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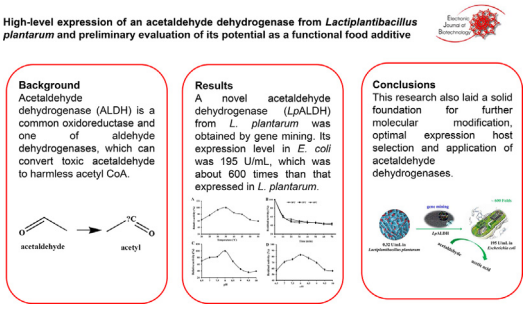
Research Article

High-level expression of an acetaldehyde dehydrogenase from *Lactiplantibacillus plantarum* and preliminary evaluation of its potential as a functional food additive

Zhe Wang [a,b], Jian-Ju He [a,b], Xin-Xin Liu [a,b], Hong-Ling Shi [a,b], Yun-Feng Lu [a,b], Jun-Ye Shi [a,b], Yun-Chao Kan [a], Lun-Guang Yao [a,b,*], Cun-Duo Tang [a,b,*]

[a] *Henan Provincial Key Laboratory of Funiu Mountain Insect Biology and Henan Provincial Engineering and Technology Center of Health Products for Livestock and Poultry, Nanyang Normal University, Nanyang 473061, People's Republic of China*
[b] *College of Life Science and Agricultural Engineering, Nanyang Normal University, Nanyang 473061, People's Republic of China*

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Acetaldehyde dehydrogenase (ALDH) is a common oxidoreductase and one of the aldehyde dehydrogenases, which can convert toxic acetaldehyde to harmless acetyl CoA.
Results: A novel acetaldehyde dehydrogenase (*Lp*ALDH) from *Lactiplantibacillus plantarum* was obtained by gene mining and then was analyzed by a series of bioinformatics software. Phylogenetic tree results showed that *Lp*ALDH was highly homologous with acetaldehyde dehydrogenase derived from *Pediococcus* sp., and their sequence similarity was 62.9%. Moreover, it was successfully expressed in *Escherichia coli* BL21, its expression level was 195 U/mL, which was about 600 times than that expressed in *L. plantarum*. After purification by affinity chromatography, the specific activity of re*Lp*ALDH was 1709 U/mg. Its temperature optimum was 35°C, and the optimal reaction pH was 8.0. Moreover, the purified *Lp*ALDH showed good stability in simulated gastrointestinal fluids, which indicated that it had great potential in functional food additive field.
Conclusions: This research also laid a solid foundation for further molecular modification, optimal expression host selection and application of acetaldehyde dehydrogenases.
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* Corresponding authors.
E-mail addresses: kanyunchao@163.com (L.-G. Yao), tcd530@126.com (C.-D. Tang).

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1. Introduction

Ethanol is usually consumed with meals, and its modest consumption is beneficial for human health [1,2]. However, excessive consumption may cause alcoholic liver diseases, such as steatohepatitis, fibrosis, cirrhosis and so on [1,3,4]. Ethanol is first oxidized by alcohol dehydrogenase to acetaldehyde, and then, it is oxidized by acetaldehyde dehydrogenase to acetic acid, which is eventually converted to acetyl CoA [5,6]. Acetaldehyde can bind readily to proteins, RNA, and DNA, which is mutagenic and categorized as group 1 carcinogen [5,6]. Therefore, its quick metabolism is important to reduce its risk of health damage [3]. Regrettably, more than 40% of East Asian people cannot convert toxic acetaldehyde to nontoxic acetic acid because of having no aldehyde dehydrogenase activity, which will lead to the toxic acetaldehyde accumulation and increase the risk of health damage [1,7]. Aldehyde dehydrogenase (ALDH, EC 1.2.1.3) is a family of NAD(P) dependent enzyme, whose molecular mass is about 50–60 kDa [8]. Currently, more than 500 ALDHs have been isolated from organisms, and they can be grouped into 24 distinct classes: ALDH1∼ALDH24 [9]. Among them, ALDH2 plays the most important role in ethanol metabolism. ALDH2-deficient people are more sensitive to ethanol; a small amount of ethanol can cause severe consequences like tachycardia, nausea, long-lasting headache and facial flushing [1]. After consuming alcohol, adding exogenous aldehyde dehydrogenase can significantly accelerate the eliminating rate of acetaldehyde, which can reduce the harmful effects of alcohol consumption on human health [10]. In the past decades, many researches have indicated that administrating erythrocyte-encapsulated ALDH could significantly reduce blood aldehyde level [11]. Moreover, many studies have reported that the ALDH secreted by intestinal microflora could effectively alleviate alcoholic liver injury [12,13].

To date, many ALDHs have been characterized from bacteria, plants, yeasts, humans, fish, horses, rats and so on [14]. Nevertheless, the commercial acetaldehyde dehydrogenases are mainly derived from animal liver or plants, which have high production cost, complex extraction step and low production efficiency. While the production of ALDHs by fermentation of microorganism has the advantages of low cost, short cycle and less investment, it has gradually become the main industrial production process of ALDHs [15]. With the development of biotechnology and bioengineering, more and more studies on the heterologous expression of ALDHs have been reported. Among them, the most successful hosts are *Escherichia coli*, *Lactococcus lactis* and *Bacillus subtilis* [10,13,16]. Unfortunately, there are still many challenges in the widespread application of acetaldehyde dehydrogenase, mainly including low catalytic activity, poor stability, strong immunogenicity and poor tolerance to gastrointestinal environment. It will be an effective strategy to break through the above bottlenecks to obtain acetaldehyde dehydrogenase mutants with better properties by genetic engineering and protein engineering techniques.

Lactobacillus plantarum, belonging to the lactic acid bacteria, is widely applied in the food and feed additives industry [17,18]. In addition, it is generally regarded as safe (GRAS) organism in many applications, such as an abundant gene repository for industrially used enzymes. In our previous study, a strain of *L. plantarum* with high yield of acetaldehyde dehydrogenase was screened, and its acetaldehyde dehydrogenase activity in fermentation supernatant was 0.3∼0.5 U/mL [19]. Meanwhile, the *L. plantarum* strain has good tolerance to high concentration of ethanol and acetaldehyde. Regrettably, its growth rate is very slow, hence, it is difficult to extract acetaldehyde dehydrogenase from *L. plantarum* to meet the large-scale production [19,20]. Using modern molecular biology techniques to excavate novel enzyme gene, achieve heterologous protein production in the appropriate expression system, which is beneficial to further application of industrial enzyme [21]. Moreover, a high-activity phytase gene from *L. plantarum* was cloned and expressed in *E. coli*, and its expression level was increased by 800 times compared with the original strain in our previous study [19]. Therefore, we aimed to use gene mining to obtain novel acetaldehyde dehydrogenase gene from *L. plantarum*, and conduct bioinformatic analysis on the target sequence. Then, to realize its heterologous expression by using appropriate expression plasmid and host. And then, its potential as a functional food additive was analyzed. This study is expected to establish solid theoretical foundation for further molecular modification, expression host optimization and practical application of acetaldehyde dehydrogenase.

2. Materials and methods

2.1. Reagents and kits

Commonly molecular biology reagents and kits, mainly including restriction enzymes, DL 2,000 DNA Marker, DNA Ligation Kit Ver.2.1 and PrimeSTAR® HS DNA Polymerase and so on, were purchased as previously reported [22]. Acetaldehyde, β-mercaptoethanol (BME) and all other chemicals were purchased from Solarbio (Beijing China).

2.2. Strains, vectors and culture media

E. coli BL21/pET28a and *E. coli* BL21 preserved by our laboratory were cultured in LB liquid medium and used for heterologous expression [23]. *L. plantarum* CICC 20038 preserved by our laboratory was cultured in MRS medium and used for PCR amplifying acetaldehyde dehydrogenase gene [19].

2.3. Gene cloning and sequence analysis of acetaldehyde dehydrogenase

Lactiplantibacillus plantarum and acetaldehyde dehydrogenase were taken as key words to search for potential *Lactiplantibacillus plantarum* acetaldehyde dehydrogenase in NCBI, a novel potential aldehyde dehydrogenase (*Lp*ALDH, WP_003641903) from *L. plantarum* was searched in NCBI, and its corresponding genome sequence number is NZ_CP097175.1. The upstream and downstream primers for amplification of the *Lp*ALDH gene, *Lp*ALDH-F: CGGGATCCATGGGACAAAATGCTGTT and *Lp*ALDH-R: CCGCTCGAGT TATCCTTGAGCCTTTTC, were designed and synthesized by Suzhou Hongxun Biological Technology Co., LTD (Suzhou, China). The genomic DNA of *L. plantarum* CICC 20038 was extracted, and the target genes were obtained by PCR amplification with *Lp*ALDH-F and *Lp*ALDH-R primers, and the target genes were ligated into the pET28a plasmid with the same double digestion by *Bam*H I and *Eco*R I, respectively. Based on the sequencing results of plasmid with the inserted gene, the amino acid sequence of the target enzyme was deduced by DNAMAN. Then, bioinformatics analysis was performed on the target enzyme by modern bioinformatics tools [19,21,24]. ProtParam tool was used to predict theoretically physical and chemical parameters of target enzyme. EasyModeller 2.0 and Modeller 9.9 were used for multi-template homology modeling of three-dimensional (3D) structure. PyMOL was used for 3D structure observation and analysis. DNAMAN was used for sequence analysis. ClustalX2 and MEGA6.0 were used to carry out multiple sequence alignment and phylogenetic tree construction.

2.4. Expression and purification of acetaldehyde dehydrogenase

The *E. coli* BL21/pET28a and *E. coli* BL21/pET28a-*LpALDH* strains containing acetaldehyde dehydrogenase encoding gene were cultured as described method previously [22,25]. The cultured cells of fermentation liquor were harvested by centrifugation and then lysed by high-pressure homogenization, and the cell lysate was purified to homogeneity by One-Stop His-Tagged Protein Miniprep Pack.

2.5. Enzyme activity and protein assays

The determination of acetaldehyde dehydrogenase activity was slightly modified based on the reported method [10]. The reaction mixture contained pH 8.0 100 mM Tris-HCl buffer, 2.0 mM NAD^+, 30 mM KCl, 2.0 mM acetaldehyde and 0.1 mM β-mercaptoethanol in a final volume of 1.0 mL. After the reaction mixture was preheated at 35°C for 5 min, 0.1 mL diluted enzyme solution was added. The oxidation activity was assayed spectrophotometrically at 35°C by monitoring the absorbance change of NADH at 340 nm within 1 min. For all assays, enzyme activity was defined as one unit representing the increase of 0.001 of OD_{340} per minute under the above determination condition. The protein concentration and sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the acetaldehyde dehydrogenase were performed using the reported method [26]. Meanwhile, its apparent molecular mass was estimated by Quantity One software [27].

2.6. Temperature characteristics of the recombinant enzyme

The optimal reaction temperature of the recombinant acetaldehyde dehydrogenase was determined at the standard determination condition as above, except the temperature ranging from 20 to 55°C. To investigate thermostability of the recombinant acetaldehyde dehydrogenase, it was preincubated at 30, 35 and 40°C for 0∼70 min, respectively, and then, its residual activity was measured at optimal reaction temperature.

2.7. pH characteristics of the recombinant enzyme

The optimal reaction pH of the recombinant acetaldehyde dehydrogenase was determined by the standard activity determination method as above described in buffer varied pH values from 6.5 to 10. To investigate its pH stability, aliquots of the recombinant enzyme were preincubated at 0°C for 1.0 h in varied pH values from 6.5 to 10.0, and its residual activity was assayed at optimal reaction temperature and pH.

2.8. Optimal K^+ concentration of recombinant enzyme

It has been reported that the catalytic activity of acetaldehyde dehydrogenase is influenced by K^+ concentration [28]. To evaluate the effect of K^+ concentration on the catalytic activity of the recombinant enzyme, its catalytic activity was measured at optimal reaction temperature and pH with varied K^+ concentrations from 0.1 to 0.4 mol/L.

2.9. Kinetic parameters for the recombinant enzyme

The kinetic parameters for the recombinant enzyme on acetaldehyde were determined as described previously [22]. The catalytic activity of the recombinant enzyme was measured at optimal reaction temperature, pH and K^+ concentrations with varied acetaldehyde concentrations from 0.25 to 2.0 mmol/L. Each measurement was executed three times. The apparent kinetic data for the enzyme exhibiting no substrate inhibition were calculated using the Michaelis-Menten equation, or when substrate inhibition was observed using the equation: $v = V_{max} / (1 + K_m/S + S/K_i)$ [29]. All calculations were performed by Origin 2018.

2.10. Stability of recombinant enzymes in simulated gastrointestinal fluids

To investigate the tolerance of recombinant acetaldehyde dehydrogenase to gastric and intestinal fluids when used as food additive, its stabilities in simulated gastrointestinal fluids were measured. The simulated gastrointestinal fluids were prepared according to the reported method with slight modification [30]. Simulated gastric fluid (SGF) was consisted of 10 g/L pepsin, 2 g/L NaCl and 100 mM pH 2.0 citric acid-phosphate buffer, while the simulated intestinal fluid (SIF) was consisted of 10 g/L trypsin and 100 mM pH 6.8 phosphate buffer. The stability of recombinant enzymes in simulated gastrointestinal fluids was examined by mixing the recombinant enzymes with the above SGF and SIF at ratio of 1:1, respectively, and incubated at 37°C for 160 min, and then, its residual activity was measured at optimal reaction temperature, pH and K^+ concentrations.

2.11. Alcohol tolerance test of recombinant E. coli BL21 strain

To investigate the tolerance of recombinant *E. coli* BL21 strain to alcohol, its stress tolerance test was measured. The recombinant *E. coli* BL21 strain containing *Lp*ALDH was picked and cultured for 14 h in tubes containing 4 mL LB medium with 50 μg mL^{-1} kalamycin at 37°C and 200 rpm, and then, the cultures were transferred into flasks containing 100 mL fresh LB medium containing 50 μg mL^{-1} kalamycin. After cultivation at 37°C for 2.5 h, these cultures were divided into two large groups (A and B), each of which was divided into five groups (1–5). The group A was not added IPTG, and only added alcohol to final concentrations of 0%-8% (groups 1–5), while the group B was added IPTG to a final concentration of 0.1 mM and added alcohol to final concentrations of 0%-8% (groups 1–5). Then, they were induced for 20 h at 16°C, and their OD_{600} values were measured at the 10th and 20th hours to determine their alcohol tolerance.

3. Results and discussion

3.1. Gene cloning of acetaldehyde dehydrogenase

Genomic DNA of *L. plantarum* was used as template for PCR amplification, and the amplified products were detected and analyzed by agarose gel electrophoresis (Fig. 1). As shown in Fig. 1, the fragment length of amplified products was about 1500 bp, which was basically consistent with the expected theoretical length. The purified amplified products were digested with *Bam*H I and *Eco*R I, and then ligated into the digested pET28a plasmid by same restriction enzymes. And then, the actual gene sequence of *Lp*ALDH was obtained by sequencing, and its amino acid sequences were also obtained by DNAMAN software. The alignment result showed that there was a difference of 2 bases between *Lp*ALDH actual gene sequence and reported gene sequence (NZ_CP097175.1) by NCBI, but the amino acid sequences were completely consistent with that reported one (WP_003641903) by NCBI.

3.2. Sequence analysis of acetaldehyde dehydrogenase

The inferred amino acid sequence of *Lp*ALDH was predicted on ProtParam for its theoretical physicochemical properties. And the results showed that the theoretical isoelectric point and molecular

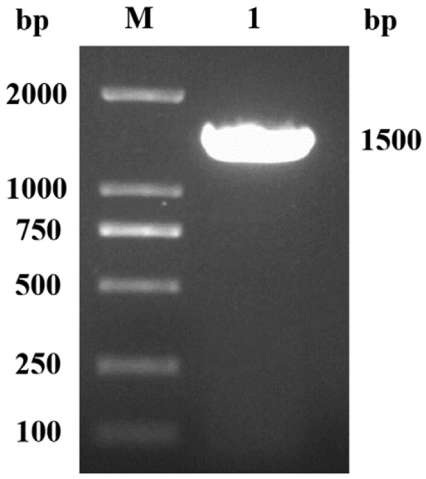

Fig. 1. The agarose gel electrophoresis analysis for PCR products of *Lp*ALDH. M: 2000 bp DNA ladder marker; 1: PCR products of *Lp*ALDH.

mass of *Lp*ALDH were 6.33 and 48676.76 Da, which the instability index II was 24.47. Moreover, its half-life in *E. coli* and yeast can reach more than 10 h and 20 h, respectively, indicating that the *Lp*ALDH may be a relatively stable protein. The amino acid sequence of *Lp*ALDH was taken as a probe for BLAST analysis, and 10 chosen acetaldehyde dehydrogenase sequences were searched. After multiple sequence alignment with ClustalX2, a phylogenetic tree of these acetaldehyde dehydrogenases was constructed by MEGA6.0, and the results are shown in Fig. 2. Based on phylogenetic tree analysis, it can be seen that *Lp*ALDH was more closely related to the *Pe*ALDH, an acetaldehyde dehydrogenase from *Pediococcus* sp., which has 62.9% sequence identity to *Lp*ALDH. While *Lp*ALDH had low homology with human acetaldehyde dehydrogenase (*Ha*ALDH) from *Homo sapiens*, their sequence identity was only 33.3%. Based on these results, *Lp*ALDH could be identified as a novel acetaldehyde dehydrogenase, suggesting that its enzymatic properties may be significantly different from those of previously reported acetaldehyde dehydrogenases.

Taking the amino acid sequence of *Lp*ALDH as a probe for BLAST analysis in Protein Data Bank (PDB), 3 crystal structures with the highest sequence identity with *Lp*ALDH were searched, whose PDB numbers were 3MY7, 5J78 and 3K9D, respectively. Among them, the acetaldehyde dehydrogenase (*Vp*ALDH, 3MY7) from *Vibrio parahaemolyticus* had the highest sequence identity with *Lp*ALDH (44.8%). The *Lp*ALDH was analyzed with them in multiple sequence alignment, and the results were shown in Fig. 3. It has been clearly reported that the four amino acid residues related to catalysis in *Pt*ALDH were His166, Cys273, Glu362, and His394 [31], and the results of multiple sequence alignment showed that the amino acid residues at these four sites are identical for the four compared acetaldehyde dehydrogenases, indicating that the four sites of acetaldehyde dehydrogenase are highly conservative. Moreover, the corresponding four residues in *Lp*ALDH were His144, Cys251, Glu342 and His374. The identities between *Lp*ALDH and the three templates are less than 65%, which is too low to ensure the facticity and accuracy of predicted 3D structure by single template homology modeling [32,33]. To ensure the facticity and accuracy of predicted 3D structures of *Lp*ALDH, three crystal structures with PDB number of 3MY7, 5J78 and 3K9D were used as templates to conduct multi-template homologous modeling by EasyModeller 2.0 and Modeller 9.9 software. And then, the predicted 3D structures were optimized. The predicted 3D structure of *Lp*ALDH was compared with that of *Pt*ALDH by PyMOL software, and the results are shown in Fig. 4. As shown in Fig. 4, the spatial positions of His144, Cys251, Glu342 and His37 of *Lp*ALDH were relatively close to each other, and the spatial positions of the four residues corresponding to *Pt*ALDH also largely overlapped, indicating that the 3D structures were still highly conserved despite the large differences in their primary structures.

3.3. Expression and purification of acetaldehyde dehydrogenase

To evaluate the expression level and enzymatic properties of *Lp*ALDH, the recombinant *E. coli* strain containing *Lp*ALDH encoding gene was picked and incubated as above method. The recombinant *Lp*ALDH (re*Lp*ALDH) was purified by Ni-chelating affinity chromatography. And the purified re*Lp*ALDH was examined by the SDS-PAGE analysis (Fig. 5). As shown in Fig. 5**,** the re*Lp*ALDH

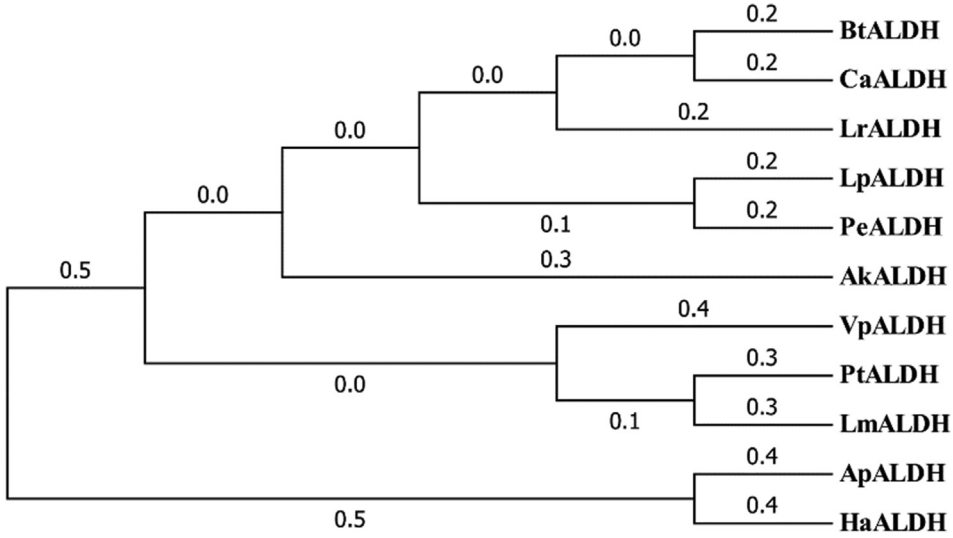

Fig. 2. The phylogenetic tree analysis of typical acetaldehyde dehydrogenases. *Bt*ALDH, ALDH of *Bacillus thuringiensis* (EEM39793.1); *Ca*ALDH, ALDH of *Carnobacterium sp.* CP1 (ALV22986.1); *Lr*ALDH, ALDH of *Lacticaseibacillus rhamnosus* (KM061556.1); *Lp*ALDH, ALDH of *Lactiplantibacillus plantarum* (this study); *Pe*ALDH, ALDH of *Pediococcus* sp. (HB047068.1); *Ak*ALDH, ALDH of *Apilactobacillus kunkeei* (KOY71851.1); *Vp*ALDH, ALDH of *Vibrio parahaemolyticus* (PDB: 3MY7); *Pt*ALDH, ALDH of *Parageobacillus thermoglucosidasius* (PDB: 5J78); *Lm*ALDH, ALDH of *Listeria monocytogenes* (PDB: 3K9D); *Ap*ALDH, ALDH of *Acetobacter pasteurianus* (WP_012813250.1); *Ha*ALDH, ALDH of *Homo sapiens* (AAP36614.A).

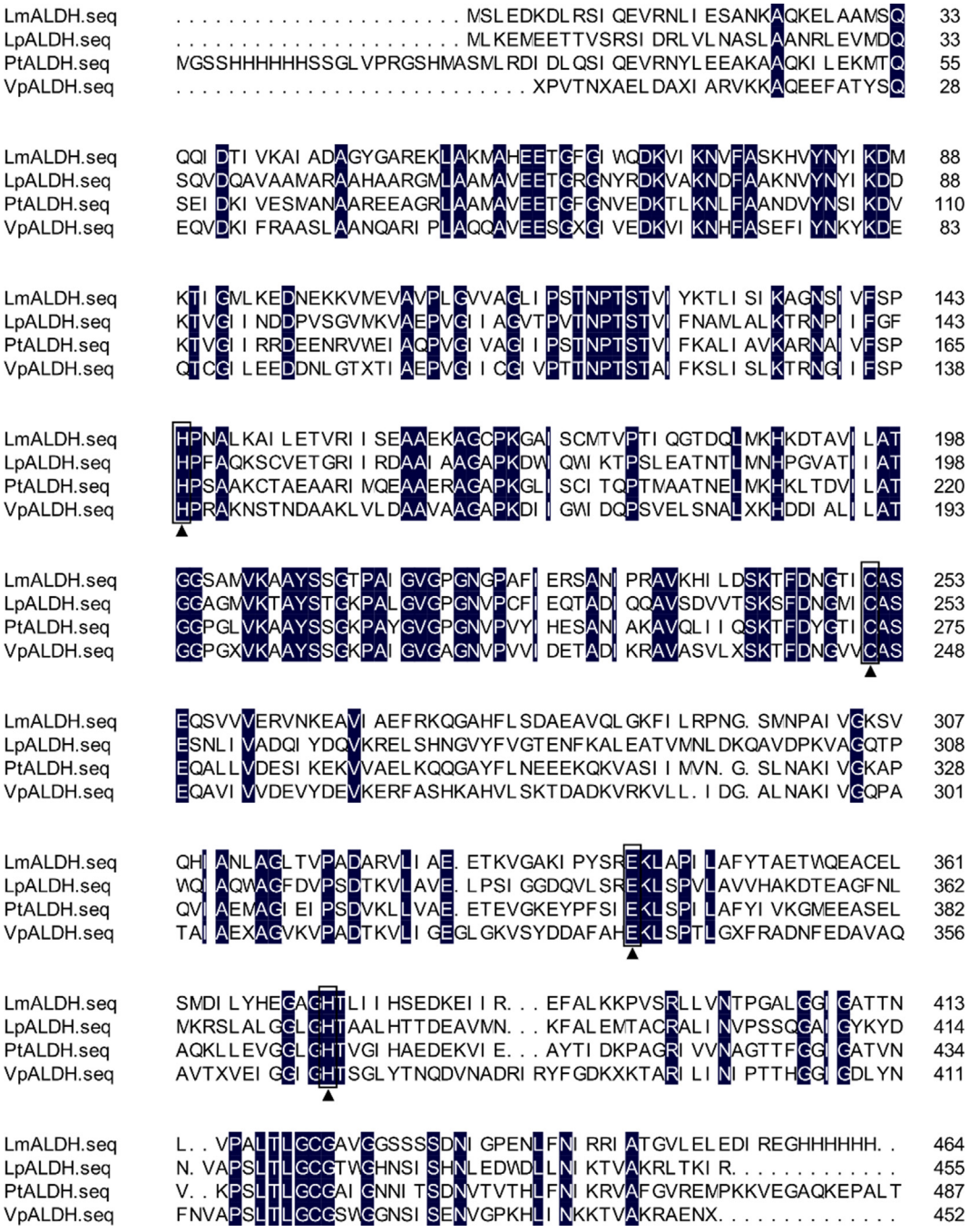

Fig. 3. The multiple sequence alignments of *Lp*ALDH with the other representative acetaldehyde dehydrogenases. *Lm*ALDH, ALDH of *Listeria monocytogenes* (PDB: 3K9D); *Lp*ALDH, ALDH of *Lactiplantibacillus plantarum* (this study); *Pt*ALDH, ALDH of *Parageobacillus thermoglucosidasius* (PDB: 5J78); *Vp*ALDH, ALDH of *Vibrio parahaemolyticus* (PDB: 3MY7). The four amino acids labeled by triangle are the key amino acid residues related to catalysis.

was successfully expressed in soluble form. Furthermore, the results indicated that the re*Lp*ALDH was purified to homogeneity with apparent molecular mass of 50 kDa, which was consistent with its theoretical molecular mass of 48676.76 Da. After measurement and conversion, the dehydrogenase activity in fermentation liquor was 195 U/mL, which was about 600 times than that expressed in original strain. Meanwhile, the expression level was about 2-fold that ALDH from *Issatchenkia terricola* expressed in *Bacillus subtilis* [10]. And the specific activity of the re*Lp*ALDH toward acetaldehyde was 1709 U/mg, which was significantly higher than that previously reported [15,16], and had greater potential for application.

3.4. Temperature characteristics of the reLpALDH

The optimal reaction temperature and temperature stability of the re*Lp*ALDH were measured according to the above method, and the results of temperature characteristics are shown in Fig. 6. As shown in Fig. 6A, the reaction rate of re*Lp*ALDH increases with the increase of temperature in the range of 20~35°C, and the reaction rate decreases after exceeding 35°C. The optimal reaction temperature of *Lp*ALDH is 35°C, which is close to the optimal growth temperature of the bacterium and the physiological temperature of human, indicating that it may have good temperature adaptability when it is used as a functional food additive. Regret-

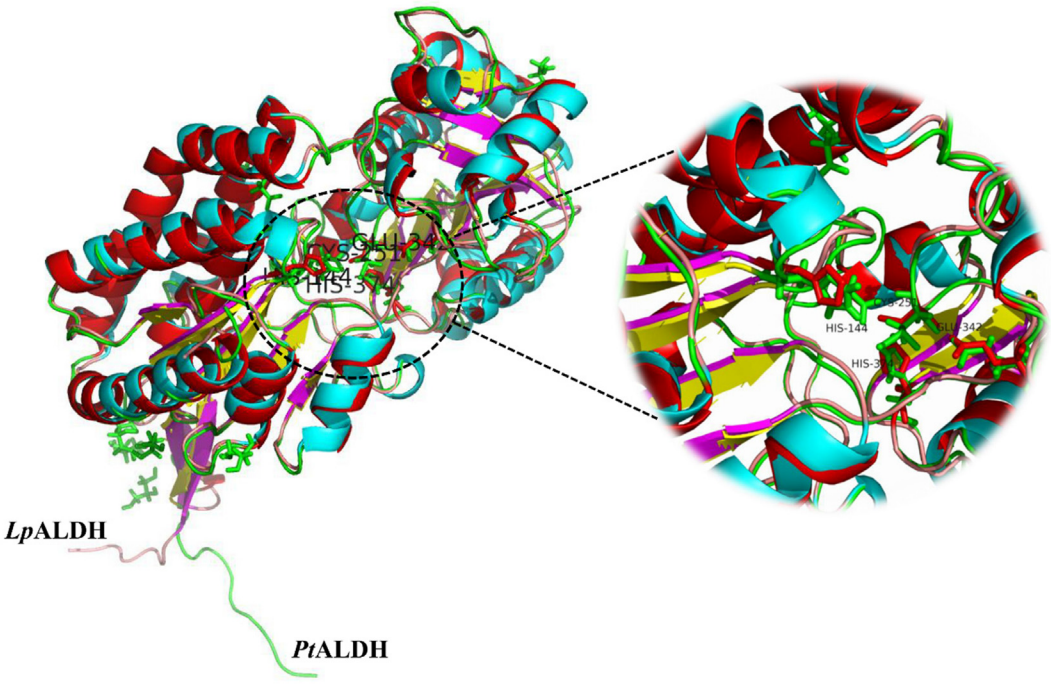

Fig. 4. Superimposition for the predicted 3D structures of *Lp*ALDH and *Pt*ALDH. Superposition of the *Lp*ALDH (red) and *Pt*ALDH (green) active site residues showing the similarity in position of His 144/166, Glu 342/362, His 374/394, and Cys 251/273.

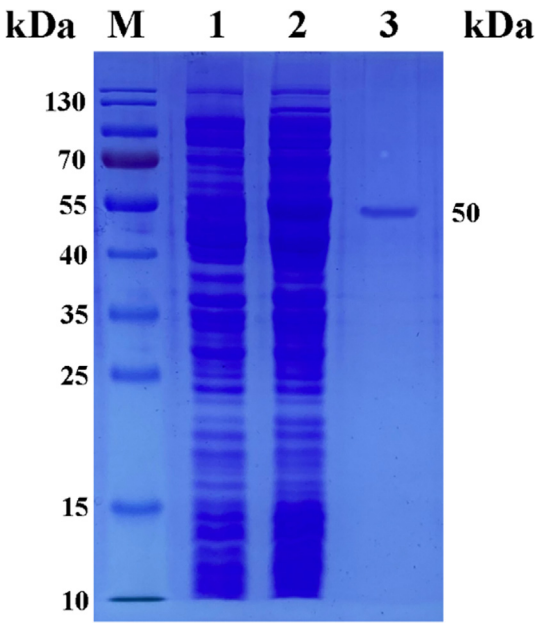

Fig. 5. The SDS-PAGE analysis for the recombinant acetaldehyde dehydrogenase. M: PageRuler Prestained Protein Ladder; 1: lysate supernatants of *E. coli* BL21/pET28a; 2: lysate supernatants of *E. coli* BL21/pET28a-*Lp*ALDH; 3: purified products of re*Lp*ALDH.

tably, the temperature stability of *Lp*ALDH is poor. As can be seen from Fig. 6B, the residual activity is basically only about 50% after incubating at 30, 35 and 40°C for 70 min, which may limit its application in functional food additive field. In our subsequent studies, it is still necessary to properly improve its temperature stability by means of rational design or directed evolution to meet the needs of actual production and application [32].

3.5. pH characteristics of the reLpALDH

The pH characteristics of the re*Lp*ALDH were shown in Fig. 6. pH of buffer too high or too low will lead to changes in spatial structure of the enzyme, thus reducing its catalytic activity [8]. As shown in Fig. 6C, the pH value of buffer can significantly affect the catalytic activity of the re*Lp*ALDH. It exhibited the highest catalytic activity at pH value of 7.0 to 8.0, which is basically consistent with the human physiological pH, indicating that the re*Lp*ALDH has good pH adaptability when it is used as a functional food additive. As shown in Fig. 6D, the re*Lp*ALDH has good pH stability and can retain more than 70% residual activity between pH 7 and 9, indicating that the re*Lp*ALDH can theoretically tolerate the pH environment of human intestinal solution and blood when it is used as a functional food additive [30].

3.6. Optimal K^+ concentration of the reLpALDH

The optimal K^+ concentration of re*Lp*ALDH was determined using the above method, and the results are shown in Fig. 7. The re*Lp*ALDH exhibited the highest catalytic activity at K^+ concentration of 0.25 to 0.3 mol/L, and the relative activity could be above 80%. And it decreased significantly when the K^+ concentration was lower than 0.25 mol/L or higher than 0.3 mol/L. The results showed that the re*Lp*ALDH was also a K^+ -dependent acetaldehyde dehydrogenase, and its activity was significantly affected by K^+ concentration, too low concentration of K^+ cannot fully play its prosthetic group role, while too high concentration of K^+ will cause significant inhibition on its activity. If the concentration of K^+ in the application environment can be properly controlled when it is used as a functional food additive, its ability to eliminate acetaldehyde will be further improved.

3.7. Kinetic parameters of the reLpALDH

The kinetic parameters of the re*Lp*ALDH toward acetaldehyde were determined according to the above method, and the nonlin-

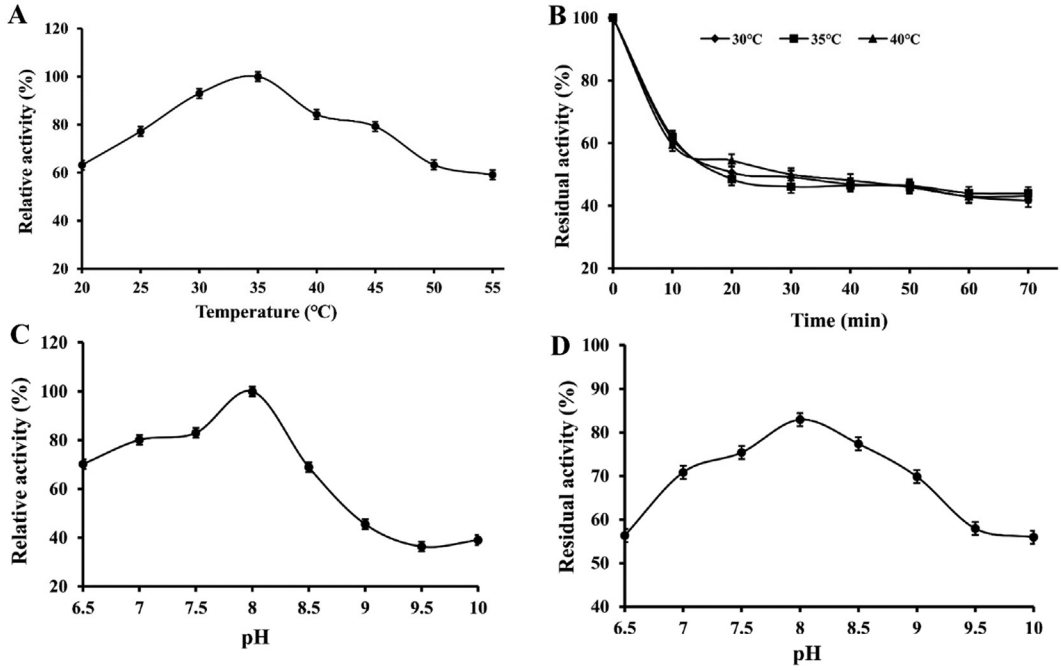

Fig. 6. The temperature and pH characteristics of the *reLp*ALDH. (A) The optimal reaction temperature of the *reLp*ALDH. The optimal reaction temperature of the re*Lp*ALDH toward acetaldehyde was determined under the standard assay conditions as above, except temperatures ranging from 20 to 55°C. **(B) The thermostability of the re*Lp*ALDH.** The residual enzyme activity was measured at its optimal reaction temperature after being preincubated at 30, 35 and 40°C for 0∼70 min, respectively. **(C) The pH optimum of the re*Lp*ALDH.** The pH optimum of the re*Lp*ALDH was assayed by the standard activity assay method as stated above except the reaction pH values ranging from 6.5 to 10.0. **(D) The pH stability of the re*Lp*ALDH.** The residual activities were assayed under the optimal reaction temperatures and pH values after preincubating at 0°C for 1.0 h in varied pH values from 6.5 to 10.0.

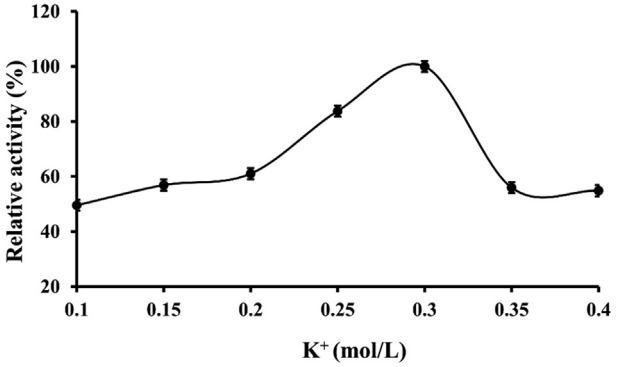

Fig. 7. The optimal K^+ concentration of the re*Lp*ALDH. The optimal K^+ concentration of the re*Lp*ALDH was measured at optimal reaction temperature and pH value with varied K^+ concentrations from 0.1 to 0.4 mol/L.

ear fitting curve for the kinetic parameters of the *reLp*ALDH was performed by Origin 2018, as shown in Fig. 8. It exhibited high catalytic efficiency and affinity toward acetaldehyde, its V_{max} and K_m values were 334.47 μmol·min^{-1}·mg^{-1} and 0.04 mM. Compared with the reported recombinant human ALDH1B1 [34], its K_m value is basically consistent with what has been reported (669 nmol·min^{-1}·mg^{-1}), and its V_{max} value is about 500 folds than that has been reported (0.05 mM). After the conversion, its K_{cat} and K_{cat}/K_m values were 271.35 s^{-1} and 6957.66 s^{-1}·mM^{-1}, indicating that the reLpALDH has great potential in the rapid removal of acetaldehyde.

3.8. Stability of reLpALDH in simulated gastrointestinal fluids

To investigate the tolerance of recombinant acetaldehyde dehydrogenase to gastric and intestinal fluids when used as food additive, its stabilities in simulated gastrointestinal fluids were measured, and the results are shown in Fig. 9. Under the above conditions, the residual activity of re*Lp*ALDH could still maintain at more than 50% after incubating 160 min, which was basically consistent with the result of temperature stability, and indirectly indicated that the re*Lp*ALDH had a good tolerance to pepsin and trypsin in this environment, and could well adapt to the future application environment.

3.9. Alcohol tolerance test of E. coli BL21/pET28a-LpALDH

The alcohol tolerance test of *E. coli* BL21/pET28a-*LpALDH* was determined according to the above method, and the results were shown in Table 1. As shown in Table 1, the induced *E. coli* BL21/

Table 1
The OD_{600} values of *E. coli* BL21/pET28a-*LpALDH* strains in different culture conditions and time.

Time (h)	0% alcohol		2% alcohol		4% alcohol		6% alcohol		8% alcohol	
	A	B	A	B	A	B	A	B	A	B
0	1.025	1.084	1.009	1.019	1.099	1.014	1.010	1.011	1.020	1.050
10	1.982	1.959	1.886	1.931	1.761	1.899	1.259	1.455	1.135	1.258
20	2.494	2.512	1.874	1.994	1.685	1.874	1.231	1.384	1.082	1.207

The group A was not added IPTG, and the group B was added IPTG to a final concentration of 0.1 mM.

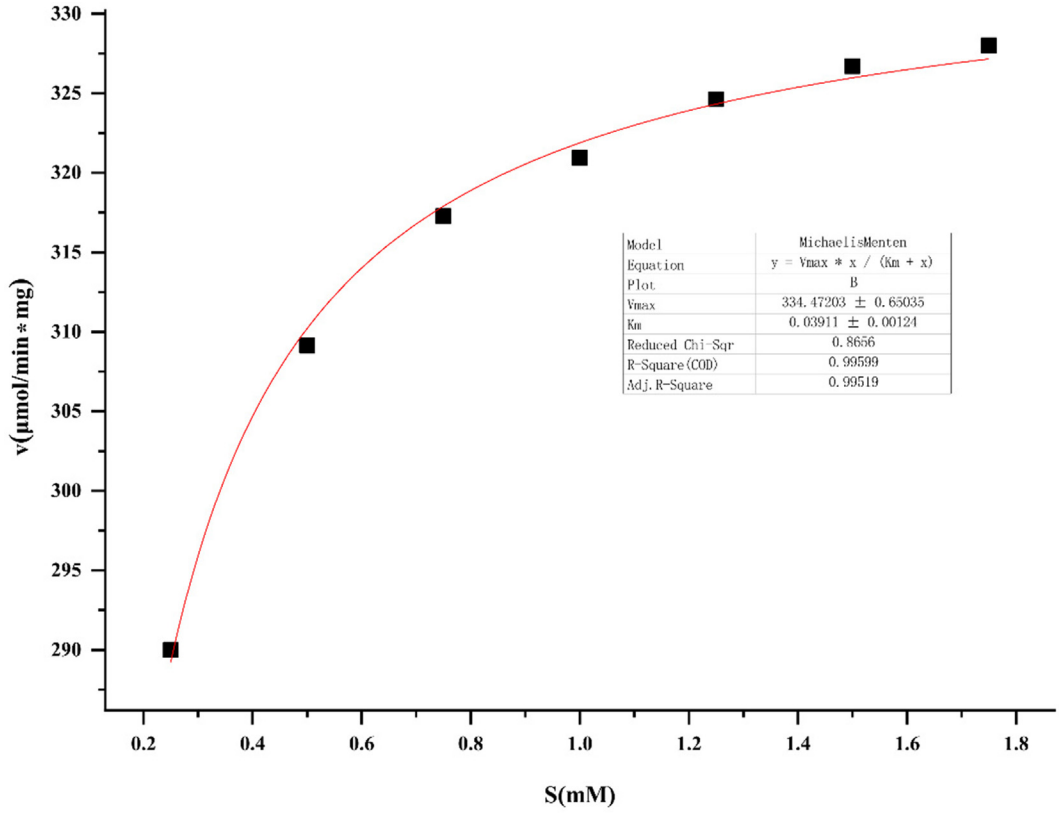

Fig. 8. The nonlinear fitting curve for the kinetic parameters of the re*Lp*ALDH. The catalytic activity of the recombinant enzyme was measured at optimal reaction condition with varied acetaldehyde concentrations from 0.25 to 2.0 mmol/L. All calculations were performed by Origin 2018.

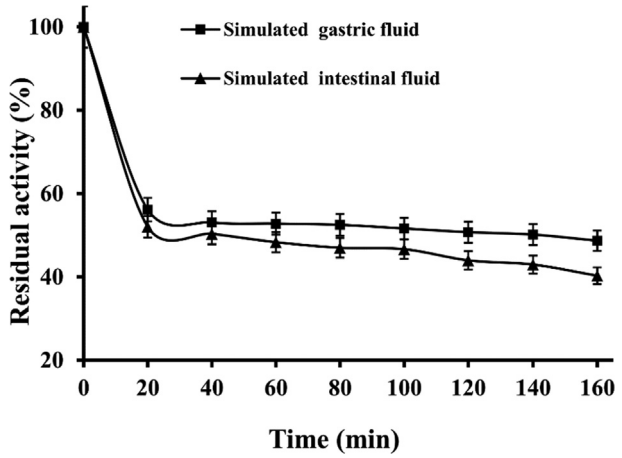

Fig. 9. The stability of re*Lp*ALDH in simulated gastrointestinal fluids. The stability of re*Lp*ALDH in simulated gastrointestinal fluids was examined by mixing the re*Lp*ALDH with the SGF and SIF at ratio of 1:1, respectively, and incubated at 37°C for 160 min, and then, the residual activity was measured at the optimal reaction conditions.

pET28a-*LpALDH* had better alcohol tolerance than the uninduced *E. coli* BL21/pET28a-*LpALDH*, which also showed that overexpression *LpALDH* could improve the alcohol tolerance of *E. coli* BL21.

4. Conclusions

In conclusion, a novel acetaldehyde dehydrogenase (*Lp*ALDH) from *L. plantarum* was obtained by gene mining and then was analyzed by a series of bioinformatics software. Phylogenetic tree results showed that *Lp*ALDH was highly homologous with acetaldehyde dehydrogenase derived from *Pediococcus* sp., and their sequence similarity was 62.9%. Moreover, it was successfully expressed in *E. coli* BL21, its expression level was 195 U/mL, which was about 600 times than that expressed in original strain. After purification, the specific activity of purified *Lp*ALDH was 1709 U/mg. Its temperature optimum was 35℃, and optimal reaction pH was 8.0. Moreover, the purified *Lp*ALDH showed good stability in simulated gastrointestinal fluids, which indicated that it had great potential in the functional food additive field. This study also established a solid theoretical basis for further molecular modification, optimal expression host selection and application of acetaldehyde dehydrogenases.

Author contributions

- Study conception and design: Z Wang, C Tang
- Data collection: J He, X Liu, J Shi
- Analysis and interpretation of results: Y Lu, H Shi
- Draft manuscript preparation: C Tang, L Yao, Z Wang
- Revision of the results and approved the final version of the manuscript: Y Kan

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Conflict of interest

The authors declare that they have no competing interests.

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