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Full Length Research Paper

Cloning and characterization of thermostable-deoxy-D-ribose-5-phosphate aldolase from *Hyperthermus butylicus*

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The 2-deoxy-D-ribose-5-phosphate aldolase gene from *Hyperthermus butylicus* was subcloned, overexpressed in *Escherichia coli* and purified to apparent homogeneity. Analysis of the sequence of gene revealed an open reading frame (ORF) of 672 base pairs encoding 237 amino acids predicted to yield a protein of molecular mass 26.4 kDa. The encoded protein was overexpressed in *E. coli* and purified to apparent homogeneity. The enzyme activity is optimal at pH 5.5 and 80°C. For 2-deoxyribose-5-phosphate, the apparent K_m was calculated to be 0.15 ± 0.01 mM. The recombinant protein was heat stable; no activity loss was observed even after incubation at 90°C for 10 min. In addition, the thermophilic enzyme also showed a remarkable resistance to acetaldehyde; it retained more than 70% activity after exposure for 8 h to 300 mM acetaldehyde at 25°C.

Key words: 2-Deoxy-D-ribose-5-phosphate aldolase, thermophiles, aldol condensation, acetaldehyde resistance.

INTRODUCTION

Aldolases are a specific group of lyases that catalyzes the reversible stereoselective addition of a donor compound (nucleophile) onto an acceptor compound (electrophile). These enzymes are categorized based on their requirement for nucleophiles. So-called Class I aldolases need no cofactor, as their reaction mechanism of donor activation involves the formation of a Schiff base at a conserved lysine residue in the active site of the enzyme. Class II aldolases are dependent on a metal that acts as a Lewis acid and activates the donor substrate (Samland and Sprenger, 2006). 2-Deoxy-D-ribose-5-phosphate aldolases (DERA; EC 4.1.2.4) are Class I aldolases active in DNA salvage pathways in many microorganisms; these aldolases catalyze reversible aldol reactions between natural acetaldehydes and D-glyceraldehyde-3-phosphate to form 2-deoxyribose-5-phosphate (DRP) (Machajewski and Wong, 2000). The ability of DERAs to generate chiral centers in the resulting aldol adducts makes them powerful tools for the syntheses of rare sugars or sugar-derived compounds (Barbas et al., 2002). For example, a DERA from *Escherichia coli* is able to catalyze sequential aldol reactions involving three aldehyde substrates, resulting in the formation of two carbon-carbon bonds with concomitant generation of two chiral centers (Gijsen and Wong, 1994). DERAs have been used to produce statins with unique side chains (Douglas and Overman, 2004). Statins are some of the best selling drugs in the world, accounting for nearly $20 billion in

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Abbreviation: DERA, 2-Deoxy-D-ribose-5-phosphate aldolase; **DRP,** 2-deoxy-D-Ribose-5-Phosphate; **ORF,** open reading frame; **TPI,** triose-phosphate isomerase; **GDP,** glycerol-3-phosphate dehydrogenase; $DERA_{Hbu}$, *Hyperthermus butylicus* 2-deoxy-D-ribose 5-phosphate aldolase; **LB,** lysogeny broth; **IPTG,** isopropyl-β-D-thiogalactopyranoside; **PEG,** polyethylene glycol; **SDS-PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **DMSO,** dimethyl sulfoxide.

annual sales (Maggon, 2005; Patel, 2006). To date, DERAs from microorganisms, including *Bacillus cereus*, *E. coli K12, Klebsiella pneumoniae, Lactobacillus plantarum, Salmonella typhimurium, Streptococcus mutans GS-5, Yersinia* sp. *EA015, Aeropyrum pernix, Pyrobaculum aerophilum* and *Thermotoga maritima*, have been studied (Pricer and Horecker, 1960; Hoffee, 1968; Sgarrella et al., 1992; Gijsen and Wong, 1994; Horinouchi et al., 2003; Sakuraba et al., 2003; Han et al., 2004; Sakuraba et al., 2007; Kim et al., 2009).

The stability of enzymes is often an issue for industrial applications, where high concentrations of organic substrates are usually desired to increase volumetric productivity (Panke et al., 2004; Pollard and Woodley, 2007). Enzymes with high thermal stability often also have high stability in the presence of denaturing agents such as detergents and organic solvents (Burton et al., 2002; Cowan, 1997; Egorova and Antranikian, 2005). For example, most proteases from extremophilic archaea are stable in high concentrations of detergents and denaturing agents even at high temperature. Serine alkaline proteases from thermophilic archaea are used as additives for laundering as these enzymes resist denaturation. Esterases from *Aeropyrum pernix*, *Pyrobaculum calidifontis* and *Sulfolobus tokodaii* exhibit high activities and thermostability in high concentrations of acetonitrile and dimethyl sulfoxide (Antranikian et al., 2005). Several thermostable aldolases possess enhanced stability in organic solvents (Hao and Berry, 2004).

The hyperthermophilic *Hyperthermus butylicus* was isolated in 1990 from the sea floor of a solfataric habitat off the coast of the island of São Miguel Azores where temperatures reach 112℃ (Zillig et al., 1990). The whole genome was sequenced, annotated and analyzed in 2007 (Brugger et al., 2007). In this report, we cloned, expressed, purified and biochemically characterized the DERA from *H. butylicus*. Purified recombinant DERA showed heat stability and resistance to a high concentration of acetaldehyde. We anticipate that *H. butylicus* aldolase will be an attractive enzyme for use as a biocatalyst for industrial applications.

MATERIALS AND METHODS

Materials

The pET-303/CT-His vector was obtained from Invitrogen (Paisley, UK). The *E. coli* strain BL21-CodonPlus (DE3)-RIL was purchased from Stratagene (La Jolla, CA). DRP, triose-phosphate isomerase (TPI), and glycerol-3-phosphate dehydrogenase (GDP) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of reagent grade.

Gene cloning and multiple sequence alignment

The nucleotide sequence encoding DERA from the thermophilic microorganism *H. butylicus* was obtained from the EMBL Nucleotide Sequence Database (http://www.ebi.ac.uk/embl/Access/). The *H. butylicus* DERA is abbreviated as $DERA_{Hbu}$. The DNA encoding full length $DERA_{Hbu}$ was synthesized by Sangon (Shanghai, China). An *Xba* I restriction site was introduced at the 5' end before the initiation codon and an *Xho* I restriction site was introduced at the 3' end overlapping the termination codon. The sequences were digested with restriction enzymes (*Xba* I and *Xho* I) and ligated into the expression vector pET303/CT-His linearized with *Xba* I and *Xho* I to give an expression vector carrying the desired $DERA_{Hbu}$ coding region.

In order to identify the conserved residues in $DERA_{Hbu}$, the amino acid sequences of DERAs from representative thermophilic organisms *A. pernix*, *P. aerophilum* and *T. maritima* were aligned using ClustalW (http://ebi.ac.uk/clustalw/). The amino acid sequences were obtained from UniProtKB/TrEMBL Database. The accession numbers of the sequences were as follows: *A. pernix*, Q9Y948; *P. aerophilum*, Q8ZXK7; and *T. maritima*, Q9X1P5.

Protein expression, purification and molecular mass determination

The expression vector carrying the desired DERA sequence was introduced into the *E. coli* strain BL21-CodonPlus (DE3)-RIL. Cells were grown in 1 mL lysogeny broth (LB) medium at 37℃ for 1 h. The cells (10 – 200 μL) were plated on LB plates containing 100 mg/L of ampicillin and incubated at 37℃ overnight to obtain transformants. The transformants were cultivated in LB medium containing 100 mg/L of ampicillin at 37℃ until the optical density at 600 nm reached 0.6. Expression was induced by adding isopropyl-β-D- thiogalactopyranoside (IPTG) at the final concentration of 0.5 mM and cultivation was continued for an additional 5 h at 37℃. Cells were harvested by centrifugation at 15,000 x *g* for 5 min and suspended in 100 mM sodium phosphate (pH 7.5), 200 mM sodium chloride and freeze-thawed three times. Supernatant was incubated with Ni-NTA agarose (Qiagen, Hilden, Germany) for 1 h at 4℃ and the mixture loaded onto a chromatography column. The column was washed with buffer containing 100 mM sodium phosphate (pH 7.5), 200 mM sodium chloride and 10 mM imidazole. The C-terminal His-tagged DERA was eluted from the column with the same buffer containing 500 mM imidazole. The protein was dialyzed overnight against 20 mM sodium phosphate (pH 7.5) and concentrated with polyethylene glycol (PEG) 20000 and then freeze-dried. Enzyme powders were stored at -20℃. The purified enzymes were assayed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie brilliant blue G-250 staining and the protein concentration was determined by the Bradford method.

The molecular mass of the purified enzyme was determined by analytical gel filtration on a Superdex 200 column (2.6 × 62 cm; Amersham Biosciences) pre-equilibrated with 50 mM Tris-HCl buffer (pH 8.0) containing 0.2 M NaCl. The molecular mass of DERA was compared with retention time of four standard proteins including lysozyme (14 kDa), chymotrypsin (25 kDa), maltose binding protein (43 kDa) and bovine serum albumin (68 kDa).

Enzyme activity assay

The DERA cleavage activity was measured by following the oxidation of nicotinamide adenine dinucleotide (NADH) in a coupled assay converting glyceraldehyde-3-phosphate, one of DRP cleavage products, to glycerol 3-phosphate by TPI and GDP. The reaction mixture contained 100 mM sodium acetate buffer (pH 5.5), 0.1 mM NADH, 0.4 mM DRP, 11 U triose-phosphate isomerase, 4 U glycerol-3-phosphate dehydrogenase and various concentrations of $DERA_{Hbu}$. The assay was initiated by addition of DERA and the decrease in NADH level was monitored at 340 nm using an ultraviolet-visible spectrophotometer (Shimadzu Co., Kyoto, Japan). The unit (U) activity was defined as the amount of DERA required to catalyze the cleavage of 1 μmol of DRP per minute. Kinetic

Table 1. Summary of the purification process of *H. butylicus* DERA from 1 litre of *E. coli* BL21-CodonPlus (DE3)-RIL harboring pET–DERA.

Step	Protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Purification fold	Yield (%)
Crude cell extract	413	84	0.2	—	100%
NI-NTA affinity chromatography	63	34	0.5	2.5	40.4%

properties of DERA were examined in 100 mM sodium acetate buffer (pH 5.5) at 25°C. Five concentrations of DRP, ranging from 0.04 to0.4 mM were used to determine reaction rates. The apparent Michaelis-Menten constants for DRP cleavage reactions were determined from the double reciprocal Lineweaver-Burk plots of the reaction rate. Each activity assay was repeated at least twice, with each replicate performed on a different day.

Effects of pH on enzyme activity and stability

The buffers used to determine effects of pH were sodium acetate (0.1 M, pH 3.0 to pH 6.0), imidazole-HCl (0.1 M, pH 6.0 to pH 7.5), triethanolamine-HCl (0.1 M, pH 7.5 to pH 8.5), glycine-NaOH (0.1 M, pH 8.5 to pH 11.0) and Na_2HPO_3-NaOH (0.1 M, pH 11.0 to pH 13.0). The pH optimum of DERA was determined by analysis of DRP cleavage in pH from 3.0 to 11.0. The effect of pH on enzyme stability was determined by comparing the relative activity of the enzyme (at 0.5 mg/ml) incubated in pH ranging 3.0 to 13.0 at 25°C for 30 min.

Effects of temperature on enzyme activity and stability

The optimum temperature of DERA was measured using the DRP cleavage assay with a slight modification. After the reaction mixture containing 100 mM sodium acetate buffer (pH 5.5), 0.4 mM DRP and a known concentration of DERA was incubated in the range of 20 to 100°C for 1 min, DERA was removed. The reduction reaction was then carried out at 25°C for 30 min in the presence of TPI and GDP and the decrease of NADH was monitored. To determine the effect of temperature on enzyme stability, DERA (0.5 mg/mL) was incubated for 10 min at different temperatures, after which the residual activities were assayed.

Effects of organic solvents and acetaldehyde on enzyme stability

The effect of organic solvents on DERA activity was also analyzed using the standard assay method in the presence of varying concentrations of acetonitrile, dimethyl sulfoxide (DMSO), 1,4-dioxane, tetrahydrofuran, methanol and isopropanol at 25°C. The relative activity was compared with the DERA activity in the absence of the organic solvent. To examine the effect of acetaldehyde on enzyme stability, 0.5 mg/ml DERA in 100 mM sodium acetate buffer (pH 5.5) containing 300 mM acetaldehyde was incubated at 25°C for various intervals. The acetaldehyde was removed from the enzyme solution using a centrifugal filter device (Microcon YM-10; Millipore). The resulting DERA was diluted to 0.2 mg/mL with 100 mM sodium acetate (pH 5.5) and the residual activity was analyzed.

RESULTS AND DISCUSSION

Cloning, expression and purification

The expression vector carrying the $DERA_{Hbu}$ gene was constructed by ligating the polymerase chain reaction (PCR)-amplified gene into the plasmid pET-303/CT-His; the resulting plasmid, pET-DERA, was transformed into *E. coli* BL21-CodonPlus (DE3)-RIL cells to yield a high-level expression of recombinant protein. The recombinant protein containing a His-tag was purified to homogeneity using a Ni-NTA column. The details of the purification results are shown in Table 1. The molecular weight of DERA measured by SDS-PAGE analysis was 26 kDa (Figure 1a), similar to the molecular weight predicted from the sequence of 26.4 kDa. The native molecular mass of the enzyme determined by gel filtration is 52 kDa (Fig. 1b). This indicates that the enzyme consists of two subunits with identical molecular mass. *E. coli* DERA also has a dimer structure (Protein Data Bank code 1JCL) composed of two identical subunits, which is most common for DERA.

Multiple sequence alignment

The $DERA_{Hbu}$ protein (A2BLE9) was aligned with representative DERA proteins of thermophilic microorganisms, including *A. pernix* (Q9Y948), *P. aerophilum* (Q8ZXK7) and *T. maritima* (Q9X1P5). A comparison of amino acid sequence of $DERA_{Hbu}$ with other DERAs demonstrated that $DERA_{Hbu}$ is 29, 28 and 26% identical to those of *A. pernix*, *T. maritima* and *P. aerophilum*, respectively. The amino acid sequences had low homology; however, the catalytic residue Lys131 of $DERA_{Hbu}$, essential in forming the Schiff-base with the aldehydic substrate, was conserved in all DERAs. The active site residues Asp97 and Lys197 of $DERA_{Hbu}$, known to be important in proton relays, were also conserved in all DERAs (Figure 2) (Sakuraba et al., 2007).

Optimum pH and pH stability

Properties of the purified recombinant $DERA_{Hbu}$ were investigated using DRP as a substrate. Under the assay conditions used in our study, the enzyme exhibited its highest activity at pH 5.5 with more than 65% activity retained between pH 5.0 and 6.0 (Figure 3a); this is a more acidic pH optimum than that exhibited by other DERAs. DERAs from *P. aerophilum* and *T. maritima* exhibited highest activity at pH 6.0 and 7.0, respectively (Sakuraba et al., 2007). The activity of $DERA_{Hbu}$

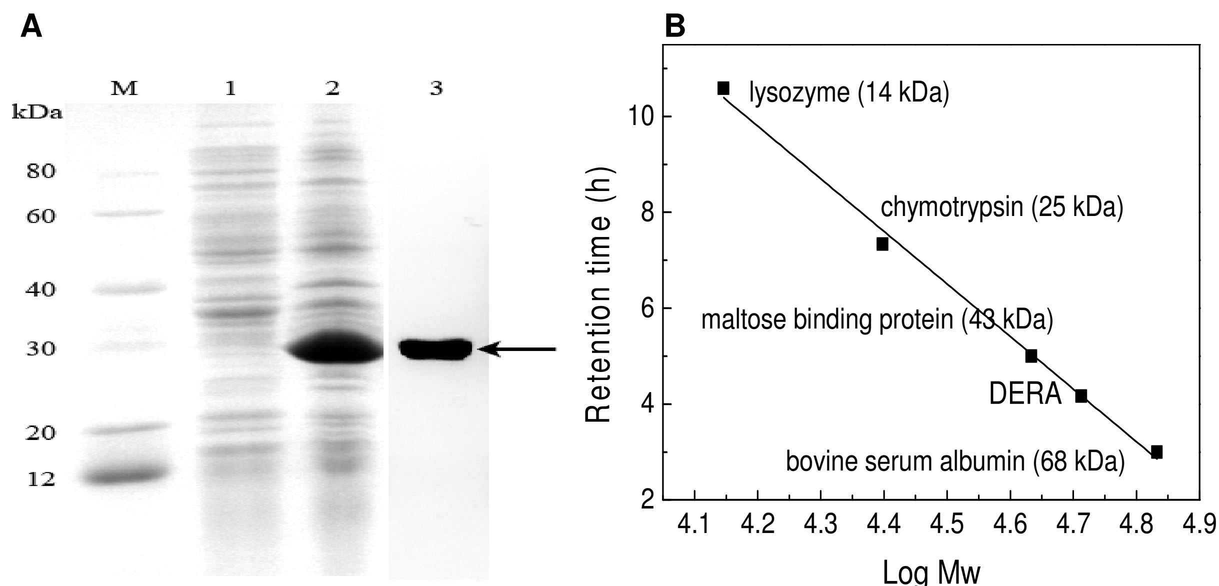

Figure 1. SDS-PAGE analysis of recombinant DERA from *H. butylicus*. (A) M, Molecular weight marker; Lane 1, uninduced crude extract; Lane 2, induced crude extract; Lane 3, purified $DERA_{Hbu}$ after NI-NTA affinity chromatography. (B) Gel filtration analysis of recombinant DERA: molecular mass of DERA was compared with the values of retention time of lysozyme (14 kDa), chymotrypsin (25 kDa), maltose binding protein (43 kDa) and bovine serum albumin (68 kDa).

decreased sharply below pH 5.0 and above pH 7.0. The stability of the enzyme after incubation at various pH values is shown in Figure 3b.The enzyme was extremely stable over a wide range of pH levels; after heating at pH levels ranging from 4.0 to 11.0 for 30 min at 50℃, the enzyme showed no loss of activity. Compared with other thermophilic DERAs, $DERA_{Hbu}$ show higher stability under acidic conditions (Sakuraba et al., 2007). The DERA purified in this study is highly pH-stable (over the pH range of 4.0-11.0), suggesting that $DERA_{Hbu}$ will be stable under different pH conditions encountered in industrial applications.

Optimum temperature and thermal stability

The effect of temperature on enzyme activity at pH 5.5 is shown in Figure 4. The highest activity was observed at 80℃. The enzyme exhibited similar activity at temperatures between 70 to 80℃. The activity sharply decreased below 70 and above 80℃. The thermal stability of DERA was determined after incubation at various temperatures. After heating the protein at 90℃ for 10 min, no loss of activity was observed for $DERA_{Hbu}$ (Figure 4).
After incubation at 100℃ for 10 min, the enzyme retained 80% residual activity. These results showed that $DERA_{Hbu}$ is a remarkably heat stable enzyme.

Basic kinetic constants for $DERA_{Hbu}$

Initial rate kinetics for the aldol cleavage reaction catalyzed by $DERA_{Hbu}$ was determined at various concentrations of DRP by fitting the data to the Michaelis-Menten equation. The apparent K_m value of the enzyme was 0.15 ± 0.01 mM. The apparent K_m values of *P. aerophilum* DERA and *T. maritima* were 0.060 and 0.20, respectively (Sakuraba et al., 2007). Compared to K_m value of 9.1 mM for mesophilic DERA from *Yersinia* sp., the reported thermophilic DERAs have significantly higher substrate affinity with K_m values of no more than 0.2 mM (Kim et al., 2009).

Effects of organic solvents and acetaldehyde on enzyme stability

The effect of organic solvents on $DERA_{Hbu}$ activity was determined. $DERA_{Hbu}$ showed moderate stability against organic solvents (Table 2). For example, the enzyme retained over 60% activity in methanol and dimethyl-sulfoxide, but lost activity completely in 20% acetonitrile. Since no data are available for stability of DERAs from other organisms to organic solvents, a comparison of the data is not possible.

$DERA_{Hbu}$ retained more than 75% DRP cleavage activity after exposure for 8 h to 300 mM acetaldehyde at 25℃ (Figure 5). No further loss of activity was observed after 1 h as most of the acetaldehyde was converted to lactol. These results indicted that the enzyme was highly resistant to acetaldehyde and that acetaldehyde was an excellent substrate for the sequential aldol reaction. This is consistent with previous findings. Thermophilic DERAs from *P. aerophilum* and *T. maritima* also show a

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A.pernix       ........MLPSARDILQQGLDRLGS.PEDLASRIDSTLI    31
T.maritima     MIEYRIEEAVAKYREFYEFKPVRESAGIEDVKSAIEHTNL    40
H.butylicus    ..........MSESFFCRFG.......VSEIASRIDHAVL    23
P.aerophilum   ..............................MIHLVDYALI    10
Consensus                                             l

A.pernix       SPRATEEDVRNLVREASDYGFRCAVLTPVYTVKISGLAEK    71
T.maritima     KPFATPDDIKKLCLEARENRFHGVCVNPCYVKLAREELEG    80
H.butylicus    KPWSSVSELEKAIRELEELNLRCLIISPTHLRLAR...EK    60
P.aerophilum   KPYLTVDEAVAGARKAEELGVAAYCVNPIYAPVVR..PLL    48
Consensus       p                         p

A.pernix       LGVKLCSVIGFPLGQAPLEVKLVEAQTVLEAGATELDVVP   111
T.maritima     TDVKVVTVVGFPLGANETRTKAHEAIFAVESGADEIDMVI   120
H.butylicus    TNKCLGVVVGFPFGYSTIEAKIKELEDSIALGAQEIDYVA   100
P.aerophilum   RKVKLCVVADFPFGALPTASRIALVSRLAEV.ADEIDVVA    87
Consensus             v  fp g                 a e d v

A.pernix       HLSLGP....EAVYREVSGIVKLAKSYGAVVKVILEAPLW   147
T.maritima     NVGMLKAKEWEYVYEDIRSVVESVK..GKVVKVIIETCYL   158
H.butylicus    NTQLLLAGRTEEYLNEIRAAITICRDSGVKCKVIIEAPAL   140
P.aerophilum   PIGLVKSRRWAEVRRDLISVVGAAG..GRVVKVITEEPYL   125
Consensus                                 g   kvi e

A.pernix       DDKTLSLLVDSSRRAG..ADIVKTSTGVYTKGGDPVTVFR   185
T.maritima     DTEEKIAACVISKLAG..AHFVKTSTGFGTGGATAEDVHL   196
H.butylicus    PRNLLVEIVEKIAMMDPHPDYIKTSTGYGPRPTYVEDVYL   180
P.aerophilum   RDEERYTLYDIIAEAG..AHFIKSSTGFAEEAYAARQGNP   163
Consensus                            k stg

A.pernix       LAS............LAKPLGMGVKASGGIRSGIDAVLAV   213
T.maritima     MKW............IVG.DEMGVKASGGIRTFEDAVKMI   223
H.butylicus    IDQTLR........RIGKRDEIGIKAAGGIREGLQAAAML   212
P.aerophilum   VHSTPERAAAIARYIKEKGYRLGVKMAGGIRTREQAKAIV   203
Consensus                            g k ggir    a

A.pernix       GAGADIIGTSSAVKVLESFKSLV.                   236
T.maritima     MYGADRIGTSSGVKIVQ.......                   240
H.butylicus    LAGADVIGTSTPRQVIETYKELCR                   236
P.aerophilum   DAIGWGEDPARVRLGTSTPEALL.                   226
Consensus
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Figure 2. Amino acid sequence alignment of DERAs from *H. butylicus* (A2BLE9), *A. pernix* (Q9Y948), *P. aerophilum* (Q8ZXK7), and *T. maritima* (Q9X1P5). Gaps, indicated by dashes, were introduced into the sequences to maximize homology. Residues important for catalysis as discussed in the text are shown in triangles.

significant resistance to high concentration of acetaldehyde, retaining more than 50% DRP cleavage activity for hours to 300 mM acetaldehyde (Sakuraba et al., 2007). Activity of DERAs from these species toward acetaldehyde were less than that of *H. butylicus* DERA, as it took 3 h before no further activity loss was observed for these enzymes under the same experiment condition. In contrast, the mesophilic counterpart DERA from *E. coli* is almost completely inactivated after exposure to acetaldehyde for 2 h under the same conditions (Sakuraba et al., 2007). Our findings, together with previous reports, support the hypothesis that thermostable DERAs are promising candidates for industrial applications (Burton et al., 2002; Cowan, 1997; Egorova and Antranikian, 2005), as the key issue faced in statin synthesis is poor tolerance of DERA toward high

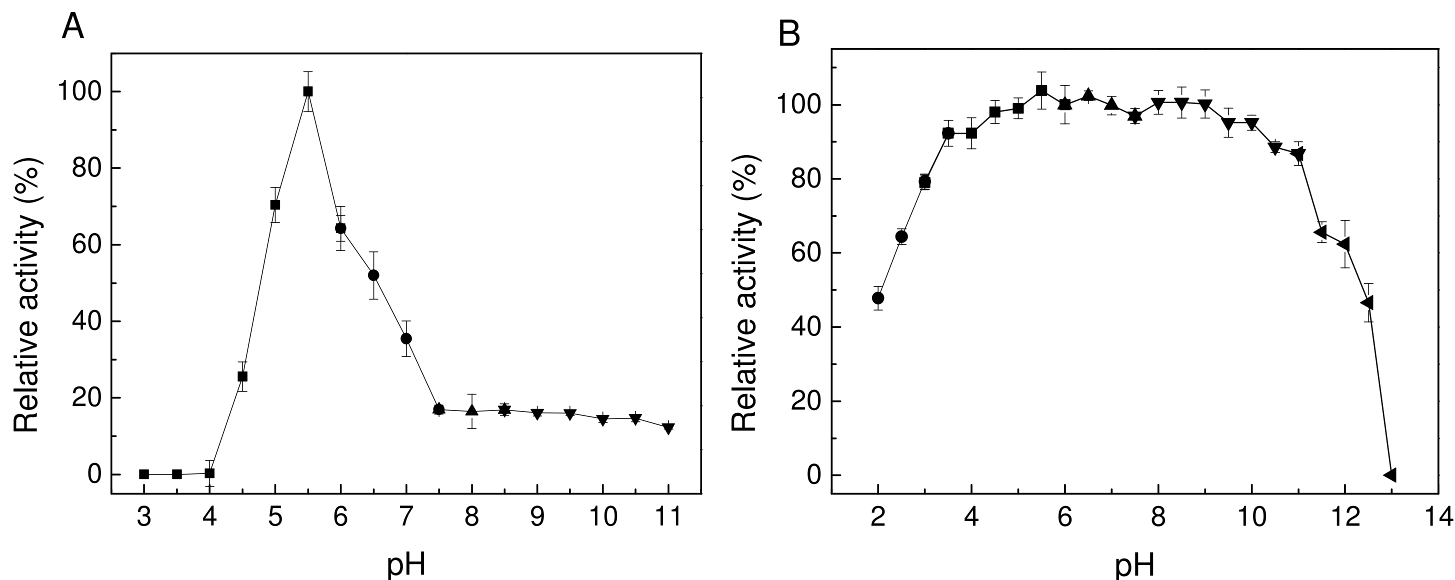

Figure 3. Optimum pH and pH stability of *H. butylicus* DERA. (A) The optimum pH was performed at a variety of pH levels by determining cleavage of the DRP at 25℃. Solid squares, pH 3.0 - 6.0 (sodium acetate buffer); solid circles, pH 6.0 - 7.5 (imidazole-HCl buffer); uptriangles, pH 7.5 - 8.5 (triethanolamine-HCl buffer); downtriangles, pH 8.5 -11.0 (glycine-NaOH buffer); (B) The enzyme was incubated for 30 min at 50℃ in buffers of various pH levels, after which the remaining activity was assayed. Solid circles, pH 2.0 - 4.0 (Gly-HCl buffer), solid squares, pH 3.0 - 6.0 (sodium acetate buffer); solid circles, pH 6.0 - 7.5 (imidazole-HCl buffer); uptriangles, pH 7.5 - 8.5 (triethanolamine-HCl buffer); downtriangles, pH 8.5 - 11.0; lefttriangles (glycine-NaOH buffer), pH 11.5 - 13.0 Na2HPO3-NaOH.

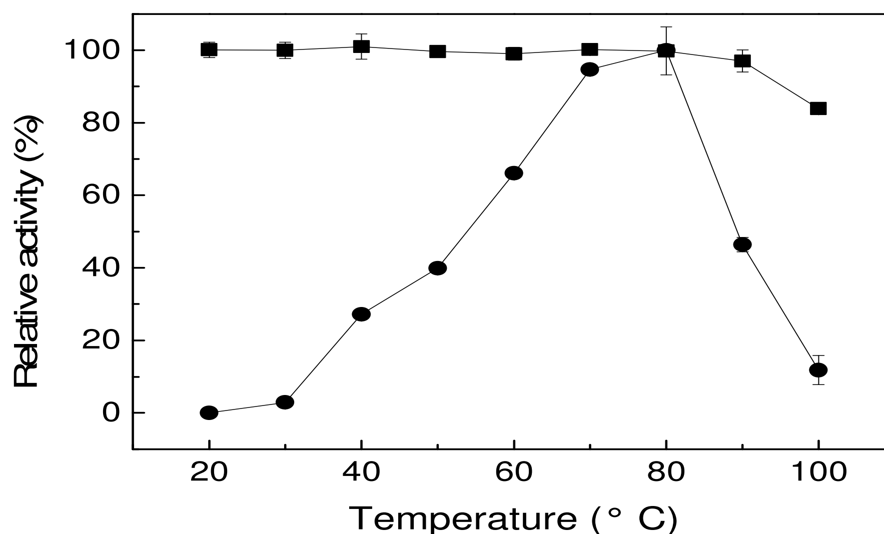

Figure 4. Effects of temperature on activity (●) and stability (■) of *H. butylicus* DERA. Effect of temperature was determined using 100 mM in sodium acetate buffer (pH 5.5). Thermostability was determined after incubation for 10 min at the indicated temperatures in 100 mM in sodium acetate buffer (pH 5.5).

concentrations of acetaldehyde substrate.

In conclusion, we have reported the cloning, expression and characterization of DERA from *H. butylicus*. In industrial applications, use of DERAs has been hindered by their poor resistance toward high concentrations of acetaldehyde. $DERA_{Hbu}$ showed thermostability as well as high stability in high concentrations of acetaldehyde. We expect that $DERA_{Hbu}$ will be useful in manufacturing settings where high concentrations of acetaldehyde are necessary. We are currently investigating use of $DERA_{Hbu}$ in synthesis of statin side chains and this data will be reported in due course.

Table 2. Activity of $DERA_{Hbu}$ in organic solvent.

Organic solvent	Relative activity (%)[a]	
	5% solvent	20% solvent
Methanol	89%	67%
Acetonitrile	64%	0%
Isopropanol	72%	11%
Tetrahydrofuran	72%	0%
1,4-Dioxane	78%	28%
Dimethyl sulfoxide	78%	61%

[a]The activity of purified $DERA_{Hbu}$ was measured in the standard assay buffer in the indicated concentration of organic solvent; activity is relative to $DERA_{Hbu}$ without organic solvent.

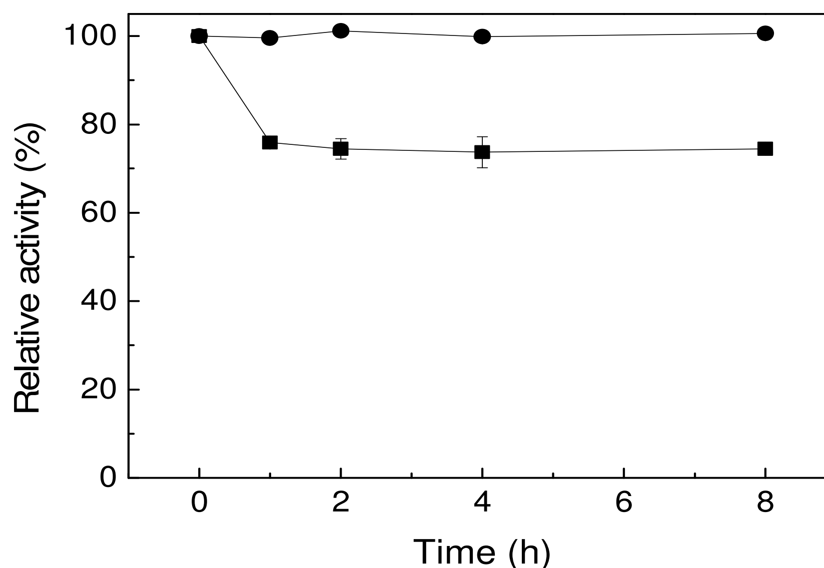

Figure 5. Effect of acetaldehyde on enzyme stability. The enzyme was incubated at 25°C in the presence of 300 mM acetaldehyde and the DRP cleavage activity was assayed at appropriate intervals. Filled squares, in the presence of 300 mM acetaldehyde; filled circles, without acetaldehyde.

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