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Asymmetric anti-Prelog reduction of ketones catalysed by *Paracoccus pantotrophus* and *Comamonas* sp. cells via hydrogen transfer

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ABSTRACT

A broad range of ketones including methyl-aryl-, methyl-alkyl-, cyclic and sterically hindered ketones were reduced to the corresponding anti-Prelog alcohols with moderate to excellent stereoselectivities by employing lyophilised cells of *Paracoccus pantotrophus* DSM 11072 and *Comamonas* sp. DSM 15091 via hydrogen transfer. The reduction equivalents were provided using 2-propanol as a hydride donor. For instance, acetophenone was reduced to the corresponding (*R*)-enantiomer with >99% ee.

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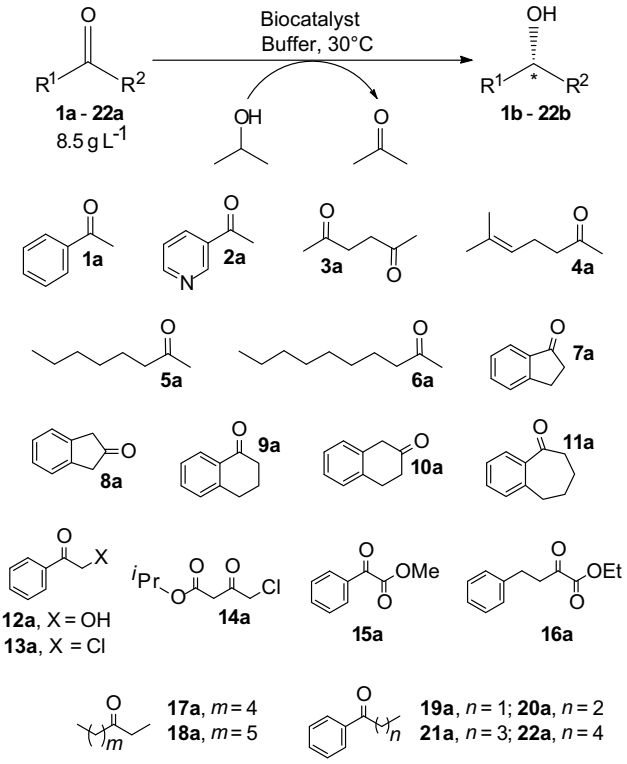
1. Introduction

Biocatalysis has recently gained increasing importance for the preparation of enantiopure compounds required for pharmaceutical, agrochemical and food industry.[1] In this context, enantiopure alcohols are very frequently needed as chiral synthons. Methods for their preparation, such as kinetic resolutions or dynamic kinetic resolutions,[2] have been widely employed leading to the acylated alcohol. Reduction of ketones to the corresponding enantiopure alcohols has been achieved by chemical (metal) catalysts[3] or biocatalytic methods in order to obtain quantitative yields. Alcohol dehydrogenases (ADHs) are found in many microbial strains catalysing the stereoselective reduction of the carbonyl group leading to the corresponding (*S*)-or (*R*)-alcohol.[4] However, most of these biocatalysts follow 'Prelog's rule',[5] thus the (*S*)-alcohol is usually obtained assuming that the smaller substituent of the ketone has the lower CIP priority. Only a few 'anti-Prelog'-(*R*)-specific biocatalysts have been described.[6]

When using cells as the catalyst, the reducing equivalents are mostly provided by the metabolic pathways by employing fermenting cells and glucose, or a huge excess of cells is needed. Both cases lead to unwanted side-reactions or competing opposite selectivities leading to lower ees, since many enzymes are active under these conditions as well.[7] Only a few examples have been reported, where cells are combined with other reducing agents, such as 2-propanol (Scheme 1).[8] An additional advantage of employing this hydride donor is that many undesired enzymes are deactivated in the presence of 2-propanol. Herein, we report two novel bacterial strains that accept 2-propanol as a hydrogen donor for the preparation of 'anti-Prelog' alcohols at elevated substrate concentrations.



Scheme 1. Asymmetric reduction of ketones via biocatalytic hydrogen transfer.

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2. Results and discussion

By testing more than 50 lyophilised cells from bacteria and yeasts for the reduction of acetophenone **1a** (Scheme 1) as a model substrate at 15% v/v of 2-propanol as a co-substrate, six positive strains leading to the conversion of >5% were identified (Table 1). However, only *Paracoccus pantotrophus* DSM[9] 11072 and *Comamonas* sp. DSM 15091 showed (*R*)-stereopreference. According to the literature,[10] *Yarrowia lipolytica* usually displays (*R*)-stereo-preference as well, but in our screening several *Yarrowia* strains showed only low activity (conv. ~1%). Repetition of the experiments with 1% v/v 2-propanol did not lead to significant improvements.

Table 1
Active strains for the reduction of acetophenone (**1a**, 8.5 g L^{-1}) via hydrogen transfer

Biocatalyst	Cosubstrate[a]	c[b] (%)	ee[b] (%)
Arthrobacter sp. DSM 7325	2-Propanol	6	>99 (*S*)
Candida parapsilosis DSM 70125	2-Propanol	7	>99 (*S*)
Paracoccus pantotrophus DSM 11072	2-Propanol	16	>99 (*R*)
Pseudomonas sp. DSM 12877	2-Propanol	6	>99 (*S*)
Sphingomonas sp. DSM 11094	2-Propanol	5	>99 (*S*)
Comamonas sp. DSM 15091	2-Propanol	67	>99 (*R*)
Ralstonia sp. DSM 9750	Ethanol	8	>99 (*S*)
Pseudomonas sp. DSM 12877	Ethanol	11	>99 (*S*)
Sphingomonas sp. DSM 11094	Ethanol	8	>99 (*S*)

[a] 15% v/v for 2-propanol; 10% v/v for ethanol.
[b] Measured by GC on a chiral stationary phase.

It was noticed that the stereoselectivity of *P. pantotrophus* depended on the medium used for cell cultivation. For instance, lower stereoselectivities (90% ee) were obtained in cases where the cells were grown on DSMZ medium 1 or Luria broth, instead of the complex medium used here which led to >99% ee.

As an alternative hydrogen source, ethanol[11] was tested with the same bacterial strains and yeasts. Using 10% v/v ethanol instead of 2-propanol as a co-substrate, we identified three active strains showing conversion higher than 5% (Table 1); all of them transformed acetophenone to the enantiopure (*S*)-enantiomer (ee >99%). However, the 'anti-Prelog' strains identified with 2-propanol showed no significant activity with ethanol.

In a concluding screening, 31 fungi were tested at 15% v/v and 1% v/v of 2-propanol concentration. Only one active fungus namely *Mortierella alpina* ATCC 8978 was identified, which showed a conversion of just 1%.

Therefore, we decided to continue the study with *P. pantotrophus* DSM 11072 and *Comamonas* sp. DSM 15091 strains, since they displayed a promising anti-Prelog activity with acetophenone. In the next step, the pH of the Tris buffer (50 mM) was optimised. For that purpose, lyophilised cells of both strains were rehydrated in 50 mM Tris buffer at varied pHs, and assayed with **1a** and 2-propanol (Fig. 1). While *P. pantotrophus* showed an optimum at pH 7.0, *Comamonas* sp. showed the best activity between pH 7.0 and 8.0.

The co-substrate 2-propanol fulfils two functions: on the one hand, it provides the hydrogen equivalents, while on the other hand, it improves the solubility of the substrate in the aqueous phase; nevertheless it can also lead to deactivation of the biocatalyst. To obtain a rough idea about its effect, the concentration of 2-propanol was varied and the conversion was determined (Fig. 2). *P. pantotrophus* showed the highest conversion at 5% v/v and *Comamonas* sp. at 10% v/v, although the latter strain was active over a broad concentration range of 2-propanol. Obviously, 2-propanol was required for the biotransformations, since without it almost no conversion was observed.

After optimisation of the reaction parameters, various types of substrates were transformed with both strains. In the first set of

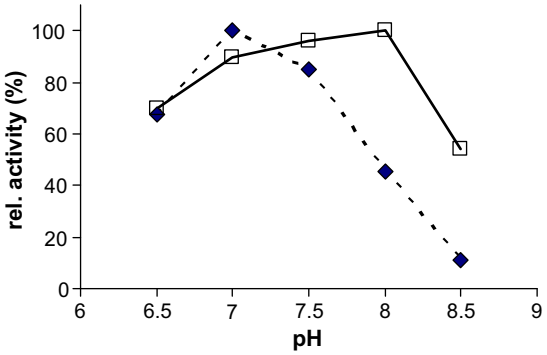

Figure 1. Reduction of **1a** (8.5 g L^{-1}, t = 24 h) with 2-propanol (15% v/v) and lyophilised cells of *Paracoccus pantotrophus* DSM 11072 (♦) and *Comamonas* sp. DSM 15091 (□) at varied pHs.

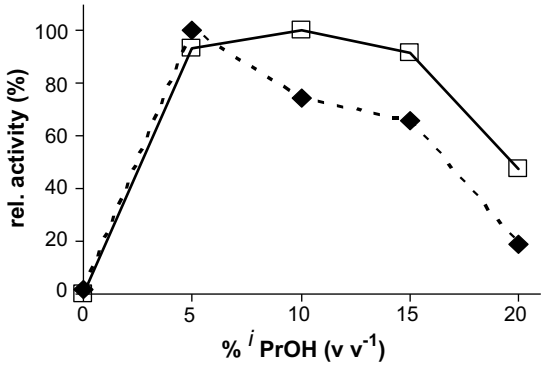

Figure 2. Reduction of **1a** (t = 24 h) at varied 2-propanol concentration in Tris buffer, pH 7.5, employing *Paracoccus pantotrophus* DSM 11072 (♦) and *Comamonas* sp. DSM 15091 (□).

experiments, various methyl ketones were reduced (Table 2): aromatic ketone **1a** and heteroaromatic ketone **2a**, aliphatic ketones **4a**–**6a** and a diketone such as 2,5-hexanedione **3a** were reduced with both strains, yielding the corresponding (*R*)-alcohols with good to excellent conversions (69–94%) and stereoselectivities (58–99%) after 24 h. *Comamonas* sp. led to higher conversions in most cases, however, *P. pantotrophus* displayed better stereoselectivities. Diketone **3a** was reduced to the corresponding (*R*,*R*)-diol,

Table 2
Reduction of methyl ketones **1a**–**6a** employing lyophilised cells of *P. pantotrophus* or *Comamonas* sp. and 2-propanol (t = 24 h)

Biocatalyst	Substrate	c[a] (%)	ee[a] (%)
Paracoccus pantotrophus	**1a**	69	>99 (*R*)
Paracoccus pantotrophus	**2a**	93	>99 (*R*)
Paracoccus pantotrophus	**3a**	60[b]	66 (*R*,*R*)[c]
Paracoccus pantotrophus	**4a**	82	91 (*R*)
Paracoccus pantotrophus	**5a**	79	84 (*R*)
Paracoccus pantotrophus	**6a**	82	94 (*R*)
Comamonas sp.	**1a**	77	98 (*R*)
Comamonas sp.	**2a**	94	97 (*R*)
Comamonas sp.	**3a**	92[d]	94 (*R*,*R*)[c]
Comamonas sp.	**4a**	94	58 (*R*)
Comamonas sp.	**5a**	92	58 (*R*)
Comamonas sp.	**6a**	92	87 (*R*)

[a] Measured by GC on a chiral stationary phase.
[b] In addition, 11% of hydroxyketone intermediate **3c** was obtained.
[c] No trace of any other diastereomer could be detected, thus >99% de.
[d] No hydroxyketone was detected.

and the corresponding intermediate hydroxyketone[12] was only detectable for *P. pantotrophus* (11% at 24 h).

In another set of substrates, cyclic ketones **7a**–**11a** were tested (Table 3). It can be concluded that the activity depended on the position of the carbonyl moiety within the ring. Thus, if the ketone was at position 1 or α of the cyclic system (**7a**, **9a** and **11a**), reductions proceeded slowly, if at all, leading to low conversion even after 24 h; however, if the carbonyl group was at position 2 or β (**8a**, **10a**), conversions were much better and in the case of β-tetralone **10a**, the corresponding (*R*)-**10b** alcohol was obtained with 99% conversion and excellent enantiomeric excess (98%), employing *Comamonas* sp. The low conversion for the ketones at the α-position could be attributed to steric hindrance compared to the β-position.

Table 3
Reduction of cyclic ketones **7a**–**11a** employing lyophilised cells of *P. pantotrophus* or *Comamonas* sp. and 2-propanol (*t* = 24 h)

Biocatalyst	Substrate	*c*[a] (%)	ee[a] (%)
Paracoccus pantotrophus	**7a**	0	n.d.
Paracoccus pantotrophus	**8a**	13	—
Paracoccus pantotrophus	**9a**	0	n.d.
Paracoccus pantotrophus	**10a**	63	92 (*R*)
Paracoccus pantotrophus	**11a**	3	n.d.
Comamonas sp.	**7a**	20	n.d.
Comamonas sp.	**8a**	61	—
Comamonas sp.	**9a**	8	n. d.
Comamonas sp.	**10a**	99	98 (*R*)
Comamonas sp.	**11a**	3	n.d.

n.d. not determined due to low conversion.
[a] Measured by GC on a chiral stationary phase.

Continuing to study the substrate spectrum of these anti-Prelog strains, we tested various ketones with two bulky substituents ('bulky–bulky' ketones) (Table 4). The acetophenone derivatives **12a** and **13a** were compared: one with a hydroxy- and one with a chloro group at the ω-position. ω-Chloro acetophenone **13a** reacted much faster than **12a**, to give the corresponding (*S*)-alcohol **13b** with good stereoselectivity (94% *P. pantotrophus*). Derivatives of **13b** are frequently employed as intermediates in the synthesis of pharmaceuticals, for example, in the treatment of obesity and depression.[13] Another α-chloro ketone **14a** was reduced to the anti-Prelog product with excellent ee (>99%) and conversion (99%, *P. paracoccus*). For *Comamonas* sp., the biotransformation of **14a** was stopped after 4 h since longer reaction times led to the degradation of the alcohol catalysed by the organism. Two α-ketoesters **15a**–**16a** were tested, since α-hydroxyesters are valuable compounds;[4f,g,14] for instance, alcohol **16b** is an important intermediate for ACE inhibitors.[15] Both ketones were converted with moderate to good selectivity to the corresponding (*S*)-α-hydroxyesters (72% for **15b** and *Comamonas*, 85% for **16b** with *P. pantotrophus*). In the case of **15a** with *Comamonas* sp., an additional 7% of the corresponding isopropyl ketoester was formed, probably due to transesterification caused by the presence of lipase(s) or esterase(s) in the lyophilised cell preparation. The three ethyl ketones tested, **17a**–**19a**, were converted with good activity and excellent stereoselectivity to afford enantiomerically pure (*R*)-**17b**–**19b**.

Table 4
Reduction of 'bulky–bulky' ketones **12a**–**22a** employing lyophilised cells of *P. pantotrophus* or *Comamonas* sp. and 2-propanol (*t* = 24 h)

Biocatalyst	Substrate	*c*[a] (%)	ee[a] (%)
Paracoccus pantotrophus	**12a**	4	n.d.
Paracoccus pantotrophus	**13a**	96	94 (*S*)[b]
Paracoccus pantotrophus	**14a**	99	>99 (*S*)[b]
Paracoccus pantotrophus	**15a**	41	29 (*S*)[b]
Paracoccus pantotrophus	**16a**	98	85 (*S*)[b]
Paracoccus pantotrophus	**17a**	71	>99 (*R*)
Paracoccus pantotrophus	**18a**	70	>99 (*R*)
Paracoccus pantotrophus	**19a**	17	>99 (*R*)
Paracoccus pantotrophus	**20a**	1	n.d.
Paracoccus pantotrophus	**21a**	0	n.d.
Paracoccus pantotrophus	**22a**	0	n.d.
Comamonas sp.	**12a**	29	n.d.
Comamonas sp.	**13a**	>99	71 (*S*)[b]
Comamonas sp.	**14a**	99[c]	93 (*S*)[b]
Comamonas sp.	**15a**	80	72 (*S*)[b,d]
Comamonas sp.	**16a**	98	31 (*S*)[b]
Comamonas sp.	**17a**	84	>99 (*R*)
Comamonas sp.	**18a**	84	>99 (*R*)
Comamonas sp.	**19a**	53	89 (*R*)
Comamonas sp.	**20a**	13	47 (*R*)
Comamonas sp.	**21a**	17	54 (*R*)
Comamonas sp.	**22a**	19	53 (*R*)

n.d. not determined.
[a] Measured by GC or HPLC on a chiral stationary phase.
[b] Switch in CIP priority.
[c] Conversion at 4 h.
[d] In addition, 7% of the corresponding 2-propyl ketoester was formed.

Finally, a set of bulky 1-phenyl-1-alkanones **20a**–**22a** with different alkyl chain lengths were tested to study the influence of the length of the alkyl chain on the conversion (Fig. 3). For *P. pantotrophus*, a clear decrease of activity was observed when methyl **1a** was exchanged with an ethyl chain **19a**, finding no conversion for substrates possessing a larger alkyl chain **20a**–**22a**. In contrast, this effect was less pronounced for *Comamonas* sp., thus substrates **21a** and **22a** were accepted and reduced to the corresponding (*R*)-alcohols. Additionally, a change in stereoselectivity was observed too. For acetophenone **1a**, good conversion and ee were achieved (Table 2), for propiophenone **19a** moderate conversion but good selectivity was obtained (Table 4), while for bulkier ketones low conversions and moderate ees were measured (Table 4). This could be explained by the presence of at least two different ADHs with a different substrate pattern and stereopreference. Thus, (at least) one ADH which could accept 'small-bulky' ketones with good anti-Prelog preference could reduce efficiently substrates **1a** and **19a**, while another ADH(s) with better affinity for 'bulky-bulky' substrates, but worse or even reverse stereoselectivity transforms substrates **20a**–**22a**.

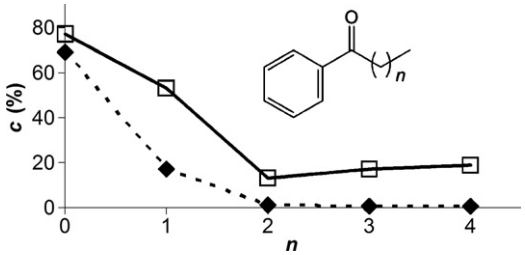

Figure 3. Effect of the alkyl chain length on the conversion of 1-phenyl-1-alkanones (**1a**, **19a**–**22a**, *t* = 24 h) in Tris buffer (50 mM) and 2-propanol for: *Paracoccus pantotrophus* DSM 11072 (♦); *Comamonas* sp. DSM 15091 (□).

3. Conclusions

We have identified two bacterial strains that reduce ketones to the corresponding anti-Prelog alcohols via biocatalytic hydrogen transfer using 2-propanol as a hydrogen source, resembling a Meerwein–Ponndorf–Verley reaction. *P. pantotrophus* DSM 11072 and *Comamonas* sp. DSM 15091 usually showed very good activities and selectivities towards a broad spectrum of 'small–bulky', cyclic and 'bulky–bulky' ketones. In general, the first strain showed a better stereoselectivity than the second one. Purification of the

enzymes responsible for these biotransformations is ongoing in our laboratories.

4. Experimental

4.1. General

Ketones **1a**–**22a**, alcohol **8b**, racemic alcohols **1b**, **4b**–**7b**, **9b**, **10b**, **12b** and **15b**–**19b**, (*R*)- and (*S*)-**13b**, (*R*)- and (*S*)-**20b**, diol **3b** and hydroxyketone **3c** were commercially available, either from Sigma–Aldrich–Fluka (Vienna, Austria) or from Lancaster (Frankfurt am Main, Germany). Racemic compounds **2b**, **11b**, **14b**, **21b** and **22b** were synthesised by conventional reduction from the corresponding ketones ($NaBH_4$, MeOH, room temperature).[8c] All other reagents and solvents were of the highest quality available. Conversions, enantiomeric excesses and absolute configurations were determined using GC analysis as previously described.[16]

Lyophilised cells of *P. pantotrophus* DSM 11072 and *Comamonas* sp. DSM 15091 were obtained by cultivating the organism in 250 mL of a complex medium (10 g L^{-1} yeast extract, 10 g L^{-1} bacteriological peptone, 10 g L^{-1} glucose, 2 g L^{-1} NaCl, 0.15 g L^{-1} $MgSO_4{\cdot}7H_2O$, 1.3 g L^{-1} NaH_2PO_4, 4.4 g L^{-1} K_2HPO_4 and distilled water) in one litre baffled shake flasks at 30 °C at 120 rpm. After three days, the cells were harvested by centrifugation (8000 rpm, 20 min), washed with phosphate buffer (50 mM, pH 7.5), shock frozen in liquid nitrogen and lyophilised.

4.2. Experimental procedures

4.2.1. Screening of lyophilised cells for the reduction of acetophenone

Lyophilised cells (20 mg) were rehydrated in Tris/HCl buffer (600 μL, 50 mM, pH 7.5) for 30 min at 30 °C and 120 rpm on a rotary shaker in an Eppendorf vial (1.5 mL). Then, 2-propanol (105 μL, 15% v/v) or ethanol (67 μL, 10% v/v) and acetophenone (**1a**, 6 μL) were added. Reactions were shaken at 30 °C and 120 rpm for 24 h, and stopped by extraction with ethyl acetate (2 × 0.5 mL). The organic layer was separated from the cells by centrifugation (2 min, 13,000 rpm) and dried over Na_2SO_4. Conversion and enantiomeric excess of **1b** were determined by chiral GC analysis.

4.2.2. Optimisation of pH and 2-propanol concentration

Experiments were performed as described in Section 4.2.1, using the appropriate pH and 2-propanol concentration.

4.2.3. General method for the biocatalytic reduction of ketones employing lyophilised cells of *P. pantotrophus* DSM 11072

Lyophilised cells of *P. pantotrophus* (20 mg) stored at 4 °C were rehydrated in Tris/HCl buffer (600 μL, 50 mM, pH 7) for 30 min at 30 °C and 120 rpm on a rotary shaker in an Eppendorf vial (1.5 mL). Then, 2-propanol (32 μL, 5% v/v) and the corresponding ketones **1a**–**22a** (6 μL or 6 mg) were added. Reactions were shaken at 30 °C and 120 rpm for 24 h, and stopped by extraction with ethyl acetate (2 × 0.5 mL). The organic layer was separated from the cells by centrifugation (2 min, 13,000 rpm) and dried over Na_2SO_4. Conversions and enantiomeric excesses of the corresponding alcohols were determined by chiral GC or HPLC analysis.

4.2.4. General method for the biocatalytic reduction of ketones employing lyophilised cells of *Comamonas* sp. DSM 15091

Lyophilised cells of *Comamonas* sp. (20 mg) stored at 4 °C were rehydrated in 600 μL of Tris/HCl buffer (50 mM, pH 8) for 30 min at 30 °C and 120 rpm on a rotary shaker in an Eppendorf vial (1.5 mL). Then, 2-propanol (67 μL, 10% v/v) and the corresponding ketones **1a**–**22a** (6 μL or 6 mg) were added. Reactions were shaken at 30 °C and 120 rpm for 24 h, and stopped by extraction with ethyl acetate (2 × 0.5 mL). The organic layer was separated from the cells by centrifugation (2 min, 13,000 rpm) and dried over Na_2SO_4. Conversions and enantiomeric excesses of the corresponding alcohols were determined by chiral GC or HPLC analysis.

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