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Lyophilised yeasts: easy-to-handle biocatalysts for stereoselective reduction of ketones

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Abstract

The use of lyophilised yeasts as biocatalysts for the reduction of carbonyl compounds has been studied. First, a comparison of the performances of fresh and lyophilised cells of seven yeasts was performed using ethyl acetoacetate and acetophenone as typical substrates. Lyophilised cells gave from low to high conversions but, with a few exceptions, always good enantioselectivity, and they were then employed for the reduction of structurally different carbonyl compounds. Highly enantioselective reduction of carbonyls was often achieved, even with aromatic ketones. Both enantiomers of the alcohols were, in most cases, obtained with high enantiomeric excess by simply choosing a suitable yeast.

1. Introduction

The asymmetric reduction of carbonyl compounds by microbial dehydrogenases is a well recognised method for the preparation of chiral alcohols. Biocatalysts can catalyse stereoselective reductions with high selectivity and, additionally, they work under mild and safe conditions.[1] These methods offer great potential for the production of a number of chiral fine chemicals which have a relevance in different areas such as the pharmaceutical and agrochemical industries. The use of whole microbial cells is particularly advantageous for carrying out the desired reduction since they do not require addition of co-factors for their regeneration. *Saccharomyces cerevisiae* has been employed for a number of asymmetric reductions.[2–4] Other microbial species, such as bacteria (*Bacillus stearothermophilus*,[5] *Gluconobacter oxydans*,[6] various Lactobacilli,[7,8] *Thermoanaerobium brockii*,[9] *Thermoanaerobium ethanolicus*[10]) and fungi (yeasts[11,12] and *Geotrichum candidum*[13,14]), possess dehydrogenases which have been successfully employed for the stereoselective reduction of carbonyls.

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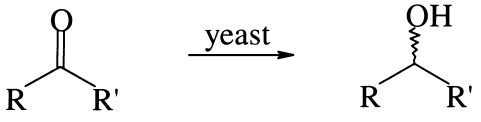
However, there is still a need for new biocatalysts that are able to perform highly stereoselective reactions, and the potential of microbial bio-reductions appears to be still far from being fully exploited. The reasons why an extensive use of microbial cells as catalysts has not yet been adequately accepted are mostly related to the demand of reliable preparation techniques capable of producing high yields of the desired products and to the low familiarity with the techniques of applied microbiology. The use of dry cells may partially circumvent these problems by making available biocatalysts easy to use without any microbiological facilities. Besides the easily available *S. cerevisiae*, acetone powder of the fungus *G. candidum* has proven to be effective in the asymmetric reduction of different ketones furnishing the corresponding (*S*)-alcohols with excellent selectivity.[15] Therefore, it is attractive to look for other microbial species to be used as dry cells and which are also able to furnish the opposite enantiomer.

The aim of this work was the evaluation of lyophilised yeasts as easy-to-handle biocatalysts for stereoselective reduction of various prochiral carbonyls (Scheme 1). The yeasts were selected on the basis of previous studies, which have shown their ability to perform highly selective reductions.[10,16,17]



1.	R = Me,	R' = CH_2COOEt	**9**.	R = CH_2Cl,	R' = CH_2COOEt
2.	R = Ph,	R' = Me	**10**.	R = Ph,	R' = Et
3.	R = Me,	R' = Et	**11**.	R = Ph,	R' = *n*-Propyl
4.	R = Me,	R' = *n*-Propyl	**12**.	R = Ph,	R' = CH_2Br
5.	R = Me,	R' = *n*-Butyl	**13**.	R=*o*-BrC_6H_4	R' = Me
6.	R = Me,	R' = *n*-Pentyl	**14**.	R = Ph	R' = $CH_2CH_2NO_2$
7.	R = Me,	R' = *n*-Hexyl	**15**.	R = Ph,	R' = $CH_2CH_2CH_2NO_2$
8.	R = Et,	R' = CH_2COOEt			

Scheme 1.

2. Results and discussion

Ethyl acetoacetate and acetophenone were used as typical substrates to compare the performances of fresh and lyophilised cells. The reduction of these substrates have been thoroughly studied with *S. cerevisiae* and other yeasts,[2,3,7,18–20] also on a preparative scale.[21–23] Table 1 reports the molar conversion and enantiomeric excesses obtained after 24 h in biotransformations performed with the same cell concentration.

The conversions generally decreased when lyophilised cells were used, but only in a few cases did this occur in a dramatic manner. As expected, the reduction of **1** is accelerated by the β-oxo group and much higher yields are always obtained.[1] On the other hand, the enantiomeric excesses increased in some cases with the use of lyophilised cells.

Table 1
Reduction of acetophenone and ethyl acetoacetate with fresh and lyophilised cells of yeasts. Molar conversion (%) and enantiomeric excess (%) after 24 h

	1 (ethyl acetoacetate)				2 (acetophenone)			
	Fresh		Lyophilised		Fresh		Lyophilised	
Yeast	conv.	e.e.	conv.	e.e.	conv.	e.e.	conv.	e.e.
Candida utilis	> 98	80 (*S*)	> 98	90 (*S*)	30	95 (*S*)	15	> 98 (*S*)
Kluyveromyces marxianus	95	< 5 (*R*)	90	10 (*R*)	10	35 (*R*)	< 5	15 (*R*)
Pichia etchellsii	> 98	80 (*R*)	> 98	> 98 (*R*)	90	95 (*R*)	80	97 (*R*)
Pichia fermentans	95	75 (*S*)	80	75 (*S*)	<5	-	< 5	-
Pichia glucozyma	> 98	75 (*S*)	80	55 (*S*)	60	90 (*S*)	30	85 (*S*)
Pichia minuta	> 98	85 (*S*)	80	75 (*S*)	40	95 (*S*)	20	> 98 (*S*)
Saccharomyces cerevisiae	90	> 98 (*S*)	20	75 (*S*)	<5	-	< 5	-

Lyophilised cells were stored and used over six months without noticeable loss of activity. The reduction of various prochiral carbonyls is reported in Table 2. Structurally different compounds were chosen to check the substrate specificity of the yeasts.

The yeasts followed Prelog's rule in the reduction of 2-octanone **7**, the only exception being *Pichia etchellsii*. It has already been observed that *P. etchellsii* is able to reduce C4–C8 methylketones to the corresponding (*R*)-alcohols,[16] while *S. cerevisiae* gives poor yields and low enantiomeric excess of the (*S*)-alcohols, following Prelog's rule.[24,25] The attack from the *re*-face became less predominant as the difference between the small group (methyl) and the large one decreased. With *Candida utilis* and *Pichia fermentans*, the opposite enantiomer was obtained with shorter chain ketones as the difference of size between the two substituents decreased.

The reduction of β-keto esters **1** and **8** generally occurred with high yields and enantioselectivity, while large decreases of the molar conversions with **9** are probably due to the poor stability of the substrate. In this latter case, lower stereoselectivity was also observed, probably as a consequence of the smaller differentiation between the large group and the small group.

The reduction of different aromatic ketones **10**–**15** was also performed. Simple aromatic ketones are usually very poor substrates for Bakers' yeast-mediated reductions, often furnishing very low yields of the desired chiral alcohol.[18,20] Only *P. etchellsii*, *P. glucozyma* and *P. minuta* gave the reduction of **10** and **11** with notable molar conversions, although with different trends. *Pichia etchellsii* furnished lower yields when increasing the size of the aliphatic substituent from methyl to propyl, while *P. glucozyma* and *P. minuta* allowed for good conversions also in the reduction of **10** and **11** (95 and 50%, respectively).

The aromatic ketones **12** and **13** are both substrates of interest for producing chiral intermediates. The alcohol obtained by reduction of **12** can be transformed into the corresponding enantiomerically pure epoxide. It has been reported that Bakers' yeast reduction of **12** furnished the (*R*)-enantiomer (ee ≥80%).[19] Compound **12** was not very stable and in some cases the substrate disappeared without alcohol formation (*P. etchellsii* and *P. glucozyma*), while with *Kluyveromyces marxianus* and *P. fermentans* very high enantioselectivity was achieved. Prelog's rule was followed in all cases.

Table 2. Reduction of compounds **3–15** with lyophilised yeasts. Molar conversion (%) and enantiomeric excess (%) after 24 h

Substrate	*C. utilis*		*K. marxianus*		*P. etchellsii*		*P. fermentans*		*P. glucozyma*		*P. minuta*		*S. cerevisiae*	
	Conv.	e.e.	Conv.	e.e.	Conv.	e.e.	Conv.	e.e.	Conv.	e.e.	Conv.	e.e.	Conv.	e.e.
3	30	35 (*R*)	25	>98(*S*)	85	75 (*R*)	70	10 (*R*)	55	<5 (*S*)	80	<5 (*S*)	< 5	-
4	70	< 5(*R*)	20	55 (*S*)	80	90 (*R*)	65	50 (*R*)	55	10 (*S*)	65		15	>98(*S*)
5	90	55 (*R*)	60	65(*S*)	80	80 (*R*)	40	90 (*S*)	75	95 (*S*)	55	90 (*S*)	20	>98(*S*)
6	25	>98(*S*)	50	40(*S*)	75	75 (*R*)	15	95 (*S*)	65	>98(*S*)	65	80 (*S*)	< 5	
7	55	>98(*S*)	25	90 (*S*)	65	75 (*R*)	15	>98(*S*)	25	85 (*S*)	20	95 (*S*)	< 5	-
8	> 98	85 (*S*)	> 98	85 (*S*)	> 98	10 (*S*)	85	60 (*S*)	> 98	60 (*S*)	> 98	>98(*S*)	> 98	80 (*S*)
9	40	30 (*R*)	60	60 (*R*)	40	90 (*R*)	30	< 5	65	25 (*R*)	30	50 (*S*)	< 5	
10	< 5	-	< 5	-	10	>98(*R*)	< 5	-	40	<98(*S*)	35	>98(*S*)	< 5	-
11	< 5	-	< 5	-	< 5	-	< 5	-	95	95 (*S*)	50	>98(*S*)	< 5	-
12	40	50 (*R*)	> 98	75 (*R*)	15	45 (*R*)	45	>98(*R*)	10	25 (*R*)	10	>98(*R*)	15	70 (*R*)
13	55	>98(*S*)	35	>98(*S*)	15	>98(*S*)	20	>98(*S*)	70	>98(*S*)	35	>98(*S*)	10	>98(*S*)
14	90	70 (*S*)	80	50 (*R*)	85	70 (*S*)	50	< 5	85	60 (*S*)	75	90 (*S*)	90	45 (*S*)
15	80	65 (*S*)	95	50 (*R*)	90	80 (*S*)	35	< 5	80	45 (*S*)	85	90 (*S*)	45	80 (*S*)

Enantioselective reduction of **13** gave access to chiral cyclic boronates after introduction of the boronic acid on the enantiomerically pure alcohol. The reduction of **13** occurred always with high enantioselectivity yielding the corresponding enantiomerically pure (*S*)-alcohol. The biotransformations showed the same stereobias observed with fresh cells of *S. cerevisiae*[26] and acetone powder of *G. candidum*.[15] Reduction of nitro derivatives **14** and **15** followed behaviours similar to that observed with fresh cells,[17] furnishing high molar conversions in the ketone reduction.

3. Conclusion

In conclusion, this work shows that lyophilised yeasts can be employed as enantioselective biocatalysts for carbonyl reduction. In a few cases, dry cells were more effective than fresh cells in terms of activity, but this drawback is largely recompensed by the easiness of working with lyophilised cells since enantiomeric excesses were quite similar. Although a wider range of substrates needs to be investigated to fully evaluate the steric and electronic effects affecting yields and stereoselectivity of yeast-mediated reduction, the use of lyophilised yeasts is an easy and efficient procedure available for organic chemists without any skill in the techniques of applied microbiology. This method is simple and also suited for small-scale screening aimed at the selection of the 'right' yeast for a given substrate just by evaluating a restricted number of strains, often furnishing both enantiomers of the desired alcohol.

4. Experimental

4.1. Material

Substrates **1**–**13** were from Aldrich, while **14**–**15** were synthesized as described before.[27]

4.2. Microorganisms, media and culture conditions

The microorganisms were from CBS (Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands), DPVPG (Dipartimento di Biologia Vegetale Perugia, Italy) and MIM (Microbiologia Industriale Milano, Italy). They were cultured in 3.0 L fermenters with 1.0 L of malt broth pH 6.0 for 48 h at 27°C and agitation speed 100 rpm. The amounts of lyophilised cells obtained were:

Candida utilis CBS 621 8.0 g/L
Kluyveromyces marxianus CBS 397 3.4 g/L
Pichia etchellsii CBS 2011 5.8 g/L
Pichia fermentans IMAP 2770 5.1 g/L
Pichia glucozyma CBS 5766 5.1 g/L
Pichia minuta CBS 1708 3.0 g/L
Saccharomyces cerevisiae type II Sigma 5.9 g/L

Fresh cells from submerged cultures were centrifuged and washed with 0.1 M phosphate buffer, pH 7.0. Washed cells were either directly used or lyophilised.

4.3. Biotransformation conditions

Reductions were carried out in 10 mL screw-capped test tubes with a reaction volume of 5 mL with cells (50 g L^{-1}, dry weight) resuspended in 0.1 M phosphate buffer with 5% glucose, pH 7.0. After

45 min of incubation, neat substrate (20 mM) was added and the incubation continued for 24 h under magnetic stirring.

4.4. Analytical methods

Alcohol and ketone concentrations were determined by gas-chromatographic analysis on a Carlo Erba Fractovap GC equipped with a hydrogen flame ionization detector. The column (diameter 3 mm, length 2000 mm) was packed with Carbowax 1540 (10% on Chromosorb 80–100 mesh). Samples (0.2 ml) were taken at intervals and added to an equal volume of an internal standard (1-heptanol) solution in methanol.

The stereochemical outcome of the transformations was expressed as enantiomeric excess (ee) of the major enantiomer. The alcohols obtained by reduction of compounds **1–13** were extracted with ethyl ether, dried and transformed into the corresponding butyrate esters by reaction with butyryl chloride in dry CH_2Cl_2 with 2% pyridine.[5] The nitro-alcohols were analyzed as described before.[6] The enantiomeric composition was determined by gas-chromatographic analysis using a chiral capillary column (diameter 0.25 mm, length 25 m, thickness 0.25 μ, DMePeBeta-CDX-PS086, MEGA, Legnano, Italy). The absolute configuration was determined by comparison with authentic samples from Aldrich or obtained as reported.

References

1. *Selective Biocatalysis. A Synthetic Approach*; Poppe, L.; Novak, L., Eds.; VCH Publishers: New York, 1992.
2. Servi, S. *Synthesis* **1990**, 1.
3. Ward, P. P.; Young, C. S. *Enzyme Microb. Technol*. **1990**, *12*, 482.
4. Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49.
5. Bortolini, O.; Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Guerrini, A.; Medici, A. *J. Org. Chem*. **1997**, *62*, 1854.
6. Adlercreutz, P. *Enzyme Microb. Technol*. **1991**, *13*, 9.
7. Maconi, E.; Aragozzini, F. *Appl. Microbiol. Biotechnol*. **1989**, *33*, 29.
8. Hummel, W. *Appl. Microbiol. Biotechnol*. **1990**, *34*, 15.
9. Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc*. **1986**, *108*, 162.
10. Zheng, C.; Pham, V. T.; Phillips, R. *Catal. Today* **1994**, *22*, 607.
11. Aragozzini, F.; Maconi, E.; Craveri, R. *Appl. Microbiol. Biotechnol*. **1986**, *24*, 175.
12. Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Medici, A.; Pedrini, P.; Gardini, F.; Lanciotti, R. *Tetrahedron* **1996**, *52*, 3547.
13. Azerad, R.; Buisson, D. *Microbial Reagents in Organic Chemistry*; Servi, S., Ed.; Kluwer Academic: Dordrecht, 1992; pp. 421–440.
14. Nakamura, K.; Inoue, Y.; Ohno, A. *Tetrahedron Lett*. **1995**, *36*, 265.
15. Nakamura, K.; Matsuda, T. *J. Org. Chem*. **1998**, *63*, 8957.
16. Molinari, F.; Bertolini, C.; Aragozzini, F. *Biocatalysis and Biotransformations* **1998**, *16*, 87.
17. Molinari, F.; Occhiato, E. G.; Aragozzini, F.; Guarna, A. *Tetrahedron: Asymmetry* **1998**, *9*, 1389.
18. MacLeod, R.; Prosser, H.; Fikentscher, L.; Lanyi, J.; Mosher, H. S. *Biochemistry* **1964**, *3*, 838.
19. Sih, C. J.; Chen, C. S. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 570.
20. *Biotransformation in Preparative Chemistry*; Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. Academic Press: London, 1989.
21. Kometani, T.; Yoshii, H.; Kitatsuji, E.; Nishimura, H.; Matsuno, R. *J. Ferment. Bioeng*. **1993**, *76*, 33.
22. Kometani, T.; Morita, Y.; Furui, H.; Yoshii, H.; Matsuno, R. *J. Ferment. Bioeng*. **1994**, *77*, 13.
23. Cheng, C.; Ma, J. H. *Proc. Biochem*. **1996**, *31*, 119.
24. Prelog, V. *Pure Appl. Chem*. **1964**, *9*, 119.
25. Heidlas, J.; Engel, K. H.; Tressl, R. *Enzyme Microb. Technol.* **1991**, *13*, 817.
26. Resnick, S. M.; Torok, D. S.; Gibson, D. T. *J. Org. Chem.* **1995**, *60*, 3546.
27. Guarna, A.; Occhiato, E. G.; Spinetti, L. M.; Vallecchi, M. E.; Scarpi, D. *Tetrahedron* **1995**, *51*, 1775.