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# Environmental DNA as a source of a novel epoxide hydrolase reacting with aliphatic terminal epoxides

Michael Kotik[a,*], Václav Štěpánek[a], Helena Marešová[a], Pavel Kyslík[a], Alain Archelas[b]

[a] Laboratory of Enzyme Technology, Institute of Microbiology, Academy of Sciences of the Czech Republic, v.v.i., Vídeňská 1083, 142 20 Prague 4, Czech Republic
[b] Institut des Sciences Moléculaires de Marseille 2, Biosciences, UMR CNRS 6263, University of Aix-Marseille, Avenue Escadrille Normandie Niemen, 13397 Marseille Cedex 20, France

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ABSTRACT

We describe a convenient method for amplification of novel epoxide hydrolase-encoding genes directly from the metagenome. In a first step, small specific regions of putative epoxide hydrolase genes were amplified by using PCR with degenerate consensus primers specific for prokaryotic epoxide hydrolases, and environmental DNA as template. In a second step, the sequence obtained from one randomly selected epoxide hydrolase gene fragment served as the starting point for genome-walking PCR. This technique enabled us to recover a complete novel epoxide hydrolase gene with a GC content of 64.7%. A database search revealed that this novel gene was 44% and 43% identical to two putative epoxide hydrolases from *Ralstonia metallidurans* and *Ralstonia eutropha,* respectively, at the amino acid level, the highest among all orthologs searched. The gene, which encodes a polypeptide with a molecular mass of 34 kDa, was cloned and overexpressed in *Escherichia coli*. The recombinant enzyme showed hydrolyzing activity toward aliphatic terminal epoxides with chain lengths ranging from 6 to 10 carbon atoms. In all cases, the enantioselectivity of the enzyme was low. Determination of the regioselectivity coefficients $\alpha_R$ and $\alpha_S$ revealed that the oxirane ring was attacked almost exclusively at the non-substituted carbon of the *R*-epoxide. The preference for attack at the non-substituted ring carbon of the *S*-epoxide was dependent on the chain length of the substrate and ranged from 55% to 78%, resulting in a partially enantioconvergent reaction.



## 1. Introduction

Epoxide hydrolases (EHs) are common enzymes found in many microorganisms, plants, insects, and mammals [1,2]. They catalyze the hydrolysis of epoxides to their corresponding vicinal diols. Most EHs appear to have an α/β hydrolase fold [3]. However, different topologies have been revealed recently for some EHs [4,5]. EHs have been studied extensively for their role in detoxification reactions, signaling molecule synthesis, and the metabolism of specific carbon sources [6,7].

EHs are also studied for their potential use in chiral organic chemistry. Enantioselective EHs have gained considerable interest in biotechnology and chemistry as biocatalysts for processes using kinetic resolution of racemic epoxides, resulting in the formation of optically pure epoxides with a theoretical yield of 50%. These enantiopure epoxides can serve as convenient building blocks for the asymmetric synthesis of various bioactive compounds [8–10]. Another interesting class of EHs converts racemic epoxides in an enantioconvergent reaction to optically pure diols with a theoretical yield of 100% [11,12].

Various EH activities have been found in many types of microorganisms including bacteria, fungi, and yeast [13–17]. In several cases, the determined enantioselectivities were sufficiently high to permit the use of EHs in biotechnological applications [18–22]. EHs that do not have high enantioselectivity for a particular substrate can be improved by genetic engineering or directed evolution [23–26].

Instead of screening for EH activities, we can obtain genes that encode putative EHs by database analysis of existing genome sequences using EH-specific sequence motifs [2]. The use of such specific, conserved regions in PCR-based techniques with environmental DNA (eDNA) as template has enabled the discovery of novel genes encoding a specific class of enzymes from unknown or uncultivable microorganisms [27,28].

In this paper, we describe a convenient PCR-based method that is suitable for cloning of novel EH-encoding genes from the metagenome. Using this method, a novel EH gene was isolated directly from eDNA and overexpressed in *Escherichia coli*. We

* Corresponding author. Tel.: +420 2 4106 2428; fax: +420 2 4172 7021.
*E-mail address:* kotik@biomed.cas.cz (M. Kotik).

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**Table 1**
PCR primer sequences

| Name of primer[a] | Sequence | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Core region[b] | | | | | |
| | | **G** | **Y** | **G** | **F** | **S** | |
| Def205 | 5′-CTNCGN | GGN | TAY | GGN | TTY | TCN | GAYAARCC-3′ |
| | | **G** | **H** | **D** | **W** | **G** | |
| | 5′- | GGN | CAY | GAY | TGG | GGN | GCGGCTGTTGCTGC-3′ |
| Der250 | 3′- | CCN | GTR | CTR | ACC | CCN | CGCCGACAACGACG-5′ |
| Bf1 | 5′-TGATCGCCGATGTGATCGGTTTGTTG-3′ | | | | | | |
| Ar2 | 5′-CGTGCGGCGCGTTCAATATGACCA-3′ | | | | | | |
| Br2 | 5′-TTCCGATGCCGTATTGATCCAACAAAC-3′ | | | | | | |
| EHf | 5′-CAAGACAGACTCGAAATCGCCCACGAC-3′ | | | | | | |
| EHr | 5′-GGCGAAGTGGTCAATCTAGACGCGTTCCGCAAG-3′ | | | | | | |

[a]Primers designated De are degenerate EH-consensus primers used to amplify internal EH gene fragments. Primer names containing the letter "f" are forward primers, those containing the letter "r" are reverse primers. Primers EHf and EHr were used to amplify the full-length gene from eDNA. Primer EHr introduced an Xba I restriction site (underlined) downstream of the gene. The following primer pairs were used for two genome-walking steps (see Section 2): Step 1: Def205-C1, Bf1-C2; Step 2: Ar2-C1, Br2-C2. The linker-specific primers C1 and C2 were obtained from TaKaRa. [b]The amino acid consensus sequences encoded at the core positions of the EH-consensus primers are shown.

also report the kinetic properties of this novel EH, which should be useful in expanding the repertoire of EHs with potential biotechnological applicability.

## 2. Experimental

### 2.1. Chemicals and strains

All racemic epoxides were obtained from Fluka, Acros, Merck or Aldrich. Enantiopure epoxides with an enantiomeric excess of 98–99% were obtained from Aldrich. The vicinal diols were purchased (Aldrich, Fluka) or prepared from the corresponding epoxides by acid hydrolysis. Cloning experiments were conducted with the *E. coli* strain TOP10 (Invitrogen). The *E. coli* strain BL21 (GE Healthcare Bio-Sciences) was used for overexpression experiments.

### 2.2. Design of EH-specific degenerate primers

EH gene sequences from prokaryotes were obtained from GenBank at the National Center for Biotechnology Information (NCBI). Alignments of genes-encoding EHs were performed using CLUSTAL V in the MegAlign module of the Lasergene software package (DNASTAR Inc.). Alignment of 31 prokaryotic sequences-encoding EHs (including putative ones) revealed three highly conserved sites; two of them were targets for EH-specific primer design: (1) the sequence G-F/Y-G-X-S, which corresponds to the previously established conserved motif G-X-small-X-S/T and (2) G-G/H-D-W-G [2]. For design of degenerate EH-specific primers, we followed the design principles described by Rose et al. to some extent [29]. According to this design strategy, primers that are targeted to consensus sequences of a family of related genes consist of two regions, a 5′ non-degenerate consensus clamp and a 3′ degenerate core, which spans the highly conserved amino acids. The entire sequence of a degenerate EH-specific primer was based on the consensus amino acid sequences of site (1) or (2) and their flanking regions, obtained by sequence alignment (Table 1).

### 2.3. Extraction and purification of environmental DNA

The blending method of Gabor et al. [30] was used to extract the bacterial biomass and its total DNA from a biofilter installed in a sewage disposal plant for communal waste (Ružomberok, Slovakia). The biofilter was composed primarily of soil and wood chips (1 cm × 3 cm) as adsorbents for the microorganisms. The DNA was purified using a High Pure PCR Product Purification Kit (Roche) followed by phenol–chloroform extraction.

### 2.4. Cloning of EH gene fragments with consensus primers

Purified eDNA was used as template for amplification of EH gene fragments using degenerate EH-consensus primers. PCR reactions were performed with 1 U of *Taq* DNA polymerase (Fermentas) in 25-μL mixtures containing 200 μM of each of the four dNTPs, 2 mM $MgCl_2$, 50 ng eDNA, and 4 μM of the forward and reverse primers Def205 and Der250 (Table 1). Thirty-five cycles of 94 °C (45 s), 55 °C (45 s), and 72 °C (45 s) were performed. Amplified gene fragments of approximately 150 bp were isolated from an agarose gel using the Qiaex II Agarose Gel Extraction Kit (Qiagen) and ligated into pGEM-T Easy (Promega) for sequencing. All sequences were obtained by

the dideoxy chain-terminating method using an automated DNA sequencer ABI PRISM 3100 (Applied Biosystems).

### 2.5. Genome-walking PCR

The purified eDNA was partially restricted with Sau3A I, and DNA fragments of 0.8–5.0 kb were isolated from an agarose gel as described above. An unphosphorylated double-stranded Sau3A I linker (TaKaRa) was ligated to the restricted eDNA. After precipitation, the modified eDNA was used for two genome-walking steps [31]. The first PCR of each step was conducted using the linker-specific primer C1 (TaKaRa) and a walking primer (Def205 or Ar2; see Table 1). A diluted aliquot of DNA products obtained in the first PCR served as template in a second PCR with the linker-specific primer C2 (TaKaRa) and a second gene-specific walking primer (Bf1 or Br2; see Table 1). PCR mixtures (25 μL) contained 400 μM of each of the four NTPs, 3 μM of a degenerate primer or 0.4 μM of a non-degenerate primer, and 20–100 ng of modified eDNA as template. One unit of Pwo SuperYield DNA polymerase and GC-rich resolution solution (Roche) or 1 U of Herculase Enhanced DNA polymerase (Stratagene) and 5% DMSO were added to the PCR mixtures containing the appropriate buffers. Reactions were initiated at 95 °C (2 min), followed by 35 cycles of 94 °C (30 s), 55–64 °C (45 s), and 72 °C (90 s) with a final incubation at 72 °C for 10 min. PCR products were purified by agarose gel electrophoresis and used as templates in a PCR test reaction for the presence of EH-specific sequences using primers that were designed using sequences obtained from previous PCR reactions. Templates that yielded PCR products of the expected size in the test reaction were cloned into pGEM-T Easy for sequencing. After two steps of genome-walking, the sequence of the full-length EH gene could be predicted.

### 2.6. Cloning of the full-length gene

Primers EHf and EHr (Table 1), which were specific for the flanking regions of the putative gene, allowed for amplification of the entire gene using Herculase Enhanced DNA polymerase and the original purified eDNA as template, which was further treated as follows. Purified eDNA was moderately sheared (30 s, 70 kPa) with a nebulizer (Stratagene) and extracted from an agarose gel. The resulting PCR product encoding the complete gene was restricted with Xba I and ligated into the vector pSE420 (Invitrogen) prepared as previously described [32], generating the plasmid pSEEH9.

### 2.7. Nucleotide sequence accession number

The complete nucleotide sequence of the novel EH-encoding gene is available in GenBank under the accession number EU216566.

### 2.8. Overproduction and ammonium sulfate precipitation of recombinant EH

Cultures of *E. coli* BL21 (pSEEH9) were grown in shaking flasks for 12 h at 28 °C in 100 mL of Luria-Bertani (LB) broth supplemented with 100 μg mL$^{-1}$ of ampicillin. Seventy milliliters of LB broth containing ampicillin and IPTG (final concentration 200 μM) were added, and the biomass was harvested after 6 h of cultivation at 28 °C. The cells were washed with 40 mM phosphate buffer (pH 7.5) containing 3 mM 2-mercaptoethanol and 1 mM EDTA (buffer A), resuspended in the same buffer and disrupted by sonication. Cell debris was removed by centrifugation at 8500 × *g* for 20 min, and saturated ammonium sulfate solution was added to the supernatant until a saturation level of 40% was reached. The precipitate was removed by centrifugation, and the supernatant was saturated to 85% of ammonium sulfate and centrifuged. The EH was stored in the refrigerator as an ammonium sulfate precipitate prior to use.

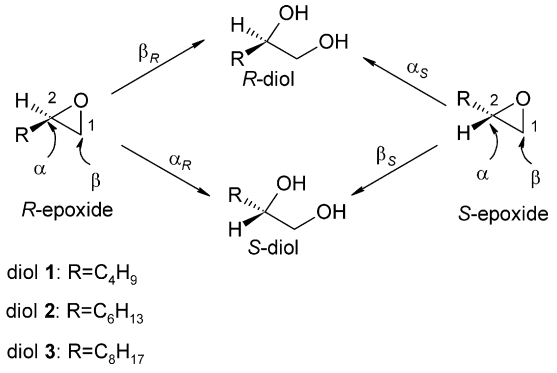


**Fig. 1.** Attack of the oxirane ring can occur on the terminal carbon atom ($C_1$), resulting in a diol with a retained configuration (β-attack), or on the carbon atom bearing the alkyl substituent ($C_2$), resulting in a diol with an inverted configuration (α-attack) [39,11,40]. The following relationships hold: $\alpha_R + \beta_R = 100\%$, and $\alpha_S + \beta_S = 100\%$. Epoxide substrates used in this study: epoxide **1**, 1,2-epoxyhexane; epoxide **2**, 1,2-epoxyoctane; epoxide **3**, 1,2-epoxydecane.

### 2.9. Characterization of recombinant EH

Thermal stability of the recombinant EH was determined by incubation of the enzyme at various temperatures ranging from 20 to 70 °C for 30 min in buffer A. Samples were chilled on ice before assaying them under standard conditions at 25 °C. The substrate range of the enzyme was analyzed with a set of epoxides (*tert*-butyl glycidyl ether, styrene oxide, epoxycyclooctane, and epoxide **3** [Fig. 1]) in a single GC run using a CP-Chirasil Dex column (Chrompack) and the following temperature profile: 50 °C for 5 min; 5 °C min$^{-1}$ to 170 °C; 170 °C for 3 min. In addition, hydrolyzing activity toward benzyl glycidyl ether and allyl glycidyl ether was analyzed by GC as described previously [33]. Biotransformation reactions were carried out at 25 °C in Erlenmeyer flasks with glass stoppers containing buffer A, 10% ethanol, 2.5–5 mM epoxides **1**–**3** (Fig. 1), and an appropriate amount of ammonium sulfate-precipitated EH. The reactions were complete within 1–2 h. Four hundred microliter-samples were withdrawn at various time points of the reaction (spanning the complete conversion of both epoxide enantiomers) and saturated with NaCl. The residual epoxide and formed diol were extracted by ethyl acetate. Absolute configurations were established using the previously determined elution order of epoxide and diol enantiomers [34] (C.A.G.M. Weijers, personal communication). Quantification of extracted epoxide and diol enantiomers was conducted by chiral GC (see the Supplementary material for the GC conditions used). The enantiomeric ratio *E* together with the regioselectivity coefficients $\alpha_R$ and $\alpha_S$ were determined by a non-linear data fitting program (Scientist, MicroMath Inc.) using Eqs. (1) and (2). Eq. (1) is derived from Sih's equation expressing a relationship between *E*, extent of conversion *c*, and the enantiomeric excess of the residual epoxide $ee_s$ [35].

$$c = 1 - [(1 - ee_s) \cdot (1 + ee_s)^{-E}]^{1/(E-1)} \quad (1)$$

Eq. (2) defines a relationship between the regioselectivity coefficients $\alpha_R$ and $\alpha_S$, extent of conversion *c*, the enantiomeric excess of the formed diol $ee_p$, and $ee_s$ [11].

$$ee_p = \alpha_S - \alpha_R + (1 - \alpha_S - \alpha_R) \cdot ee_s \cdot (1 - c) \cdot c^{-1} \quad (2)$$

Protein concentrations were determined using the BCA Protein Assay Kit (Pierce) with bovine serum albumin as the standard.

## 3. Results and discussion

### 3.1. Cloning of a novel EH gene using eDNA

Alignment of 31 prokaryotic EH sequences revealed three highly conserved regions: (1) the consensus sequence G-F/Y-G-X-S, (2) the active-site consensus sequence G-G/H-D-W-G containing the nucleophilic aspartate, and (3) the H-G-W/F-P motif [2]. Regions (1) and (2) were used for design of the degenerate EH-specific primers Def205 and Der250. With these degenerate consensus primers, we amplified fragments of putative EH genes directly from eDNA. Similarly, careful design of consensus primers allowed amplification of lipase-specific DNA fragments using eDNA as template [28].

The amplification products (Fig. 2) were cloned, five of which were randomly selected for sequence analysis. Using a BLASTP search at NCBI, four unique translated sequences with homology to putative EHs were found (Fig. 3). All four sequences in the NCBI database appeared to be EHs, because all of them contained the EH-specific consensus region H-G-W/F-P. They also contained two additional EH-specific regions, which conformed to the previously established EH-specific motifs G-X-small-X-S/T and G-G/H-D-W-G to a high degree [2]. The sequence of clone no. 4 (Fig. 3) was selected for further cloning experiments. Two genome-walking primers (Def205 and Bf1, Fig. 4), which were based on the insert sequence of this clone, were used in combination with the linker-primers C1 and C2 and eDNA as template to amplify a downstream segment of the EH-gene corresponding to clone no. 4. Sequence analysis of the three PCR products generated revealed that the large 800-bp fragment was composed of two smaller fragments (300 and 500 bp) separated by a Sau3A I restriction site. A second step of genome-walking PCR was performed with walking primers Ar2 and Br2 in order to assess the region upstream of the known sequence. An 800-bp fragment was obtained that spanned one-third of the putative EH gene, including the upstream located flanking region. The sequences obtained allowed assembly of the entire putative EH gene. In order to amplify the full-length gene, primer EHf (specific for the 5′ end of the gene) was paired with primer EHr (introducing an Xba I restriction site downstream of the TGA stop codon) in a final PCR with nebulized eDNA as template.

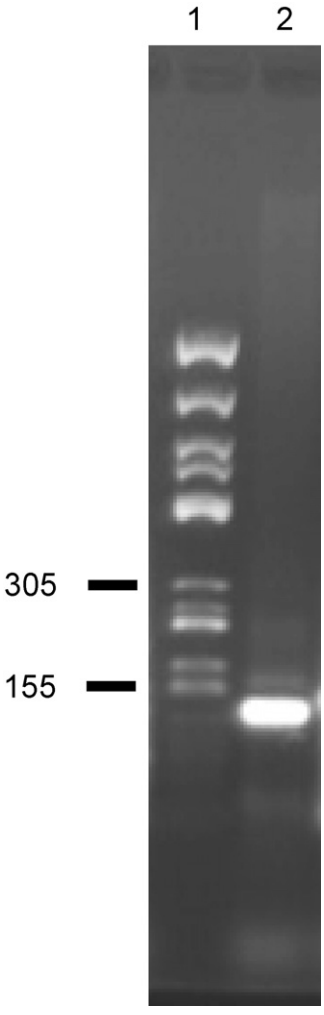


**Fig. 2.** PCR products of approximately 150 bp obtained with primer pair Def205-Der250 using eDNA isolated from a biofilter (Lane 2). Lane 1: DNA standard.


**Fig. 3.** Amino acid sequence analysis of PCR products generated using the primer pair Def205-Der250 with purified eDNA as template. The sequences of the primers were not included in the sequence analysis. For each sequence, one GenBank accession number is given, representing the sequence with the highest score obtained in a BLASTP search using the indicated amino acid sequence as query. Identical amino acids are represented by thick vertical bars. Positive amino acids are represented by thin vertical bars. EAS73267: α/β hydrolase fold protein (according to GenBank) from *Psychroflexus torquis* ATCC 700755, most likely an EH given that the sequence contains the EH-specific regions HGFP, GYNKS, and GHDWG [2]. YP_641600: EH-like protein (according to GenBank) from *Mycobacterium* sp. MCS, the sequence contains the EH-specific regions HGWP, GYGFS, and GGDWG. YP_001208347: putative α/β hydrolase fold protein (according to GenBank) from *Bradyrhizobium* sp. ORS278, probably an EH considering that the sequence contains the EH-specific regions HGWP, GYGTT, and GYDWG. YP_001208347: putative EH (according to GenBank) from *Arabidopsis thaliana*, the sequence contains the EH-specific regions HGFP, GYGDS, and GHDWG.

The possibility that the assembled sequence is a chimera of several homologous genes and, therefore, does not constitute a true single gene extant in the eDNA sample was eliminated by the fact that a full-length gene of nearly the same nucleotide sequence was amplified using the original eDNA sample and primers derived from the flanking regions of the assembled putative gene. The sequence of the EH gene that was amplified directly using eDNA differed by 10 nucleotides from the predicted sequence obtained by assembly of the individual gene fragments. Interestingly, these differences did not change the amino acid sequence of the protein. A BLASTP search revealed that this protein shared 44% identity with a putative EH from *Ralstonia metallidurans* CH34, and 43% identity with a putative EH from *Ralstonia eutropha* JMP134, the highest among all proteins searched. Other putative EHs with identities higher than 40% included those from other *Ralstonia* strains.

The GC content of the cloned gene was calculated to be 64.7%, which is significantly higher than the average of known genomic GC contents [36]. The novel EH is composed of 301 amino acids and contains the EH-specific regions HGFP, GYNLS, and GHDWG, which includes the nucleophilic aspartate in the active site of the enzyme. The molecular mass of the EH gene product was calculated to be 33,702 Da.

### 3.2. Overexpression and characterization of the novel EH

SDS-PAGE of the ammonium sulfate precipitate revealed a prominent band corresponding to a protein with a molecular mass of approximately 34 kDa (see Fig. S1 in the Supplementary

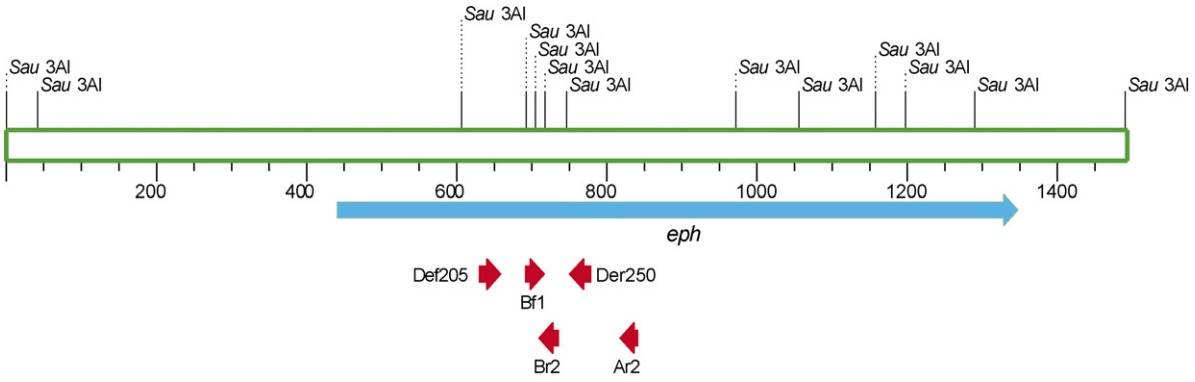


**Fig. 4.** Schematic representation of the cloned EH-encoding gene (*eph*) including sequenced flanking regions. The arrows indicate the annealing sites of EH consensus primers (Def205 and Der250) and the genome-walking primers. Sau3AI restriction sites are represented by vertical lines.

material). *E. coli* BL21 (pSEEH9) cells had EH activity and hydrolyzed to the corresponding vicinal diols short to long-chain aliphatic terminal epoxides (Fig. 1). Control cells harboring only the parent vector pSE420 lacked EH activity. It appeared that the enzyme did not accept as substrates *meso*-epoxides or terminal epoxides with non-aliphatic substituents, because no enzymatic hydrolysis of epoxycyclooctane or *tert*-butyl glycidyl ether, allyl glycidyl ether, benzyl glycidyl ether, and styrene oxide was detected. The access tunnel to the active site of the enzyme is likely modeled in such a way that only aliphatic terminal epoxides are accepted as substrate.

The pH profile of the enzymatic reaction revealed maximum enzyme activity at pH 7.5. Further, the enzyme showed 50% of its maximum activity at pH 5.8 and 8.3. Thermal stability of the EH was examined by incubating the enzyme at various temperatures in buffer A for 30 min. Under these conditions, the EH lost half of its activity at 43 °C. No loss of activity was determined at incubation temperatures up to 30 °C.

The specific activity of the enzyme was compromised with the increase in alkyl chain length (Table 2), suggesting that the solubility of the epoxide in the biotransformation mixture and its availability to the biocatalyst may play a role in determining specific enzymatic activity. The enzyme preferentially hydrolyzed the *R*-enantiomer of the epoxide, as was found previously for EHs from various yeast strains when tested with aliphatic epoxides [15,34,37]. The absolute configuration of the main diol product was determined to be *R* for all tested epoxides **1**–**3**. The experimentally determined $ee_p$ and $ee_s$ values and the curves obtained by curve fitting using Eqs. (1) and (2) are shown for all three biotransformation reactions (see Fig. 5 for epoxides **1** and **2**, and Supplementary material [Fig. S2] for epoxide **3**). The enantioselectivity of the enzyme was rather low for all investigated epoxides (Table 2). The regioselectivity data, which were determined by the non-linear fitting program, were confirmed by chiral GC analysis of the diol products for the reaction with epoxide **2** using enantiopure epoxides as substrates (Table 2). Calculation of the $\alpha_R$-values revealed that the fraction of *R*-epoxides attacked at the substituted carbon atom $C_2$ (see Fig. 1) was close to zero (Table 2). Consequently, the vast majority of *R*-epoxide molecules was attacked at position $C_1$, leading to the almost exclusive formation of *R*-diols. On the other hand, the determined $\alpha_S$-value, which represents the fraction of *S*-epoxide attacked at position $C_2$, was dependent on the epoxide used and varied between 22% and 45% (Table 2). The enzyme showed a mixed regioselectivity with the slower reacting *S*-epoxide, resulting in the formation of both *R*- and *S*-diol from *S*-epoxide. Therefore,

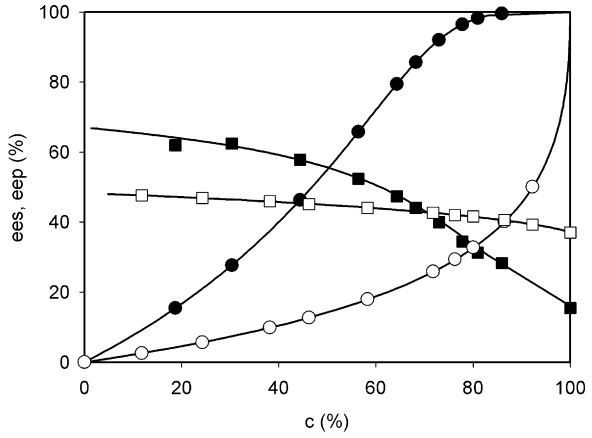


**Fig. 5.** Determined $ee_p$ (square symbols) and $ee_s$ values (round symbols) together with the fitted curves for representative biotransformation reactions of epoxide **1** (filled symbols) and epoxide **2** (open symbols) are shown. The curves were obtained using the non-linear fitting program Scientist with Eqs. (1) and (2) and the fitting parameters *E*, $\alpha_S$, and $\alpha_R$. The data are represented as a function of the conversion ratio *c* calculated from Eq. (1) after curve fitting. The values for *E*, $\alpha_S$, and $\alpha_R$ (determined from three biotransformation reactions) are given in Table 2.

**Table 2**
Hydrolysis of aliphatic 1,2-epoxides using the novel recombinant EH

| Epoxide substrate | Abs. config. of residual epoxide[a] | Diol product | | *E*-value | Regioselectivity[c] | | Specific activity[d] |
|---|---|---|---|---|---|---|---|
| | | Abs. config.[a] | $ee_p$ (%)[b] | | $\alpha_S$ (%) | $\alpha_R$ (%) | |
| **1** | (*S*) | (*R*) | 16 ± 1 | 5.9 ± 0.2 | 22 ± 2 | 6 ± 2 | 42 |
| **2** | (*S*) | (*R*) | 40 ± 2 | 1.5 ± 0.1 | 45 ± 3 (47) | 5 ± 2 (7) | 38 |
| **3** | (*S*) | (*R*) | 28 ± 1 | 2.1 ± 0.1 | 29 ± 2 | 1 ± 1 | 16 |

[a] Absolute configurations (abs. config.) of the residual epoxide enantiomer and the main diol enantiomer are indicated.
[b] Enantiomeric excess of the diol product determined at 100% conversion.
[c] The numbers in brackets indicate regioselectivity coefficients determined by using *R*-epoxide **2** (for determination of $\alpha_R$) or *S*-epoxide **2** (for determination of $\alpha_S$) as a substrate.
[d] The specific activity is given in nmol $min^{-1}$ $mg^{-1}$.

the proportion of attack at each of the two ring carbon atoms of *S*-epoxide was dependent on chain length of the substrate. The overall reaction with racemic epoxide as the substrate was partially enantioconvergent, leading to non-racemic diol products at the end of the enzymatic conversion with enantiomeric excesses that were significantly higher than zero (Table 2).

## 4. Conclusion

eDNA has been established as a rich source of genes encoding novel EHs from uncultured microorganisms [38]. Screening of eDNA expression libraries for specific enzyme activities is one method of obtaining the corresponding genes. Another approach is the use of PCR and degenerate primers targeted to conserved sequences in order to amplify internal fragments of genes directly from eDNA. By using genome-walking techniques, putative genes can be assembled. The full-length genes can then be amplified directly from eDNA in a single PCR using primers specific for the 5′- and 3′-end of the predicted gene. As reported in this paper, the latter approach has led to the recovery of a complete EH gene from eDNA by applying PCR techniques together with EH-specific consensus primers. The newly identified EH showed rather low homology to other putative or known EHs. It hydrolyzed aliphatic terminal epoxides containing 6–10 carbon atoms with low enantioselectivity. In all cases, diol formation occurred in a partially enantioconvergent reaction.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2008.05.018.

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