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Synthesis of enantiopure epoxide by 'one pot' chemoenzymatic approach using a highly enantioselective dehydrogenase

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ABSTRACT

Enantiopure α-phenethyl alcohols, including aromatic halohydrins, are important chiral building blocks. One of the best approaches to synthesise α-phenethyl alcohols is asymmetric reduction of prochiral ketones by alcohol dehydrogenases (ADHs). The obtained enantiopure halohydrin could be directly used to produce enantiopure epoxide through a base-induced ring-closure reaction, which is an attractive 'one pot' chemoenzymatic method for producing high-yield epoxide. In this study, a novel medium-chain dehydrogenase (KcDH) from *Kuraishia capsulate* CBS1993 was identified and characterised to show its broad substrate scope and excellent enantioselectivity. KcDH showed activities on 25 substrates of the 26 tested aromatic ketones and heteroaryl ketones, with an enantiomeric excess (ee) >99% and the highest relative activity observed with *para*-nitro acetophenone. Due to its high enantioselectivity for α-haloketones, a chemoenzymatic method for the synthesis of enantiopure styrene oxide (SO) and phenyl glycidyl ether (PGE) was developed through a base-induced ring-closure reaction on enantiopure halohydrin obtained with KcDH. (*R*)-SO and (*S*)-PGE were obtained in 86% and 94% analytical yield, respectively, and both epoxides were obtained with ee >99%. Thus, our results suggested that KcDH may be a promising biocatalyst for the production of multiple enantiopure α-phenethyl alcohols and epoxides.

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Introduction

Optically pure aromatic secondary alcohols, such as α-phenethyl alcohol and its substitutive derivatives, represent an important class of chiral building blocks that are versatile intermediates for the synthesis of a vast number of bioactive compounds, including agrochemicals, β-blockers, HIV-protease inhibitors and anti-asthmatic agents.[1] Additionally, the important intermediate halohydrin can be converted into epoxide by adding alkali into the reaction medium after reduction.[2] Epoxides are also attractive synthons used for fine organic synthesis due to their chemical versatility.[3]

Asymmetric reduction of prochiral ketones using ADHs is an attractive synthesis approach to producing α-phenethyl alcohols, because it gives a 100% theoretical yield. Examples of industrial ADHs include those from *Thermoanaerobacter brockii* ATCC 53556,[4] *Rhodococcus ruber* DSM 44541,[5] and *Rhodococcus erythropolis* DSM43297,[6] with each demonstrating excellent properties of enantioselectivity and substrate scope. Among these reported ADHs, those from *R. ruber* DSM 44541 and *Lactobacillus kefir* were successfully used in the synthesis of (*R*)-1,2-epoxyoctane and (*S*)-1-octene oxide, respectively, using a base-induced ring-closure reaction.[7,8]

Expanding the ADH toolbox remains an important topic in redox biocatalysis. Based on the increased availability of public genome information, many putative ADHs can be obtained from Genbank.[9] In this study, one putative ADH from *Kuraishia capsulate* CBS1993 (KcDH) was heterologously overexpressed in *Escherichia coli*, and its catalytic properties were studied in detail, revealing a broad substrate specificity and excellent enantioselectivity. Furthermore, due to its high enantioselectivity to halohydrin, we performed a chemoenzymatic assay to synthesise two representative epoxides, which showed promising results.

Materials and methods

Materials

All ketones, styrene oxide (SO), phenyl glycidyl ether (PGE) and 1-phenylethane-1,2-diol and 3-phenoxy-1,2-propanediol used in this study are commercially available. The racemic sec-alcohol standards of acetophenone-substitutive derivatives were obtained by the reduction of ketone with 1.2 equiv of $NaBH_4$ in 1% NaOH. The four chloroalcohols were synthesised by reduction with corresponding ketones in the presence of $NaBH_4$ and $CaCl_2$, with excess

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HCl added during the reaction according to literature.[10] The genome of *Kuraishia capsulate* CBS1993 was stored in our lab. *E. coli* DH5α and *E. coli* BL21 (DE3) cells were grown in Luria–Bertani (LB) medium and used as the cloning and expression hosts, respectively. Column SB-AQ (4.6 mm × 250 mm; Agilent Technologies, Santa Clara, CA, USA) was used for conversion-ratio determination. The enantiomeric excess (ee) of sec-alcohol products PGE and SO were analysed by high-performance liquid chromatography (HPLC) or gas chromatography (GC) equipped with a chiral column: Chiralcel OB-H column, OD-H column (250 mm × 4.6 mm; Daicel Corp., Hyogo, Japan), Supelco fused-silica capillary column β-Dex 120 (Supelco, 30 m × 0.25 mm × 0.25 μm; Sigma–Aldrich, St. Louis, MO, USA,) and CP-Chirasil-Dex CB (30 m × 0.25 mm × 0.25 μm; Agilent Technologies).

Cloning, expression and purification of the KcDH gene

The full-length KcDH gene (accession number: CDK24134.1) flanked by *Nde*I and *Hind*III restriction enzyme sites was amplified by polymerase chain reaction using forward (5′-GGAATTCCATATGTCTGCTCTCTCCAAAACCCAGG-3′) and reverse primers (5′-CCCAAGCTTTCAGCCCGCGGGGTGGTTCTCC-3′). The obtained DNA fragment was digested and ligated into the correspondingly digested pET-28a(+) plasmid. The resulting recombinant plasmid was then transformed into BL21 (DE3) cells for KcDH expression. A single transformant was cultured at 37 °C for 12 h, and then transferred to 100 mL fresh LB medium supplemented with kanamycin (50 μg/mL) and cultured at 37 °C. The culture was induced by the addition of 0.1 mM isopropyl β-D-1-thiogalactopyranoside after reaching an OD_{600} of 0.6. After induction at 20 °C for 20 h, cells were harvested by centrifugation at 8500*g* for 10 min, and resuspended in 50 mM Tris–HCl (pH 8.0). After disrupting the cells by sonication and removing cell debris/inclusion bodies by centrifugation, the soluble cell-free extract was filtered (0.22-μm filter; EMD Millipore, Billerica, MA, USA) and loaded onto a nickel column pre-equilibrated with 50 mM Tris–HCl (pH 8.0) binding buffer. After washing with the binding buffer, the bound recombinant enzyme was eluted by applying binding buffer with increasing concentrations of imidazole (20–200 mM). Pure KcDH was obtained following the addition of 200 mM imidazole. The expression and purity of the protein were identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 12% gels.

Determination of enzyme activity and kinetic parameters

KcDH activity was assayed spectrophotometrically at 35 °C by measuring the change in absorbance at 340 nm of NADH in 1 min. The standard assay for the reduction reaction was performed by adding 4 μL of 200 mM α-chloroacetophenone to 196 μL of preheated assay mixture containing 14 μg KcDH and 0.5 mM NADH in 50 mM potassium phosphate buffer (pH 7.0). The assay for oxidation activity was performed by adding 16 μL of 200 mM isopropanol to 184 μL of preheated assay mixture containing 4.2 μg KcDH and 0.5 mM NAD in 50 mM potassium phosphate buffer (pH 7.0). The kinetic parameters of α-chloroacetophenone and isopropanol were determined by measuring initial velocities at different substrate concentrations using a Lineweaver–Burk plot (1/v vs 1/[S]).

Effects of pH, temperature and metal ions on KcDH activity

The optimal pH for purified KcDH activity was investigated by measuring its activity in 0.1 M of the following buffers with pH ranging from 4 to 10.5: citrate buffer (pH 4.0, 5.0 and 6.0), phosphate buffer (pH 6.0, 7.0 and 8.0), Tris–HCl buffer (pH 8.0, 9.0 and 9.5) and carbonic buffer (pH 10.5). The effects of temperature on KcDH activity were examined over a temperature range of 25 °C to 50 °C in 50 mM potassium phosphate buffer (pH 7.0).

$CaCl_2$, $CoCl_2$, $CuCl_2$, $FeCl_3$, $MgCl_2$, $MnCl_2$, $NiCl_2$ and $ZnCl_2$ were used to test the effects of metal ions, and EDTA was used to test whether KcDH requires essential metal ions to maintain its activity. The influences of various metal ions and EDTA on enzyme activity were investigated by pre-incubating the enzyme (70 μg/ml) at a certain concentration of each different compound in 50 mM potassium phosphate buffer (pH 7.0) for 20 min at 35 °C. The enzyme activity was estimated using the previously described standard assay protocol. Relative activity was expressed as a percentage of the activity in the absence of any test compound.

Substrate scope and enantioselectivity determination

The relative activities of 26 substrates were measured using the previously described assay protocol with adjusted ratio of enzyme and substrate concentration. The α-chloroacetophenone activity was assumed 100%.

Enantioselectivity was determined by examining the reduction of aromatic ketones using an NADH-regeneration system consisting of the purified KcDH and glucose dehydrogenase (GDH) from *Bacillus subtilis* CGMCC 1.1398. The 1-mL reaction mixture contained 0.5 mM NAD^+, 10 mM ketone, 1 U KcDH, 50 mg glucose and 2 U GDH in 50 mM potassium phosphate buffer (pH 7.0). After 16 h, the reaction sample was equally separated into two parts, with one terminated by adding an equal volume of methanol, followed by HPLC analysis to determine the conversion ratio, and the other extracted with ethyl acetate, followed by ee analysis. Methods used for analysing chiral products using HPLC or GC are described in Supplementary Table S1.

Epoxidation after reduction

α-Chloroacetophenone and 3-chloro-1-phenoxy-2-propanone were selected as typical substrates for chemoenzymatic epoxidation. Resting *E. coli* cells containing KcDH (50 mg/mL) were used as catalysts for this procedure, and 5% isopropanol was used as the co-substrate for NADH regeneration. After reduction, 25 μL of 6 M NaOH was added to the 1-mL reaction medium (containing 10 mM product) to a final NaOH concentration of 146 mM. Three samples were collected at different time points (5, 15 and 30 min), and epoxidation was terminated by adjusting the pH between 8.0 and 9.0. The analytical yield was measured by HPLC analysis with the solvents methanol and H_2O at a ratio of 2:3. The methods used to assay the ee of SO and PGE are illustrated in Supplementary Table S1.

Scale-up preparation of (*R*)-SO and (*S*)-PGE

The reaction started at 30 °C by adding 624 mg α-chloroacetophenone or 738 mg 3-chloro-1-phenoxy-2-propanone into 100 ml PBS buffer (pH7.6) containing 100 mg/ml KcDH resting cell, 1 ml isopropanol, and 20 mg NAD. After bio-reduction was completed, 2.5 ml of 6 M NaOH was added for epoxidation. The epoxidation was terminated after 30 min by neutralisation with 6 M HCl. The product SO and PGE was extracted with hexane three times. After drying over anhydrous sodium sulphate, solvents were removed under vacuum. The obtained SO and PGE were analysed by chiral HPLC and GC, and the spectral data are presented in Supplemental material.

Results and discussion

A novel biocatalyst, KcDH from *Kuraishia capsulate* CBS1993, was identified by genomic data mining and cloned, characterised,

and overexpressed in *E. coli*. KcDH belongs to the medium-chain dehydrogenase/reductase super family, which contains two types of proteins: metalloproteins containing zinc and nonmetalloproteins without zinc.[11] KcDH contains 341 amino acids and has two typical Zn-binding regions that coordinate catalytic Zn (Cys45, Ser47, His67, Asp158) and structural Zn (Cys99, Cys102, Cys105, Cys113), respectively, implicating KcDH as a Zn-dependent enzyme. The protein exhibiting the highest homology was a secondary alcohol dehydrogenase from *Candida auris*, which shared 49% amino acid sequence identity. After purification, KcDH enantioselectivity was first tested on α-chloroacetophenone, and showed ee >99%, which encouraged further investigation.

The optimal temperature and pH for activity were determined as 35 °C and pH 8.0, respectively (Fig. 1), with KcDH showing the highest activity level in phosphate buffer. Under optimal reaction conditions, the specific activity of KcDH on α-chloroacetophenone was measured at 0.38 U/mg. KcDH only used the less expensive NADH instead of NADPH as a cofactor, which lowered the cost associated with bioprocessing. NADH can be regenerated via simple hydrogen transfer using isopropanol as the hydrogen donor, making the reaction system less complex as compared to the system using GDH and glucose. Therefore, we investigated the ability of KcDH to reduce 10 mM α-chloroacetophenone with 5% isopropanol, and observed complete conversion. Furthermore, the kinetic parameters associated with α-chloroacetophenone and isopropanol were obtained, indicating a K_m and V_{max} for α-chloroacetophenone of 0.33 mM and 0.39 μmol min^{-1} mg^{-1}, respectively, and a K_m and V_{max} for isopropanol of 1.5 mM and 4.13 μmol min^{-1} mg^{-1}, respectively. These data suggested isopropanol as a potential co-substrate for use in reduction reactions catalysed by KcDH.

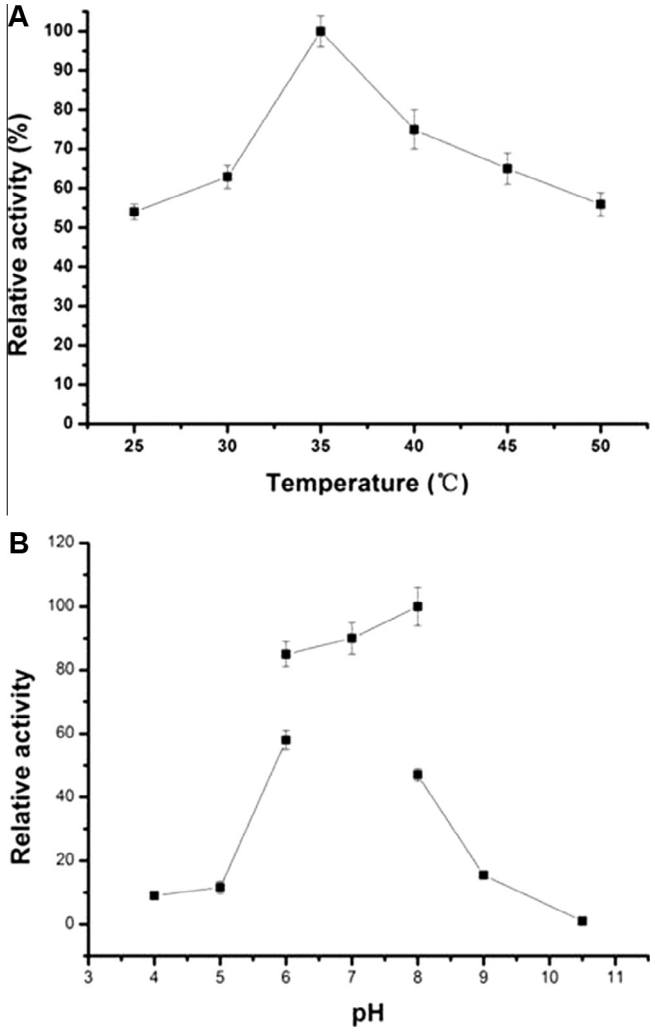


Figure 1. Effects of temperature and pH on KcDH activity. (A) The activities of TpEH1 were tested at different temperatures. The value at 35 °C was set as 100%. (B) pH ranging from 4 to 10.5: citrate buffer (pH 4, 5 and 6); phosphate buffer (pH 6, 7 and 8); Tris–HCl buffer (pH 8, 9 and 9.5); and carbonic buffer (pH 10.5). The value at pH 8.0 was set as 100%. All experiments were performed in triplicate.

Metal ions may exhibit substantial effects on KcDH activity, therefore, we investigated the effects of metal ions and EDTA on enzyme activity (Table 1). Among the nine compounds tested, KcDH displayed a slightly higher activity following the addition of Mg^{2+} and Mn^{2+}. There were no obvious differences on KcDH activity when increasing the concentration of Mg^{2+} and Mn^{2+} to 5 mM. However, the presence of 1 mM Co^{2+}, Cu^{2+} and Zn^{2+} considerably diminished enzyme activity, and more serious inhibitions were observed by increasing their concentrations to 5 mM. Fe^{3+} and Ni^{2+} completely inhibited KcDH activity, and Ca^{2+} demonstrated no effect on KcDH activity.

The presence of the chelating agent EDTA also diminished enzyme activity. The activity of KcDH decreased with the increasing concentration of EDTA and was totally inhibited in 100 mM EDTA (Fig 2), suggesting that KcDH required metals to sustain the enzyme activity (Fig. 2). As a predicted Zn-dependent enzyme, the loss of essential metal ions was assumed as the explanation for the observed reduction in activity.[12] All the tested reagents have no effect on enantioselectivity.

To map the substrate spectrum of KcDH, various ketones with diverse structures were employed (Table 2), with four types of substrates corresponding to high-value enantiopure alcohols investigated. Type I substrates consisted of α-substituted phenyl ethyl ketones .The derivatives of type I products are building blocks used for many adrenergic drugs, such as salbutamol, sotalol and nifenalol.[13] For these drugs, the (*R*)-enantiomer exhibits much higher physiological activity as compared to the (*S*)-enantiomer. Type II substrates contain acetophenone ring-substitutive derivatives, the products of which are generally used as building blocks for selective histamine H1-antagonists[14] and antisepsis agents.[15] Type III substrates represent heteroaryl ketones, of which the (−)-(*S*)-4-pyridylethanol is used for the synthesis of multiple β_3-blockers. The (*R*)-alcohols of type IV substrates are important precursors for adrenergic β-blockers.[16] After epoxidation, (*R*)-1-chloro-3-phenoxy-2-propanol is converted into (*S*)-PGE (with a switch in Cahn-Ingold-Prelog priority), which is used for the synthesis of adrenergic β-blockers.

The relative activity and ee of the products are illustrated in Table 2. KcDH showed activity on 25 of the 26 substrates tested, with relative activities ranging from 0% to 1779%, suggesting that different influences on enzyme activity were caused by various substituents. It was notable that all of the products were obtained

Table 1
Effect of metal ions and additives on reductase activity

Metal ions	1 mM		5 mM	
	Relative activity (%)	ee (%)	Relative activity (%)	ee (%)
Control	100	>99	100	>99
Ca^{2+}	100	>99	100	>99
Co^{2+}	16	>99	0	–
Cu^{2+}	2.5	–	0	–
Fe^{3+}	0	–	0	–
Mg^{2+}	108	>99	109	>99
Mn^{2+}	119	>99	116	>99
Ni^{2+}	0	–	0	–
Zn^{2+}	15	>99	5	–

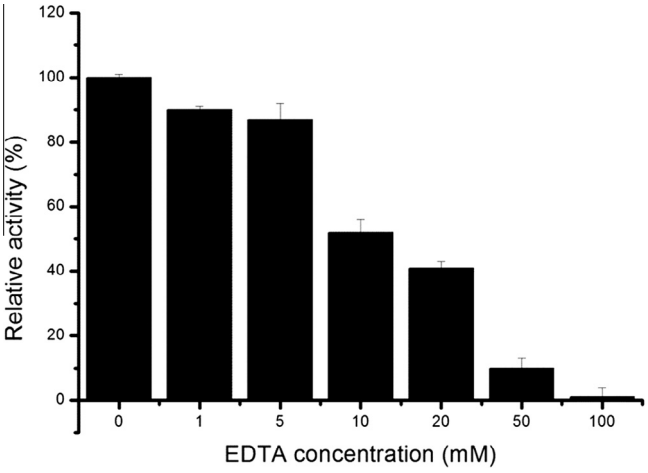


Figure 2. KcDH activity assay of samples treated with different concentrations of EDTA.

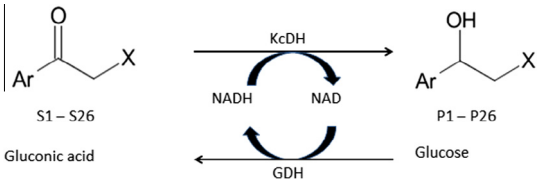
with ee >99% after complete conversion. The high enantioselectivity and wide substrate scope demonstrated that KcDH is an excellent biocatalyst for the production of optically pure alcohols. Based on our results, we observed that KcDH activity increased gradually according to the substituents in the order *ortho-* < *meta-* < *para-*. Additionally, the strong electron-withdrawing groups, such as the nitro group, resulted in a much higher activity relative to the other substituents. We inferred that electronic and steric effects of phenyl-substituents caused the phenomenon together. The typical result was that *para*-nitro acetophenone demonstrated the highest activity, while no activity was detected for *ortho*-nitro acetophenone.

Some previous reports discussed the impact of phenyl substituents on catalytic efficiency. Dunming et al. investigated 24 different ketoreductases and 15 acetophenone derivatives, introducing the Hammett substituent constant and using regression analysis to explain the correlation of electronic effects of ring substituents and enzyme activity. They found that the electron-withdrawing characteristic of the substituent enhanced the activity of certain ketoreductases,[17] which was in agreement with our results.

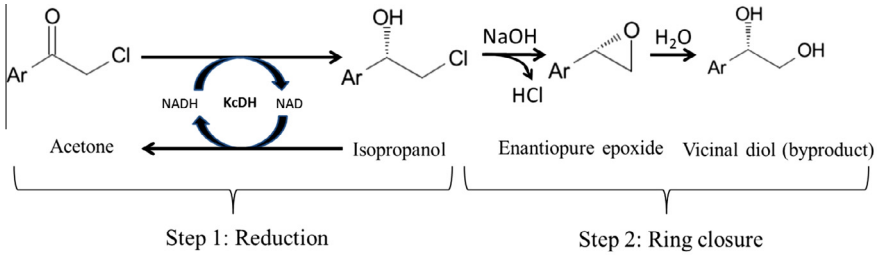
Enantiopure SO and PGE are two representative high-value epoxides.[3] The two corresponding optically pure epoxides, (*R*)-SO and (*S*)-PGE, are the bioactive enantiomers required for the synthesis of adrenergic receptor agonists and β-blockers, respectively. Many chemical-based asymmetric synthesis methods for these terminal epoxides have been described in recent years, such as Jacobsen asymmetric epoxidation and hydrolytic kinetic resolution.[18] However, these elegant chemical methods suffer from potentially toxic heavy-metal-based catalysts, very low reaction temperatures (−20 °C),[19] low ee,[20] or low yield,[21] especially for preparation of enantiopure SO.

An alternative approach involves the biocatalytic asymmetric reduction of α-chloroketone, coupled with chemical ring-closure under basic conditions as a second step (Scheme 1). After biocatalytic reduction of α-chloroacetophenone and chloro-1-phenoxy-2-propanone, optically pure halohydrins could be easily converted into epoxides by adding excessive base and maintaining its ee. We developed this chemoenzymatic method using KcDH, based on its high enantioselectivity, and obtained two epoxides with a high ee and in a high yield. To increase the epoxide yield, NaOH concentration and reaction time for ring closure of halohydrin were investigated. The optimal results are illustrated in Figure 3. The epoxide yield reached maximum after 30 min at 146 mM NaOH. Under these conditions, the analytical yield of (*R*)-SO was 86%, with ee >99% and the analytical yield of (*S*)-PGE was 94%, with ee >99%. Because the addition of base catalysed (*R*)-SO hydrolysis without strict regioselectivity,[22] vicinal diols were produced as by-products, with only 30% ee and 13% analytical yield. In order to explore the application potential of the chemoenzymatic approach, we further performed this chemoenzymatic method with 40 mM substrate concentration in 100 ml volume. The (*R*)-SO was obtained with 350 mg in nearly enantiopure form (98.0% ee) and the

Table 2



Substrate	X	Ar	Relative activity (%)	ee (%), Absolute configuration	Substrate	X	Ar	Relative activity (%)	ee (%), Absolute configuration
I					S15	H	4′-Me-Ph	280	>99, (*S*)
S1	Cl	Ph	100	>99, (*R*)	S16	H	2′-NO_2-Ph	0	—
S2	Cl	4′-Cl-Ph	180	>99, (*R*)	S17	H	3′-NO_2-Ph	762	>99, (*S*)
S3	Cl	4′-MeO-Ph	120	>99, (*R*)	S18	H	4′-NO_2-Ph	1779	>99, (*S*)
II					S19	H	3′-MeO-Ph	98	>99, (*S*)
S4	H	Ph	108	>99, (*S*)	S20	H	4′-MeO-Ph	116	>99, (*S*)
S5	H	2′-Cl-Ph	90	>99, (*S*)	III				
S6	H	3′-Cl-Ph	200	>99, (*S*)	S21	H	Pyridin-2-yl	77	>99, (*S*)
S7	H	4′-Cl-Ph	240	>99, (*S*)	S22	H	Pyridin-3-yl	30	>99, (*S*)
S8	H	2′-Br-Ph	176	>99, (*S*)	S23	H	Pyridin-4-yl	156	>99, (*S*)
S9	H	3′-Br-Ph	240	>99, (*S*)	S24	H	Thiophen-2-yl	12	>99, (*S*)
S10	H	4′-Br-Ph	460	>99, (*S*)	IV				
S11	H	3′-F-Ph	128	>99, (*S*)	S25	Cl	Ph-O	220	>99,(*R*)
S12	H	4′-F-Ph	212	>99, (*S*)	S26	Cl	4-Me-Ph-O	108	>99,(*R*)
S13	H	2′-Me-Ph	40	>99, (*S*)					
S14	H	3′-Me-Ph	240	>99, (*S*)					



Scheme 1. Synthesis of enantiopure epoxide by chemoenzymatic approach. 'one-pot two-steps' cascade-reaction consisting of a biocatalytic reduction of α-halo ketones (step 1) and in situ base-induced ring closure to furnish the corresponding epoxide (step 2). The remaining base catalyses the hydrolysis of epoxide which results in the byproduct vicinal diol.

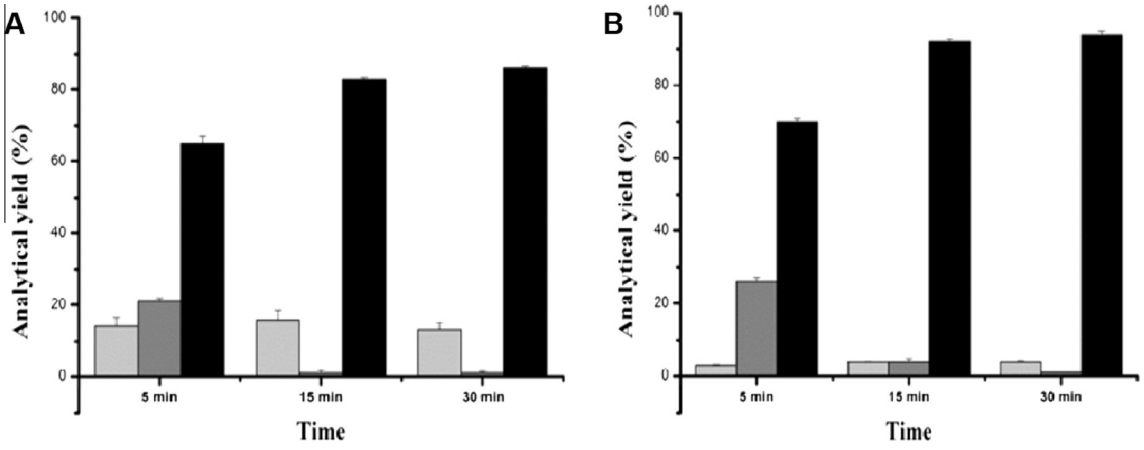


Figure 3. Analytical yield of enantiopure SO and PGE. (A) Analytical yield of three components during SO epoxidation: 1-phenylethane-1,2-diol (light grey); (*R*)-2-chloro-1-phenylethanol (grey); (*R*)-SO (black). (B) Analytical yield of three components during PGE epoxidation: 3-phenoxy-1,2-propanediol (light grey), (*R*)-1-chloro-3-phenoxy-2-propanol (grey); (*S*)-PGE (black).

isolated yield was 73.1%. The (*S*)-PGE was obtained with 498 mg (99.2% ee) and the isolated yield was 83.0%. The enantiopure epoxide could be easily extracted from reaction medium using hexane and the byproduct remained in aqueous phase,[23] which made the downstream purification much easier.

Reduction of α-chloroacetophenone coupled with isopropanol for NADH regeneration could lead to quasi-quantitative conversion. During this investigation, we found that only 2.6 equiv of isopropanol was enough for >99% conversion of the substrate, which further lowered the cost of this procedure. Furthermore, this 'one pot, two steps' procedure using inexpensive alkali for epoxidation was less complex as compared to previous methods using two-enzyme cascades.[24] The two-enzyme cascade using ADH and halohydrin dehalogenase to produce epoxides must overcome equilibrium issues by adding anion exchanging resins, and requires excellent organic tolerance for both enzymes.

Besides ADHs, biocatalysts, including epoxide hydrolase,[25] halohydrin dehalogenases,[26] cytochrome P450[27] and styrene monooxygenase,[28] were developed as alternatives for chemical methods. Among these enzymes, epoxide hydrolase suffered a limitation of 50% theoretical yield, and halohydrin dehalogenases suffered an equilibrium problem, requiring the removal of halide ions from the reaction medium.[24] Although cytochrome P450 displayed 100% theoretical yield, poor operational stability and low activity remain a major challenge. The styrene monooxygenase from *Pseudomonas* sp. strain VLB120 was a promising biocatalyst that showed bioprocesses superior to two chemical-process alternatives, and slightly inferior to a third. The use of an additional toxic bis(2-ethylhexyl)phthalate phase was the major drawback of this process.[29] Although few reports using ADH to produce enantiopure epoxide exist,[8,30,31] promising results were obtained. The best report involved using ADH from *Lactobacillus kefir*, which was used to prepare 1 M (*S*)-1-Octene oxide by adding 10 equiv of NaOH after 1-Bromo-2-octanone was reduced to halohydrin. These results indicated that this chemoenzymatic method using ADH was a promising approach.

Conclusion

Here, the novel enzyme KcDH was expressed and identified, and the basic catalytic properties were studied using α-chloroacetophenone as a substrate. KcDH showed a wide substrate scope and excellent enantioselectivity for 25 substrates, demonstrating potential for the production of enantiopure α-phenethyl alcohols and aromatic halohydrins. Furthermore, we optimised the 'one-pot, two-steps' chemoenzymatic approach to produce enantiopure epoxides, obtaining desirable analytical yield and ee. Although the substrate scope and ee for KcDH was satisfactory, the quantified enzyme activity was still low. Improving KcDH activity through protein engineering, and diminishing by-product formation will constitute our future work on this process.

^{1}H NMR data of the synthesised enantiopure products

(*R*)-2-Chloro-1-phenylethanol: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50–7.27 (m, 5H, Ar), 4.90 (dd, J = 8.6, 2.4 Hz, 1H, CH), 3.74 (dd, J = 11.2, 3.3 Hz, 1H, CH_2Cl), 3.65 (dd, J = 11.1, 9.0 Hz, 1H, CH_2Cl), 2.69 (s, 1H, OH).

(*R*)-1-Chloro-3-phenoxy-2-propanol: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (t, J = 7.8 Hz, 2H, Ar), 7.00 (t, J = 7.3 Hz, 1H, Ar), 6.93 (d, J = 8.1 Hz, 2H, Ar), 4.27–4.17 (m, 1H, CH), 4.15–4.03 (m, 2H,

OCH_2), 3.76 (ddd, J = 29.5, 11.2, 5.4 Hz, 2H, CH_2Cl), 2.81 (d, J = 3.2 Hz, 1H, OH).

(*S*)-Phenyl glycidyl ether: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (t, J = 7.7 Hz, 2H, Ar), 7.09–6.80 (m, 3H, Ar), 4.22 (dd, J = 10.9, 2.6 Hz, 1H, OCH_2), 3.97 (dd, J = 10.9, 5.6 Hz, 1H, OCH_2), 3.39–3.33 (m, 1H, CH), 2.91 (t, J = 4.2 Hz, 1H, CH_2), 2.83–2.62 (m, 1H, CH_2).

(*R*)-Styrene oxide: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52–7.11 (m, 5H, Ar), 3.86 (s, 1H, CH), 3.15 (dd, J = 4.7, 4.7 Hz, 1H, CH_2), 2.80 (dd, J = 5.2, 2.0 Hz, 1H, CH_2).

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.01.048.

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