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Cloning, Expression, and Characterization of a Novel (*S*)-Specific Alcohol Dehydrogenase from *Lactobacillus kefir*

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Abstract A gene encoding a novel (*S*)-specific NADH-dependent alcohol dehydrogenase (LK-ADH) was isolated from the genomic DNA of *Lactobacillus kefir* DSM 20587 by thermal asymmetric interlaced-polymerase chain reaction. The nucleotide sequence of (*S*)-LK-ADH gene (*adhS*) was determined, which consists of an open reading frame of 1,044 bp, coding for 347 amino acids with a molecular mass of 37.065 kDa. After a BLAST similarity search in GenBank database, the amino acid sequence of (*S*)-LK-ADH showed some homologies to several zinc containing medium-chain alcohol dehydrogenases. This novel gene was deposited into GenBank with the accession number of EU877965. *adhS* gene was subcloned into plasmid pET-28a(+), and recombinant (*S*)-LK-ADH was successfully expressed in *E. coli* BL21(DE3) by isopropyl-β-D-1-thiogalactopyranoside induction. Purified enzyme showed a high enantioselectivity in the reduction of acetophenone to (*S*)-phenylethanol with an ee value of 99.4%. The substrate specificity and cofactor preference of recombinant (*S*)-LK-ADH were also tested.

Keywords *Lactobacillus kefir* · TAIL-PCR · (*S*)-specific alcohol dehydrogenase · NADH dependent

Introduction

Dehydrogenases are enzymes belonging to the class of oxidoreductases. Within this class, alcohol dehydrogenases (E.C.1.1.1.1, also known as keto-reductase) represent an important group of biocatalysts due to their ability to stereospecifically reduce prochiral carbonyl compounds. Alcohol dehydrogenases (ADH) can be used efficiently in the synthesis of optically active alcohols, which are key building blocks for the fine chemicals industry [1, 2].

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From a practical point of view, alcohol dehydrogenases that use NAD/NADH as cofactors are particularly important because the formate/formate dehydrogenase system has been well established to regenerate the cofactor efficiently. On the other hand, however, current available cofactor-regeneration systems for NADP/NADPH-dependent enzymes are much less efficient.

Various alcohol dehydrogenases which are valuable for organic synthesis have been described previously[3–8], among which the most frequently used are the commercially available ADH from yeast, horse liver, and *Thermoanaerobium brockii*. However, stability and substrate specificity of these enzymes have restricted their technical and industrial applications. Only a few enzymes which are able to reduce ketones with bulky side chains, such as acetophenone and its derivatives, have been reported so far. A gene coding for an alcohol dehydrogenase was identified in *Pseudomonas fluorescens* DSM 50106 [9] and was functionally expressed in *Escherichia coli*. This recombinant enzyme converted cyclic ketones to the corresponding alcohols while using NADH as cofactor. In addition, another novel NADH-dependent alcohol dehydrogenase (RE-ADH) was isolated from *Rhodococcus erythropolis* [10].

In this paper, we describe the cloning and sequencing of an *adhS* gene from *Lactobacillus kefir* DSM 20587 and the comparison of the deduced amino acid sequence to those expressed from other alcohol dehydrogenase genes. Expression and purification of this (*S*)-specific NADH-dependent alcohol dehydrogenase and its substrate specificity as well as enantioselectivity are also reported.

Materials and Methods

Materials, Bacterial Strains, Plasmid, and Medium

Restriction endonuclease, LA Taq DNA polymerase, phenol/chloroform and T_4 DNA ligase were purchased from TaKaRa (Dalian, China). *E. coli* DH5α (maintained in Shanghai Institute of Pharmaceutical Industry, SIPI) was used as a host for standard cloning experiments and was grown in Luria Bertani (LB) medium. *E. coli* BL21 (DE3) (purchased from Novagen, Madison, WI, USA) was used for alcohol dehydrogenase expression and the strain bearing plasmid pET-28a(+) (Novagen, Madison, WI, USA) were grown in LB supplemented with 50 μg kanamycin/ml.

Molecular Techniques

Genomic DNA from *L. kefir* DSM 20587 was extracted according to the established protocols [11]. Restricted digestion and PCR were performed following instruction by the suppliers (TaKaRa). Transformation of plasmids into *E. coli*, agarose gel electrophoresis, and gel purification of DNA were performed according to standard procedures [12]. pMD19-T vector (TaKaRa) was used for cloning of PCR products. DNA sequencing was carried out by Invitrogen biotechnology company (China).

Cloning of the Alcohol Dehydrogenase Gene

A pair of degenerate oligonucleotide primers for amplification of an internal fragment of *adhS* gene were designed based on the alignment and conserved block search of amino acid sequences of six alcohol dehydrogenases from *Lactobacillus* (GenBank accession numbers

ABJ63420, ABJ64152, ABJ64046, NP_786231, NP_785248, CAD65227): 5′ primer(P1), 5′-GGHCAYGAAGSHDYHGGMAWBGT-3′ and 3′ primer(P2), 5′-VCCRASSSCRCCR KYMCC-3′. The 5′ and 3′ primers are located within the corresponding zinc-binding consensus and cofactor binding domain, respectively.

PCR was performed using genomic DNA from *L. kefir* DSM 20587 as template. Twenty microliters reaction mixture contained 0.2 μg genomic DNA, 1.0 μM of each primers, 0.2 mM of each dNTP, 1.5 U LA Taq DNA polymerase, 2.5 mM $MgCl_2$, and 1×LA Buffer. The PCR amplification protocol consisted of a denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 30 s, and a final hold for an extra 10 min at 72°C. The product was gel purified and inserted into pMD19-T then transformed into *E. coli* DH5α for sequencing.

Furthermore, 5′ and 3′ flanking region of the internal fragment were amplified by the thermal asymmetric interlaced PCR (TAIL-PCR). Briefly, TAIL-PCR utilizes three nested specific primers in consecutive reactions together with a set of arbitrary degenerate (AD) primers which have lower T_m (melting temperature), so that the relative amplification efficiencies of specific and nonspecific products can be thermally controlled [13]. In the primary reaction, one low-stringency PCR cycle is conducted after five high-stringency PCR cycles to create one or more annealing sites for the AD primer on the targeted sequence. Specific product is then preferentially amplified over nonspecific ones by 15 thermal asymmetric super PCR cycles. Since the specific primers are nested, specific product of TAIL-PCR has the characteristic that the second round product is shorter than the first round product, and the third round product is shorter than the second round product.

In this study, three nested specific primers (SPs), SP1-SP3/SP1′-SP3′, were designed based on the internal sequence and combined with a primer bank of 13 arbitrary degenerate primers (ADs) to amplify the 5′ and 3′ flanking region, respectively. Arbitrary degenerate primers were designed based on conserved amino acid sequence for proteins that are prevalent in all species. Thirteen ADs we used here have been previously reported in scientific papers [13, 14]. The nucleotide sequences of SPs and ADs are listed in Table 1. Cycling parameters for the primary, secondary, and tertiary rounds of TAIL-PCR are shown in Table 2. In the primary round of TAIL-PCR, the specific primer (SP1 or SP1′) and 13 degenerate primers (ADs) were used (Table 1). Product of primary reaction was diluted 200-fold and used as template for the secondary reaction. In the secondary round, the specific primer (SP2 or SP2′) and the same degenerate primers (ADs) were used. Product of this round was also diluted 200-fold and used as template for the tertiary reaction. The obtained 5′ and 3′ flanking regions together with the internal fragment were assembled, and the full sequence was analyzed by ContigExpress project software (Vector NTI; Invitrogen).

Based on the analyzed sequence, the full *adhS* gene was PCR amplified by using *L. kefir* DSM 20587 genomic DNA as template and two primers [sense(P3) (5′- ATGAAAT CAACCATTTTTGTAAAACC -3′) and antisense(P4) (5′- CTATTTTTGAGCGACAA CCAAC -3′]. The PCR amplification was carried out in a reaction mixture described as above, and the reaction protocol consisted of a denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 1 min, elongation at 72°C for l.5 min, and a final hold for an extra 10 min at 72°C. The PCR product was inserted into plasmid pMD19-T to generate plasmid pYGadh. pYGadh was then transformed into *E. coli* DH5α for DNA sequencing and analyzing.

The nucleotide sequence of the *adhS* gene has been deposited to the GenBank databases under accession no. EU877965.

Table 1 Primers used in TAIL-PCR.

Primer name	Sequence
SP1	5′TTCGGCTTGATAACCAGCACTAA3′
SP2	5′AACCAGCACTAAAGTTGTCGCTATGA3′
SP3	5′TGGTGCGATTACAAAGTCTCCCG3′
SP1′	5′CGTTACCAGCATGCCGAGTG3′
SP2′	5′CATGCCGAGTGGTCACTGGTTAA3′
SP3′	5′ATGCTGCCCGTGTCGCCAAC3′
AD1	5′NGTCGASWGANAWGAA3′
AD2	5′TGWGNAGWANCASAGA3′
AD3	5′AGWGNAGWANCAWAGG3′
AD4	5′STTGNTASTNCTNTGC3′
AD5	5′NTCGASTWTSGWGTT3′
AD6	5′WGTGNAGWANCANAGA3′
AD7	5′CAWCGNCNGANASGAA3′
AD8	5′TCSTNCGNACNTWGGA3′
AD9	5′WCAGNTGWTNGTNCTG3′
AD10	5′TCTTNCGNACNTNGGA3′
AD11	5′TTGNAGNACNANAGG3′
AD12	5′GTNCGASWCANAWGTT3′
AD13	5′NTCAGSTWTSGWGWT3′

Construction of Expression Plasmid pETadh

To facilitate the construction of an expression plasmid, a pair of specific primers was designed as follows: sense 5′-GCGGGATCCATGAAATCAACCATTTTTGT-3′ (*Bam*HI site underlined) and antisense 5′-GCGAAGCTTCTATTTTTGAGCGACAACC-3′ (*Hin*dIII site underlined). The obtained PCR product was digested with *Bam*HI and *Hin*dIII and ligated into pET-28a(+) to obtain the expression plasmid pETadh. *E. coli* BL21 (DE3) transformed with pETadh was used as the host for recombinant LK-ADH expression.

Table 2 Cycling parameters of TAIL-PCR.

Reaction	Number of cycles	Thermal settings
Primary	1	95°C, 5 min
	5	94°C, 30 s; 54°C, 1 min; 72°C, 2.5 min
	1	94°C, 30 s; 25°C, ramping to 72°C in 3 min; 72°C, 2.5 min
	15	94°C, 30 s; 54°C, 1 min
		94°C, 30 s; 54°C, 1 min
		94°C, 30 s; 44°C, 1 min; 72°C, 2.5 min
	1	72°C, 10 min; 4°C hold
Secondary	15	94°C, 30 s; 54°C, 1 min
		94°C, 30 s; 54°C, 1 min
		94°C, 30 s; 44°C, 1 min; 72°C, 2.5 min
	1	72°C, 10 min; 4°C hold
Tertiary	15	94°C, 30 s; 54°C[a], 1 min
		94°C, 30 s; 54°C[a], 1 min
		94°C, 30 s; 44°C, 1 min; 72°C, 2.5 min
	1	72°C, 10 min; 4°C hold

[a] To amplify the 5′ flanking region, the annealing temperature of tertiary round of TAIL-PCR is set to 54°C; To amplify the 3′ flanking region, the annealing temperature of tertiary round of TAIL-PCR is set to 60°C

Expression and Purification of Recombinant LK-ADH

E. coli BL21 (DE3)/pETadh cells were grown at 37°C in LB medium supplemented with 50 μg kanamycin/ml. Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.2 mM for induction when the cell density (OD_{600}) was 0.6 measured by a spectrophotometer (UltraUV 7700, Amersham), followed by further growth for 16 h at 27°C. The cells were harvested by centrifugation (12,000×*g*, 10 min) and resuspended in 100 mM potassium phosphate buffer (pH 6.0) at a ratio of 1 g cells per 2.5 ml buffer. Cells were disrupted by sonication at 4°C for 5 s at 5 s interval for a total of 3 min and debris was removed by centrifugation (12,000×*g*, 20 min).

The obtained crude extract was applied to a Ni–NTA chelating affinity column (Shanghai Shenergy Biocolor BioScience&Technology) equilibrated with 20 mM Tris–HCl (pH 8.0) and 500 mM NaCl. The bound enzyme was eluted by applying a stepwise gradient concentration of imidazole from 20 to 200 mM. Fractions containing the eluted LK-ADH were pooled, and the protein was then dialyzed overnight at 4°C in a dialysis bag (13,000 M.W. cutoff) against 100 mM potassium phosphate buffer, pH 6.0.

Enzyme Activity Assay

Alcohol dehydrogenase activity was determined spectrophotometrically at 340 nm (ε_{340}= 6.22 mM^{-1} cm^{-1}) using acetophenone as substrate. Activity was measured at 30°C in a total volume of 1 ml reaction mixture containing 10 mM acetophenone in 100 mM potassium phosphate buffer (pH 6.0), 0.25 mM NADH, and 0.2 ml of enzyme solution. The reaction was started by the addition of the enzyme. One unit of LK-ADH activity was defined as the amount of enzyme that converted 1 μmol of NADH per minute.

Enantioselectivity and Gas Chromatography Analysis

The enantioselectivity of LK-ADH was determined as follows: The reaction mixture containing 15 μl NADH (0.2 mM), 7.7 μl 2-propanol (0.1 M), 1.2 μl acetophenone (10 mM) (Sigma-Aldrich), 176 μl potassium phosphate buffer (100 mM; pH 6.0, supplement with 1 mM $MgCl_2$), and 800 μl LK-ADH was incubated for 4 h at 30°C. Then, 200 μl sample was extracted with 200 μl chloroform, and the organic phase was analyzed by gas chromatography (Agilent 6890) using a CP-Chirasil-DEX CB column (25 m; diameter 25 μm). Temperature program, 5 min at 60°C, then 5°C/min to 195°C. Column flow, 1.3 ml/min; gas, helium; injection volumes, 1 μl, detection, FID.

The enantiomeric excess (ee) was calculated according to the integral area of the converted products.

Results

Sequence Analysis of the Cloned *adhS* and Alignment of *adhS* with other *adh*

An internal fragment (369 bp) of the LK-ADH gene was PCR-amplified from the genomic DNA of *L. kefir* DSM 20587 by using P1 and P2 as primers. 5′ and 3′ flanking region (1,112 bp and 1,660 bp in length, respectively) of this fragment were then obtained by TAIL-PCR (see "Materials and Methods" section for detailed PCR protocol). The electrophoresis of both PCR products are shown in Fig. 1. The flanking regions were

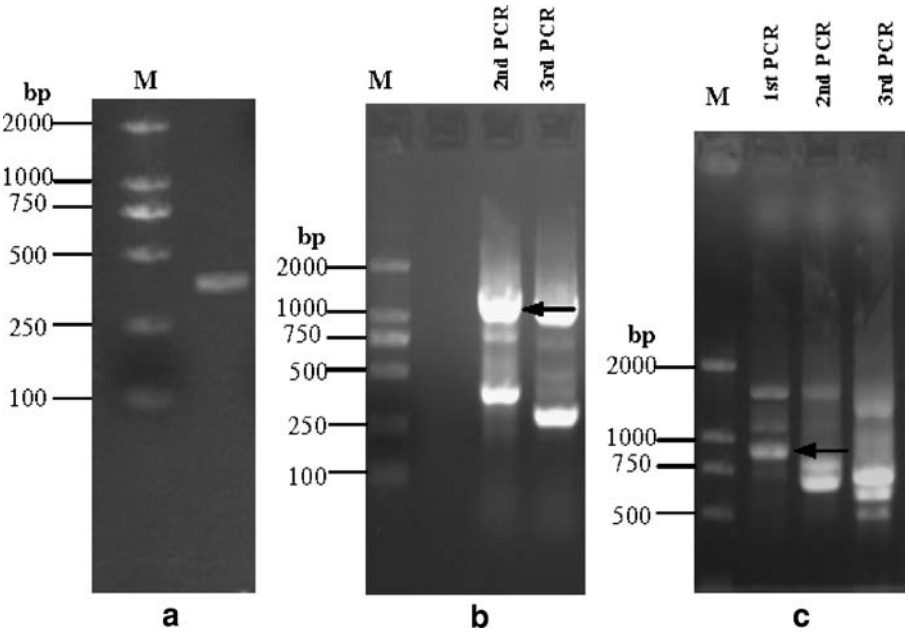

Fig. 1 Gel electrophoresis of PCR products. **a** amplification of the 369-bp internal fragment of *adhS* gene. **b** TAIL-PCR result of 3′ flanking region, product indicated by an *arrow*. **c** TAIL-PCR result of 5′ flanking region, product indicated by an *arrow*. *M* DNA Marker DL2, 000

assembled with the 369-bp fragment to obtain the full length gene. Nucleotide sequence analysis revealed that it contained an open reading frame (ORF) which started with ATG and ending with a TAG stop codon. This 1,044 bp ORF (named as *adhS* and the corresponding protein as LK-ADH) encoded 347 amino acid residues with a deduced molecular mass of 37.065 kDa (see Fig. 2).

BLAST similarity search of GenBank (http://www.ncbi.nlm.nih.gov.cn/BLAST) showed that there are many zinc-containing alcohol dehydrogenases (medium- and long-chain ADHs) similar to LK-ADH. The highest amino acid sequence similarities were found with threonine dehydrogenase-related Zn-dependent dehydrogenase (LB-ADH) from *Lactobacillus brevis* ATCC 367 (GenBank accession number YP_795041) and a zinc-containing alcohol dehydrogenase (LR-ADH) from *Lactobacillus reuteri* 100-23 (GenBank accession number ZP_01273263) which are 78% and 72% identical, respectively. The amino acid sequence alignment is shown in Fig. 2.

Thus, *adhS* was identified as a novel alcohol dehydrogenase from *L. kefir* and was deposited into GenBank under accession number EU877965.

Expression of *adhS* in *E. coli* and Purification of the Recombinant Enzyme

LK-ADH can be efficiently expressed in *E. coli* BL21 (DE3) by IPTG induction at 27°C for 16 h. SDS-PAGE analysis showed a band corresponding to 41 kDa, consistent with the deduced molecular mass (Fig. 3a). The recombinant protein was about 60% of the total protein of the cell, according to the gray-scale scanning.

Since the recombinant protein has a poly-His tag, it was purified on a Ni–NTA chelate affinity chromatography column and eluted with a stepwise gradient concentration of

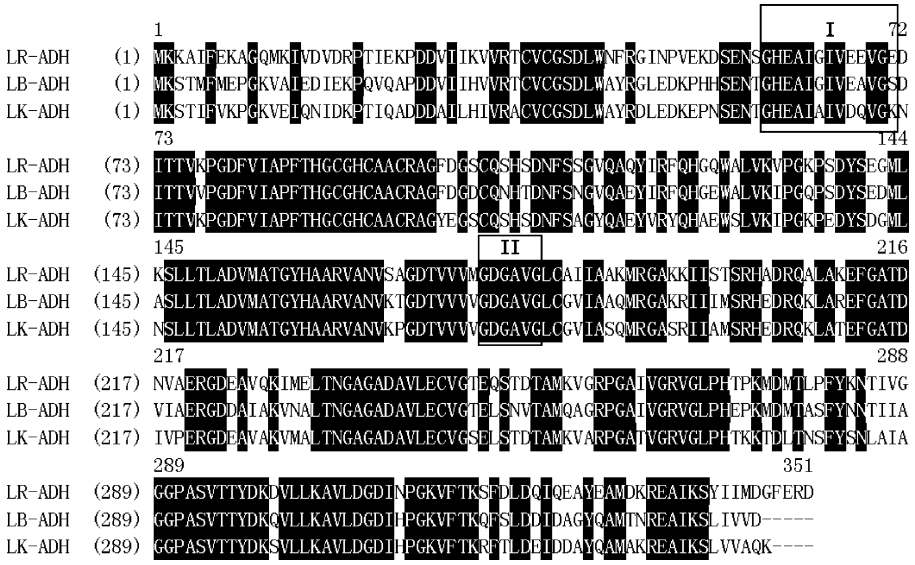

Fig. 2 Sequence alignment of LK-ADH from *L. kefir* DSM 20587 with the Threonine dehydrogenase related Zn-dependent dehydrogenase (LB-ADH; accession number YP_795041) from *Lactobacillus brevis* ATCC 367 and with a zinc-containing alcohol dehydrogenase from *Lactobacillus reuteri* 100-23 (LR-ADH; accession number ZP_01273263). Identical amino acid residues are in *black*. Box I, zinc-binding motif; Box II, $NAD(P)^+$ binding motif

imidazole. About 4.2 mg purified LK-ADH was obtained from 1 L culture of *E. coli* BL21 (DE3)/*pETadh*. The eluted LK-ADH protein was dialyzed against potassium phosphate buffer to eliminate the imidazole then tested for alcohol dehydrogenase activity. The recombinant protein was purified to apparent homogeneity (Fig. 3b) in SDS-PAGE, and

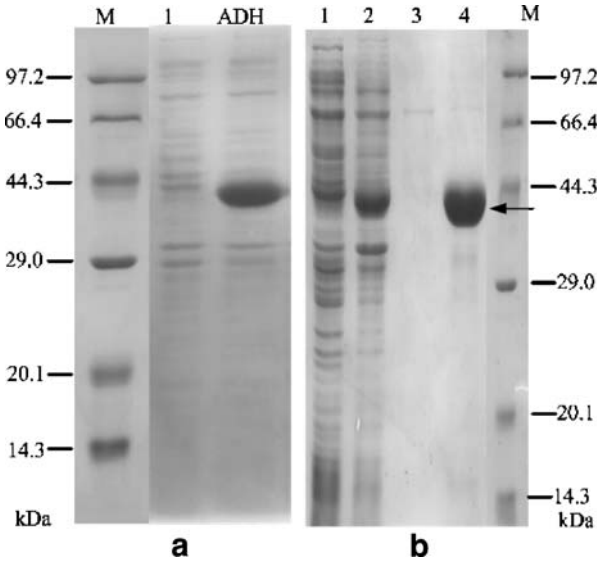

Fig. 3 SDS-PAGE analysis of the expression and purification of the LK-ADH protein. **a** Analysis of IPTG-induced cultures. *M* Molecular weight standards (kDa), *1* crude cell extract of *E. coli* BL21 (DE3)/*pET28a*, *ADH* crude cell extract of recombinant *E. coli* BL21 (DE3)/*pETadh*. **b** Analysis of fractions eluted by stepwise gradient concentration of imidazole from Ni-NTA chelate affinity chromatography column (*lanes 1–4*). LK-ADH protein is indicated by an *arrow*. SDS-polyacrylamide gels were stained with Coomassie blue

Table 3 Purification of recombinant LK-ADH.

Step	Protein (mg)	Total activity (U)	Specific activity (U/mg)	Yields (%)	Purification factor (fold)
Crude extract	72.9	109.3	1.5	100	1
Affinity chromatography	4.2	28.1	6.7	25.7	4.5

the purification factor was calculated as 4.5-fold, while the specific activity of purified LK-ADH was 6.7 U/mg for the reduction of acetophenone (Table 3).

Coenzyme Preference and Substrate Specificity

To determine the preference of cofactors, NADH and NADPH (0.25 mM) were used in the LK-ADH activity assay with 10 mM acetophenone. The specific ADH activity for NADH

Table 4 Substrate specificity of LK-ADH[a].

Reduction substrate[b]	Relative activity (%)[c]	Oxidation substrate[b]	Relative activity (%)[d]
Acetophenone	100	2-Propanol	100
Formaldehyde	0	Ethanol	0
Acetaldehyde	0	1-Propanol	0
Propionaldehyde	20	Methanol	0
n-Butyraldehyde	10	1-Butanol	0
Isobutyraldehyde	16	2-Butanol	75
n-Valeraldehyde	733	tert-butyl alcohol	54
n-Hexyl aldehyde	829	1-Pentanol	16
n-Heptyl aldehyde	755	2-Pentanol	130
Benzaldehyde	421	1-Hexanol	44
Phenylacetaldehyde	334	1-Heptanol	56
Salicylaldehyde	120	Stearyl alcohol	0
O-Chlorobenzaldehyde	330	Polyethylene Glycol200	0
Citral	180	1,2-propanediol	0
Cinnamaldehyde	928	1,4-Butanediol	0
Ethyl acetoacetate	309	Glycerol	0
Acetone	0	Benzyl alcohol	0
Acetylacetone	0	β-Phenylethanol	19
2-Butanone	0	Cyclopentanol	13
3-Pentanone	17	Cyclohexanol	0
p-Cl-acetophenone	440		
Cyclopentanone	12		
Cyclohexanone	0		
4-Hydroxy-4-methyl-2-pentanone	120		

[a] The activity was measured at 30°C as described in the "Materials and Methods" section

[b] The concentration of each substrate was 10 mM

[c] Reductive reaction was measured by the NADH consumption and relative rates were calculated by defining the activity for acetophenone as 100%

[d] Oxidative reaction was measured by the NADH formation and relative rates were calculated by defining the activity for 2-propanol as 100%

was 7.23×10^{-2}U/ml, while the activity for NADPH was only 0.90×10^{-2} U/ml, with an obvious discrepancy of 8.0-fold. This result indicated that LK-ADH from *L. kefir* DSM 20587 preferably used NADH as a cofactor.

The substrate specificity of LK-ADH was also examined either in reduction or in oxidation reaction (Table 4). This enzyme showed relatively high activity against all the tested aldehydes. Ketones with bulky side chains, such as acetophenone and its derivative *p*-Cl-Acetophenone were also found to be effectively transformed to their corresponding alcohols by recombinant LK-ADH. However, LK-ADH showed relatively less activity in oxidation of alcohols including methanol, ethanol, propanol, and butanol.

Enantioselectivity

The enantioselectivity of LK-ADH was tested as described in the "Materials and Methods" section. After 4 h, more than 95% of the substrate had been converted. GC analysis result was shown in Fig. 4; acetophenone (rt: 5.623 min) area=23.88; (*R*)-phenylethanol (Sigma-Aldrich) (rt: 7.634 min) area=1.42; (*S*)-phenylethanol (Sigma-Aldrich) (retention time 7.884 min): area=462.28 (Fig. 4). The enantiomeric excess (ee) value was calculated as (462.28−1.42)/(462.28+1.42)×100%=99.4%. This novel LK-ADH showed S-specific activity in the reduction of acetophenone.

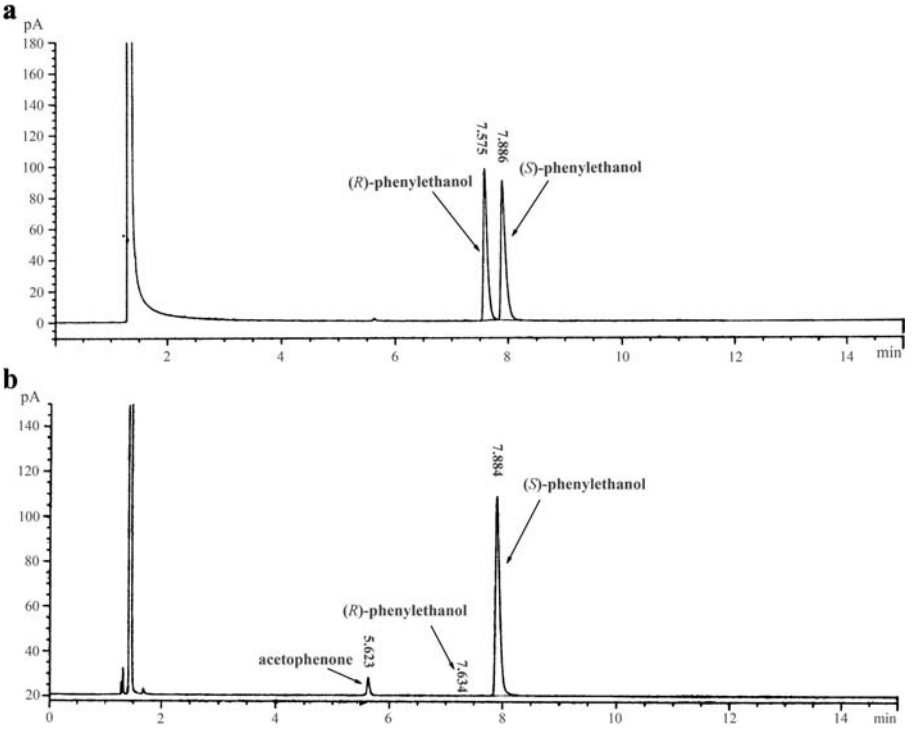

Fig. 4 GC analysis of the product in enantioselective reduction of acetophenone. **a** (*S*)-phenylethanol and (*R*)-phenylethanol standard (Sigma-Aldrich). **b** Reaction mixture. The retention times for acetophenone, (*R*)-phenylethanol and (*S*)-phenylethanol were 5.623, 7.634, and 7.884 min, respectively

Discussion

To our knowledge, there are two conventional methods for novel gene cloning. To obtain the full length sequence, either the enzyme needs to be purified, partially sequenced, and PCR performed to obtain the ORF or a genomic library (cDNA library for eukaryotic genes) prepared and the gene cloned by probe-oriented hybridization. Here, we describe a new method for cloning a novel *adhS* gene from *L. kefir* genomic DNA. Combining regular PCR and TAIL-PCR, we successfully cloned the full length *adhS* gene from *L. kefir* DSM 20587. We also believe that this method is applicable for cloning the genes encoding other novel and potentially valuable enzymes.

The $NAD(P)^+$-dependent ADH family can be classified into three groups [15]; zinc-dependent long-chain ADHs (approximately 385 amino acid residues) including alcohol dehydrogenase II from *Zymomonas mobilis* and alcohol dehydrogenase IV from *Saccharomyces cerevisiae* belong to group I; zinc-dependent medium-chain ADHs (approximately 350 residues) including alcohol dehydrogenases I, II, and III from *Saccharomyces cerevisiae* belong to group II; Ribitol dehydrogenase from *Klebsiella aerogenes* belongs to group III which are zinc-independent short chain ADHs (approximately 250 residues). According to homology analysis and the primary structure of the protein, it could be suggested that LK-ADH belongs to the family of $NAD(P)^+$-dependent medium-chain zinc-dependent alcohol dehydrogenases, group II. In general, these enzymes typically contain a catalytically active zinc-binding domain which was also characterized in LK-ADH (see Fig. 2 Box I, GHEAIAIVDQVGK). An $NAD(P)^+$ binding motif (GDGAVG, Box II) was identified in LK-ADH as well which was consistent with the GXGXXG fingerprint pattern of the cofactor binding domain [15].

An NADH-dependent phenylacetaldehyde reductase (PAR) was isolated from a styrene-assimilating *Corynebacterium* sp. strain. By using this enzyme, a practical hydrogen-transfer bioreduction process was established to produce (*S*)-1-phenylethanol and other chiral alcohols from the corresponding ketones [16]. In this study, a similar reaction mixture containing 0.1 M 2-propanol as a hydrogen donor without additional coenzyme regeneration system such as formate/FDH and glucose/GDH was adopted and (*S*)-1-phenylethanol was also successfully converted from acetophenone by recombinant LK-ADH.

(*R*)-specific alcohol dehydrogenase from *L. kefir* has been reported several years ago [17]. The purified enzyme also showed a high transformation activity and enantioselectivity. This is quite promising for biotransformation because this report, together with our study, shows that *L. kefir* has at least two alcohol dehydrogenases which both have high transforming ability and enantioselectivity but different substrate specificity [18]. The recombinant LK-ADH could be a potential catalyst for production of chiral alcohols.

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