A novel carbonyl reductase with anti-Prelog stereospecificity for the production of *t*-butyl 6-cyano-(3*R*, 5*R*)-dihydroxyhexanoate

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Abstract

A novel gene (*crc1*) from *Candida boidinii* was cloned and then overexpressed in a recombinant strain BL21(DE3)/pET30a-*crc1* of *Escherichia coli*. The resulting carbonyl reductase was prepared through fermentations using the recombinant strain. The purified enzyme showed an NADPH-dependent activity and specific activity was 4.65 U/mg using *t*-butyl 6-cyano-(5*R*)-hydroxy-3-oxohexanoate (ATS-6) as substrate. The enzyme was optimally active at 35 °C and pH 7, respectively. The apparent K_m and V_{max} of the enzyme for ATS-6 are 1.5 mM and 21.1 $\mu\text{mol}/\text{min mg}$, respectively, indicating excellent anti-Prelog stereospecificity. Under the optimum condition, *t*-butyl 6-cyano-(3*R*,5*R*)-dihydroxyhexanoate (ATS-7) was prepared with the enzyme with high *d.e.* value (99.9%) and good conversion (94%) in 4 h, indicating high stereoselectivity and conversion efficiency in biotransformation of ATS-6 to ATS-7.

Keywords Biocatalysis · Carbonyl reductase · Characterization · Enzyme activity · Expression

Introduction

Carbonyl reductases (EC 1.1.1.148) catalyze the reduction of a variety of carbonyl compounds to their corresponding hydroxy derivatives. They belong to a family of short-chain dehydrogenases/reductases and are widely distributed in many organisms, especially microorganisms (Forrest and Gonzalez 2000). Carbonyl reductase is an attractive tool for the preparation of optically active alcohols containing at least one asymmetric center due to their chemo-, regio-, and enantioselectivities (Nakamura et al. 2003; Ye et al. 2011). However, most carbonyl reductases catalyzing asymmetric reductions generally follow the Prelog's rule, while there are

limited carbonyl reductases with anti-Prelog stereoselectivity responsible for transformation of chiral alcohols (Itoh 2014; Liang et al. 2013; Shah et al. 2018).

t-Butyl-6-cyano-(3*R*, 5*R*)-dihydroxyhexanoate (ATS-7) containing two hydroxyl groups is a key precursor for atorvastatin, widely applied for treating hypercholesterolaemia. In commercial production, chiral alcohols are mainly prepared chemically, but often suffer from high cost, poor atom economy due to a complicated synthetic route, and poor safety together with heavy metal pollution (Liljeblad et al. 2009). Biocatalysis using isolated enzymes or whole-cell systems was focused because of the advantage of stereoselectivity, mild reaction conditions, non-toxicity and no residue of heavy metal (Huang et al. 2010). Carbonyl reductases are the best biocatalysts for this reduction reaction (Fig. 1). So far, only a few microorganisms such as *Saccharomyces cerevisiae* (Giver et al. 2013), *Pichia angusta* (Reeve 1997), *Pichia caribbica* (Sheng et al. 2013), *Pichia guilliermondi* (Mao et al. 2012), and *Rhodotorula glutinis* (Luo et al. 2016) have been isolated for the transformation of *t*-butyl 6-cyano-(5*R*)-hydroxy-3-oxohexanoate (ATS-6) to ATS-7. Some carbonyl reductases from above strains have been selected for the preparation of ATS-7. They have exhibited high catalytic activity and high selectivity for the asymmetric synthesis (Luo et al. 2016; Wu et al. 2015; Xiao et al. 2013). But there are some problems, such as high cost, low stability of

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Fig. 1 Bioreduction of *t*-butyl 6-cyano-(5*R*)-hydroxy-3-oxohexanoate (ATS-6) to ATS-7 by carbonyl reductase

enzymes and biocatalytic efficiency. Furthermore, there are few carbonyl reductases catalyzing transformation of ATS-6 to ATS-7 following the anti-Prelog's rule (Wu et al. 2015). Therefore, screening for a new type of carbonyl reductase with anti-Prelog stereopreference is still a valuable and necessary strategy to improve achievement of chiral side chain of atorvastatin.

We previously isolated a strain of *Candida boidinii* CGMCC 9446 that possesses carbonyl reductase activity responsible for the transformation of ATS-6 to ATS-7 (Jin et al. 2017). The objective of the present study was to clone, express, purify and characterize this carbonyl reductase. The results revealed that the carbonyl reductase has high stereoselectivity, making it a promising biocatalyst for asymmetric synthesis of chiral alcohol.

Materials and methods

Materials

Candida boidinii CGMCC 9446 (Jin et al. 2017) was used for the preparation of the gene (*crc1*) and its protein (Crc1) of carbonyl reductase. *Escherichia coli* BL21 (DE3) and plasmid pET30a (Generay, China) were used for the over-expression of the recombinant *crc1*. *t*-Butyl-6-cyano-(5*R*)-hydroxy-3-oxohexanoate (ATS-6, 99.9% *e.e.*) and *t*-butyl 6-cyano-(3*R*, 5*R*)-dihydroxyhexanoate (ATS-7) were purchased from J&K Scientific Ltd, China. All other biologicals and chemicals were of analytical grade purity and commercially available.

Construction and expression of Crc1

Genomic DNA of *C. boidinii* CGMCC 9446 was extracted by a DNA extraction kit (Promega, USA). The forward primer 5'-CGCCATATATGACAGGCGCA-3' and reverse primer 5'-GCGGATCCTTACGTGGTCCG-3' were synthesized by Generay Biotechnology Corp., China, and then used for the PCR amplification of *crc1* from genomic DNA with Phusion DNA polymerase using the manufacturer's instruction (Thermo Scientific, USA). Purification of PCR products, restriction enzyme digestion, and ligation were carried out using standard techniques with Gel Extraction Kit (Omega Biotek, USA), FastDigest restriction enzymes

and T4 DNA ligase (Thermo Scientific, USA) following the protocols of the manufacturers. The gene was constructed on plasmid pET30a, and then was transformed into strain BL21(DE3) to obtain a recombinant strain BL21(DE3)/pET30a-*crc1* (BL21-pET30a-*crc1*) of *E. coli*. Extraction of plasmid was performed with Axyprep Plasmid Miniprep Kit (Axygen, China). The gene *crc1* in the plasmid pET30a-*crc1* was sequenced by Generay Biotechnology Corp, and then the deduced amino acid sequence was aligned with the reference genes obtained from the GenBank database by the multiple-sequence alignment software CLUSTAL X Version 2.0.

The recombinant strain was first inoculated in 1 mL lysogeny broth (LB) containing 50 µg kanamycin/mL at 37 °C for 7–10 h. The culture was then transferred to 50 mL LB (50 µg kanamycin/mL) in a 250-mL flask. The cells were grown at 37 °C and 250 rpm for about 2 h to reach an OD₆₀₀ of 0.6, followed by optimization of protein expression at different temperatures, with different IPTG concentrations and for different induction times (see Supplementary Fig. 3). Under the optimal condition, expression of Crc1 was induced and the resulting cells were collected by centrifugation (3500g, 10 min). Cell pellets were resuspended in an appropriate buffer as resting cells, and cell density was recorded by measuring OD₆₀₀ value.

Purification of Crc1

For a large amount of preparation of the strain BL21-pET30a-*crc1*, the cell was inoculated into flasks containing 70 mL LB (50 µg kanamycin/mL). After 8 h cultivation, the cultures were then inoculated into 15-L fermentor containing 7 L fermentation medium (1% glucose, 1.5% peptone, 0.5% yeast extract, 0.3% K₂HPO₄, 0.2% MgSO₄, 0.2% citric acid, 0.4% (NH₄)₂SO₄, pH 7) and cultured at 37 °C, 300 rpm for 10 h. pH was maintained at 7 using 25% (v/v) NH₄OH. When OD₆₀₀ value reached about 1, protein expression was induced at 28 °C by the addition of 1 mM IPTG. After induction, cells were harvested by centrifugation at 4 °C, 8000g for 10 min. Cells were suspended in 50 mM sodium phosphate (pH 7) and then disrupted by a nano-homogenizer machine (ATS, Canada). Cell lysate was collected via centrifugation at 9000g for 30 min and used as crude enzyme of Crc1.

Protein purification was performed using an AKTA prime system. Crc1 was purified using a HisTrap FF column (5 mL, GE Healthcare, USA). The column was pre-equilibrated with 50 mL of 50 mM sodium phosphate buffer, 300 mM NaCl, 5 mM imidazole, pH 7. Cell lysate was loaded at 1 mL/min. Crc1 was eluted with 50 mM sodium phosphate buffer, 300 mM NaCl, 150 mM imidazole, pH 7. Fractions containing Crc1 were collected and dialyzed with 50 mM sodium phosphate buffer (pH 7) for desalting. The purified enzyme was analyzed by SDS-PAGE and used for enzymatic assays. Protein concentration was estimated using a bicinchoninic acid protein assay kit.

Enzyme activity assay

Enzyme activity was determined in a reaction containing 100 mM sodium phosphate buffer (pH 7), 2 mM ATS-6, and 0.5 mM NADPH. The reduction of ATS-6 was calculated by measuring the decrease in absorption of NADPH at 340 nm and 25 °C. One unit (U) of activity is defined as the amount of enzyme that consumes 1 μ mol NADPH per min under the above assay conditions.

Kinetic analysis

The purified Crc1 was used for kinetic analysis. The reaction rate was determined with ATS-6 from 0.05 to 2.25 mM and NADH or NADPH at 0.001–1.0 mM at 25 °C in 100 mM sodium phosphate buffer (pH 7). Kinetic parameters were calculated from Lineweaver–Burk plots of enzymatic reaction rates (Wang et al. 2011).

Effects of temperature, pH, metal ions and organic solvents

Enzymatic activity and stability were determined at various temperatures from 10 to 55 °C and pH values from 4 to 9.5 in the presence or absence of metal ions or organic solvents. The residual enzyme activity was tested under the standard assay condition.

Biosynthesis of ATS-7

The crude enzyme of Crc1 was used as biocatalyst in the synthesis of ATS-7. Biotransformation reaction was performed in flasks at 300 rpm. A 20 mL reaction comprised 0.8 M ATS-6, 1 M glucose and 120 μ M NADP in 100 mM sodium phosphate buffer (pH 7). The crude enzymes of Crc1 (equivalent to 12 g DCW cells/L) and glucose dehydrogenase (equivalent to 6 g DCW cells/L) were added to the flask, respectively, and the reaction was performed for 7 h at 25 °C. Samples were taken periodically and supernatant was prepared by centrifugation at 3500g for 10 min.

HPLC analysis

The production of ATS-7 was determined by reverse-phase HPLC. A column packed with Hypersil ODS-2 C18 column (2.5 μ m; 4.6 mm $\times$ 250 mm) (Elite, China) was developed with acetonitrile–water ($v/v = 1:3$), at 1 mL/min at 35 °C. ATS-6 and ATS-7 were detected by UV absorption at 210 nm.

The values of enantiomeric excess (*e.e.*) and diastereomeric excess (*d.e.*) of ATS-7 were detected with chiral HPLC. A column packed with SDMPB130718(5) (2.5 μ m; 4.6 mm $\times$ 250 mm) (Guangzhou Research & Creativity, China) was developed with hexane–ethanol ($v/v = 3:1$), at 1 mL/min. ATS-7 was detected using evaporative light-scattering detector (ELSD) at an air flow of 1.5 L/min.

The retention times for ATS-7 and ATS-6 were 13.1 min and 14.2 min, respectively, on the reverse-phase HPLC column, and the retention times for ATS-7, and its (3*S*, 5*S*)-enantiomer, (3*S*, 5*R*)-non-enantiomer, (3*R*, 5*S*)-non-enantiomer were 7.2 min, 7.7 min, 8.0 min, and 8.6 min, respectively, on the chiral column. The yield of ATS-7 was calculated using the formula: $\text{yield} = (1 - \text{MS}/\text{MS}_0) \times 100\%$, where MS is the number of mole of ATS-6 in product and MS_0 is the number of mole of ATS-6 added. The *e.e.* value of ATS-7 was defined as the ratio of $(A_{[3R, 5R]} - A_{[3S, 5S]}) / (A_{[3R, 5R]} + A_{[3S, 5S]}) \times 100\%$, and the *d.e.* value was defined as the ratio of $(A_{[3R, 5R]} - A_{[3S, 5R]}) / (A_{[3R, 5R]} + A_{[3S, 5R]}) \times 100\%$, where $A_{[3R, 5R]}$, $A_{[3S, 5S]}$ and $A_{[3S, 5R]}$ are the peak areas of ATS-7 and the corresponding (3*S*, 5*S*)-enantiomer and (3*S*, 5*R*)-non-enantiomer, respectively.

Results and discussion

Amino acid sequence comparison of carbonyl reductase

A novel gene (*crc1*) was identified previously from *C. boidinii* CGMCC 9446 (Jin et al. 2017). The gene *crc1* included one complete open reading frame with a length of 936 bp coding for 311 amino acid residues of carbonyl reductase. The deduced amino acid sequence (Crc1) was compared with other carbonyl reductases such as Cmc1 from *Kluyveromyces marxianus* (GenBank accession no. AB183149.1) (Kataoka et al. 2006) and Cr1 from *Saccharomyces cerevisiae* (GenBank accession no. NP_010159.1) (Wu et al. 2015) used for asymmetric biosynthesis of ATS-7. A higher level of identity was found with the sequences of Cmc1 (86% similarity) than Cr1 (20% similarity), and one conserved sequence of NADPH-binding motif GXXGXXA was found between residues 3 and 9 of the Crc1 sequence (Supplementary

Fig. 1). So, it could be deduced that Crc1 might be a novel NADPH-dependent enzyme useful for biotransformation of chiral alcohol.

Optimization of Crc1 expression

For heterologous expression of the carbonyl reductase, 936 bp open reading frame of *crc1* was amplified by PCR from *C. boidinii* CGMCC 9446 (Supplementary Fig. 2a). Then a recombinant strain BL21(DE3)/pET30a-crc1 (BL21-pET30a-crc1) was constructed and the expression of Crc1 was investigated in *E. coli*. The enzyme activity was increased gradually during the fermentation and showed close correlation with the biomass of the recombinant cells. SDS-PAGE revealed a band with estimated molecular weights of 35 kDa in the recombinant cell (Supplementary Fig. 2b), which was nearly identical to the predicted molecular mass (35 kDa) of Crc1.

Temperature, IPTG concentration and induction time play important roles during protein expression. To reach an optimal expression, different temperatures, IPTG concentrations and induction times were investigated. It was found that protein expression reached the highest specific activity of 0.78 U/mg at 28 °C by the addition of 1 mM IPTG after 8-h induction (Supplementary Fig. 3).

Fermentation of the recombinant strain BL21-pET30a-crc1

To further test the potential enzymatic activity of the recombinant strain BL21-pET30a-crc1 at a scale-up condition, a fermentation of 7 L was performed. It was observed that the amount of glucose was decreased sharply after 20 h meaning much nutrition was consumed and the cells grew quickly (Fig. 2). After the initial amount of glucose was depleted, glucose was fed at a constant flow rate of 2 g/L, and final concentration of 1 mM IPTG was also added to induce protein expression at 28 °C. After 3 h of induction, catalytic activity was tested and the result showed that *crc1* was successfully expressed in *E. coli* at the scale-up condition. In 48 h of the cultivation, the biomass of the recombinant cells and the specific activity of Crc1 reached maxima with 22.7 g DCW/L and 0.65 U/mg (Fig. 2), which were increased by 115% and 270%, respectively, compared with those without glucose feeding. After this time, the biomass and the enzymatic activity did not change significantly, indicating that the equilibration period of cell growth arrival (Fig. 2).

Enzyme purification of Crc1

After fermentation, the cells were harvested and disrupted using a high-pressure homogenizer. The supernatant was obtained as crude enzyme extract with specific activity of 0.48 U/mg protein (Table 1). The crude enzyme was purified

Fig. 2 Fermentation curve of the recombinant BL21(DE3)/pET30a-crc1 of *Escherichia coli* under a pH-state feeding strategy. Dry cell weight (DCW, closed triangle), glucose concentration (closed circle)

and enzyme activity (closed square) of the recombinant strains were detected, respectively, during fermentation

Table 1 Purification of Crc1 from the recombinant BL21(DE3)/pET30a-crc1 of *Escherichia coli*

Step ^a	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Recovery (%)	Purification
Crude extraction	96.4	200	0.48	100	1.0
HisTrap column purification	56.2	12	4.65	58	9.7

^aThese activities were measured at 25 °C

using His-tagged protein purification system. The yield of the purified enzyme was 58% with specific activity of 4.65 U/mg and 9.7-fold purification (Table 1). SDS-PAGE of the purified enzyme also revealed that Crc1 had been purified successfully (Supplementary Fig. 2c). So the purified enzyme was used to investigate biochemical properties of Crc1.

Characterization of Crc1

Kinetic parameters of Crc1 for the substrate (*t*-butyl 6-cyano-(5*R*)-hydroxy-3-oxohexanoate, ATS-6) and the coenzyme (NADH or NADPH) were investigated. It showed that Crc1 preferred NADPH and the apparent Michaelis constant (K_m) was 0.01 mM. No activity on NADH was observed indicating that the carbonyl reductase possesses NADPH-dependent activity. Crc1 showed kinetic parameters of K_m (1.5 mM) and maximum reaction rate (V_{max} , 21.1 $\mu\text{mol}/\text{min mg}$) for ATS-6, indicating good anti-Prelog stereospecificity. So far, ketoreductases with anti-Prelog stereospecificity from yeasts are still limited (Table 2). V_{max} of Crc1 was excellent among these ketoreductases indicating that Crc1 has a prominent reaction rate for the production of the chiral alcohol compared with other ketoreductases from yeasts (Table 2). In addition, Crc1 is the first reported type of carbonyl reductase

with anti-Prelog stereospecificity from *C. boidinii* useful for ATS-7 formation.

Appropriate reaction temperature and pH are required to achieve the best catalytic efficiency. Effect of temperature on enzymatic activity of Crc1 was studied by performing the enzyme activity assay at 10–55 °C (Fig. 3a). The activity increased as temperature increased from 10 to 35 °C. However, the activity decreased rapidly when temperature was above 35 °C. Thermal stability of Crc1 is depicted in Fig. 3b. About 60% of enzymatic activity was maintained after 30 min of incubation at 35 °C. However, the activity of Crc1 was completely lost after incubation at 55 °C for 30 min. The activity of Crc1 was monitored from pH 4 to 9.5 (Fig. 3c). The optimum pH for Crc1 was 7, with 100% of the activity. The pH stability profile showed that Crc1 was highly stable in acidic environment and more than 80% of the activity was maintained when exposed to pH 5.5–7.5 for 60 min at 25 °C (Fig. 3d).

Effects of various metal ions on the activity of Crc1 are shown in Table 3. The enzyme displayed a higher activity in the presence of Ca^{2+} , K^+ , Na^+ and Mn^{2+} and a strong inhibition in the presence of Cu^{2+} and Fe^{3+} . The metal-chelating reagent EDTA slightly reduced the activity indicating non-metal dependence of Crc1, different from many representative carbonyl reductases (He et al. 2014). Effects of organic solvents on enzyme activity showed that ethanol

Table 2 Representative NADPH-dependent ketoreductases with anti-Prelog stereoselectivity from yeasts

Enzyme	LEK	CgKR2	CPADH	CR1	Crc1
Source	<i>Lodderomyces elongisporus</i>	<i>Candida glabrata</i>	<i>Candida parapsilosis</i>	<i>Saccharomyces cerevisiae</i>	<i>Candida boidinii</i>
Specific activity (U/mg)	0.18	10.6	2.58	105	4.65
Substrate	COBE	OPBE	PED	ATS-6	ATS-6
Product	(<i>R</i>)-CHBE	(<i>R</i>)-HPBE	(<i>S</i>)-PED	ATS-7	ATS-7
K_m (mM)	37.01	0.1	5.83	0.25 ^a	1.52
V_{max} ($\mu\text{mol}/\text{min mg}$)	0.67	18.5	18.3	–	21.1
Temperature (°C)	35	45	35	20	35
pH	6	6	4.5	7	7
Conversion (%)	100	91	–	99%	94%
<i>d.e.</i> (%)	99	99	99	99.5	99.9
References	Wang et al. (2014)	Shen et al. (2012)	Nie et al. (2011)	Wu et al. (2015)	This work

COBE ethyl 4-chloro-3-oxobutanoate, (*R*)-CHBE ethyl (*R*)-4-chloro-3-hydroxybutanoate, OPBE ethyl 2-oxo-4-phenylbutyrate, (*R*)-HPBE ethyl (*R*)-2-hydroxy-4-phenylbutyrate, PED 1-phenyl-1,2-ethanediol

^aThe value was obtained using ethyl 3-methyl-2-oxobutanoate as substrate (Ishihara et al. 2004)

Fig. 3 Effects of temperature and pH on the activity and the stability of Crc1. **a** The optimal temperature was determined by assaying the activity at 10–55 °C in 100 mM sodium phosphate buffer (pH 7). **b** The thermostability was evaluated by measuring the residual activity at 25 °C after the enzyme was incubated at the above temperatures for 0.5 h in 100 mM sodium phosphate buffer (pH 7). **c** The optimal

pH was determined by measuring the activity in 100 mM citric acid–sodium citrate buffer (pH 4–6, closed square), sodium phosphate buffer (pH 6.5–8, closed triangle) and glycine–NaOH buffer (pH 8.5–9.5, closed circle). **d** The pH stability was analyzed by measuring the residual activity after the enzyme was pretreated with the above buffers at 25 °C for 1 h

Table 3 Effects of various metal ions on the activity of Crc1

Metal ion	Concentration (mM)	Relative activity ^b (%)
Control ^a	—	100.0 ± 0.6
MnSO ₄	5	149.2 ± 5.1
NaCl	5	128.4 ± 4.2
CaCl ₂	5	115.1 ± 3.9
KCl	5	114.4 ± 4.6
EDTA	5	89.8 ± 2.6
MgSO ₄	5	40.5 ± 1.6
CuSO ₄	5	7.2 ± 0.8
FeCl ₃	5	2.2 ± 0.4

^aThe metal ion was absent from treatment. The specific activity of the control was 4 U/mg

^bEffect of metal ion on activity was assayed by measuring the residual activity under the standard condition after the enzyme was incubated at 25 °C for 1 h with each reagent. All reactions were performed in triplicate

at 5% (v/v) had the least inhibition leaving by 73% of the activity, and Crc1 was very unstable in most of the tested agents (Table 4).

Biocatalysis with Crc1

Compared with pure ATS-7 as a control, asymmetric reduction of ATS-6 (99.9% *e.e.*) to ATS-7 by the biocatalysis of Crc1 in the reaction mixture was monitored by reverse-phase HPLC under the optimized condition (Supplementary Fig. 4), and catalytic property of Crc1 was explored. It was found that there was no peak of the corresponding (3*S*, 5*S*)-enantiomer and (3*S*, 5*R*)-non-enantiomer of *t*-butyl 6-cyano-(3*R*, 5*R*)-dihydroxyhexanoate (ATS-7), resulting in the highly enantiomeric product of 99.9% *d.e.* by chiral HPLC of ATS-7. The time course of the production of ATS-7 is shown in Fig. 4. The highest yield of 56 mM product could be achieved within 4 h and then the

Table 4 Effects of various organic solvents on the activity of Crc1

Organic solvent	Concentration (%, v/v)	Relative activity ^b (%)
Control ^a	–	100.0 ± 0.4
Ethanol	5	73.6 ± 3.1
Isopropanol	5	41.1 ± 2.9
Acetonitrile	5	37.7 ± 2.4
Dimethyl sulfoxide	5	32.6 ± 2.8
Tetrahydrofuran	5	24.1 ± 1.1
Pyridine	5	19.8 ± 1.4

^aOrganic solvent was the absence from the treatment. The specific activity of the control was 4 U/mg

^bEffect of organic solvent on activity was assessed by measuring the residual activity under the standard condition after the enzyme was incubated at 25 °C for 1 h with each reagent. All reactions were performed in triplicate

plateau emerged. The value of *d.e.* remained at 99.9% and the conversion reached 94%.

Recently, preparations of ATS-7 were reported with wild-type cells of *R. glutinis* (Luo et al. 2016), or *E. coli* recombinant strains overexpressing carbonyl reductases from *S. cerevisiae* (Wu et al. 2015) and *R. glutinis* (Xiao et al. 2013). It was found that the production rate of ATS-7 (14 mM/h) using Crc1 from *C. boidinii* in this work was greatly higher than that (1.15 mM/h) with wild-type cells of *R. glutinis* (Luo et al. 2016), while lower than that (21.8 mM/h) with engineered cells containing carbonyl reductase from *S. cerevisiae* (Wu et al. 2015) in 1 h. It was also found that Crc1 could

improve enantioselectivity during the formation of ATS-7 and reach 99.9% *d.e.* in 4 h, which is higher than that (approximately 99% *d.e.*) of other biocatalysts (Luo et al. 2016; Wu et al. 2015; Xiao et al. 2013). Furthermore, Crc1 showed the highest stereospecificity among these ketoreductases from yeasts used for productions of other chiral alcohols (Table 2). It could be concluded that Crc1 has excellent anti-Prelog stereospecificity for the substrate and it is potentially useful for asymmetric synthesis of chiral alcohols.

Conclusions

In summary, a novel gene *crc1* encoding carbonyl reductase was found from *C. boidinii* CGMCC 9446, and the expression, purification and characterization of the enzyme were performed in this study. The carbonyl reductase was found to be a non-metal-dependent but NADPH-dependent enzyme with anti-Prelog stereoselectivity. The enzyme was characterized using *t*-butyl 6-cyano-(5*R*)-hydroxy-3-oxohexanoate (ATS-6) as substrate, showing kinetic parameters of K_m (1.5 mM) and V_{max} (21.1 μmol/min mg) for the substrate. Under the optimum condition of the enzyme, the highest yield of 56 mM *t*-butyl 6-cyano-(3*R*, 5*R*)-dihydroxyhexanoate (ATS-7) was achieved with high conversion (94%) and high optical activity (99.9% *d.e.*) in 4 h, indicating Crc1 is a promising enzyme useful for producing stereoisomerically pure ATS-7.

Fig. 4 Time course of enzymatic reduction of ATS-6 to ATS-7. Conversion (closed triangle) and *d.e.* value (closed circle) were detected and calculated during the biocatalysis, respectively

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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