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Anti-Prelog Microbial Reduction of Prochiral Carbonyl Compounds

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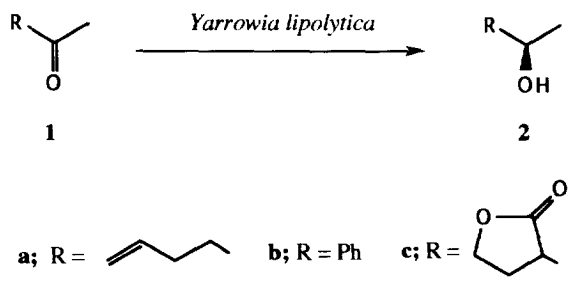
Abstract: *Yarrowia lipolytica* strains isolated from various habitats were tested in the reduction of prochiral carbonyl groups. The anti-Prelog reduction (*R*-enantiomer) is observed with different yields and enantiomeric excesses depending on the structure of the ketones **1a-e**.

Microbial reduction of prochiral carbonyl groups to prepare chiral alcohols is wide spread and very efficient. [1-4] Baker's yeast [2,3] and other microrganisms[5] are currently used to reduce a full range of carbonyl compounds. Most of these reductions generally follow the Prelog's rule[6] affording the S-enantiomer.

In this paper *Yarrowia lipolytica*, a relatively recent yeast species, was tested in the microbial reduction of prochiral carbonyl groups. The choice of this microrganism is due to the substantial differences to other yeasts:[7] *Yarrowia lipolytica* , in fact, uses oils and fats as the sole carbon source[8] and is distributed over a wide range of food systems. In this work a wide screening of *Yarrowia lipolytica* strains, isolated from various habitats, has been made in the reduction of structurally different ketones **1a-c** and an anti-Prelog reduction is described (Scheme).



The results of the screening are summarized in Figures **1-3**, where, for each ketone, the yields and the corresponding enantiomeric excesses of the R-alcohol obtained by reduction with *Yarrowia lipolytica* strains,

labeled Y (□), isolated and characterized from commercial chilled food,[9] *Yarrowia lipolytica* strains, labeled PO (◇), isolated from superficial water of lagoons of Po river delta, [7] *Yarrowia lipolytica* strains, labeled RO (△), isolated from commercial light butter,[10] and *Yarrowia lipolytica* strains, labeled A, B, C, D. (○), isolated from irradiated poultry meat[7] are reported.

Fig. 1. Reduction screening of 5-hexen-2-one

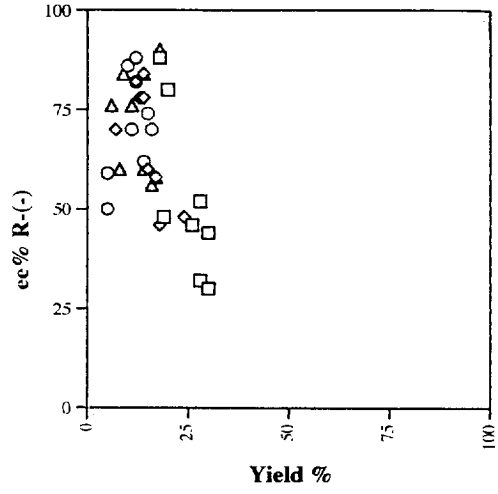


Fig. 2. Reduction screening of acetophenone

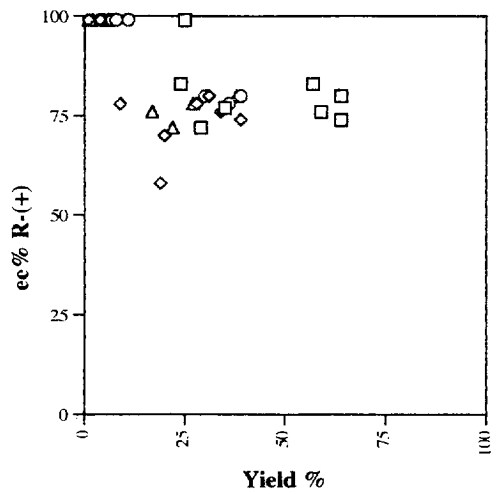
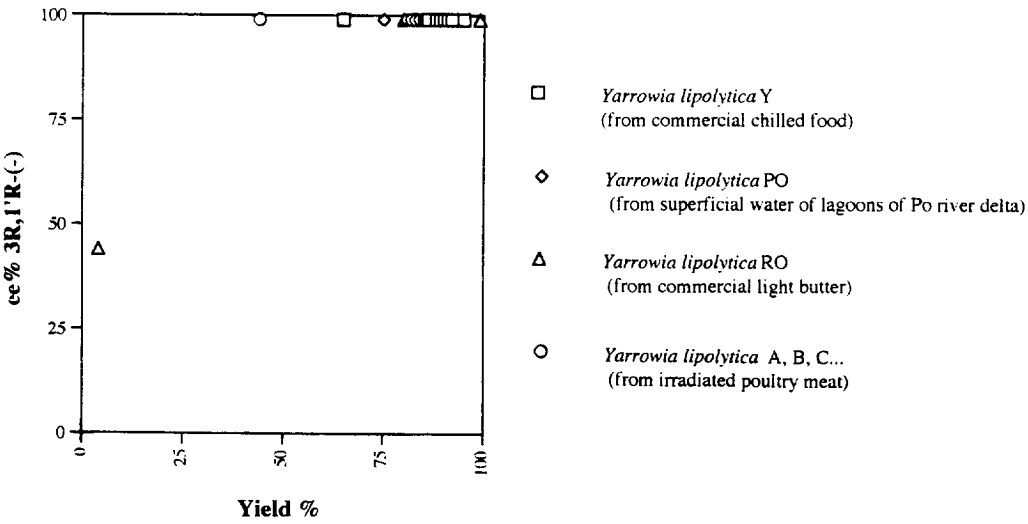


Fig. 3. Reduction screening of α-acetyl-γ-butyrolactone



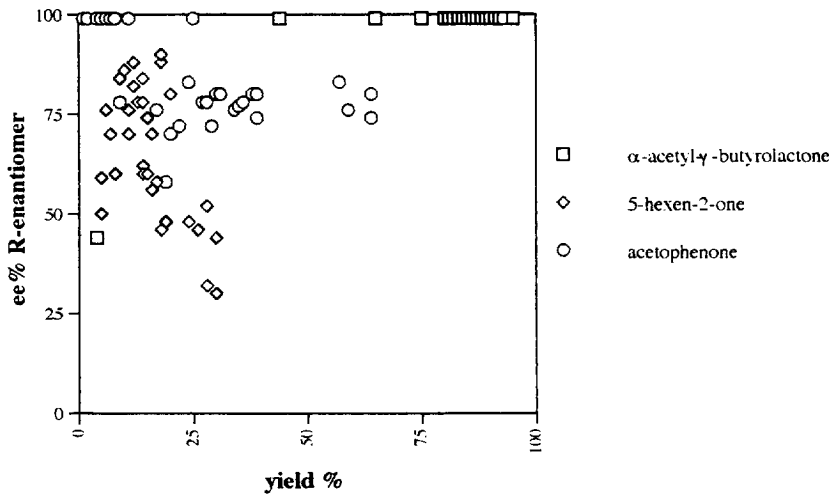
The reduction screening are carried out on analytical scale. Forthy individual strains of *Yarrowia lipolytica* (10 for each habitat) were grown in Saboraud dextrose broth for 48 h at 26°C in the presence of small amount of the selected substrate in order to induce or activate the production of particular enzymes during the growth phase.

To the grown culture the ketones **1a-c** (10 mg) were added and the incubation was continued for 24 h in the same conditions. The crude reduction products are analyzed by GLC on a chiral column using cyclohexanone as internal standard.

As observed in Figure 1, the reduction of 5-hexen-2-one **1a** affords generally low yield (5-30%) of the corresponding R-alcohol but in the most cases the enantiomeric excesses are up to 50%. For the acetophenone **1b** (Fig. 2) the reduction yields are higher (25-70%) and the enantiomeric excesses also are generally up to 75%. With the enolizable α-acetyl-γ-butyrolactone **1c** (Fig. 3) in all cases the 3R,1'R-enantiomer is obtained with excellent yields and ee.

The Figure 4, moreover, reports the results of the reduction screening of the ketones **1a** (◇), **1b** (○) and **1c** (□) not distinguishing among the strains of *Yarrowia lipolytica* from various habitats.

Fig. 4. Reduction with *Yarrowia lipolytica* of ketones **1a-c**

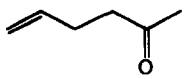
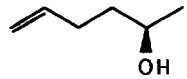
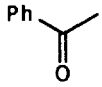
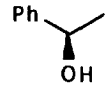
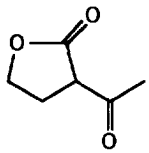
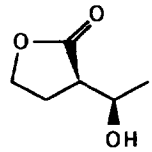
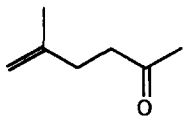
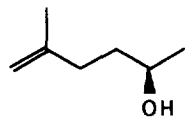
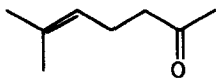
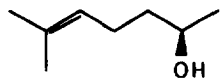


From these data we can point out that the reductions are not much affected by the habitats from which the *Yarrowia* strains are isolated but much more by the structure of compounds. Moreover, it is worth mentioning that the R-enantiomer is always produced with good ee while the reduction of the ketones **1a-c** with baker's yeast affords the S-enantiomer.

On the basis of this screening, some strains have been selected and the reactions have been carried out on preparative scale (0.2 g of ketone) and in order to confirm this behaviour, together with the ketones **1a-c**, 5-methyl-5-hexen-2-one **1d** and 6-methyl-5-hepten-2-one **1e** are also reduced. As summarized in the Table the results of the screening are substantially confirmed.

The reduction of the hexenone **1a** affords poor yields of the R-enantiomer **2a** (14-30%) with quite good enantiomeric excesses (48-88%). Better yields (25-64%) are obtained by the reduction of acetophenone **1b** with excellent ee of the R-alcohol **2b** (80-100%). On the other hand the enolizable α-acetoxy-γ-butyrolactone **1c** is quantitatively reduced to the pure (3R,1'R)-enantiomer. In this case the reaction is diastereoselective as well as enantioselective. Finally very good results are obtained in the reduction of the hexenone **1d** and heptenone **1e** that afford the corresponding R-alcohols **2d** and **2e** in high yields (69-81%) with good enantiomeric excesses (60-95%).

Table. Reduction with *Yarrowia lipolytica* of ketones **1a-e**.

ketone	*Yarrowia lipolytica*[a]	alcohol (yield %)	ee[b] % (abs. conf.)[c]
			
1a	Y5	**2a** (30)	48 (*R*)
1a	PO5	**2a** (14)	88 (*R*)
			
1b	Y3	**2b** (25)	100 (*R*)
1b	Y10	**2b** (64)	80 (*R*)
			
1c	Y5	**2c** (95)	100 (3*R*,1'*R*)
1c	PO5	**2c** (90)	100 (3*R*,1'*R*)
			
1d	1A	**2d** (69)	66 (*R*)
1d	RO3	**2d** (81)	60 (*R*)
			
1e	Y10	**2e** (70)	95 (*R*)

[a] The *Yarrowia lipolytica* cultures belong to DPVA collection. [b] Determined by GLC on chiral column by comparison with the racemic compound. [c] Absolute configuration is determined by comparison of the sign of the specific rotation with the reported value (see experimental).

In conclusion the microbial reduction with *Yarrowia lipolytica*, a recent yeast species, can be a choice to the use of Baker's yeast affording the anti-Prelog alcohol (R-enantiomer).

EXPERIMENTAL

General. The 40 individual strains, belonging to *Yarrowia lipolytica* cultures of DPVA collection, analyzed were isolated from the following habitat: habitat 1, commercial chilled food[9] (*Yarr. lip.* labeled Y); habitat 2, superficial water of lagoons of Po river delta (Italy)[7] (*Yarr. lip.* labeled PO); habitat 3, commercial light butter[10] (*Yarr. lip.*labeled RO); habitat 4, irradiated poultry meat[7] (*Yarr. lip.* A, B, C....).

Enantiomer separation has been made on Megadex 5 column (25 m X 0.25 mm) containing dimethyl-*n*-pentyl-β-cyclodextrine in OV 1701 from Mega s.n.c: carrier gas: helium (0.8 atm). For reduction of **1a**: temp. 80-150°C (0.5°C/min); retention time in min: **1a** 4.59 , (S)-**2a** 7.87, (R)-**2a** 8.06. For reduction of **1b**: temp. 130-200 °C (2°C/min); retention time in min: **1b** 5.32, (R)-**2b** 7.11, (S)-**2b** 7.32. For reduction of **1c**: temp. 100-200 °C (0.5°C/min); retention time in min: **1c** 16.69, (3R,1'R)-**2c** (as trifluoroacetyl derivative) 23.33, (3S,1'S)-**2c** (as trifluoroacetyl derivative) 23.97. For the reduction of **1d**: temp. 90-200°C (0.5°C/min); retention time in min: **1d** 5.85; (S)-**2d** 9.63, (R)-**2d** 9.85. For the reduction of **1e**: temp. 80-200°C (1.5°C/min) (after acetylation with acetic anhydride and pyridine) **1e** 3.88, (S)-**2e** 5.96, (R)-**2e** 6.72.
The ketones **1a-e** are commercially available.

Screening of *Yarrowia lipolytica* reduction of the ketones 1a-c on analytical scale. The culture medium (8 mL of Saboraud dextrose broth) is inoculated with a spore suspension of the *Yarrowia lipolytica* strains and grown for 48 h in the presence of small amounts of the selected substrate solution (20 μL) (the solution is prepared dissolving 0.1 g of the selected substrate in 1 mL of DMF). To a culture is added a further 80 μL of the substrate solution and the incubation continued for a further 24 h at 26 °C. The suspension is removed by centrifugation, the mixture is extracted with diethyl ether and dried over anhydrous Na_2SO_4. The crude reduction products are analyzed by GLC on a chiral column using cyclohexanone as internal standard. The results of the screening are reported in Fig. 1-3.

***Yarrowia lipolytica* reduction of the ketones 1a-e on preparative scale.** The reaction is carried out as above starting from 150 mL of culture medium, inoculated with the appropriate spore suspension, and 0.2 g of the selected ketone dissolved in 2 mL of DMF. After 24 h the reaction mixture was extracted with diethyl ether (250 mL) with a continous liquid liquid extractor, dried over anhydrous Na_2SO_4 and concentrated under vacuum. Chromatography of the crude reaction mixture (silica gel, petroleum ether/diethyl ether 80/20) gave the purified alcohols **2a-d** (see Table). The enantiomeric excesses are determined by GLC on chiral column. The absolute configurations of the alcohols **2a-e** was determined comparing the sign of their specific rotation with those of the literature: (R)-5-hexen-2-ol **2a** $[\alpha]_D$ = -12.1 (c 4.6, $CHCl_3$),[11] (R)-1-phenylethanol **2b** $[\alpha]_D$ = 41 (c,5.1, $CHCl_3$),[11] (3R,1'R)-α-hydroxyethyl-γ-butyrolactone **2c** $[\alpha]_D$ = - 40.6 (c 1.8, $CHCl_3$),[12] 5-methyl-5-hexen-2-ol **2d** $[\alpha]_D$ = -18.58 ($CHCl_3$),[13] (R)-6-methyl-5-hepten-2-ol **2e** $[\alpha]_D$ = -14.5 (c 1.3, EtOH).[11]

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