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A genomic search approach to identify carbonyl reductases in *Gluconobacter oxydans* for enantioselective reduction of ketones

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The versatile carbonyl reductases from *Gluconobacter oxydans* in the enantioselective reduction of ketones to the corresponding alcohols were exploited by genome search approach. All purified enzymes showed activities toward the tested ketoesters with different activities. In the reduction of 4-phenyl-2-butanone with *in situ* NAD(P)H regeneration system, (S)-alcohol was obtained with an e.e. of up to 100% catalyzed by Gox0644. Under the same experimental condition, all enzymes catalyzed ethyl 4-chloroacetoacetate to give chiral products with an excellent e.e. of up to 99%, except Gox0644. Gox2036 had a strict requirement for NADH as the cofactor and showed excellent enantiospecificity in the synthesis of ethyl (R)-4-chloro-3-hydroxybutanoate. For the reduction of ethyl 2-oxo-4-phenylbutyrate, excellent e.e. (>99%) and high conversion (93.1%) were obtained by Gox0525, whereas the other enzymes showed relatively lower e.e. and conversions. Among them, Gox2036 and Gox0525 showed potentials in the synthesis of chiral alcohols as useful biocatalysts.

Key words: carbonyl reductase; enantioselective reduction; chiral alcohols

Chiral alcohols with additional functional groups play an increasingly important role as building blocks for the synthesis of enantiomeric pure pharmaceuticals, agrochemicals, and natural products. Optical active secondary alcohols, especially chiral aryl alcohols and ketoesters, are widely used as intermediates. For example, (S)-4-phenyl-2-butanol is used as a precursor for antihypertensive agents (labetalol) and spasmolytics or anti-epileptics(emepronium)).[1,2] Ethyl (S)-4-chloro-3-hydroxybutanoate ((S)-CHBE) is used for production of the HMG-CoA reductase inhibitors(statins),[3] while (R)-CHBE is useful for the synthesis of biologically and pharmacologically important materials: (R)-carnitine, (R)-4-amino-3-hydroxy-butayric acid, and (R)-4-hydroxy-2-pyrrolidone.[4] Another chiral ketoester, enantiopure ethyl 2-hydroxy-4-phenylbutyrate is common precursor in the manufacture of a variety of angiotensin-converting enzyme inhibitors including benazepril, cilazapril, and enalapril.[5,6] Preparing these chiral alcohols by asymmetric reduction of carbonyl substrates via biocatalyst is an efficient and practical way.[7] These biocatalysts, such as carbonyl reductases, have drawn considerable attention due to not only the advantage of their high enantioselectivity and regioselectivity, but also economic viability and environment friendliness.[8,9] Carbonyl reductases widely used in asymmetric reduction can achieve up to 100% theoretical yield.[8,10] Up to now, many carbonyl reductases have been obtained either by culture-based strain enrichment or by a metagenomic screening with different substrate specificities and stereoselectivities.[11] However, the former excludes a large fraction of microbial organisms in environments and the latter is time consuming and laborious. In comparison with traditional approaches, genome mining has become more effective and promising which involves searching new enzymes from databases with those of known enzymes.[12] Some new carbonyl reductases have been obtained in this way from many species.[13]

In the current study, we characterized novel enzymes based on the genome of *Gluconobacter oxydans*, which is commonly applied in many industries for its abilities of stereoselective and regioselective oxidoreduction.[14,15] The *G. oxydans* 621H genome revealed the presence of 75 open reading frames (ORFs) that encoded putative dehydrogenases/oxidoreductases based on sequence analysis.[16] Among these, a number of ORFs had been expressed and functionally determined, some of which showed important biological function and great biotechnological interest. Gox1615 was

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Abbreviations: BsGDH, *Bacillus subtilis*-derived glucose dehydrogenase; CHBE, ethyl 4-chloro-3-hydroxybutanoate; COBE, ethyl 4-chloroacetoacetate; CsKR, Ketoreductase from the *Cyanobacterium Synechococcus* sp. Strain PCC 7942; e.e., enantiomeric excess; fabG, 3-ketoacyl-acyl carrier protein reductases; HPBE, ethyl 2-hydroxy-4-phenylbutyrate; HPLC, high-performance liquid chromatography; IPTG, isopropyl β-D-thiogalactopyranoside; NAD(P)H, Nicotinamide adenine dinucleotide (phosphate) reduced tetrasodium salt; OPBE, ethyl 2-oxo-4-phenylbutyrate; ORFs, open reading frames.

classified as an NADP-dependent glycerol dehydrogenase that can be applied in the kinetic resolution of glyceraldehyde and in the production of enantiopure L-glyceraldehyde on a preparative scale.[17] Gox2181, as a polyol dehydrogenase, had potential applications in asymmetric oxidation of sugars and polyols for the synthesis of certain rare ketoses.[18] Considering the huge utilization potentiality, we noticed that 21 putative carbonyl reductases maybe catalyze the reversible oxidation of alcohol to aldehydes or ketones, therefore we cloned some of these genes and obtained their corresponding enzymes for determining their chemo-, regio-, and stereoselectivities. The results of this study can promote the application of *G. oxydans* in many industries.

Material and methods

Chemicals and micro-organism cultivation. Ketones and chiral alcohol standard samples were purchased from Sigma–Aldrich and TCI companies. All other chemicals used were commercially available and of analytical grade.

G. oxydans DSM2343 was cultured in yeast–sorbitol medium containing 8% (w/v) sorbitol and 2% (w/v) yeast extract at 30 °C. Cells were harvested by centrifugation at 8000 g for 10 min and used for the total genomic DNA preparation according to the cetyltrimethyl ammonium bromide extraction protocol.[19] *Escherichia coli* DH5α, Rosetta (DE3), and BL21 (DE3) were routinely cultured in Luria–Bertani medium. Ampicillin, kanamycin, and chloramphenicol were added to the medium at 100, 50, and 25 μg mL^{-1}, respectively, when necessary. Recombinant *Bacillus subtilis*-derived glucose dehydrogenase (BsGDH) was prepared as previously described.[20]

Gene cloning and expression of carbonyl reductases. All DNA manipulations and bacterial transformations were carried out according to standard protocols.[21] The putative carbonyl reductases gene gox2036 (YP_192428.1), gox0525 (YP_190959), gox0644 (YP_191077.1), gox1615 (YP_192012.1), gox1598 (YP_191995.1), gox1462 (YP_191867.1), and gox0290 (YP_190729.1) were amplified by polymerase chain reaction (PCR). The primers, vectors, and bacterial strains used in this study were listed in Table 1. The amplified products were digested with relative double restriction enzymes and then ligated to vectors. The constructed plasmids were introduced into *E. coli,* BL21 (DE3) or Rosetta (DE3). The positive clones were screened by PCR amplification and further verified by sequencing. The *E. coli* host carrying the recombinant plasmid was induced with isopropyl β-D-thiogalactopyranoside (IPTG) ranging from 0.1 to 1.0 mM and induced at 25, 30, and 37 °C to obtain soluble recombinant enzymes. The cell-free extract was prepared after lysis of the cell in a 20 mM phosphate buffer by ultrasonication at 4 °C. Cell debris was removed by centrifugation. The supernatant was used to determine the enzyme expression by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the enzyme concentration was determined by the Bradford method. The soluble enzymes were purified by Ni-NTA agarose according to the operation manual (Qiagen, Hilden, Germany), and enzyme activity was assayed.

Activity assay and determination of kinetic parameters. The enzyme activity toward the reduction of ketones was determined by spectrophotometrically measuring the oxidation of NAD(P)H at 340 nm ($\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$) in the presence of an excess

Table 1. Primers, test vectors and strains used.

Gene	Primer sequence	Test vectors	Strains
gox2036	F: 5′-gaattcATGTCCCTTTCTGGAAAAA-3′ R: 5′- aagcttTCAGCGGAAAACGAGAC-3′	pET28a	BL21(DE3)
gox0525	F: 5′-gaattcATGACCCACAGAGTAGCGC-3′ R: 5′-aagcttTCAGGCGACGAGACCGCC-3′	pET28a	BL21(DE3)
gox0644	F: 5′-catatgAGCGCCTCGTCACAGGTTC-3′ R: 5′-aagcttATATCAGAATTTCGCCGTAT-3′	pSE380 or pET32a/28a	BL21(DE3)
gox1615	F: 5′-ggatccAGCGCCGCATCCGACACCA-3′ R: 5′-ctcgagATATCAGTCCCGTGCCGGGG-3′	pET32a	BL21(DE3)
gox1598	F: 5′-ggatccCTGGGGGCTTCCCCCCCATT-3′ R: 5′-ctcgagTCAGGGAGCCGGGTTGGAG-3′	pET32a/28a	BL21(DE3)/Rosetta (DE3)
gox1462	F: 5′-ggatccCAGTATCGTCAGCTTGGTC-3′ R: 5′-ctcgagTCATTTTTTCGTTTCAAGGGCC-3′	pET32a/28a	BL21(DE3)/Rosetta (DE3)
gox0290	F: 5′-gtcgacATGAAAACAGTCACACTCAGGA-3′ R: 5′-gcggccgcGATCATGGCGAGAGATACCT-3′	pET32a	Rosetta (DE3)

amount of ketones. The change in absorbance of NAD(P)H was monitored at 340 nm using an ultraviolet-visible spectrophotometer with a temperature-controlled cuvette holder (Shimadzu Co., Kyoto, Japan). One unit (U) activity was defined as the amount of enzymes required to catalyze the oxidation of 1 μmol NAD(P)H per minute at 30 °C. The data were expressed as U/mg of protein. The standard reaction mixture contained 100 mM phosphate buffer (pH 7.0), 0.1 mM NAD(P)H, 5 mM substrate, and enzyme solvent in a total volume of 1 mL. The reaction was initiated by adding 20 μL solvent containing 10 μg–40 μg of enzymes. Blanks without the enzyme were carried out for each substrate, and data were collected in triplicate. Protein concentrations were determined with the bicinchoninic acid assay using bovine serum albumin as a standard.

NAD(P)H or NADH was assayed under standard reaction conditions for the study of coenzyme dependence. A range of substrates from 1 to 20 mM concentration was assayed under the standard reaction conditions for the study of kinetics. Apparent values of Michaelis constant (*K*m) and *k*cat were calculated by fitting the data into Michaelis–Menten equation using the SigmaPlot (Systat Software Inc., San Jose, CA, USA). All reactions followed Michaelis–Menten-type kinetics.

Enantioselective reduction of ketones. The enantioselectivity of the enzymes was determined by examining the reduction of aryl ketones, ethyl 4-chloroacetoacetate (COBE), and ethyl 2-oxo-4-phenylbutyrate (OPBE) using an NAD(P)H regeneration system consisting of BsGDH and glucose. The general procedure was as follows: D-glucose (0.5%), recombinant BsGDH (10 U), $NAD(P)^+$ (0.1 mM), the recombinant cell (30 g L^{-1}, wet weight), and ketone solvated in ethanol [1 g L^{-1}, 10%(v/v)] were mixed in a potassium phosphate buffer (10 mL, 100 mM, pH 7.0). The mixture was shaken at 30 °C for 12 h.[22] Upon termination of the reaction, each sample was extracted twice with equivalent ethyl acetate. The organic layer was removed, dried, diluted in the mobile phase, and then subjected to chiral high-performance liquid chromatography (HPLC) to determine the conversion and enantiomeric excess (e.e.). Chiral HPLC analysis was performed on an Agilent 1100 series HPLC system with a UV detector.[23] Chiral CHBE was analyzed on a chiracel OB-H column (Daicel, Japan) at λ210 nm using hexane/2-propanol (90/10, v/v) as eluent at a flow rate of 0.8 mL min^{-1} and a temperature of 25 °C. Chiral HPBE and 4-phenyl-2-butanol were analyzed on a chiracel OD-H column at λ210 nm and λ254 nm using hexane/2-propanol (98/2, v/v) as eluent at a flow rate of 1.0 mL min^{-1} and a temperature of 30 °C. Authentic (relevant) standards were used for peak identification, and quantification was based on the peak area that was suitably calibrated with standards of known concentration.

Results and discussion

Selection of carbonyl reductases and analysis of the sequences

According to the genome of *G. oxydans* 621H, 21 ORFs were predicted to be putative carbonyl reductases by sequence analysis and possibly reduce carbonyl substrates.[16] Three of them, Gox0458, Gox1458, and Gox1538, were previously expressed in *E. coli* and had no activities of aldo/keto reduction and alcohol/aldehyde oxidation (Schweiger PB, unpublished results). In our previous study, the gox1139, gox1034, gox0601, and gox2371 genes were heterologously overexpressed in *E. coli*, and their corresponding proteins had no activities toward ketones. The Gox2378 and Gox0950, predicted to locate on the membrane, were insoluble expressed in *E. coli*, as same as Gox0716. The insoluble proteins had no detected activity with tested ketones after detergent treatment. Gox1899 is an aldehyde reductase that can efficiently catalyze the reduction of aldehydes while Gox2181 can oxidize polyol as polyol dehydrogenase. Both of them showed no activity toward ketones.[18,24] Gox0646 had a very narrow substrate spectrum, exclusively exhibited activities toward diketones rather than ketoester, hydroxy ketone, and aryl ketone.[25] Gox0644 and Gox1615 displayed broad substrate specificities and asymmetric reduction of aliphatic ketones to produce chiral α-hydroxycarbonyls.[26,27]

Furthermore, analysis using a nonredundant Basic Local Alignment Search Tool (BLAST) in GenBank and protein databases revealed that Gox2036 shared 50% and 54% of amino acid identities with diacetyl reductase from *Klebsiella terrigena* and *Corynebacterium glutamicum*, respectively.[28] Gox0525 exhibited a similar domain of the 3-ketoacyl-acyl carrier protein reductase gene (fabG), which was responsible for the reduction of prochiral ketones. The FabG homolog from *E. coli* can reduce ethyl 4-chloroacetoacetate; meanwhile, the recombinant FabG from *Synechococcus* sp. can asymmetrically reduce various prochiral ketones with good to excellent enantioselectivities.[13,29] BLAST-P analysis revealed that the amino acid sequences of Gox1462, Gox1598, and Gox0290 had putative conserved domains of the aldo–keto reductase superfamily. The analyzed carbonyl reductases shared a key catalytic tetrad for substrate binding, composed of four amino acids: N, S, Y, and K residues in Gox0525 and Gox2036, and D, Y, K, and H residues in Gox0644, Gox1615, Gox1598, Gox 1462, and Gox0290 (Fig. 1), respectively. These strict conservative residues presumably formed the framework for a proton relay system according to previous mutagenetic and structural studies on reaction mechanism.[30,31] Rather, the specificity and stereoselectivity were probably defined by the geometry of the active site, resulting from X-ray crystallographic studies.[32] Therefore, the enzyme set, consisting of Gox2036, Gox0525, Gox1615, Gox0644, Gox1462, Gox1598, and Gox0290, was assembled so as to investigate their substrate selectivity and stereoselectivity toward the reduction of ketones.

Expression and protein purification

These ORFs were amplified from the genomic DNA of *G. oxydans* DSM2343, and the amplified DNA fragments were cloned and successfully expressed in *E. coli*. The pSE380, pET28a, and pET32a vectors were used to carry genes to express in *E. coli* for obtaining soluble recombinant enzymes. Gox1598 and

Fig. 1. Sequence alignment of amino-acid sequences of putative carbonyl reductases.

Notes: The key catalytic tetrad of N, S, Y and K residues found in Gox0525 and Gox2036, and D, Y, K and H residues found in Gox0644, Gox1615, Gox1598, Gox1462 and Gox0290 are marked with *. CsKR, Ketoreductase from the *Cyanobacterium Synechococcus* sp. Strain PCC 7942 (ABB56716.1); ScCR, NADH-dependent reductase from the *Streptomyces coelicolor* (NP_631416.1).

Gox1462 obtained a soluble expression using Rosetta (DE3) rather than BL21 (DE3) as the expressive host. The pET32a vector was more suitable for protein expression in soluble fraction than pET28a when used to carry the genes of gox1598 and gox1462. Gox0644 was expressed in soluble recombinant protein in BL21 (DE3) by inserting gox0644 into pSE380, whereas the recombinant pET28a or pET32a carrying Gox0644 formed an inclusion body in BL21 (DE3) during IPTG induction. The optimal expression conditions were screened by varying inducer (IPTG) concentrations (0.1–1 mM) and culture temperatures (25, 30, and 37 °C). Gox2036 and Gox 0525 were induced by 0.5 mM IPTG at 30 °C for 6 h; others were induced by 0.25 mM IPTG at 25 °C overnight. Finally, all enzymes were successfully expressed in soluble form in *E. coli*. The percentage of recombinant target enzymes in crude cell extract was more than 30% and was quantified by Bandscan analysis software. The recombinant enzymes were purified to homogeneity using a Ni–NTA affinity chromatography for the evaluation of enzymatic properties (Fig. 2).

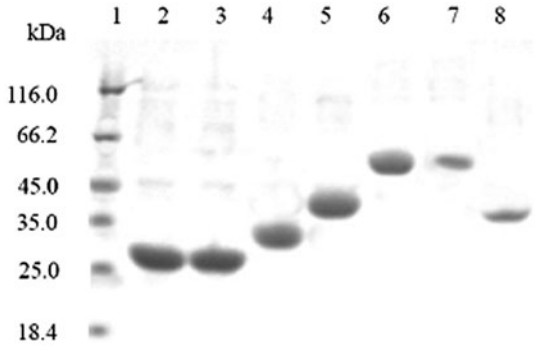

Fig. 2. SDS-PAGE analysis of purified enzymes. The purified proteins were resolved by SDS-PAGE on a 12% polyacrylamide gel and stained with Coomassie Brilliant Blue G-250.

Notes: Lane 1, molecular mass standard; Lane 2, purified Gox2036; Lane 3, purified Gox0525; Lane 4, purified Gox0644; Lane 5, purified Gox1615; Lane 6, purified Gox1598; Lane 7, purified Gox1462; Lane 8, purified Gox0290.

Substrate and coenzyme specificity

It is reported that carbonyl reductases had abilities to asymmetrically reduce carbonyl compounds to produce optically active alcohols, differing in stereospecificities and substrate specificities.[10] Therefore, the enzymes were evaluated for their abilities to reduce various ketones and ketoesters with diverse side groups.

As shown in Table 2, all enzymes showed activities toward OPBE, ethyl pyruvate, and COBE, all of which belonged to ketoesters containing α-carbonyl or

Table 2. Substrate spectrum of carbonyl reductases from *G. oxydans*.

Substrate	Gox2036 activity	Gox0525 activity	Gox0644 activity	Gox1615 activity (U/mg)	Gox1598 activity	Gox1462 activity	Gox0290 activity
Acetophenone	1.29	2.1	2.39	NMA[a]	NMA	NMA	NMA
1-phenyl-2-propanone	3.53	0.6	2.48	NMA	NMA	NMA	NMA
4-phenyl-2-butanone	8.89	2.0	3.72	NMA	NMA	NMA	NMA
1-phenyl-1,2-propanedione	8.01	10.1	70.4	NMA	NMA	NMA	NMA
2,3-butanedione	121.2	32.4	63.4	6.91	0.34	NMA	4.56
2,3-pentanedione	39.8	21.5	51.9	15.4	0.21	NMA	3.60
Ethyl benzoylacetate	2.62	3.9	NMA	NMA	1.06	4.1	2.08
Ethyl pyruvate	6.7	3.7	54.6	1.12	2.89	3.0	3.14
OPBE	3.44	7.15	4.5	2.31	2.16	28.8	4.06
COBE	3.07	2.82	5.71	2.92	0.21	5.69	1.30
Ethyl phenylacetate	NMA	NMA	NMA	NMA	0.20	13.7	2.08

[a]NMA, no measurable activity.

Table 3. Kinetic parameters.

Enzyme	4-phenyl-2-butanone			COBE			OPBE		
	*K*m (mM)	*k*cat (s^{-1})	*k*cat/*K*m ($M^{-1}s^{-1}$)	*K*m (mM)	*k*cat (s^{-1})	*k*cat/*K*m ($M^{-1}s^{-1}$)	*K*m (mM)	*k*cat (s^{-1})	*k*cat/*K*m ($M^{-1}s^{-1}$)
Gox2036	0.34	0.04	120	0.77	1.08	1.40×10^3	1.47	0.42	290
Gox0525	1.71	0.51	300	1.27	5.20	4.09×10^3	2.97	51.04	1.719×10^4
Gox0644	13.6	1.02	70	79.01	48.68	620	0.81	1.57	1.94×10^3
Gox1615	NMA [a]	NMA	NMA	6.76	4.11	610	3.73	25.69	6.89×10^3
Gox1598	NMA	NMA	NMA	1.58	1.11	2.37×10^3	0.47	0.96	2.04×10^3
Gox1462	NMA	NMA	NMA	2.30	0.83	360	2.21	2.30	1.04×10^3

[a]NMA, no measurable activity.

β-carbonyl groups. Gox2036, Gox0525, and Gox0644 reduced aryl ketones, whereas others showed no activities. The reduction of aliphatic diketone can be detected using all enzymes, except Gox1462 (Table 2). Gox1462 exhibited no detectable activities on the tested aryl and aliphatic compounds. Gox2036, Gox0525, and Gox0644 displayed broad substrate acceptances, including aryl, aliphatic, α- and β-carbonyl ketones.

Kinetic parameters (*K*m, *k*cat, and *k*cat/*K*m) were determined for the three important ketones by nonlinear regression using a Sigma Plot program for each enzyme (Table 3). Gox0525 showed useful specificity for 4-phenyl-2-butanone, COBE, and OPBE, with *k*cat/*K*m values of 300, 4.09×10^3, and $1.719\times10^4\ M^{-1}\ s^{-1}$, respectively. Gox2036, Gox0525, and Gox0644 exhibited low-catalytic efficiency for the reduction of 4-phenyl-2-butanone. The apparent *K*m value of Gox2036 for COBE was 0.77 mM, which is lowest in the test enzymes. The *K*m value is similar to that of BYueD (0.70 mM) from *Bacillus* sp.ECU001327)[33] and much lower than those of ARII (1.49 mM) from *Sporobolomyces salmonicolor*, S1 (4.6 mM) from *Candida magnolia*, and PsCRI (4.9 mM) and PsCRII (3.3 mM) from *Pichia stipitis*.[34] These results demonstrated that the Gox2036 could reach its maximal reactive velocity at a lower substrate concentration, which is important because high concentrations of substrate can destabilize the enzyme. Although Gox1598 had the lowest *K*m of 0.47 mM, its *k*cat was also low at $0.96\ s^{-1}$. That illustrated Gox1598 had the strongest affinity for OPBE. However, only a few numbers of substrate molecules turned over per enzyme molecule per second.

In terms of coenzyme specificity, Gox2036 was strictly an NADH-dependent enzyme, whereas the others were NADPH-dependent enzymes. For industrial utility, NADH cofactor specificity would be preferable over NADPH, due to the greater stability and one-tenth to one-fifth price of the former.[35]

Enantioselective reductions of ketones

BsGDH was successfully expressed in *E. coli* to build the cofactor-regenerating system because of its high activity toward both NAD^+ and $NADP^+$.[36] In this study, BsGDH/glucose as an external NAD(P)H regeneration system was used to analyze the enantioselectivity of the enzyme.

The results of three representative reactions using 4-phenyl-2-butanone (aryl ketone), COBE (β-keto ester), and OPBE (α-ketoester and aryl group) as the substrates were summarized in Fig. 3. Gox2036 demonstrated moderate stereoselectivity (S-type, 56% e.e.) toward 4-phenyl-2-butanone, but excellent enantioselectivity (approximately 100%) was obtained

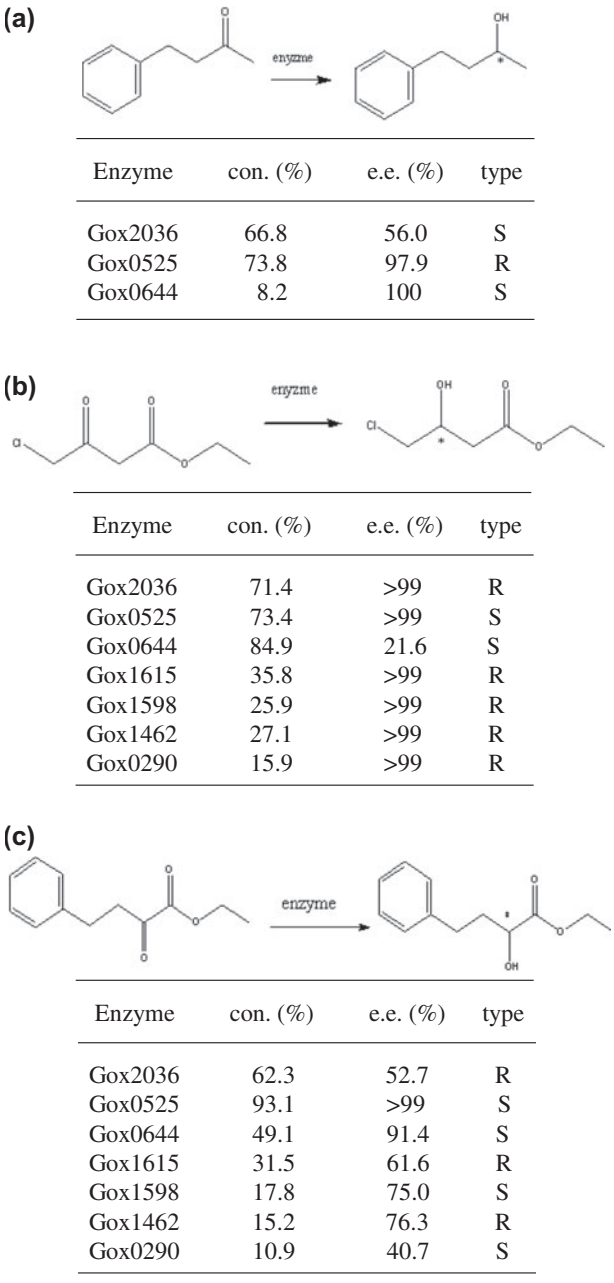

Fig. 3. Asymmetric reduction of ketones and ketoesters catalyzed by carbonyl reductases *from G. oxydans* in the presence of BsGDH/glucose with an additionally coupled NAD(P)H regeneration system.
Notes: (a) 4-phenyl-2-butanone; (b) COBE; and (c) OPBE.

for the bioreduction of acetophenone and 1-phenyl-2-propanone.[37] Gox0644 produced an (S)-type alcohol with 100% e.e. and the lowest conversion (8.2%). By contrast, Gox0525 produced an R-type alcohol with 97.9% e.e. and 73.8% conversion (Fig. 3(a)). All the tested recombinant enzymes catalyzed COBE with an excellent e.e. of up to 99%, except Gox0644. R-CHBE was obtained by Gox2036, Gox1615, Gox1598, Gox1462, and Gox0290 while Gox0525 and Gox0644 afforded the configuration as S. Gox2036, Gox0525, and Gox0644 had higher conversions than the other tested enzymes with regard to CHBE production (Fig. 3(b)). Excellent e.e. values (>99%) were obtained for OPBE reduction by Gox0525, whereas other enzymes showed relatively low e.e. A 93.1% conversion of (S)-HPBE production was obtained by Gox0525. Gox0644 had high enantioselectivities for the reduction of 4-phenyl-2-butanone and OPBE (100 and 91.4%); however, it showed relatively low conversions (8.2 and 49.1%)(Fig. 3(c)).

Conclusions

In this study, we rapidly characterized biocatalysts based on genome database from *G. oxydans*. Seven ORFs were successfully isolated and overexpressed as a functional recombinant protein in *E. coli*. All carbonyl reductases showed activities toward ethyl pyruvate, COBE, and OPBE. Three types of carbonyl substrates were selected to be asymmetrically reduced by these recombinant enzymes for their reductive chiral products, which can be used to synthesize biologically and pharmacologically important materials. Among the tested enzymes, Gox2036 had a strict requirement for NADH as the cofactor and showed excellent enantiospecificity in synthesis of (R)-CHBE. Gox0525 reduced tested ketones with high e.e. (from 97.9 to 99%) and conversion (from 73.4 to 93.1%), even under the unoptimized experimental conditions. Therefore, these enzymes had potentials in the synthesis of chiral alcohols as useful biocatalysts. It is noted that the Gox0525 exhibited anti-Prelog selectivity in the sense of stereochemistry outcomes.[38]

Author contribution

D. Wei and J. Lin designed the study. R. Chen carried out the bulk of the experiments. X. Liu contributed to the expression and purification of the reductase. All authors have read and approved the final manuscript.

Supplemental material

The Supplemental material for this paper is available at http://dx.doi.org/10.1080/09168451.2014.925775.

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