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ORIGINAL RESEARCH PAPER

Characterization of alcohol dehydrogenase from *Kangiella koreensis* and its application to production of all-*trans*-retinol

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Abstract A recombinant alcohol dehydrogenase (ADH) from *Kangiella koreensis* was purified as a 40 kDa dimer with a specific activity of 21.3 nmol min^{-1} mg^{-1}, a K_m of 1.8 μM, and a k_{cat} of 1.7 min^{-1} for all-*trans*-retinal using NADH as cofactor. The enzyme showed activity for all-*trans*-retinol using NAD^+ as a cofactor. The reaction conditions for all-*trans*-retinol production were optimal at pH 6.5 and 60 °C, 2 g enzyme l^{-1}, and 2,200 mg all-*trans*-retinal l^{-1} in the presence of 5 % (v/v) methanol, 1 % (w/v) hydroquinone, and 10 mM NADH. Under optimized conditions, the ADH produced 600 mg all-*trans*-retinol l^{-1} after 3 h, with a conversion yield of 27.3 % (w/w) and a productivity of 200 mg l^{-1} h^{-1}. This is the first report of the characterization of a bacterial ADH for all-*trans*-retinal and the biotechnological production of all-*trans*-retinol using ADH.

Keywords Alcohol dehydrogenase · Enzyme characterization · *Kangiella koreensis* · All-*trans*-retinal · All-*trans*-retinol

Introduction

Retinoids, such as retinal, retinol, retinyl ester and retinoic acid, are C_{20} isoprenoids metabolically derived from the oxidative cleavage of C_{40} carotenoids (von Lintig and Vogt 2000). Retinoids and their derivatives are used in foods, cosmetics, pharmaceuticals, and nutraceuticals owing to their anti-infective, anti-cancer, anti-oxidant, and anti-wrinkle properties (Semba 1999). Retinoids are applied in the treatment of dermatological conditions such as skin cancer and photoaging (Stefanaki et al. 2005). Retinal is important for development, cell differentiation, cancer prevention, and membrane and skin protection. Retinol can treat the visible signs of premature aging, such as fine lines, age spots, and other types of pigmentation (De Luca 1991; Fisher and Voorhees 1996; Semba 1999). All-*trans*-retinoic acid (tretinoin) and 13-*cis*-retinoic acid (isotretinoin) are used in the treatment of acne and certain other skin disorders (Mukherjee et al. 2006).

Commercial production of retinoids is currently by chemical synthesis using a pentadiene derivative (Chabardes 1994). However, this process has disadvantages, including complex purification steps, chemical waste

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formation, and undesirable by-product formation. Therefore, the biological production of retinoids has been attempted as an alternative to chemical synthesis.

Retinoids can be produced from glycerol by metabolically-engineered *Escherichia coli* via fermentation (Lee et al. 2012; Jang et al. 2014). β-Carotene monooxygenase (Blh) can produce retinal from β-carotene in an enzymatic conversion (Kim et al. 2010). The formed retinal can then be converted into retinol or retinoic acid by retinal-converting enzymes, including alcohol dehydrogenase (ADH), short-chain dehydrogenase (SDR), aldo–keto reductase (AKR), and aldehyde dehydrogenase (ALDH) (Fig. 1).

Kangiella koreensis, is a Gram-negative, non-motile, non-spore-forming bacterium isolated from tidal flat sediments at Daepo Beach, Yellow Sea, Korea (Han et al. 2009). In this study, the putative ADH from *K. koreensis*, which shares a high sequence identity with animal ADHs (49–79 %), was cloned and expressed in *E. coli*. We characterized the properties of *K. koreensis* ADH for all-*trans*-retinal and used the enzyme to produce all-*trans*-retinol from all-*trans*-retinal.

Materials and methods

Gene cloning, culture conditions, and enzyme expression

Genomic DNA from *K. koreensis* SW-125, *E. coli* ER2566, and pET-22b(+) were used as the sources of ADH gene, host cells, and expression vector, respectively. The gene encoding a putative ADH was amplified by PCR using genomic DNA of *K. koreensis*. Primer sequences were based on the DNA sequence of ADH from *K. koreensis* (GenBank accession number ACV27458.1). Forward (5′-GGATCCAATGTCGAACGAAGTGATTAAATG-3′) and reverse (5′-CTCGAGATAATGAATCACGCTACGAATA-3′) primers were designed to introduce *Bam*HI and *Xho*I restriction sites (underlined), respectively. The digested PCR product was cloned into *Bam*HI and *Xho*I sites of the pET-22b(+) vector and transformed into *E. coli* ER2566.

For enzyme expression, recombinant *E. coli* cells expressing ADH protein were cultivated at 37 °C with shaking at 200 rpm in a 2 l flask containing 500 ml LB medium, supplemented with 20 μg ampicillin ml^{-1}. Cultures were induced with 0.1 mM IPTG at an OD_{600} of 0.8 and incubated for 16 h at 16 °C with shaking at 150 rpm.

Amino acid sequence and phylogenetic analyses

The amino acid sequence of *K. koreensis* ADH was compared with those of ADHs from other sources using the BLAST network at the NCBI. ADHs were chosen as the characterized enzymes for animal ADHs and the enzymes with high sequence identities above 50 % for bacterial ADHs. Manually annotated and reviewed sequence data for ADHs were retrieved from the Uniprot Database. Amino acid sequence alignment and phylogenetic tree construction were performed with a

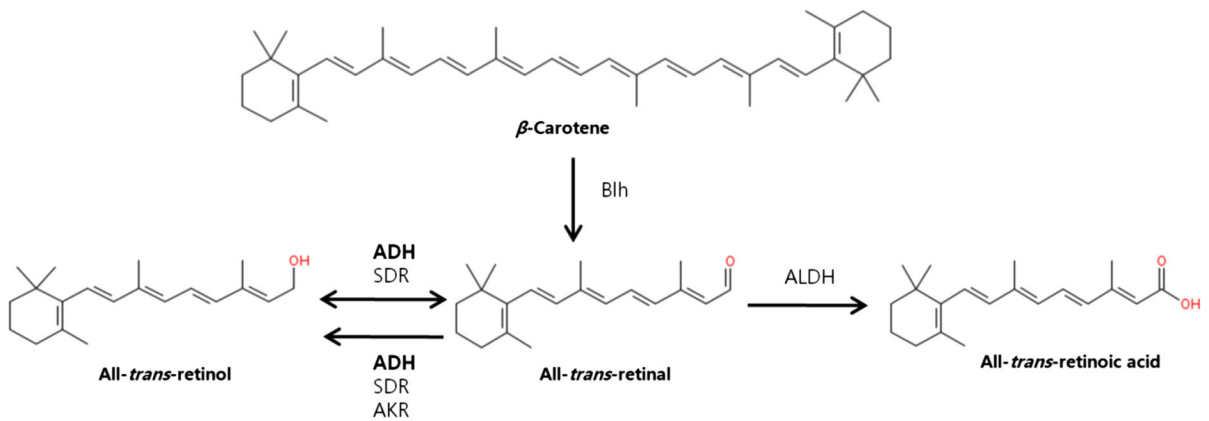


Fig. 1 Schematic representation of the formation of retinoids from β-carotenebyretinoid-producing enzymes. *Blh* β-carotene 15,15′-dioxygenase, *ADH* alcohol dehydrogenase, *SDR* short-chain dehydrogenase, *AKR* aldo–keto reductase, and *ALDH* aldehyde dehydrogenase

gap open penalty of 10 and a gap extension penalty of 0.05 using Clustal W program alignment (Kanehisa 1997). Phylogenetic tree was constructed based on the data of amino acid sequence. Metal binding site and catalytic residues were identified using our determined crystal structure of *K. koreensis* ADH (Ngo et al. 2013).

Enzyme purification

Cells were harvested from culture broth, resuspended in 50 mM phosphate buffer (pH 8.0) containing 300 mM KCl and 10 mM imidazole, and disrupted on ice using a sonicator. Unbroken cells and cell debris were removed by centrifugation at 13,000×*g* for 20 min at 4 °C, and the supernatant was applied to an immobilized metal ion affinity chromatography cartridge (Bio-Rad) equilibrated with 50 mM phosphate buffer (pH 8.0). The bound protein was eluted with a linear gradient of 10–500 mM imidazole at 1 ml min^{-1}. The active fractions were collected and loaded onto a Bio-Gel P-6 desalting cartridge (Bio-Rad) equilibrated with PIPES buffer (pH 6.5). The protein was eluted with PIPES buffer (pH 6.5) at 1 ml min^{-1} and the resulting solution was used as the purified enzyme. All purification steps using cartridges were carried out in a cold room at 4 °C with a Profinia protein purification system (Bio-Rad).

Enzyme assay

One unit of ADH activity is defined as the amount of enzyme required to liberate 1 nmol of all-*trans*-retinol per min from all-*trans*-retinal at 60 °C and pH 6.5. Unless otherwise stated, the enzymatic reaction was performed at 60 °C for 10 min in 50 mM PIPES buffer (pH 6.5) containing 0.2 mM all-*trans*-retinal and 0.5 g enzyme l^{-1} in the presence of 5 % (v/v) methanol, 1 % (w/v) hydroquinone, and 1 mM NADH. The several concentrations of all-*trans*-retinal and all-*trans*-retinol (0.01–5 mM) were used to determine the kinetic parameters and 0.2 mM substrate, including aldehyde with 1 mM $NADP^+$ or alcohol with 1 mM NADH, was used to measure the specific activity of *K. koreensis* ADH for the substrate.

Optimization of reaction conditions

The effects of pH and temperature on ADH activity for all-*trans*-retinal were examined by varying the pH from 5.5 to 7.5 at 37 °C using 50 mM MES buffer (pH 5.5–6.5) and 50 mM PIPES buffer (pH 6.5–7.5) and by varying the temperature from 40 to 70 °C in 50 mM PIPES buffer (pH 6.5). The concentration of all-*trans*-retinal as a substrate was varied from 44 to 9,570 mg l^{-1} at 1.5 g enzyme l^{-1}, and the concentration of the enzyme was varied from 0.09 to 2.5 g l^{-1} at 2,200 mg all-*trans*-retinal l^{-1}. The time course reactions were performed with 2,200 mg all-*trans*-retinal l^{-1} and 2 g enzyme l^{-1} for 3 h.

Analytical methods

An equal volume of *n*-hexane was added to each reaction solution, and the mixture was centrifuged at 13,000×*g* for 10 min at 4 °C. The supernatant was analyzed by HPLC at 350 nm with a C18 column. The product was eluted with acetonitrile/1 % ammonium acetate in water (80:20 v/v) as the mobile phase at 1.2 ml min^{-1} at 30 °C.

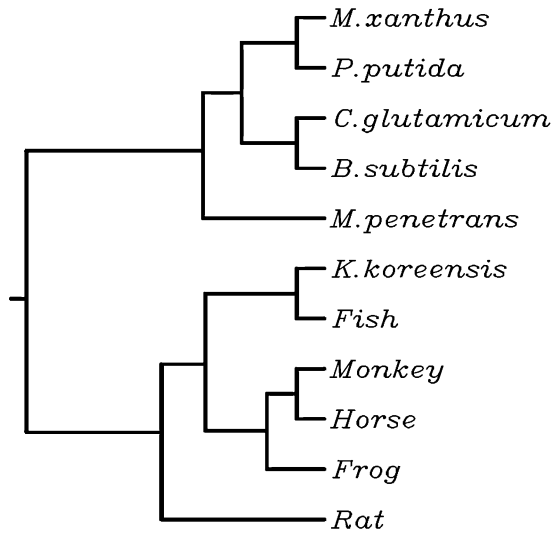
Results and discussion

Identification and characterization of the gene and protein of *K. koreensis* ADH

The gene (1,130 bp) encoding the putative *K. koreensis* ADH (Yoon et al. 2004; Han et al. 2009), having the same sequence as that reported in GenBank (accession number ACV27458.1), was cloned and expressed in *E. coli*. The expressed enzyme was purified by the immobilized metal ion affinity chromatography. The ADH showed a single band in SDS-PAGE with a molecular mass of approx. 40 kDa (data not shown), which was consistent with the calculated size (39.9 kDa) based on the 374-residue sequence plus the hexa-histidine tag. The native ADH from *K. koreensis* existed as a 40 kDa dimer with a total molecular mass of 80 kDa (Ngo et al. 2013). Animal ADHs also existed as 40 kDa dimers (Light et al. 1992; Al-Karadaghi et al. 1994).

A phylogenic tree was constructed based on the amino acid sequences of ADHs from *K. koreensis*, other bacteria, and animals (Fig. 2a). *Kangiella koreensis* ADH was more closely related to animal ADHs than to bacterial ADHs. Fish and *K. koreensis* ADHs were grouped in the same branch. The closest amino acid sequence homology of ADHs from *K. koreensis*

a



b

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                C45 T47                                 H67
Fish            LFATGVCHTDAYTLSGSDPEGLFPVILGHEGAGTVESVGEGVTKFKPGDT
Rat             VIATCVCPTDINATNPKK-KALFPVVLGHECAGIVESVGPGVTNFKPGDK
Mouse           ILATGICRTDDHIIKGSMV-SKFPVIVGHEAVGVVESVGEGVTTVRPGDK
Horse           MVATGICRSDDHVVSGTLV-TPLPVIAGHEAAGIVESIGEGVTTVRPGDK
Frog            ILASGICGSDSSVLKEIIP-SKFPVILGHEAVGVVESIGAGVTCVKPGDK
P.putida        ILYCGVCHSDIHQARNEWGIAVYPLMPGHEIIGRVTAVGDKVTAHKVGDL
M.xanthus       ILYCGVCHSDLHQVRDEWGGSRFPMVPGHEIIGRVVRVGDQVKKVKVGDM
B.subtilis      IKFSGICHSDIHSAFDEWGGGIFPMVPGHEIAGVVTAVGTKVTKLAVGDR
C.glutamicum    IKAAGICHSDIHTIRNEWGEAHFPLTVGHEIAGVVSAVGSDVTKWKVGDR
M.penetrans     IKYCGICHSDIHHGRSEWGKANYPTVMGHEIVGEVVATGNKVSKFKVGDN
K.koreensis     IVATGVCHTDAFTLSGDDPEGVFPSILGHEGGGIVESVGEGVTSVKPGDH
                :    :*  :*                *   ***  * *   *  *.    **

                      C97 C100 C103      C111
Fish            V-IPLYVPQCGECKFCKNPKTNLCQKIRI----TQGQGLLPDKTSRFTCK
Rat             V-IPFFAPQCKKCKLCLSPLTNLCGKLRNFKYPTIDQELMEDRTSRFTSK
Mouse           V-IPLFLPQCRECNACLNPEGNLCIRSDL----T-GRGVLADGTTRFTCK
Horse           V-IPLFTPQCGKCRVCKHPEGNFCLKNDL----SMPRGTMQDGTSRFTCR
Frog            V-IPLFVPQCGSCRACKSSNSNFCEKNDM----GAKTGLMADMTSRFTCR
P.putida        VGVGCMVDSCRTCEACAKDLEQYCLEGPTMTYATPDR-------------
M.xanthus       AGVGCMVDSCRTCPSCQEGLEQYCEKGNVQTYNGKEK-------------
B.subtilis      VGVGCFVDSCGECEYCLNAEEQFCTKGVVQTYNSVDY-------------
C.glutamicum    VGVGCLVNSCGECEQCVAGFENNCLRGNVGTYNSNDV-------------
M.penetrans     AGIGCMVNSCGNCDSCKVSEEQYCN-DMVVTYGGLDKF-------------
K.koreensis     V-IPLYTPECGDCKFCLSGKTNLCQKIRE----TQGKGLMPDGTTRFSIN
                .  :     .*  *  *     :  *

                                                        C174
Fish            GKQ-VFHFMGTSTFSEYTVVADISLAKVNEKAPMDKVCLLGCGISTGYGA
Rat             ERS-IYHFMGVSSFSQYTVVSEANLARVDDEANLERVCLIGCGFTSGYGA
Mouse           GKP-VQHFMNTSTFTEYTVLDESSVAKVDGAAPPEKACLIGCGFSTGYGA
Horse           GKP-IHHFLGTSTFSQYTVVDEISVAKIDAASPLEKVCLIGCGFSTGYGS
Frog            GKP-IYNLVGTSTFTEYTVVADIAVAKIDPKAPLE-SCLIGCGFATGYGA
P.putida        ----VDGSNTMGGYSSSIVVSEHFVVRIPAGMDLPSAAPILCAGITTYS-
M.xanthus       ----DGQTATQGGYSEAIVVDEAFTLKIPENLDPAAAAPLLCAGITTYS-
B.subtilis      -----DGNPTYGGYSQKIVVTDRFVVRIPDRLEMDVASPLLCAGITTYS-
C.glutamicum    -----DGTITQGGYAEKVVVNERFLCSIPEELNFDVAAPLLCAGITTYS-
M.penetrans     ----NNNEMTYGGYSNYYVVKESFAIKVPQNVPLEYIAPLFCAGITTYS-
K.koreensis     GKP-IYHYMGTSTFSEYTVLPEISLAKVNPKAPLEEVCLLGCGVTTGMGA
                          .  ::.   *: :      :          .  : *.   :  .
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and fish may be due to the horizontal transfer of ADH gene between *K. koreensis* and fish because the bacterium is isolated from marine tidal zones. Horizontal gene transfer seems to be the essential mechanism for the acquisition of new genes to adapt to new environment rather than the alteration of gene functions, and it

◀**Fig. 2** Comparison of the amino acid sequence of ADH from *K. koreensis* with those of ADHs from the fish (*Sparus aurata*), rat (*Macaca mulatta*), mouse (*Musmus culus*), horse (*Equus caballus*), and frog (*Pelophylax perezi*); and the bacterial ADHs from *Pseudomonas putida*, *Myxococcus xanthus*, *Bacillus subtilis*, *Corynebacterium glutamicum*, and *Mycoplasma penetrans*. **a** Phylogenic tree. **b** Sequence alignment. The catalytic (Thr47) are *boxed*, and the metal-binding residues (Cys45, His67, Cys97, Cys100, Cys103 Cys111, and Cys174) of *K. koreensis* ADH are highlighted in *white* on a black background. The GenBank accession numbers of ADHs from the fish, rat, mouse, horse, and frog; and the bacterial ADHs from *P. putida*, *M. xanthus*, *B. subtilis*, *C. glutamicum*, *M. penetrans* and *K. koreensis* are U84791.1, P28469.2, U20257.1, and P00327.2, O57380.3; and AAN68038.1, CAA63467.1, Q8KRC3, BAB97724.1, CAD84940.1, and ACV27458.1, respectively

has been considered as an important factor in the evolution of bacteria (Wijayawardena et al. 2013).

The amino acid sequence of *K. koreensis* ADH was compared with those of animal and bacterial ADHs (Fig. 2b). The amino acid sequence of ADH from *K. koreensis* had 70.5, 53.5, 51.1, 53.5, and 48.9 % identity with those of the fish, rat, mouse, horse, and frog, respectively. In contrast, the amino acid sequence of ADH from *K. koreensis* showed only 20.6, 24, 20.9, 24.7, and 23.5 % identity with ADHs from *Pseudomonas putida*, *Myxococcus xanthus*, *Bacillus subtilis*, *Corynebacterium glutamicum*, and *Mycoplasma penetrans*, respectively.

The cofactor-binding site and metal-binding site residues of ADH were already determined (Kang et al. 2011). Based on amino acid sequence alignment, cofactor-binding site of *K. koreensis* ADH were identified as His46, Thr47, Gly199, Leu200, Gly201, Gly202, Ile203, Asp223, Asn225, Ile269, Gly270, and Arg369. Among them, Thr47 was suggested to have a role in the reduction of all-*trans*-retinal. The metal-binding site residues of *K. koreensis* ADH were identified as Cys45, His67, Cys97, Cys100, Cys103, Cys111, and Cys174.

Substrate specificity of *K. koreensis* ADH

The specific activity of *K. koreensis* ADH in the presence of NADH followed the order acetaldehyde > furaldehyde > all-*trans*-retinal (Table 1). The specific activity of the enzyme in the presence of NAD^+ followed the order 1-butanol > 1-hexanol > 1-propanol > ethanol > all-*trans*-retinol. The K_m, k_{cat}, and k_{cat}/K_m values of *K. koreensis* ADH for all-*trans*-retinal using NADH as a cofactor were 1.8 μM, 1.7 min^{-1}, and 0.95 μM^{-1} min^{-1}, respectively. The kinetic parameters for all-*trans*-retinal were compared with those of other animal alcohol dehydrogenases (Table 2). The previously highest K_m, and k_{cat}/K_m values were reported in human dehydrogenase (ADH4) as 300 min^{-1} and 378 μM^{-1} min^{-1}, respectively, which were approx. 180- and 400-fold higher than those of *K. koreensis* ADH. The relatively poor catalytic properties of *K. koreensis* ADH mean that significant engineering is required before it can be deployed in an industrial bioprocess. To obtain a desired enzyme used in industry, the residues on or near to the active site should be modified via site-directed mutagenesis or saturated mutagenesis based on the determined structure of *K. koreensis* ADH, and then a variant with improved catalytic properties should be selected. The variant can be also achieved via DNA shuffling or error-prone PCR.

Table 1 Substrate specificity of alcohol dehydrogenase from *K. koreensis*

Substrate	Cofactor	Specific activity (unit mg^{-1})	Relative activity (%)
Acetaldehyde	NADH	66.2 ± 1	83.2 ± 1.3
Furaldehyde	NADH	24 ± 1	30.2 ± 1.3
All-*trans*-retinal	NADH	21.3 ± 0.9	26.8 ± 1.1
Butyl alcohol	NAD^+	79.6 ± 1.6	100 ± 2
Hexyl alcohol	NAD^+	61.4 ± 2.2	77.1 ± 2.8
Propyl alcohol	NAD^+	51.2 ± 0.8	64.3 ± 1
Ethyl alcohol	NAD^+	44.6 ± 0.6	56 ± 0.8
All-*trans*-retinol	NAD^+	24 ± 0.2	30.2 ± 0.3

Data represent means of three experiments with the standard deviations

Optimization of reaction conditions for the production of all-*trans*-retinol from all-*trans*-retinal by *K. Koreensis* ADH

ADH converts all-*trans*-retinal into all-*trans*-retinol using NADH as a cofactor. The formed NAD^+ is converted to NADH by glycolysis and the TCA cycle. Cytosolic NADH level responds to fluctuation of glucose level and is regulated by cellular redox situation (Hung et al. 2011). The effects of metal ions on the producing activity of all-*trans*-retinol from all-*trans*-retinal by *K. koreensis* ADH was determined. Although, Zn^{2+} was detected in the crystal structure of *K. koreensis* ADH (Ngo et al. 2013), the activity of *K. koreensis* ADH was not affected by monovalent or

Table 2 Kinetic properties of alcohol dehydrogenases for all-*trans*-retinal

Enzyme	Source	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (μM^{-1} min^{-1})	References
ADH1B2	Human	0.4	5.0	12.5	Gallego et al. (2006)
ADH2	Human	0.3	2.3	7.7	Hellgren et al. (2007)
ADH8	Frog	8.0	270	33.8	Peralba et al. (1999)
ADH4	Human	0.8	300	375	Gallego et al. (2007)
ADH	*K. koreensis*	1.8	1.7	0.94	This study

divalent cations and was not influenced by EDTA (data not shown), indicating that the enzyme was metal-independent, and Zn^{2+} was covalently bound with *K. koreensis* ADH.

The conversion rate of all-*trans*-retinal into all-*trans*-retinol by the enzyme was maximal at pH 6.5 and 60 °C (Supplementary Fig. 1). The optimal temperature of *K. koreensis* ADH for the conversion of all-*trans*-retinal to all-*trans*-retinol was higher than those of other ADHs (25–30 °C) (Peralba et al. 1999). Bioconversion at high temperatures has advantages, including reduced risk of contamination, high substrate solubility, and prevention of undesirable byproduct generation (Haki and Rakshit 2003). Retinoid conversion cannot be performed at 60 °C by fermentation using recombinant *E. coli* because *E. coli* does not grow at 60 °C. However, whole cell reaction can be applied to retinoid conversion. For example, ADH from *K. koreensis* expressed in *E. coli* can produce effectively all-*trans*-retinol from all-*trans*-retinal at 60 °C using whole cell reaction because inherent enzymes in *E. coli* still work at 60 °C. Whole cell reactions of recombinant *E. coli* harboring thermophilic enzyme at high temperature result in the denaturation of inherent enzymes and the elimination of undesired side reactions (Ninh et al. 2013). Thus, highly selective whole-cell catalysts comparable to purified enzymes have been applied to the production of metabolites (Jung et al. 2005; Kao et al. 2008; Zhang et al. 2010).

The conversion of all-*trans*-retinal into all-*trans*-retinol was assessed over 10 min by varying the concentration of all-*trans*-retinal from 44 to 9,570 mg l^{-1} (Fig. 3a). Increases in the substrate concentration led to increases in the production of all-*trans*-retinol. However, above 2,200 mg all-*trans*-retinal l^{-1}, all-*trans*-retinol production reached a plateau. The optimal enzyme concentration for all-*trans*-retinol production was investigated by varying the enzyme concentration from 0.09 to 2.5 g l^{-1} (Fig. 3b). All-*trans*-retinol production increased with increasing enzyme concentration until a plateau was reached at 2 g enzyme l^{-1}. Therefore, the optimal concentrations of substrate and enzyme were determined to be 2,200 mg l^{-1} and 2 g l^{-1}, respectively.

Production of all-*trans*-retinol from all-*trans*-retinal by *K. koreensis* ADH under the optimized conditions

The optimal reaction conditions for the production of all-*trans*-retinol from all-*trans*-retinal were 60 °C, pH 6.5, 2 g enzyme l^{-1}, and 2,200 mg all-*trans*-retinal l^{-1} in the presence of 5 % (v/v) methanol, 1 % (w/v) hydroquinone, and 10 mM NADH. Under these conditions, 2 g enzyme l^{-1} produced 600 mg all-*trans*-retinol l^{-1} after 3 h (Fig. 4), with a conversion yield of 27.3 % (w/w) and a productivity of 200 mg l^{-1} h^{-1}. Although animal ADHs convert all-*trans*-retinal into all-*trans*-retinol (Peralba et al. 1999), this is the first report on biotechnological production of all-*trans*-retinol using a bacterial ADH. All-*trans*-retinal was produced from β-carotene using Blh (Kim et al. 2010), and retinoids was produced from glycerol by metabolically engineered *E. coli* (Lee et al. 2012; Jang et al. 2014). However, ADH gene has not been introduced into retinoid producing metabolically engineered *E. coli*. Thus, *K. koreensis* ADH can be applied to all-*trans*-retinol production from β-carotene with Blh, and its gene can be introduced into retinoid producing metabolically engineered *E. coli* from glycerol.

In summary, a gene encoding the putative ADH from *K. koreensis* was cloned, expressed, and the expressed protein was purified. The properties of *K. koreensis* ADH for all-*trans*-retinal were characterized, and the production of all-*trans*-retinol from all-*trans*-retinal by this enzyme was optimized. Under the

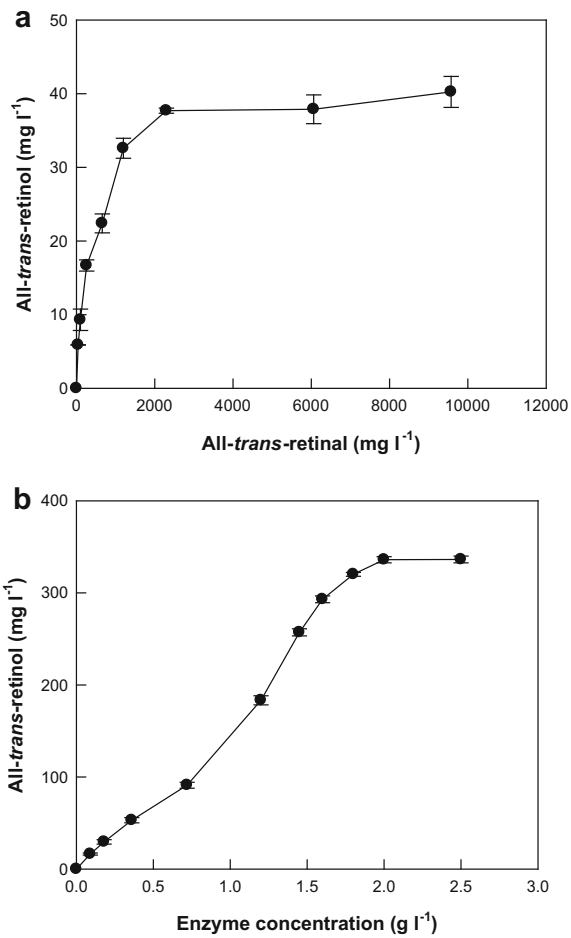

Fig. 3 Effects of substrate and enzyme concentrations on the production of all-*trans*-retinol from all-*trans*-retinal by *K. koreensis* ADH. **a** Effect of substrate concentration. The reactions were performed in 50 mM PIPES buffer (pH 6.5) containing 1.5 g enzyme l^{-1} and all-*trans*-retinal in the presence of 5 % (v/v) methanol, 1 % (v/v) hydroquinone, and 10 mM NADH by varying all-*trans*-retinal from 44 to 9,571 mg l^{-1} at 60 °C for 10 min. **b** Effect of enzyme concentration. The reactions were performed in 50 mM PIPES buffer (pH 6.5) containing 2,200 mg all-*trans*-retinal l^{-1} in the presence of 5 % (v/v) methanol, 1 % (v/v) hydroquinone, and 10 mM NADH by varying the enzyme concentration from 0.09 to 2.5 g l^{-1} at 60 °C for 10 min. Data represent the means of three experiments and *error bars* represent the standard deviation

optimized conditions, 600 mg all-*trans*-retinol l^{-1} was produced from 2,200 mg all-*trans*-retinal l^{-1} for 3 h. To the best of our knowledge, this is the first report on the characterization of a bacterial ADH for all-*trans*-retinal and the biotechnological production of all-*trans*-retinol using *K. koreensis* ADH. In addition, these findings indicate the possibility of this strategy to the production of all-*trans*-retinol from β-carotene and glycerol by biological processes.

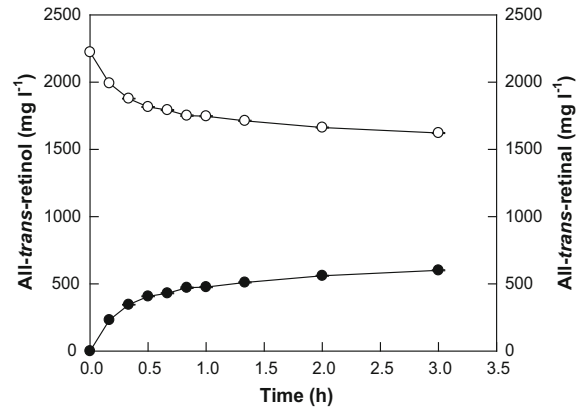

Fig. 4 Time–course reactions for the conversion of all-*trans*-retinal into all-*trans*-retinol by *K. koreensis* ADH. All-*trans*-retinal (*open circle*) and all-*trans*-retinol (*filled circle*). The reactions were performed with 2,200 mg all-*trans*-retinal l^{-1} and 2 g enzyme l^{-1} in the presence of 5 % (v/v) methanol, 1 % (v/v) hydroquinone, and 10 mM NADH at 60 °C for 3 h. Data represent the means of three experiments and *error bars* represent the standard deviation

Supporting information

Supplementary Fig. 1—Effects of pH and temperature on the activity of *K. koreensis* ADH.

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