).** When 0.31 g (2 mmol) of **1a** was subjected to the general procedure for bioreduction using *Rodothorula glutinis* CCT 2182, the product isolated was **2a** (0.30 g, 98%) as an oil; $[\alpha]_D^{25}$ -48.0$^o$ (*c* 1, cyclohexane) ($[\alpha]_D^{25}$ lit. -43.3$^o$ (*c* 1.8, cyclohexane), (*R*))[1a], giving an optical purity of 92%; IR (film) 3401; 3087; 3064; 3031; 2956; 28 95; 1494; 1453; 1426; 1248; 1200; 1085; 1064; 1012; 1064; 768; 724; 698 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 2.70 (sl, 1H); 3.61-3.68 (dd, 1H, *J* = 8.8 Hz, *J* = 11.3 Hz); 3.72-3.77 (dd, 1H, *J* = 3.3 Hz, *J* = 11.3 Hz); 4.87-4.92 (dd, 1H, *J* = 3.3 Hz, *J* = 8.8 Hz); 7.25-7.38 (m, 5H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 50.84; 73.94; 125.81; 128.24; 128.43; 139.64. MS m/z 156-158 ($M^+$).

**(*S*)-2-Chloro-1-phenyl-1-ethanol (2ª).** When 0.31 g (2 mmol) of 1a was subjected to the general procedure for bioreduction using *Geotrichum candidum* CCT 1205, the product isolated was 2a (0.27 g, 89%) as an oil; $[\alpha]_D^{25}$ +52.0$^o$ (*c* 1, cyclohexane) ($[\alpha]_D^{25}$ lit. -43.3$^o$ (*c* 1.8, cyclohexane), (*R*))[1a], giving an optical purity of > 99%; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

**(*R*)-2-Bromo-1-phenyl-1-ethanol (2b).**When 0.40 g (2 mmol) of **1b** was subjected to the general procedure for bioreduction using *Rodothorula glutinis* CCT 2182, the product isolated was **2b** (0.39 g, 97%) as an oil; $[\alpha]_D^{25}$ -44.4$^o$ (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. -38.2$^o$ (*c* 6.3, $CHCl_3$), (*R*))[1a], giving an optical purity of > 99%; IR (film) 3348; 2975; 2928; 2882; 1593; 1489; 1402; 1371; 1256; 1202; 1086; 1010; 899; 824; 770; 717 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 2.71 (sl, 1H); 3.46-3.53 (dd, 1H, *J* = 8.8 Hz, *J* = 10.6 Hz); 3.58-3.63 (dd, 1H, *J* = 3.3 Hz, *J* = 10.6 Hz); 4.87-4.92 (dd, 1H, *J* = 3.3 Hz, *J* = 8.8 Hz); 7.25-7.38 (m, 5H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 39.85; 72.95; 127.12; 128.63; 133.98; 138.48. MS m/z 200-202 ($M^+$).

**(*S*)-2-Bromo-1-phenyl-1-ethanol (2b).** When 0.40 g (2 mmol) of **1b** was subjected to the general procedure for bioreduction using *Geotrichum candidum* CCT 1205, the product isolated was **2b** (0.40 g, 99%) as an oil; $[\alpha]_D^{25}$ +40.0$^o$ (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. -38.2$^o$ (*c* 6.3, $CHCl_3$), (*R*))[1a], giving an optical purity of 90%; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

**(*R*)-2-Iodo-1-phenyl-1-ethanol (2c).** When 0.49 g (2 mmol) of **1c** was subjected to the general procedure for bioreduction using *Rodothorula glutinis* CCT 2182, the product isolated was **2c** (0.49 g, 98%) as an oil; $[\alpha]_D^{25}$ -35.0$^o$ (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. +36.3$^o$ (*c* 5.29, $CHCl_3$), (*S*))[3], giving an optical purity of 94%; IR (film) 3392; 3085; 3061; 3029; 2956; 2919; 1600; 1494; 1452; 1269; 1176; 1056; 1002; 964; 763; 745; 699 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 2.70 (sl, 1H); 3.61-3.68 (dd, 1H, *J* = 8.8 Hz, *J* = 11.3 Hz); 3.72-3.77 (dd, 1H, *J* = 3.3 Hz, *J* = 11.3 Hz); 4.87-4.92 (dd, 1H, *J* = 3.3 Hz, *J* = 8.8 Hz); 7.25-7.38 (m, 5H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 15.28; 77.28; 125.45; 128.07; 128.39; 140.82.

**(*S*)-2-Iodo-1-phenyl-1-ethanol (2c).** When 0.49 g (2 mmol) of **1c** was subjected to the general procedure for bioreduction using *Geotrichum candidum* CCT 1205, the product isolated was **2c** (0.48 g, 96%) as an oil; $[\alpha]_D^{25}$ +37.1$^o$ (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. +36.3$^o$ (*c* 5.29, $CHCl_3$), (*S*))[3], giving an optical purity of > 99%; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

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