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# Enantioselectivities of yeast epoxide hydrolases for 1,2-epoxides

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**Abstract**

Kinetic resolution of homologous series of unbranched 1,2-epoxyalkanes (C-4 to C-12), 1,2-epoxyalkenes (C-4, C-6 and C-8), a 2,2-dialkylsubstituted epoxide (2-methyl-1,2-epoxyheptane) and a benzyloxy-substituted epoxide (benzyl glycidyl ether) was investigated using resting cells of 10 different yeast strains. Biocatalysts with excellent enantioselectivity (E>100) and high initial reaction rates (>300 nmol/min/mg dry weight) were found for the 2-monosubstituted aliphatic epoxides C-6 to C-8. Yeast strains belonging to the genera *Rhodotorula*, *Rhodosporidium* and *Trichosporon* all preferentially hydrolyzed (*R*)-1,2-epoxides with retention of configuration. The epoxide hydrolases of all the yeast strains are membrane-associated. 

## 1. Introduction

Hydrolytic kinetic resolution of racemic epoxides offers a convenient route to obtain single enantiomer synthons for enantiopure fine chemicals. Since both the remaining epoxide and the diol product (employed as cyclic sulfates or sulfites) are useful as reactive intermediates, high product recovery can be achieved. Terminal epoxides are arguably the most important subclass of epoxides that serve as building blocks for organic chemistry. Asymmetric hydrolytic catalysts able to resolve these inexpensive racemates into optically active epoxides and vicinal diols have thus become an important focus of research. An efficient (salen)Co(III)(OAc) catalyst for the hydrolytic kinetic resolution of terminal C-3 to C-8 epoxides was recently developed.[1] Resolution of long-chain alkyl epoxides (C-10 to C-20) was also achieved (ees>95% and isolated yields >43%) with this catalyst.[2] Biocatalytic hydrolytic resolution of epoxides as an alternative to heavy metal catalysts has also been explored in recent years. The synthetic potential of bacterial[3,4] and filamentous fungal[5] epoxide hydrolases as chiral biocatalysts was extensively investigated. Epoxide hydrolase activity was found to be ubiquitous in these microorganisms, and the structural requirements of their epoxide substrates for chiral recognition are now well established.[6]

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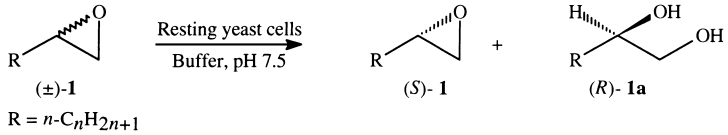
In contrast, the occurrence of these enzymes in yeasts and their enantioselectivities for epoxides of different structural classes had not been investigated until very recently. Pioneering work done by Weijers on the yeast strain *Rhodotorula glutinis* CIMW 147[7] and subsequent screening of yeasts from many different genera for epoxide hydrolase activity[8] revealed unusual enantioselectivities,[9] which differed significantly from those found in bacteria and filamentous fungi. The following results obtained with yeast epoxide hydrolases are particularly noteworthy: (1) *Rhodotorula glutinis* displayed epoxide hydrolase activity and enantioselectivity with an exceptionally broad range of structurally different substrates, which included aryl, alicyclic, *meso* and aliphatic epoxides with terminal, subterminal, *trans* and *cis* location of the epoxide moiety. In contrast, epoxide hydrolases of individual bacterial and filamentous fungal strains have stringent structural requirements for enantioselective hydrolysis of substrates, which severely limits the range of substrates that can be resolved with single strains; (2) kinetic resolution of 1,2-epoxyhexane and 1,2-epoxyoctane was achieved with exquisite enantioselectivity using yeast epoxide hydrolases. No similar enantioselectivity for unbranched 1,2-epoxyalkanes was achieved with bacterial epoxide hydrolases.[10] However, the results obtained with *Chryseomonas luteola*[11] (safety class II, which is risky to work with) suggest that this unusual enantioselectivity for 2-monosubstituted 1,2-epoxyalkanes may not be restricted to yeasts; (3) hydrolysis of most of the aliphatic substrates proceeds with unusually high initial reaction rates. Direct comparison of specific activities obtained with bacterial and fungal biocatalysts cannot be made from the available literature, but careful examination of experimental conditions published[12–15] indicates that hydrolysis of epoxides with bacterial or filamentous fungal epoxide hydrolases using similar substrate to catalyst ratios would require significantly longer reaction times; (4) high substrate concentrations (500 mM) of 1,2-epoxyhexane[8] and 1,2-epoxyoctane[9] can be used without any adverse effects on the reaction rate or enantioselectivity of yeast epoxide hydrolases. This should facilitate efficient synthesis of enantiopure epoxides on a preparative scale.

From the limited data available on yeast epoxide hydrolases, it is clear that these enzymes have promising synthetic potential. In the present study, the biocatalytic potential of yeast epoxide hydrolases is further elucidated.

## 2. Results and discussion

Asymmetric hydrolysis of the terminal epoxides (±)-**1** to (±)-**4** was achieved by employing resting cells of the different yeast strains in buffer at pH 7.5. All the strains preferentially hydrolyzed the (*R*)-1,2-epoxide to the corresponding (*R*)-*vic* diol. From the fact that the formed diol and the remaining non-hydrolyzed epoxide represent opposite enantiomeric forms, it can be assumed that the hydrolysis is proceeding with retention of configuration to a large extent. However, more detailed studies ($^{18}$O-labelling) have to be done to elucidate the reaction mechanism. Although all the substrates were hydrolyzed, both the enantioselectivities and the reaction rates were strongly influenced by both the chain length and substitution pattern of the substrates. Enantiomeric ratios given in Tables 2 and 3 were calculated based on the enantiomeric excesses of the substrates (ees), since epoxide hydrolysis does not necessarily proceed with complete regioselectivity,[16] in which case Sih's equation[17] involving the enantiomeric purity of the product (eep) is not applicable. E-values that were calculated using eep indicated that hydrolysis did not proceed with complete regioselectivity.

## 2.1. Hydrolysis of 1,2-epoxyalkanes (±)-**1** (Scheme 1)




Scheme 1. Hydrolytic kinetic resolution of a homologous range (C-4 to C-12) of 1,2-epoxyalkanes

Excellent enantioselectivities were displayed by several strains for C-6 to C-8 1,2-epoxyalkanes. The efficiency of these biocatalysts, expressed as specific activity and catalytic activity (turnover frequency), is given in Table 1. A decrease in enantioselectivity and reaction rate was displayed with both an increase and decrease in the chain length (Table 2, Fig. 1). Strains (1), (3) and (5) retained useful (E>20) enantioselectivity for the C-5 epoxide. Only strain (6) retained enantioselectivity for the C-10 epoxide, while almost no enantioselectivity was displayed by any of the catalysts for the C-12 epoxide. It should be noted, however, that the C-10 and C-12 epoxides are highly hydrophobic, and that the low reaction rates obtained were probably due to low availability of the substrate to the catalyst in the aqueous buffer, since no co-solvents were used.[18] Secondly, low effective substrate concentration has been noted to decrease the enantioselectivity of hydrolysis by epoxide hydrolases.[8,19]

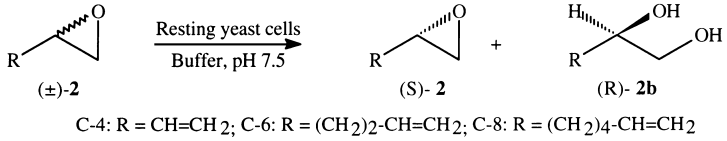
Table 1
Efficiency of purified yeast epoxide hydrolase enzymes

| **Catalyst** | ***Rhodosporidium toruloides*** **UOFS Y-0471**[a] | ***Rhodotorula* glutinis CIMW 147**[b] |
|---|---|---|
| **Substrate** | 1,2-epoxyoctane | 1,2-epoxyhexane |
| Specific activity (umol.min$^{-1}$mg catalyst$^{-1}$) | 172 | 67 |
| Turnover frequency (mol substrate processes / mol catalyst. Min$^{-1}$) | 9440 | 3015 |

a) Based on a Mr 54 000,[20] and linear reaction rate.

b) Based on a Mr 45000,[21] and linear reaction rate

## 2.2. Hydrolysis of 1,2-epoxyalkenes (±)-**2** (Scheme 2)




Scheme 2. Hydrolytic kinetic resolution of a homologous range of 1,2-epoxyalkenes

A decrease in enantioselectivity (Table 3) was observed for most strains with the introduction of an ω-double bond. As an exception, strain (1) displayed similar excellent enantioselectivity and reaction rates (Fig. 2) for 1,2-epoxyoctene as for 1,2-epoxyoctane. From the results for the 1,2-epoxyalkenes it can be observed that the enantioselectivity pattern has moved to longer chain lengths in relation to the results for 1,2-epoxyalkanes. This might be caused by the small decrease in actual chain length of epoxyalkenes in comparison with the corresponding epoxyalkanes. Of possible commercial interest will

Table 2
Enantiomeric ratios (E)[a] and initial reaction rates ($V_0$)[b] of yeast strains towards a homologous range of 1,2-epoxyalkanes (20 mM)

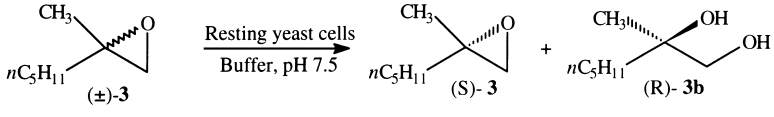
| | **Epoxide (±)-1** | **C-4** | | **C-5** | | **C-6** | | **C-7** | | **C-8** | | **C-10** | | **C-12** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Biocatalyst** | E | $V_0$ | E | $V_0$ | E | $V_0$ | E | $V_0$ | E | $V_0$ | E | $V_0$ | E | $V_0$ |
| 1 | *Rhodosporidium toruloides* UOFS Y-0471 | 8.0 | 2.9 | 23 | 10 | 100 | 35 | 100 | 366 | 100 | 134 | 2.3 | 27 | 1.1 | 50 |
| 2 | *Rhodosporidium toruloides* UOFS Y-0472 | 2.5 | 0.3 | 12 | 0.6 | 65 | 6.3 | 7.0 | 12 | 15 | 31 | 1.0 | 0.2 | 1.0 | 0.8 |
| 3 | *Rhodotorula glutinis* CIMW 147 | 8.0 | 2.3 | 20 | 7.2 | 85 | 51 | 32 | 106 | 16 | 85 | 1.0 | 40 | 1.0 | 4.6 |
| 4 | *Rhodotorula araucariae* UOFS Y-0473 | 4.5 | 4.7 | 4.0 | 10 | 97 | 18 | 13 | 37 | 200 | 55 | 2.5 | 8.3 | 1.2 | 2.3 |
| 5 | *Rhodotorula glutinis* UOFS Y-0123 | 6.0 | 2.3 | 20 | 20 | 103 | 67 | 22 | 143 | 4.0 | 250 | 1.7 | 17 | 1.0 | 9.0 |
| 6 | *Rhodotorula aurantiaca* UOFS Y-2049 | 2.5 | 0.8 | 4.5 | 1.0 | 12 | 2.1 | 30 | 15 | 20 | 3.8 | 12.0 | 11 | 1.2 | 3.2 |
| 7 | *Rhodotorula rubra* UOFS Y-0112 | 3.5 | 1.1 | 5.0 | 0.9 | 10 | 2.3 | 4.0 | 12 | 5.0 | 3.3 | 3.5 | 0.4 | 1.5 | 1.3 |
| 8 | *Rhodotorula glutinis* UOFS Y-2042 | 3.0 | 0.8 | 3.0 | 1.3 | 7.0 | 9.4 | 7.5 | 15 | 14 | 47 | 3.5 | 6.7 | 1.2 | 2.9 |
| 9 | *Trichosporon mucoides* UOFS Y-0118 | 2.0 | 1.3 | 2.0 | 1.8 | 6.5 | 2.2 | 6.0 | 15 | 24 | 16 | 3.0 | 2.2 | 1.2 | 4.0 |
| 10 | *Trichosporon jirovecii* UOFS Y-0119 | 4.0 | 0.8 | 2.0 | 3.5 | 3.6 | 12 | 7.5 | 3.3 | 10 | 19 | 3.0 | 2.9 | 1.2 | 4.0 |

[a] $E = \frac{\ln\{(1-c)(1-ees)\}}{\ln\{(1-c)(1+ees)\}}$

[b] Initial rate of epoxide hydrolysis in nmol.min$^{-1}$. mg dry weight$^{-1}$.

be the hydrolysis of the simplest allylic epoxide, 1,2-epoxy-3-butene. Enantiopure forms of this epoxide and its corresponding diol are small polyfunctional building blocks for the synthesis of a number of natural products.[22,23]

### 2.3. *Hydrolysis of 2-methyl-1,2-epoxyheptane (±)-**3** (Scheme 3)*




Scheme 3. Hydrolytic kinetic resolution of a 2,2-disubstituted 1,2-epoxyalkane, 2-methyl-1,2-epoxyheptane

A 2-alkyl-substituent resulted in a dramatic decrease in the enantioselectivity (Table 3) of all strains except for strain (8), for which the methyl-substituent improved enantioselectivity. These results contrast sharply with the results obtained for several bacterial epoxide hydrolases,[10] which require a directing methyl group in the 2-position of terminal aliphatic epoxides for enantioselectivity. The effect of the methyl group on C-2 was less pronounced for strains (6) and (8), for which moderate enantioselectivity (E=10 and 18) (Fig. 2A) was found. Reaction rates (Fig. 2B) were comparable to those of the unsubstituted C-6 to C-8 epoxides.

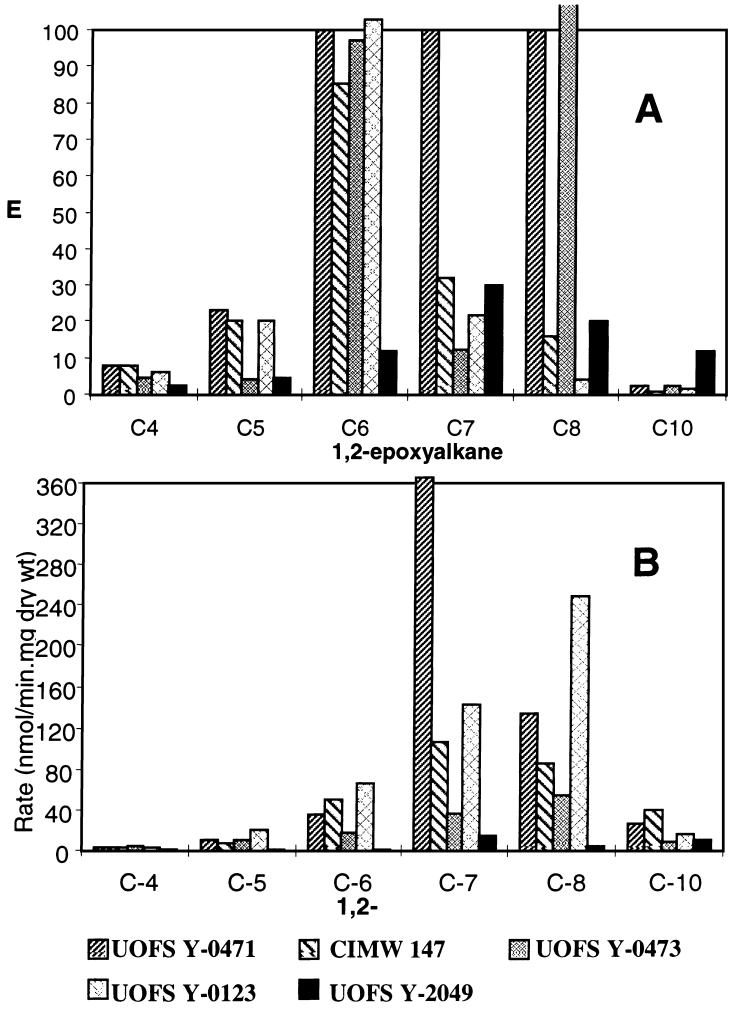
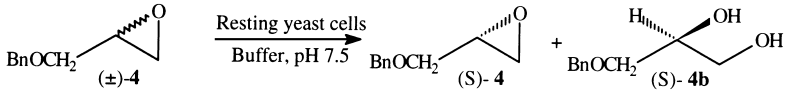



Figure 1. Enantiomeric ratio (**A**) and initial reaction rate (**B**) of the five most enantioselective yeasts towards a homologous range of 1,2-epoxyalkanes

### *2.4. Hydrolysis of benzyl glycidyl ether (±)-**4** (Scheme 4)*




Scheme 4. Hydrolytic kinetic resolution of a benzyloxy-substituted 1,2-epoxide, benzyl glycidyl ether. The absolute configuration of the formed diol is (*S*) due to a switch in CIP-nomenclature

Asymmetric hydrolysis of benzyl glycidyl ether was achieved using strains (6) and (7). Although the enantioselectivity (Table 3, Fig. 2) was too low ($E<10$) to be of practical use, this is the first report of kinetic resolution of this important building block of β-blocker drugs by epoxide hydrolases. All the strains, which were moderate ($E>10$) to excellent ($E>30$) biocatalysts for straight chain epoxides, displayed a decrease in enantioselectivity and activity upon the incorporation of a benzyloxy-substituent. It is noteworthy that the effect of both a 2-methyl- and a benzyloxy-substituent on strain (6) was less pronounced than for the other strains.

Table 3
Enantiomeric ratios (E)[a] and initial reaction rates ($V_0$)[b] of yeasts towards a homologous range of 1,2-epoxyalkenes, 2-methyl-1,2-epoxyheptane and benzyl glycidyl ether (20 mM)

| | **Epoxide** (±)- **2** | **C-4** | | **C-6** | | **C-8** | | (±)- **3** | | (±)- **4** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Biocatalyst | E | $V_0$ | E | $V_0$ | E | $V_0$ | E | $V_0$ | E | $V_0$ |
| 1 | *Rhodosporidium toruloides* UOFS Y-0471 | 2.0 | 2.0 | 14 | 32 | 100 | 168 | 5.4 | 65 | 2.6 | 7.0 |
| 2 | *Rhodosporidium toruloides* UOFS Y-O472 | 4.0 | 0.8 | 7.0 | 0.9 | 8.0 | 2.3 | 2.0 | 28 | 1.0 | 1.0 |
| 3 | *Rhodotorula glutinis* CIMW 147 | 4.0 | 2.6 | 23 | 37 | 18 | 104 | 5.6 | 39 | 2.5 | 9.0 |
| 4 | *Rhodotorula araucariae* UOFS Y-0473 | 1.0 | 0.4 | 5.0 | 1.6 | 22 | 9.7 | 2.5 | 35 | 2.0 | 4.0 |
| 5 | *Rhodotorula glutinis* UOFS Y-0123 | 2.0 | 2.1 | 21 | 30 | 11 | 66 | 3.0 | 67 | 3.0 | 30 |
| 6 | *Rhodotorula aurantiaca* UOFS Y-2049 | 1.0 | 0.6 | 5.0 | 0.9 | 20 | 7.3 | 10 | 11 | 8.5 | 14 |
| 7 | *Rhodotorula rubra* UOFS Y-0112 | 2.0 | 0.2 | 4.0 | 0.7 | 4.0 | 1.4 | 1.5 | 2.0 | 6.4 | 18 |
| 8 | *Rhodotorula glutinis* UOFS Y-2042 | 2.0 | 0.4 | 6.0 | 0.9 | 4.0 | 5.3 | 18 | 3.2 | 4.0 | 15 |
| 9 | *Trichosporon mucoides* UOFS Y-0118 | 1.0 | 1.1 | 1.0 | 1.2 | 2.0 | 1.9 | 2.0 | 1.0 | 2.0 | 1.0 |
| 10 | *Trichosporon jirovecii* UOFS Y-0119 | 1.0 | 1.2 | 7.0 | 5.0 | 12 | 4.0 | 1.0 | 3.1 | 3.0 | 1.0 |

[a] $E = \frac{\ln\{(1-c)(1-ees)\}}{\ln\{(1-c)(1+ees)\}}$

[b] Initial rate of epoxide hydrolysis in nmol.min$^{-1}$. mg dry weight$^{-1}$.

### *2.5. Cellular localization of yeast epoxide hydrolases*

The epoxide hydrolases of strains (1)[20] and (3)[21] were previously shown to be membrane-associated. No epoxide hydrolase activity could be detected in the 13 000×*g* supernatants of any of the yeast lysates after extraction of soluble enzymes with Y-PER™ yeast protein extraction reagent. Total enzyme activity was retained in the cell debris. The epoxide hydrolases of all the tested yeast strains are thus membrane-associated.

## 3. Conclusion

Kinetic resolution of a homologous range of C-4 to C-12 epoxides by various yeast epoxide hydrolases, albeit with different enantioselectivity and reaction rates, has been demonstrated. Excellent E-values and extremely high reaction rates were displayed for unbranched C-6 to C-8 epoxides by several yeast strains belonging to the genera *Rhodotorula* and *Rhodosporidium*. Hydrolytic kinetic resolution of these substrates using yeast biocatalysts may be a viable alternative to the (salen)Co(III)(OAc) catalysts developed by Jacobsen. In contrast to bacterial epoxide hydrolases, the presence of a directing group on C-2 destroys chiral recognition in most cases. A benzyloxy group resulted in loss of enantioselectivity

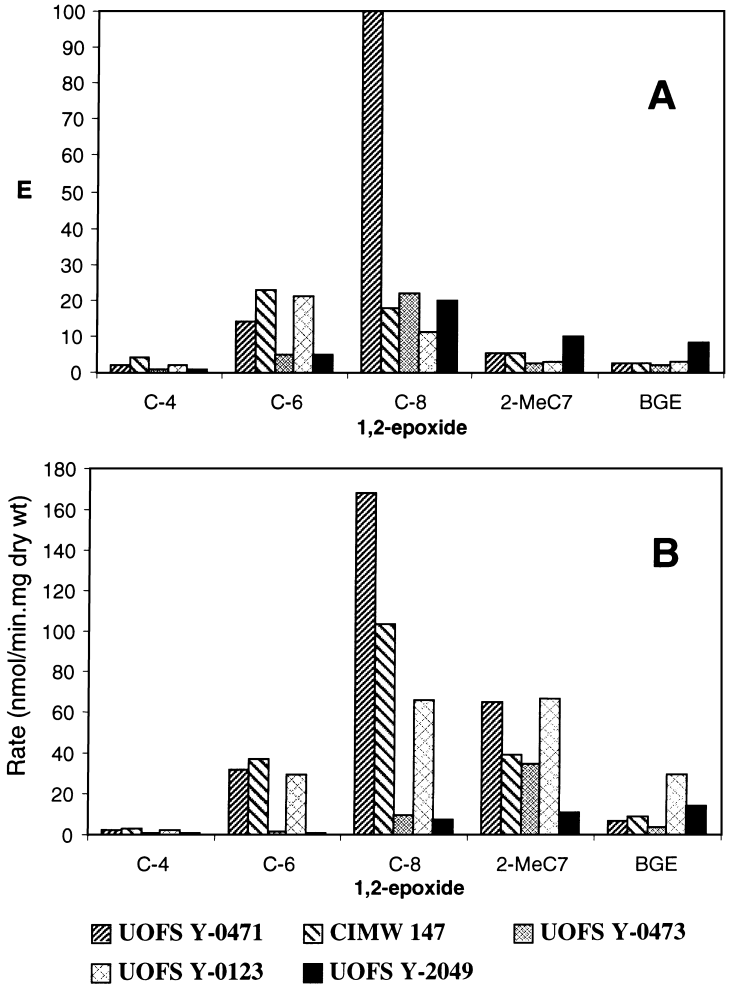



Figure 2. Enantiomeric ratio (**A**) and initial reaction rate (**B**) of the five most enantioselective yeasts towards a homologous range of 1,2-epoxyalkenes, 2-methyl-1,2-epoxyheptane and benzyl glycidyl ether

by the strains displaying high enantiopreference for straight-chain epoxides. Asymmetric hydrolysis of benzyl glycidyl ether was, however, achieved with yeast strains that displayed lower enantiopreference for straight-chain epoxides. Studies using various other aryl, alicyclic and *meso*-epoxides are in progress to assess whether these strains share with *Rhodotorula glutinis* CIMW 147 the unusually broad range of substrates[7] that can be hydrolyzed enantioselectively.

## 4. Experimental

### *4.1. General*

All yeasts were obtained from the Yeast Culture Collection of the University of the Orange Free State, except *Rhodotorula glutinis* CIMW 147, which was from the laboratory culture collection of Industrial Microbiology, AU Wageningen. Reactions were monitored and enantiomeric purities were analyzed by GLC (Hewlett–Packard 4890 equipped with FID) on fused silica cyclodextrin capillary columns (β-DEX

225 and β-DEX 120, Supelco Inc., and Chiraldex A-TA, Astec, 30 m×0.25 mm, 0.25 μm film) using $N_2$ as carrier gas. Concentrations of epoxides and diols were derived from calibration curves with heat-killed cells. Optical rotation values were measured on a Perkin–Elmer 241 polarimeter at 589 nm.

### *4.2. Epoxides **1** and formed diols **1a***

C-4, C-5, C-6, C-8 and C-12 racemic epoxides were obtained from Fluka. C-7 and C-10 epoxides were synthesized as described previously.[8,11] Chiral analysis of C-4 and C-5 epoxides was performed on β-DEX 225 (40°C isotherm), C-6 and C-7 epoxides on β-DEX 120 (45°C and 50°C isotherm), C-8 on either β-DEX 225 or Chiraldex A-TA (55°C and 52°C) and C-10 and C-12 epoxides on Chiraldex A-TA (90°C and 95°C isotherm). Absolute configurations of C-4 to C-8 epoxides were established from the previously reported data.[8] Determination of the absolute configurations of the resolved residual C-10 and C-12 epoxides was by co-injection on chiral GLC with the enantiopure (*R*) reference compounds from Aldrich. C-4, C-5, C-6, C-8 and C-12 diols were obtained from Fluka. C-7 and C-10 diols were synthesized as described previously.[11] Chiral analysis of all the diols was performed on β-DEX 120. Absolute configurations of the C-4 to C-8 diols were deduced from the previously reported data[8] and of C-10 and C-12 diols by co-injection on chiral GLC with the enantiopure reference compounds from Aldrich.

### *4.3. Epoxides **2** and formed diols **2a***

The C-4, C-6 and C-8 racemic 1,2-epoxyalkenes were obtained from Aldrich. Chiral analysis of C-4 and C-6 epoxyalkenes was performed on β-DEX 225 (50°C and 60°C isotherm) and C-8 on β-DEX 120 (60°C isotherm). The elution order of the C-6 and C-8 enantiomers was first (*R*) and second (*S*). Surprisingly, however, a reversed elution order of the C-4 enantiomers on the β-DEX 225 column was observed; first (*S*)- and second (*R*)-1,2-epoxy-3-butene was eluted from this column.

### *4.4. Absolute configurations of residual 1,2-epoxyalkenes **2***

Absolute configurations of the residual 1,2-epoxyalkenes obtained after hydrolysis of 500 mg of the appropriate epoxide by 1 g (dry weight) of *Rhodotorula glutinis* CIMW 147 cells were determined as described before for corresponding (*S*)-1,2-epoxyalkanes.[8] Chiral GLC was used for determination of ees and concentrations were derived from calibration curves. Data of chiral GLC analysis and specific optical rotation values of the residual epoxides are: epoxide (*S*)-**2**, C-4: $[\alpha]_D^{22}$ +9.1 (c=0.11, pentane; ee=37%) [lit.[24] (*R*)-**2**, C-4: $[\alpha]_D^{23}$ −0.68 (c=4.44, 2-propanol; ee=9%)] epoxide (*S*)-**2**, C-6: $[\alpha]_D^{22}$ −12.4 (c=1.61, pentane; ee=95%) epoxide (*S*)-**2**, C-8: $[\alpha]_D^{22}$ −14.0 (c=1.45, pentane; ee=90%) [lit.[25] (*R*)-2, C-8: $[\alpha]_D^{23}$ +12.2 (neat; ee>80%)].

Determination of the absolute configurations was by comparison of our results with the data reported in the literature.

### *4.5. Absolute configurations of formed alkene-1,2-diols **2a***

Absolute configurations were determined for the diols formed from hydrolysis of 500 mg of the appropriate epoxide by 1 g (dry weight) of *Rhodotorula glutinis* CIMW 147 cells as described before for corresponding (*R*)-alkane-1,2-diols.[8] Chiral GLC analysis was performed for determination of the ee values. Data of chiral GLC analysis and specific optical rotation values of the formed diols are as follows.

Diol (*R*)-**2a**, C-4: $[\alpha]_D^{22}$ +7.5 (c=4.8, methanol; ee=32%) [lit.[24] (*S*)-**2a**, C-4: $[\alpha]_D^{20}$ −43.6 (c=4.6, 2-propanol; ee>99%)] diol (*R*)-**2a**, C-6: $[\alpha]_D^{22}$ +8.3 (c=8.8, methanol; ee=70%) diol (*R*)-**2a**, C-8: $[\alpha]_D^{22}$ +12.2 (c=8.3, methanol; ee=95%).

Determination of the absolute configurations was by comparison of our results with the data reported in the literature.

### *4.6. Epoxide **3** and formed diol **3a***

(±)-2-Methyl-1,2-epoxyheptane **3** was synthesized by direct epoxidation of the corresponding alkene and the diol (±)-**3a** by acid hydrolysis of (±)-**3** as described previously.[11] Chiral analysis was performed for the diol (±)-**3a** only, on β-DEX 120 (55°C). Absolute configuration of the diol was deduced from reported data.[22]

### *4.7. Epoxide **4** and formed diol **4a***

(±)-Benzyl glycidyl ether **4** was synthesized by $BF_3 \cdot Et_2O$-catalyzed addition of benzyl alcohol onto epichlorohydrin followed by NaOH. Chiral analysis of epoxide (±)-**4** and the acetonide derivative of diol (±)-**4a** was performed on β-DEX 120 (110°C and 145°C). Absolute configurations were established by co-injection of the commercially available enantiomerically pure epoxides and diols (Fluka).

### *4.8. Cultivation and preparation of yeast cells*

Yeasts which displayed epoxide hydrolase activity were selected and grown at 30°C in 1 L shake-flask cultures containing 200 ml YM medium supplemented with 1% glucose (w/v). At late exponential phase (48–72 h) the cells were harvested by centrifugation (10 000×*g*, 10 min, 4°C), washed with phosphate buffer (50 mM, pH 7.5), centrifuged again and frozen in glycerol (10%) at −20°C in micro centrifuge tubes (1 ml cells per micro centrifuge tube). The cells could be stored for several months without significant loss of activity.

### *4.9. General procedure for hydrolysis of epoxides*

Frozen cells (1 ml) were thawed, washed with phosphate buffer (50 mM, pH 7.5) and resuspended in 4 ml buffer in 20 ml glass bottles with screw caps fitted with septa. The substrates were added neat to final concentrations of 20 mM. The mixtures were agitated on a shaking water bath at 30°C. The course of the bioconversions of epoxides was followed by withdrawing 50 μl headspace samples with a gas-tight syringe and/or by withdrawing samples (200 μl) at appropriate time intervals for longer chain epoxides. Samples were saturated with NaCl if necessary to facilitate improved extraction of the short chain diols and extracted with an equal volume of ethyl acetate. After centrifugation (3000×*g*, 2 min), the remaining epoxides and formed diols were analyzed by chiral GLC.

### *4.10. Determination of cellular localization of epoxide hydrolases*

Pelleted cells (1 ml) were resuspended in 2 ml Y-PER™ Yeast Protein Extraction Reagent (Pierce), pH 7.4, gently vortexed until the mixture was homogeneous and allowed to stand at room temperature for 1 h. Cell debris and lysates were separated by centrifugation (13 000×*g*, 10 min). The cell debris pellets were resuspended in 50 mM phosphate buffer, pH 7.4 (2 ml). 1,2-Epoxyoctane (20 μl) was added to

the cell pellets and cell lysates, and the reaction mixtures were incubated at 30°C for 6 h. The reactions were terminated by extraction with 1 ml ethyl acetate, and epoxide hydrolase activities were assayed by GC analysis of the formed diols. The effect of Y-PER™ on epoxide hydrolase activity was evaluated by incubating 1 ml pelleted cells treated with 2 ml Y-PER™ as above with 20 μl 1,2-epoxyoctane as a control.

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