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Full Length Research Paper

Study on soluble expression of glutamate dehydrogenase from tea plant in *Escherichia coli* using fusion tags

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Glutamate dehydrogenase (GDH; EC1.4.1.2) catalyses the reversible amination of 2-oxoglutarate for the synthesis of glutamate using ammonium as a substrate. Herein, a homology-based reverse transcription polymerase chain reaction (RT-PCR) strategy was employed to search for cDNAs encoding GDH from *Camellia sinensis* cv Longjing 43, an excellent variety of tea tree most suitable for processing into green tea in China. The full-length cDNA encoding GDH α-subunit, designated as *CsGDH2* (GenBank accession No. EU715396) was amplified from the tea plant. The cDNA of *CsGDH2* was 1667 bp with a 1236 bp open reading frame which encodes a 411 amino acid polypeptide. The bioinformatics analysis showed that *CsGDH2* was highly homologous to plant *GDH2* which encodes GDH α-subunit of plant. Phylogenetic tree reconstructed for GDH protein family confirmed the result because CsGDH2 was distinctly clustered into the GDH2 clade of plant. Then, to improve the expression of CsGDH2 in soluble form in *Escherichia coli*, its full-length cDNA was cloned and expressed with several different N-terminal fusion tags in *E. coli*, such as B1 immunoglobulin binding domain of protein G (GB1), glutathione-S-transferase (GST), maltose-binding protein (MBP) and N-utilization substance protein (NusA). The results reveal that without any solublizing partners, the fusion CsGDH2 was predominantly found in insoluble bodies and no soluble protein was detected by either sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) or western blot, whereas GB1, MBP and NusA tags may enhance the soluble expression of CsGDH2 to different extents. The MBP tag was found to be the best fusion partner for the soluble expression of CsGDH2. Isolation and cloning of important functional genes have significances on elucidating the molecular mechanism of high quality, yield and resistance for tea plant, as well as the genetic manipulating. This work would provide the possibility for further insights into the physiological role of GDH in nitrogen metabolism in plants.

Key words: Glutamate dehydrogenase, *Camellia sinensis*, RT-PCR, cDNA, bioinformatic analysis, soluble expression.

INTRODUCTION

Glutamate dehydrogenase (GDH; EC1.4.1.2) catalyzes the reversible oxidative deamination of L-glutamate to 2-oxoglutarate and ammonia, with nicotinamide adenine dinucleotide phosphate ($NAD(P)^+$) as a cofactor.

Theoretically, the enzyme is able to either liberate or assimilate ammonium *in vivo*. Since the conversion of these metabolites constitutes the major link between carbon (C) and nitrogen (N) metabolism in plants, GDH appears to play a pivotal role in plant metabolism. It has been demonstrated that the ammonium is predominantly assimilated via the glutamine synthetase/glutamate synthase (GS/GOGAT; EC 6.3.1.2/EC 1.4.7.1 and EC 1.4.1.14) pathway (Lea and Miflin, 1974), and the K_m for

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ammonium of GDH aminating activity is over 100 times higher than that of GS (Stewart and Larher, 1980). These results indicate that GDH plays a negligible role in assimilating ammonia in higher plants. However, it cannot be discarded that GDH could function in ammonium assimilation under certain physiological conditions (Oaks, 1995; Melo-Oliveira et al., 1996; Dubois et al., 2003). For instance, the upregulation of GDH in response to the elevated ammonium levels implies that GDH is essential in the detoxification of excess ammonium ions (Tercé-Laforgue et al., 2004a; Tercé- Laforgue et al., 2004b; Skopelitis et al., 2006). Furthermore, several experiments suggest that GDH plays catabolic role to funnel the C skeletons of glutamate into the citric acid cycle (TCA cycle) for energy production under C limited conditions (Masclaux-Daubresse et al., 2006; Miyashita and Good, 2008). However, a recent study indicates that GDH actually contributes to the control of glutamate homeostasis, but not to supply C skeletons for TCA cycle in tobacco leaf (Labboun et al., 2009).

All plant species have at least two GDH genes, each of which encodes α or β subunits, respectively. These two subunits are able to assemble randomly to form seven hexameric isoenzymes (Loulakakis and Roubelakis-Angelakis, 1991; Purnell et al., 2005). Two recent studies have revealed the functions of the GDH isoenzyme 1 (a β-subunit homohexamer) and the GDH isoenzyme 7 (an α-subunit homohexamer) by transgenic tobacco plants. Under normal growth conditions, the GDH isoenzyme 1 solely deaminates glutamate (Purnell et al., 2007), whereas the GDH isoenzyme 7 exhibits strong deaminating activity and only a very low aminating activity *in vivo* (Skopelitis et al., 2007). However, the physiological roles and regulation of the different GDH isoenzymes are still unclear up to now.

Tea plants are an important beverage crop and widely cultivated in sub-tropical and tropical regions. Since the vegetative growth and characteristic components of the tea flush are mainly determined by the amount of N supplied (Venkatesan and Ganapathy, 2004; Ruan et al., 2010), such as the production of young shoots and the content of L-theanine (N^5-ethyl-glutamine, Thea) which reduces psychological and physiological stress responses, the desired level of N are used to obtain the maximum yield and quality tea. GDH plays an important role in plant N metabolism, thus the investigation of GDH functions from tea plant is meaningful for elaboration of the physiological roles of this enzyme.

In this study, a homology-based RT-PCR strategy was employed to search for cDNAs encoding GDH from *Camellia sinensis* cv Longjing 43, a native green tea plant in China. The full-length cDNA encoding GDH α-subunit (*CsGDH2*) was cloned and expressed in *E. coli*. Considering the difficulty of eukaryotic gene expression in prokaryotic system, several fusion tags were respectively added to the N-terminus of CsGDH2 to improve the yield of soluble and functional recombinant proteins in *E. coli* (Esposito and Chatterjee, 2006; Waugh, 2005), including B1 immunoglobulin binding domain of protein G (GB1) (Bao et al., 2006), glutathione-S-transferase (GST) (Nygren et al., 1994), maltose-binding protein (MBP) (Kapust and Waugh, 1999) and N-utilization substance protein (NusA) (Davis et al., 1999). The effects of fused tags are analyzed and discussed.

MATERIALS AND METHODS

Plant material

Young and most recently emerged developing leaves from fresh shoots of *C. sinensis* cv Longjing 43 growing in normal soil condition were plucked in the tea plantation of Anhui Agriculture University. The leaves were frozen in liquid N_2 and stored at -70°C until extraction of RNA.

RNA extraction and cDNA synthesis

Total RNA was extracted with the SV Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer's instructions. Total RNA was used to synthesize the first-strand cDNA using PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Japan).

Amplification of the conserved fragment of *CsGDH2*

The degenerate primers P1 and P2 (Table 1) were used to amplify the conserved region of *CsGDH2*, which were derived from highly conserved amino acid sequence motifs of plant GDHs and designed with a computer program named COnsensus-DEgenerate Hybrid Oligonucleotide Primer (CODEHOP) (Rose et al., 1998). The conserved protein blocks were created according to the multiple sequence alignment of plant GDHs using the computer program Block Maker (http://www. bioinformatics.weizmann.ac.il/blocks/blockmkr/www/make_blocks.html). PCR was carried out in a thermal cycler (Bio-Rad iCycler) and performed by initial denaturation at 94°C for 3 min followed by 35 cycles of 30 s at 95°C, 45 s at 56°C, 1 min at 72°C with a final extension at 72°C for 10 min. The resulting PCR product was cloned into pMD19-T (TaKaRa, Japan) vector by TA cloning method and then sequenced.

Isolation of the full-length cDNA of *CsGDH2*

Rapid amplification of cDNA ends (RACE) was carried out using the Invitrogen GeneRacer kit (Invitrogen, USA). Both 5′- and 3′-RACE reactions were performed on total RNA extracted from *C. sinensis* cv Longjing43 fresh shoots. Briefly, RNA was treated with calf intestinal phosphatase (CIP) to remove the 5′-phosphates of the truncated mRNA and non-mRNA forms of total RNA. Dephosphorylated RNA was then treated with tobacco acid phosphatase (TAP) to remove the 5′ cap structure from intact full-length mRNA. A RNA oligonucleotide was ligated onto the 5′ end of the intact mRNA using T4 RNA ligase to provide a priming site for subsequent 5′ RACE. SuperScript III RT and GeneRacer Oligo (dT) provided in the kit were used for reverse-transcribing the RNA oligonucleotide-ligated mRNA to single strand cDNAs. Gene specific primers, 5′-GSP and 3′-GSP (Table 1), for the RACE PCR were designed from the conserved fragment of *CsGDH2* and amplified. The PCR product was TA-cloned into pMD19-T and

Table 1. Primers used in this study.

Primer name	Primer sequence (5′→ 3′)
P1	CGCCCGGGGCCCNATGAARGG
P2	TCACGCCCAGGGTGAANGCNCCCAT
5′-GSP	TTGCATCCAATTCCACCCTTAGCTC
3′-GSP	TGATATATATGCAAATTCTGGAGGT
SG-*Nde*I	GGAATTCCATATGAACGCTCTCGCCGCCACAAACC
SG-*Bam*HI	AATATCGCGGATCCATGAACGCTCTCGCCGCCACAAACC
ASG-*Not*I	ATAAGAATGCGGCCGCCGCTTCCCAACCCCTTAATACAGTT
GST-*Nde*I	CGGACGCCATATGTCCCCTATACTAGGTTATTGGAAAA
GST-*Bam*HI	AATATCGCGGATCCGCGTGGCACCAGACCAGAAGAATCCGATTTTGGAGGATGGTCGCC
MBP-*Nde*I	CGGACGCCATATGAAAATAAAAACAGGTGCACGCATCC
MBP-*Bam*HI	AATATCGCGGATCCGCGTGGCACCAGACCAGAAGACTTGGTGATACGAGTCTGCGCGTC
NusA-*Nde*I	CGGACGCCATATGAACAAAGAAATTTTGGCTGTAGTTG
NusA-*Bam*HI	AATATCGCGGATCCGCGTGGCACCAGACCAGAAGACGCTTCGTCACCGAACCAGCAAAT

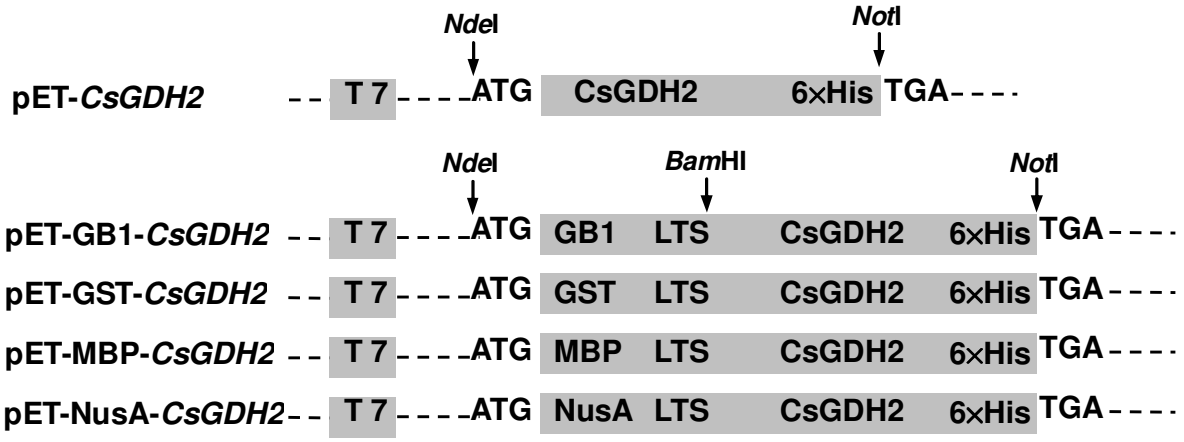

Figure 1. Schematic representation of the fusion constructs. pGBTNH contains an inducible T7 RNA polymerase promoter, the GB1-tag, a linker, a thrombin cleavage site, a multiple cloning site, and a C-terminal 6×His tag (Bao et al. 2006). T7, T7 RNA polymerase promoter; ATG, start codon; LTS, linker and thrombin site; TGA, stop codon; GB1, B1 immunoglobulin binding domain of protein G; GST, glutathione-S-transferase; MBP, maltose-binding protein; NusA, N-utilization substance protein.

sequenced. Three sequences, 5′ and 3′ ends together with the conserved fragment of *CsGDH2*, were assembled with Sequencher version 4.0.5 (Gene Codes Corporation).

Bioinformatics analysis

Amino acid sequences of plant GDHs were downloaded from GenBank via the NCBI web site (http://www.ncbi.nlm.nih.gov/). The homologues of CsGDH2 proteins were found using BLAST program (http://www.ncbi.nlm.nih.gov:/BLAST/). Multiple protein sequence alignments were carried out with ESPript 2.2 program (http://espript.ibcp.fr/ESPript/ESPript/) (Gouet et al., 1999). Phylogenetic tree was constructed using MEGA 4 software (http://www.megasoftware.net/) (Tamura et al., 2007).

Preparation of modified expression vectors

pET-GB1 (or pGBTNH) was used as the initial vector in this study. It is a pET-22b derivative expression vector harboring a 56-residue B1 immunoglobulin binding domain of *Streptococcal* protein G (GB1) as a fusion tag together with a three-residue linker sequence and a six-residue thrombin cleavage site between the restriction sites *Nde*I and *Bam*HI (Figure 1) (Bao et al., 2006). This modified expression vector was proved to be effective in enhancing the stability and solubility of the fused target protein in *E. coli*. Three other vectors, pET-GST, pET-MBP and pET-NusA with an N-terminus GST, MBP and NusA fusion tags respectively, were constructed with the backbone of pET-GB1. The open reading frame (ORF) encoding GST was amplified from pGEX-4T-1 vector (Pharmacia, USA) with primers GST-*Nde*I/GST-*Bam*HI (Table 1). ORFs encoding MBP and NusA were amplified from *E. coli* MG1655 genomic DNA with primers MBP-*Nde*I/MBP-*Bam*HI and NusA-*Nde*I/NusA-*Bam*HI (Table 1), respectively. All PCR products were excised by *Nde*I and *Bam*HI and each ligated into the same sites of pET-GB1. The resulting plasmids were denoted pET-GST, pET-MBP and pET-NusA (Figure 1). All inserts were verified by DNA sequencing.

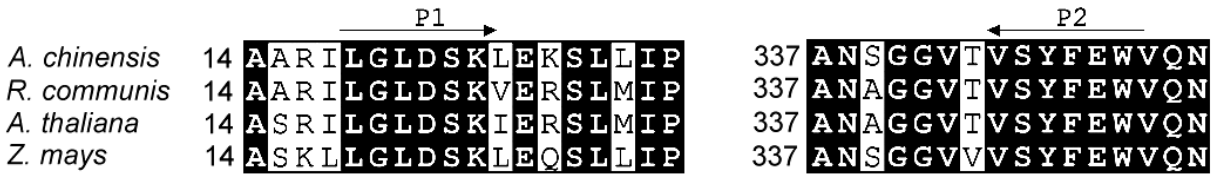

Figure 2. Block region created from conserved regions of GDH sequence from *A. thaliana* (AAB01222), *A. chinensis* (ABR45724), *R. communis* (EEF51639) and *Z. mays* (AAB80935). Amino acids used for the creation of the hybird primers P1 and P2 are marked by arrows. GDH, Glutamate dehydrogenase.

Construction of the recombinant expression vectors

The ORF of *CsGDH2* was amplified from pMD19-T-*CsGDH2* with primers SG-*Nde*I/ASG-*Not*I and SG-*Bam*HI/ASG-*Not*I (Table 1), respectively. Then, the PCR product digested with *Nde*I and *Not*I was ligated into pET-GB1 to generate pET-*CsGDH2* (Figure 1). The PCR product digested with *Bam*HI and *Not*I was inserted into the corresponding predigested pET-GB1, pET-GST, pET-MBP and pET-NusA. The recombinant plasmids were denoted pET-GB1-*CsGDH2*, pET-GST-*CsGDH2*, pET-MBP-*CsGDH2* and pET-NusA-*CsGDH2*, respectively (Figure 1). All expression vectors contained a 6 × His-tag at the C-terminus of CsGDH2 for the convenience of purification and western blot detection of the recombinant protein.

Overexpression and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

The *E. coli* Rosetta (DE3) strains (Novagen) harboring the set of expression vectors were cultured overnight in Luria-Bertani (LB) medium at 37°C, supplemented as required with 30 µg/ml kanamycin and 20 µg/ml chloramphenicol. About 1 ml of preculture was inoculated into 50 ml fresh LB medium with the same antibiotics to grow until the cell density reached an OD_{600} of 0.5 to 0.6. Then, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM to the culture with subsequent cultivation for 5 h at 37°C or 20 h at 22°C, respectively.

Cells were harvested by centrifugation and resuspended in lysis buffer (50 mM Tris-HCl, pH8.0, 150 mM NaCl, and 1 mM ethylenediaminetetraacetic acid (EDTA)). The total protein samples were collected from the cell suspension after sonication. The soluble protein samples were collected from the supernatant after removing the insoluble debris by centrifugation at 12,000 g for 15 min at 4°C. Samples were subjected to SDS-PAGE and visualized by staining with Coomassie Brilliant Blue.

Western immunobloting

SDS-PAGE gels were transferred to nitrocellulose membranes by electroblotting. The membranes were blocked for 1 h at room temperature in TBS-T (50 mM Tris-HCl pH7.5, 150 mM NaCl, 0.2% Tween-20) containing 5% nonfat milk and then washed with TBS-T for three times. His-tag polyclonal antibody (Cell Signaling Technology Inc., Beverly, MA, USA) was applied to the blots for 1 h at room temperature. After three 10 min washes with TBS-T, the blots were incubated for 1 h with alkaline phosphatase conjugated anti-rabbit IgG (Promega, Madison, WI, USA). The bound conjugate was then incubated with the Lumi-Phos^{TM} WB Chemiluminescent Substrate (Pierce Biotechnology, U.S.A.). The chemiluminescence signal, corresponding to specific antibody-antigen reaction on the blots, was visualized by exposing the blots to X-ray film for 15 min in a dark room.

RESULTS AND DISCUSSION

Cloning of the full-length cDNA of *CsGDH2*

The highly conserved protein blocks were created based on the amino acid sequence alignment of GDHs from four plants, including *Arabidopsis thaliana*, *Actinidia chinensis*, *Ricinus communis* and *Zea mays*. Then, these protein blocks were submitted to CODEHOP program for primer design (Rose et al., 1998), resulting in the sense primer P1 and the antisense primer P2 for PCR amplification (Figure 2).

As expected, a 965-bp conserved cDNA fragment was obtained by RT-PCR with P1 and P2. Based on the conserved fragment, two specific primers 5′-GSP and 3′-GSP were designed and used for 5′- and 3′-RACE PCR reactions to achieve the 5′ and 3′ ends of *CsGDH2* gene. The three fragments were assembled into the full-length of *CsGDH2* gene (GenBank accession no. EU715396). This 1667 bp gene consisted of an open reading frame beginning with the ATG start codon at base 174 and ending at the TGA stop codon at base 1409, encoding a 411 amino acid polypeptide with a predicted molecular weight of 44.8 kDa.

CODEHOPs derived from amino acid sequence motifs which are highly conserved between members of a protein family have proven to be highly effective in the identification and characterization of distantly related family members (Rose et al., 1998). Our results also confirm that CODEHOP-mediated PCR is a powerful technique to amplify new members based on the conserved motifs of known protein family.

Bioinformatic analysis of CsGDH2

A BLAST search of the available sequence databases suggested that the deduced amino acid sequence of *CsGDH2* was highly homologous to that of plant *GDH2*, a gene encoding the GDH α-subunit (Loulakakis and Roubelakis-Angelakis, 1991; Purnell et al., 2005). This search showed that CsGDH2 shared high amino acid sequence identity to GDH2s from *A. chinensis* (92%) (GenBank accession number ABR45714), *Brassica napus* (87%) (BAE45943), *A. thaliana* (85%) (AAB01222)

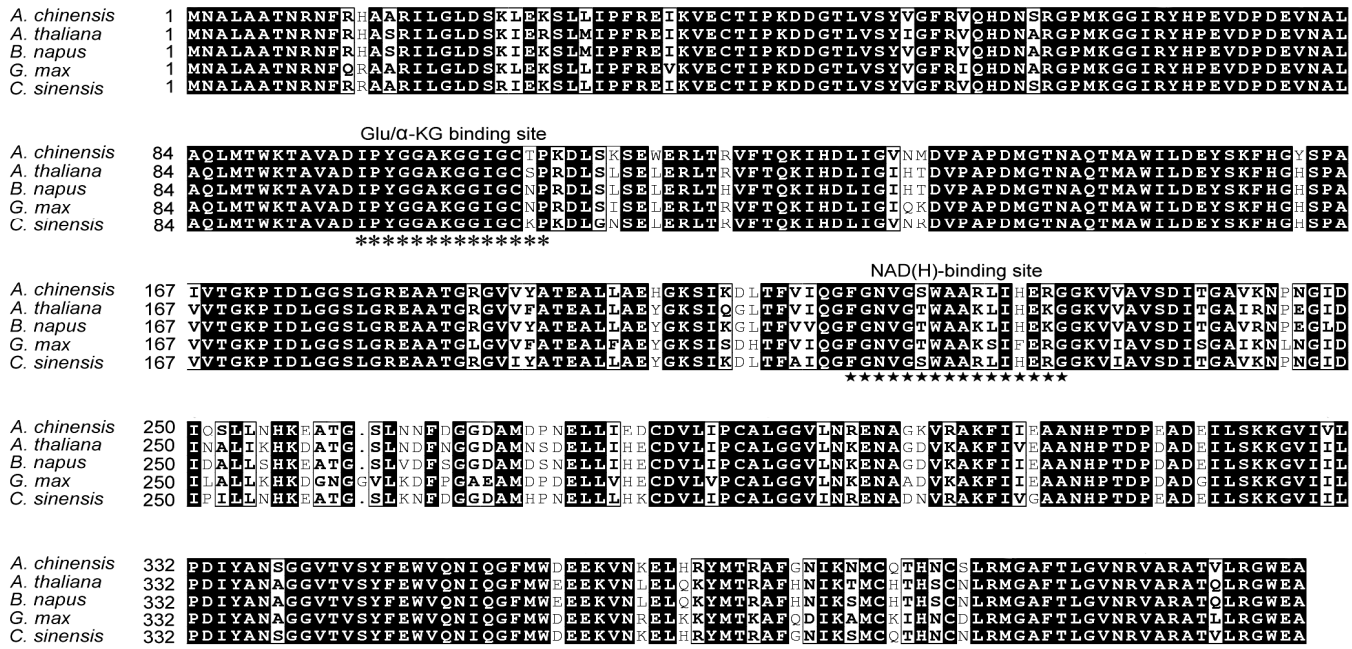

Figure 3. Multiple sequence alignment of CsGDH2 with other four plant GDH2s. The aligned GDH2s are from *C. sinensis* (ACH97123), *A. chinensis* (ABR45714), *A. thaliana* (AAB01222), *B. napus* (BAE45943) and *G. max* (CAI53674). Two conserved region in all GDH2 sequences, the Glu/α-KG binding site and NAD(H)binding site, are boxed.

and *Glycine max* (85%) (CAI53674) (Figure 3). Therefore, we may reasonably conclude that *CsGDH2* gene obtained in this study encodes GDH α-subunit of the tea plant.

Further analysis of multiple sequence alignment revealed that plant GDH2s had high similarity throughout the entire coding region (Figure 3). They all contained a Glu/α-KG binding site which was identical in other amino acid dehydrogenases (region 1 in Figure 3) and a NAD(H)-binding motif G-X-G-X_2-G-X_{10}-G (region 2 in Figure 3) which was conserved among all NAD-dependent GDHs (Inokuchi et al., 2002). This glycine-rich motif is considered to be the fingerprint of NAD-GDHs, which lies close to the adenine ribose, dictates the nature of the hydrogen bonding from the main chain to the adenine ribose moiety (Baker et al., 1992). Generally, NAD-dependent dehydrogenases have an Asp (D) or a Glu (E) at the C-terminus of the second β-strand on the nucleotide-binding domain, which forms important hydrogen bonds to the adenine ribose hydroxyl groups of the cofactor, such as the critical Glu or Asp in NAD-dependent malate dehydrogenases (Wang et al., 2011). In this study, the responding residue is Asp237 in CsGDH2 (Figure 3).

Molecular evolution analysis

A subset of protein sequences from representative species was used to reconstruct the phylogeny of GDH family by MEGA 4 using neighbor-joining method with 1000 bootstrap replicates. The phylogenetic tree clearly showed that these proteins fell broadly into two major subfamilies (I and II) with well-supported bootstrap values (Figure 4). The proteins in subfamily I are NADP(H)-dependent GDHs because they have a conserved NADP(H)-binding motif G-X-G-X_2-A-X_{10}-G, while most proteins in subfamily II are NAD(H)-dependent GDHs because they have an NAD(H)-binding motif G-X-G-X_2-G-X_{10}-G (Inokuchi et al., 2002; Qiu et al., 2009).

Subfamily II can be further divided into three groups, which contain representatives of all three domains of life (eubacteria, archaea and eukaryotes). Evidently, plant GDH2 and GDH1 (α and β subunits) are split into two clades in subfamily II. The deduced sequence of CsGDH2 was aligned with 34 other sequences and clearly clustered into the plant GDH2 clade with other GDH2 from *A. chinensis*, *Nicotiana plumbaginifolia*, *B. napus*, *A. thaliana* and *G. max* (Figure 4), suggesting that CsGDH2 could be the GDH α-subunit of tea plant.

Interestingly, phylogenetic analysis reveals that the plant GDHs was grouped with the bacterium *Thermotoga maritima* and archaeal GDHs in subfamily II. This may suggest a notably close relationship between the plant and the extremophilic enzymes. CsGDH2 shows closer relationship to *T. maritima* GDH than most other bacterial GDHs, which could be explained by the ancient horizontal gene transfer (HGT) or lateral gene transfer (LGT) between *T. maritima* and archaeal genomes (Aravind et al., 1998; Brown, 2003). The complete sequence of *T. maritima* genome has revealed that a

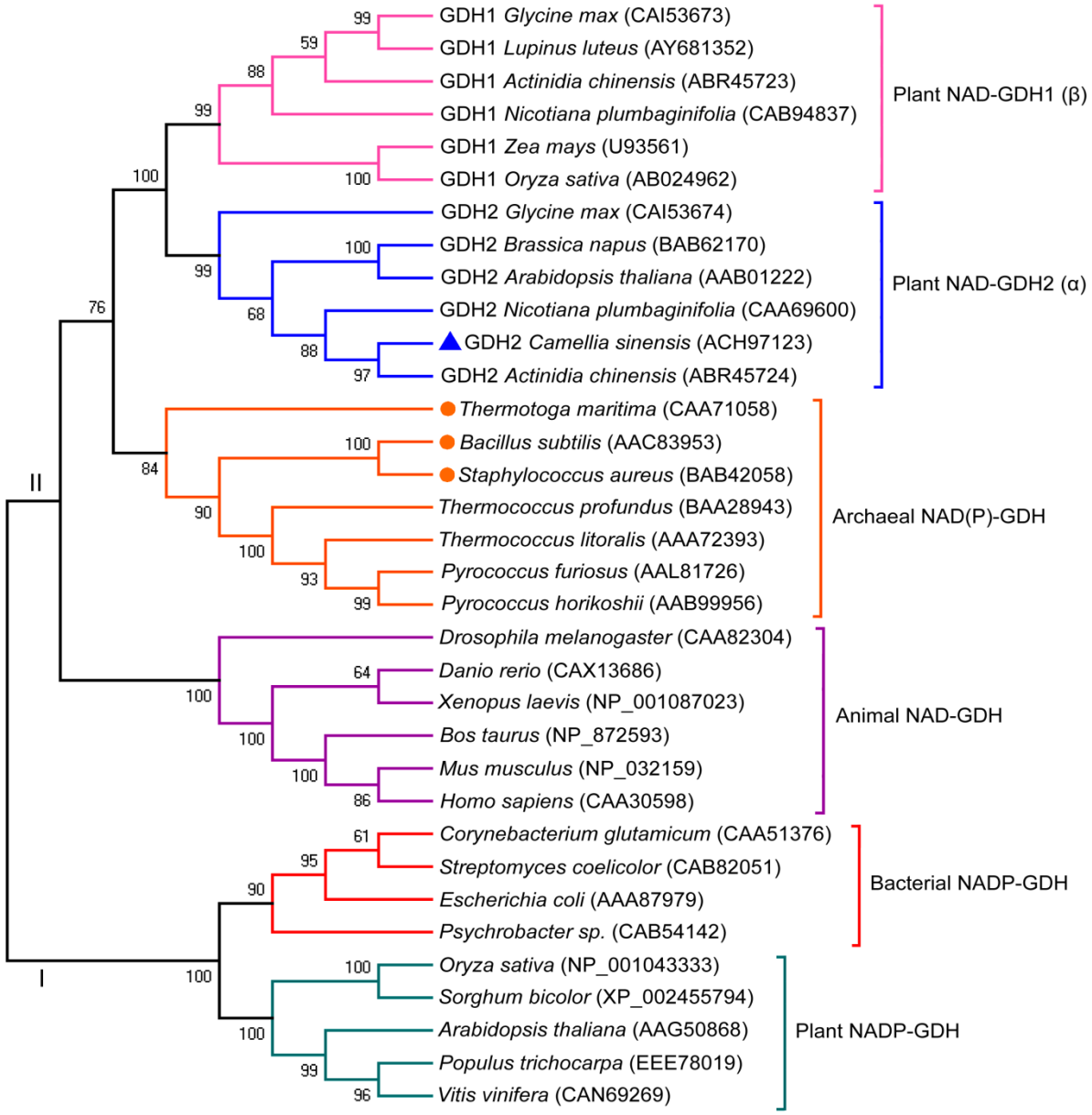

Figure 4. Phylogenetic tree of the GDH protein family generated by the neighbor-joining method. The number at each branch point represents percentage bootstrap support calculated from 1000 replicates. The evolutionary distances were calculated by Poisson correction method. 34 GDHs from various species are aligned. The GDH2 from *C. sinensis* (▲) and three bacterial GDHs (●) in the archaeal GDH clade are indicated. Protein accession number is shown in parentheses, according to the sequences deposited in GenBank and EMBL databases. GDH, Glutamate dehydrogenase.

large fraction (24%) of genes is most similar to archaeal genes than to bacterial genes (Nelson et al., 1999; Zhaxybayeva et al., 2009).

In addition, GDHs from archaea are now mixed together with those from the gram-positive bacteria *B. subtilis* and *Staphylococcus aureus* (Figure 4), suggesting a close evolutionary relationship between archaea and Gram-positive bacteria. Furthermore, *B. subtilis* NAD-GDH and *E. coli* NADP-GDH are separated into subfamily II and subfamily I, respectively, although both of them are mesophilic bacteria. It indicates that GDHs may not be used as reliable phylogenetic markers.

Detection and analysis of soluble fusion CsGDH2

To determine the expression level and product solubility of CsGDH2 with fusion tags in *E. coli*, three expression

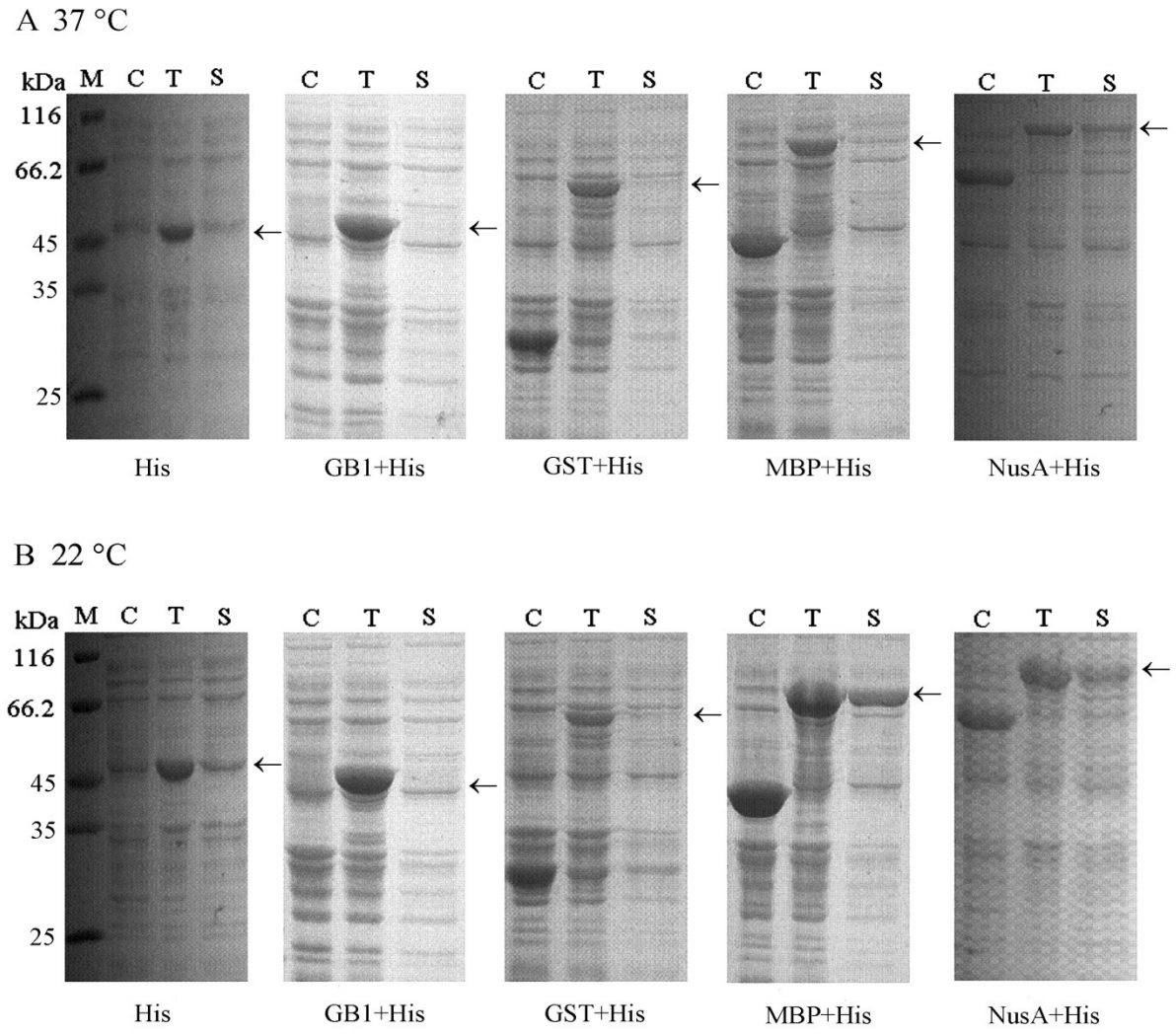

Figure 5. SDS-PAGE analysis of the overexpression of CsGDH2 together with its fusion partners at (A) 37 ℃ and (B) 22℃ in Rosetta (DE3). M, protein molecular marker; C, soluble lysate of cells expressing the empty vector after IPTG induction; T, total lysate of cells expressing recombinant plasmid after IPTG induction; S, soluble lysate of cells expressing recombinant plasmid after IPTG induction. The position of expressed CsGDH2 together with its fusion partner is shown by arrows. SDS-PAGE, Sodium dodecyl sulphate polyacrylamide gel electrophoresis.

vectors, pET-GST, pET-MBP and pET-NusA were constructed with GST, MBP and NusA fusion tags at the N-terminus using the backbone of pET-GB1. The ORF of *CsGDH2* was subcloned into these vectors to generate five different expression constructs, pET-*CsGDH2*, pET-GB1-*CsGDH2*, pET-GST-*CsGDH2*, pET-MBP-*CsGDH2* and pET-NusA-*CsGDH2* (Figure 1). All constructs were verified by DNA sequencing. Then, the five recombinant plasmids were transformed into the *E. coli* host strain Rosetta (DE3) and induced by 0.5 mM IPTG to express the fusion proteins at 37℃ and 22℃, respectively. Evidently, the total expression level of CsGDH2 was almost not affected by temperatures. High levels of recombinant CsGDH2 were achieved for all five constructs in Rosetta (DE3) at both temperatures (Figure 5). The major differences among the five constructs were the solubilizing effect of the fusion tags. When CsGDH2 was expressed only with a C-terminus 6 × His tag under both 37℃ and 22℃, the fusion protein was predominantly found in insoluble bodies and no soluble CsGDH2 was detected by either SDS-PAGE (Figure 5) or western blot (Figure 6). It was also found that the N-terminus GST fusion tag did not improve the solubility of recombinant CsGDH2 (Figure 6), even under low temperature. Although GST is proved to be a poor solubility enhancer in this present and some other studies (Dyson et al., 2004; Hammarström et al., 2006), it increased the yield of soluble proteins in several tests. This reflects the problem many fusion tags suffer from that they do not function equally well with all partner proteins (Esposito and Chatterjee, 2006). These results provide examples that it is always difficult to produce

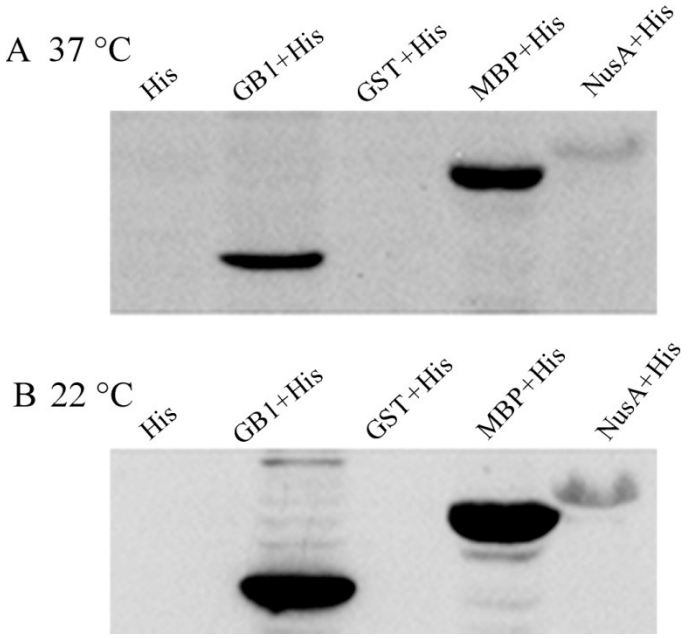

Figure 6. Western blot analysis of the overexpression of CsGDH2 together with its fusion partners at (A) 37°C and (B) 22°C. Equal amount of soluble lysate of cells harboring recombinant plasmid after IPTG induction is loaded. IPTG, Isopropyl β-D-1-thiogalactopyranoside.

soluble heterologous proteins in *E. coli*, especially the plant proteins (Esposito and Chatterjee, 2006; Tsunoda et al., 2005). Although the reason is still unclear, two factors may help explain it: (1) the rate of translation and protein folding is almost an order of magnitude faster in *E. coli* as compared with eukaryotic systems (Widmann and Christen, 2000); (2) Prokaryotic cells lack the enzymes and cellular machinery necessary to generate eukaryotic posttranslational modifications (Baneyx, 1999).

Other three fusion tags, GB1, MBP and NusA, can enhance the soluble expression of CsGDH2 in *E. coli*. Although the amount of the soluble GB1 fusion protein were so low that no band was visible on polyacrylamide gel after induction at 37 and 22°C (Figure 5), the recombinant CsGDH2 still can be detected by western blot (Figure 6). This is reasonable because GB1 has been used to enhance the expression and solubility of peptides and small proteins (Bao et al., 2006), but when fused to a larger passenger protein such as CsGDH2, its solublizing activity weakens a lot. Tags such as MBP and NusA showed a much higher amount of expressed soluble protein than GB1, especially induction at 22°C (Figures 5 and 6). In this study, MBP was the most effective tag at the N-terminus of CsGDH2 to promote protein solubility. As described elsewhere, MBP is one of the most well-studied fusion tag and N-terminal MBP fusion may frequently produce soluble proteins when the unfused partners are insoluble (Dyson et al., 2004; Kataeva et al., 2005; Busso et al., 2005). The NusA tag has also been shown to function at a similar level to MBP in producing soluble partner proteins (Schrodel et al., 2005; Turner et al., 2005). However, our results indicate that MBP is a better solubilizing agent for CsGDH2 than NusA.

In summary, we successfully isolated the full-length cDNA of a gene encoding GDH α-subunit of tea plant for the first time. *CsGDH2* was then heterologously expressed in *E. coli* with different N-terminus fusion tags to obtain soluble protein, and MBP was found to be the best fusion partner for CsGDH2. The enzymatic characterization of the soluble CsGDH2 is undergoing. Unlike malate and isocitrate dehydrogenases, the mechanisms of cofactor specificity (NAD^+ or $NADP^+$) in most redox enzymes are not well understood such as GDH. Here we report on the cloning and expression of a glutamate dehydrogenase from *C. sinensis* as a prelude to further studying the mechanisms controlling its coenzyme specificity. Also, glutamate is of central importance in plant nitrogen metabolism since the biosynthesis of all other amino acids requires this compound. Therefore, our present work would provide the possibility for a better understanding of the physiological role of GDH in tea plant.

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Abbreviations

CsGDH2, A *gdh* gene from *Camellia sinensis* encoding GDH α-subunit of plant; RT-PCR, reverse transcription polymerase chain reaction; RACE, rapid amplification of cDNA ends; ORF, open reading frame; cDNA, complementary DNA; IPTG, isopropyl β-D-1-thiogalactopyranoside; BLAST, the basic local alignment search tool; GB1, B1 immunoglobulin binding domain of protein G; GST, glutathione-S-transferase; MBP, maltose-binding protein; NusA, N-utilization substance protein; **HGT,** horizontal gene transfer; **LGT,** lateral gene transfer.

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