Ann Microbiol (2015) 65:1689–1698
DOI 10.1007/s13213-014-1008-7

ORIGINAL ARTICLE

Characterization of a novel serine hydroxymethyltransferase isolated from marine bacterium *Arthrobacter* sp. and its application on L-serine production

Junjie Huang · Lin Chen · Nan Hu · Wei Jiang · Gaobing Wu · Ziduo Liu

Received: 27 June 2014 / Accepted: 21 November 2014 / Published online: 7 January 2015

Abstract The current L-serine production relies mainly on cellular or enzymatic conversion from the precursor glycine plus a C1 compound. To date, only several reports have been published on L-serine production from glycine and methanol by methylotrophic bacteria with the serine pathway. This work aimed to isolate a novel serine hydroxymethyltransferase (SHMT) from the methanol-using *Arthrobacter* sp. and use it for L-serine production with the enzymatic conversion method. Here, A novel glyA gene was isolated from the methanol-using *Arthrobacter* sp. by thermal asymmetric interlaced PCR (TAIL-PCR), encoding a serine hydroxymethyltransferase (SHMT) with 440 amino acids, belonging to the α-family of fold type I, and pyridoxal-5-phosphate (PLP) dependent enzymes. The enzyme was stable in weakly alkali conditions, showing the optimal activity at pH 7.8 and 45 °C, and a 2.75-fold increase in activity over the corresponding enzyme of *Escherichia coli*. Two methods (resting cells reaction and enzymatic conversion) were employed to produce serine. Using glycine (133 mM) and formaldehyde (13.3 mM) as substrates to produce serine by enzymatic reaction, 93.6 mM L-serine was obtained with a 70.4 % molar conversion rate from glycine to L-serine. Thus, the characteristics of this novel strain and its enzyme suggest that it has the potential for further research and industrial use.

Keywords *Arthrobacter* sp. · SHMT · L-Serine enzymatic production · Thermal asymmetric interlaced PCR · RP-HPLC

J. Huang · L. Chen · W. Jiang · G. Wu · Z. Liu (✉)
State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
e-mail: lzd@mail.hazu.edu.cn

N. Hu
College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 211800, People's Republic of China

Introduction

Only several reports have been published on L-serine production from glycine and methanol by methylotrophic bacteria with the serine pathway (Hagishita et al. 1996; Shen et al. 2010), and all the microorganisms used in the studies reported were from the terrestrial environment. However, many other bacteria also probably have the same function. The marine realm covers 70 % of the earth's surface, and provides the largest inhabitable space for living organisms, particularly microbes (Das et al. 2006); thus isolating some novel and useful marine bacteria is feasible and important.

Serine hydroxymethyltransferase (SHMT, EC 2.1.2.1) is a ubiquitous, highly conserved pyridoxal-5-phosphate (PLP)-dependent enzyme with tetrahydrofolate (THFA) as the C_1 acceptor (Blakley 1955), and has been purified and extensively studied in animals (Jones and Priest 1976; Ulevitch and Kallen 1977), plants (Rao and Appaji Rao 1982), and bacteria (Barra et al. 1983). SHMT plays an important role in the assimilation of C_1 compounds, yielding the main L-serine intermediate. The enzymes involved in L-serine synthesis are methanol dehydrogenase (EC 1.1.1.244) and SHMT, the latter of which is coded by the *glyA* gene. The former catalyzes the oxidation of methanol to formaldehyde and the latter converts formaldehyde and glycine to L-serine by reaction. It is well-known that two molecules of glycine are needed for the synthesis of one molecule of L-serine, and the theoretical production yield is 50 % (mol/mol) (Izumi et al. 1993). If half of the C_1 units are displaced by low-cost formaldehyde, the theoretical ratio of glycine conversion may be 100 % (Izumi et al. 1993). Therefore, isolating a strain with high methanol dehydrogenase and SHMT activity is the key, and in this study, one methylotrophic strain *Arthrobacter* sp. with high SHMT activity was isolated.

The study of enzymatic properties can contribute to a better use of SHMT. In previous reports, many *glyA* genes were

obtained by shotgun technology (Hamilton et al. 1985), direct PCR amplification based on the known genome information (Schirch et al. 1985; Vidal et al. 2005), cDNA library (Byrne et al. 1992; Garrow et al. 1993), southern-blot hybridization (Miyata et al. 1993), and a genomic library constructed with the application of a probe (Shen et al. 2010). PCR amplification would be convenient only with known genome information. Otherwise the aforementioned methods would be time-consuming, and thus libraries such as EMBL and GenBank with information about various genomes and nucleic acids would facilitate the search for information about relevant genes. In this work, the partial *glyA* (P*glyA*) sequence was amplified with degenerate primers designed based on the result of multiple sequence alignment before the flanking sequences of P*glyA* were obtained by thermal asymmetric interlaced PCR (TAIL-PCR).

The current L-serine production relies mainly on cellular or enzymatic conversion from the precursor glycine plus a C_1 compound (Peters-Wendisch et al. 2005). Because there is no need to add any coenzymes PLP and tetrahydrofolate (THFA) into the reaction system, a resting cells system is a useful method to produce L-serine from glycine and methanol by methylotrophic bacteria with the serine pathway (Izumi et al. 1993; Hagishita et al. 1996). Another useful method is the enzymatic reaction, manufacturing L-serine by constructing engineering bacteria and adding coenzymes (Hsiao et al. 1988), which has been rarely reported. In China, the enzymatic reaction method is mainly used for L-serine production with the enzyme primarily from *Escherichia coli* SHMT (Sun 2000; Zuo et al. 2007)

In this paper, the two methods (resting cells reaction and enzymatic conversion) have been tried for L-serine production, and the latter method was used to evaluate the difference between the *Arthrobacter* sp. SHMT engineering bacterium and the *Escherichia coli* SHMT engineering bacterium in L-serine production.

Materials and methods

Materials

Restriction enzymes, pMD18-T vectors, T4 DNA ligases, Taq DNA polymerases, and DNA markers were purchased from TaKaRa Company (Dalian, China). Kits for plasmid extraction and DNA purification were obtained from Qiagen Company (Germany). All oligonucleotide primers (Table 1) were synthesized and all DNA fragments were sequenced by GenScript Company (Nanjing, China). All the chemicals were of HPLC grade or biotechnology grade and purchased from Sigma unless specially noted. The THFA used in the enzymatic reaction to produce L-serine was independently synthesized by our laboratory (Sun et al. 2000).

Table 1 Primers used in this study

Primer	5′ to 3′	Reference
DP-F	CTSACCAAYAARTACGC CGAGGGYT	This work
DP-R	ACCATSGGCGGSCKSGG RTCGAAGG	This work
SP1-F	GGTCGAAGATCTTGGCT GAGCGTTTG	This work
SP2-F	GAAGACGTTGTTGCCAA GGGCATC	This work
SP3-F	CGCAGGTGGAGATCACT GTGAATCG	This work
SP1-R	AACAATCATCTTCGGCT GGTGCTCG	This work
SP2-R	TGGTAGGTTTCCTCATC CACGCCGTA	This work
SP3-R	AGCTTCATGCCGTGGGT CAAATGG	This work
AD1	TGWGNAGSANCASAGA	(Liu and Whittier 1995)
AD2	AGWGNAGWANCAWAGG	(Liu and Whittier 1995)
AD3	CAWCGICNGAIASGAA	(Liu and Whittier 1995)
AD4	TCSTIGNCITWGGA	(Liu and Whittier 1995)
AD5	NGTCGASWGANAWGAA	(Liu and Huang 1998)
AD6	NTCGASTWTSGWGTT	(Liu and Huang 1998)
AD7	WGTGNAGWANCANAGA	(Liu and Huang 1998)
AD8	WCAGNTGWTNGTNCTG	(Emelyanov et al. 2006)
AD9	WGCNAGTNAGWANAAG	(Amedeo et al. 2000)
AD10	AWGCANGNCWGANATA	(Amedeo et al. 2000)
glyA-F	CGGGATCCATGAGCAACC AGACTTTTGAA	This work
glyA-R	CCGCTCGAGCTACTCGGA AACCTTTGGCA	This work

IUPAC ambiguity codes, M=A/C, R=A/G, W=A/T, S=G/C, Y=C/T, K=G/T, and N=A/G/C/T. I indicate inosine. F, forward; R, reverse. Restriction sites *Bam*H1 and *Xho*1 in primers *glyA*-F/R are underlined, respectively; Start and stop codons are in bold, respectively

Media and growth conditions

Escherichia coli strains were incubated in Luria-Bertani (LB) medium, supplemented with ampicillin (100 μg/ml) if necessary, and other bacteria were cultured in agar 2216 medium. Screening media I and II were used for the screening of strains with SHMT activity. Screening medium I consisted of methanol 1.2 % (v/v), $(NH_4)_2SO_4$ 37.84 mM, KH_2PO_4 7.34 mM, $K_2HPO_4 \cdot 3H_2O$ 13.15 mM, $MgSO_4 \cdot 7H_2O$ 0.81 mM, $MnSO_4 \cdot H_2O$ 0.012 mM, $FeSO_4 \cdot 7H_2O$ 7.19×10^{-3} mM, biotin 2.05×10^{-4} mM, thiamine hydrochloride 3.32×10^{-4} mM, $NaNO_3$ 35.30 mM, and NaCl 34.22 mM, with the pH of the mixture being adjusted to 7.5. The components listed above were also used in the mixture of screening medium II except that the concentration of methanol was increased to 5 % (w/v), and 1.5 % (w/v) agar and 0.27 M glycine were added.

Microorganism isolation

The bacteria were cultured by enrichment culture in screening culture medium I at 28 °C, and 1.2 % (v/v) methanol was added once every 2 days. After 7–10 days of growth, bacterial suspension was diluted to 10,000 times, 100,000 times, and one million times with sterile water. Then these samples were spread onto plates filled with medium II, followed by 4–5 days of incubation at 28 °C. Finally, *Arthrobacter* sp. was isolated and preserved for further use for its high SHMT activity.

Gene cloning

To amplify the P*glyA* gene, degenerate primers DP-F and DP-R (Table 1) were designed based on the result of multiple sequence alignment (Fig. 1a) of *glyA* gene sequences which belong to the genus of *Arthrobacter* (*Arthrobacter* sp. FB24 and *Arthrobacter aurescens* TC1) and were obtained from the NCBI database.

Flanking fragments of P*glyA* were amplified by TAIL-PCR (Liu and Huang 1998) using ten arbitrary degenerate primers, AD1-10 (Table 1), and six nested specific primers, SP1-3 F and SP1-3 R (Table 1), designed according to the sequencing result of P*glyA*. The cycling conditions for TAIL-PCR were listed in Table 2.

The full ORF of *glyA* was obtained from the chromosome DNA of *A. mysorens* by PCR with the forward and reverse primers (*glyA*-F and *glyA*-R, Table 1). The PCR reaction was performed as follows: one cycle at 94 °C for 4 min; 30 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min; and one final additional cycle at 72 °C for 10 min. The PCR product was gel-purified, digested with *Bam*H I and *Xho* I, and then ligated into the pGEX-6p-1 vector (Amersham Biosciences) (Lin et al. 2009). The recombinant plasmids were verified by DNA sequencing and designated as pGEX-6p-*AmglyA*.

Using the same method described above, the *glyA* gene (Gene ID: 947022) from *E. coli* was also ligated into the pGEX-6p-1 vector and designated as pGEX-6p-*EcglyA*.

Expression and purification of SHMT

To optimize the expression of SHMT, the pGEX-6p-*AmglyA* plasmids were transformed into *E. coli* BL21 (DE3) competent cells (Stratagene, USA). The transformants were cultured in Luria–Bertani broth at 37 °C until the cells reached an optical density of 0.6–0.8 at 600 nm. Then, protein expression was induced by adding IPTG to a final concentration of 1 mM. After 6–8 h, the cells were harvested and disrupted with High Pressure Homogenizer (NS100IL 2 K, Niro Soavi, Germany). SHMT of *Arthrobacter* sp. (*Am*SHMT) was purified using glutathione-S-transferase (GST)-free affinity purification method (Lin et al. 2009). SHMT of *E. coli* (*Ec*SHMT) was also purified with the same method.

Enzyme assay

Standard enzyme activity assay was conducted as described below. Moderate enzyme was added into the reaction system

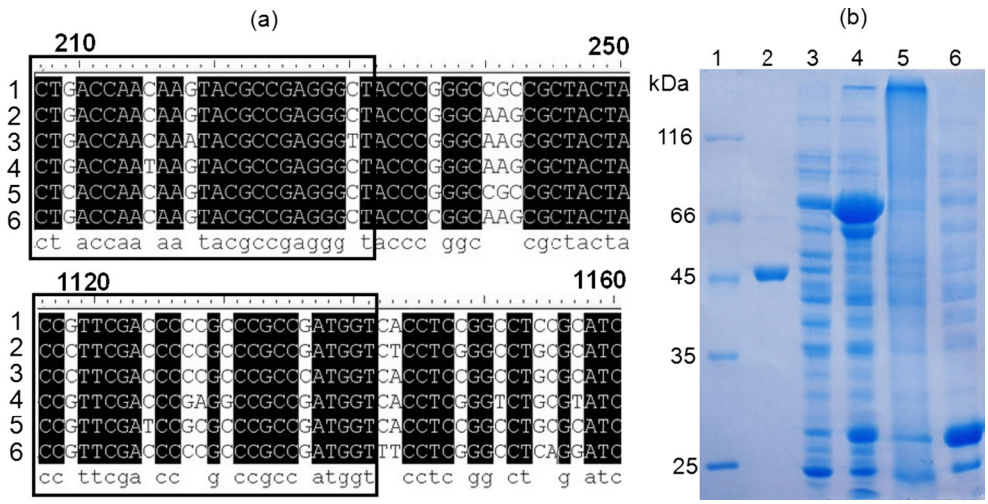


Fig. 1 Design of degenerate primers and SDS-PAGE analysis of the purified *Am*SHMT. (**a**) The *glyA* gene sequences belonged to the genus of *Arthrobacter* and were obtained from NCBI database (http://www.ncbi.nlm.nih.gov/). *Arthrobacter* sp. FB24 (GI: 116668568), 1: 830533–831870, 2: 1209958–1211265, 3: 4169907–4171268); *Arthrobacter aurescens* TC1 (GI: 119947346), 4: 1309108–1310400, 5: 4192248–4193636, 6: 4374834–4376153. The regions in the boxes were areas for degenerate primers design. (**b**) 12 % SDS-PAGE analysis of the purified *Am*SHMT, the bands in the ellipses show the GST (glutathione-S-transferase) and fusion protein (*Am*SHMT and GST). Lane1: protein marker. Lane 2: purified *Am*SHMT without GST. Lane 3: recombinant bacterium (harboring pGEX-6P-*glyA*) non-induced by IPTG. Lane 4: recombinant bacterium (harboring pGEX-6P-*glyA*) induced by 0.1 mM IPTG. Lane 5: bacterium (harboring pGEX-6p-1) non-induced by IPTG. Lane 6: bacterium (harboring pGEX-6p-1) induced by 0.1 mM IPTG. The protein molecular weight ladder is Unstained Protein Molecular Weight Marker (Fermentas, Canada)

Table 2 conditions used for TAIL-PCR

Reaction	Cycle no.	Thermal condition
Primary	1	94 °C, 2 min; 94 °C, 1 min
	5	94 °C, 30 s; 65 °C, 1 min; 72 °C, 2 min
	1	94 °C, 30 s; 25 °C, 3 min; 72 °C, 2 min
	10	94 °C, 30 s; 44 °C, 1 min; 72 °C, 2 min
	15	94 °C, 30 s; 65 °C, 1 min; 72 °C, 2 min
		94 °C, 30 s; 65 °C, 1 min; 72 °C, 2 min
		94 °C, 30 s; 44 °C, 1 min; 72 °C, 2 min
	1	72 °C, 10 min
	1	10 °C, 5 min
Secondary	1	94 °C, 5 min
	16	94 °C, 30 s; 65 °C, 1 min; 72 °C, 2 min
		94 °C, 30 s; 65 °C, 1 min; 72 °C, 2 min
		94 °C, 30 s; 44 °C, 1 min; 72 °C, 2 min
	1	72 °C, 10 min
	1	10 °C, 5 min
Tertiary	The cycle no. and thermal condition were the same as the secondary reaction.	

(1 ml, pH 7.8, sodium phosphate buffer), which contained 50 mM DL-3-phenylserine, 50 μM PLP, 1 mM Na_2EDTA (ethylene diamine tetraacetic acid), and 25 mM sodium sulfate. When cells were used, 0.03 % (w/v) cetyltrimethyl ammonium bromide (CTAB) was added. The reaction proceeded for 1 h at 30 °C, and the production of benzaldehyde was measured by its maximum absorption value at 279 nm (Zuo et al. 2007). One unit of enzyme activity was defined as the quantity of enzyme capability of releasing 1.0 μmol benzaldehyde per hour (benzaldehyde as standard). Specific activity was expressed as units/mg protein.

Enzyme characterization

The optimal pH of SHMT was determined at 30 °C in different buffers at pH 5.8–9.5, namely sodium phosphate buffer (pH 5.8–8.0) and sodium carbonate buffer (pH 8.7–9.5). The optimal temperature of SHMT was determined at 0 to 55 °C under optimal pH. The pH stability of SHMT was determined by incubating the enzyme at the optimal temperature for 3 h at different pH (pH 6.5–9.3), followed by the measurement of the enzyme activity under standard conditions. The thermostability of SHMT was determined by incubating the enzyme at 20 to 50 °C under optimal pH for 3 h and then measuring the enzyme activity under standard conditions.

Effects of the metal ions and chemical compounds on enzyme activity were determined in the standard reaction system for 1 h (Li et al. 2009).

Construction of engineering bacteria

In order to construct engineering bacteria for L-serine production, *glyA* genes from *Arthrobacter* sp. and *E. coli* were cloned into pET-15b vectors, respectively, using the same restriction enzyme cutting sites (*Nde*I and *Bam*HI). The recombinant expression plasmids were transformed into *E. coli* BL21 (DE3). The engineering bacteria were induced as described in the section of expression and purification of SHMT. The two engineering bacteria were designated as pET-15b-*AmglyA* and pET-15b-*EcglyA*, respectively.

Enzymatic reactions for L-serine production

Under the same conditions, the two engineering bacteria were inoculated, induced, cultivated, and collected. They were washed with phosphate buffer (0.2 M, pH 8.0) and centrifuged (8,000 rpm, 2 min) twice, collected (each was 3,000 mg, wet weight) and stored in a refrigerator at −80 °C for about 4 h. The bacteria were thawed at 37 °C. With phosphate buffer (0.2 M, pH 8.0), the bacteria were resuspended in the 15-ml reaction system consisting of glycine (0.133 M), formaldehyde (13.3 mM), β-mercaptoethanol (0.2 M), PLP (0.4 mM), and THFA (5 mM).

Enzymatic reactions were processed at 30 °C, 150 rpm for 24 h. A sample of 200 μl was collected every 2 h for 24 h; meanwhile, formaldehyde was added to a final concentration of 13.3 mM and pH was adjusted to 7.0–7.5. L-Serine concentration was measured by RP-HPLC with pre-column derivatization.

Sample preparation and RP-HPLC analysis

O-Phthalaldehyde (OPA) regent (4 mg/ml) and borate buffer (0.4 M, pH 10.4) were used for precolumn derivatization. Solvent A (pH 5.8) consisted of 25 mM sodium acetate buffer and tetrahydrofuran (95/5, v/v), and Solvent B of methanol (Zhao et al. 2012).

Twenty microliters of the enzymatic reaction solution was diluted to an appropriate concentration (10–100 pmol/μl) with ultrapure water. Then 100 μl of diluted enzymatic reaction solution or standard amino acid dilution (10–100 pmol/μl) was injected into a 1.5-ml centrifuge tube, followed by the addition of 600 μl of borate buffer and 300 μl OPA derivatization reagent once the timing began. After being mixed adequately, the solution was filtered through a 0.22 μm organic membrane. Five minutes later, the sample was injected into the column for RP-HPLC analysis.

L-Serine and glycine were assayed by RP-HPLC (1260 infinity quaternary LC system, Agilent Technologies) on a column of Agilent Eclipse XDB-C18 (250 mm×4.6 mm,

5 μm) as described by Jiang et al. (Jiang et al. 2013), with moderate modifications.

Results

Strain isolation

By comparing the SHMT activity of isolated strains, the strain (*Arthrobacter* sp., MCCC 1A05493) with the highest activity was isolated, whose SHMT was designated as *Am*SHMT.

DNA matching and amino acid sequence analysis

A sequence (1,574 bp) was obtained by matching the P*glyA* (936 bp) with the flanking fragments (335 and 613 bp) identified by TAIL-PCR (Fig. 2). Then the whole *glyA* gene sequence (1,323 bp) was obtained using the ORF search tool from SoftBerry (http://linux1.softberry.com/berry.phtml). The *Am*SHMT exhibited 58 % amino acid identity with the known SHMT from *E. coli* and encoded a protein with 440 amino acids and a deduced molecular mass of 47.3 kDa. A phylogenetic tree was constructed to verify further the evolutionary relationship among *Am*SHMT and other known SHMTs (Fig. 3).

The conserved active site T/ST/STTHKT/SL in all known SHMT proteins (Garrow et al. 1993) was found in *Am*SHMT (235–242) in the form of TSTTHKTL (Fig. 4). Another significant homologous sequence GQQGGP (268–273), a glycine-rich region, had been proposed to be essential for PLP binding (Usha et al. 1994). Two other well-known conserved sequences, LTNKYAEGYPGRRYYGG (61–77) and GGHLTHG (134–140) (Hong et al. 1999), were also found in *Am*SHMT.

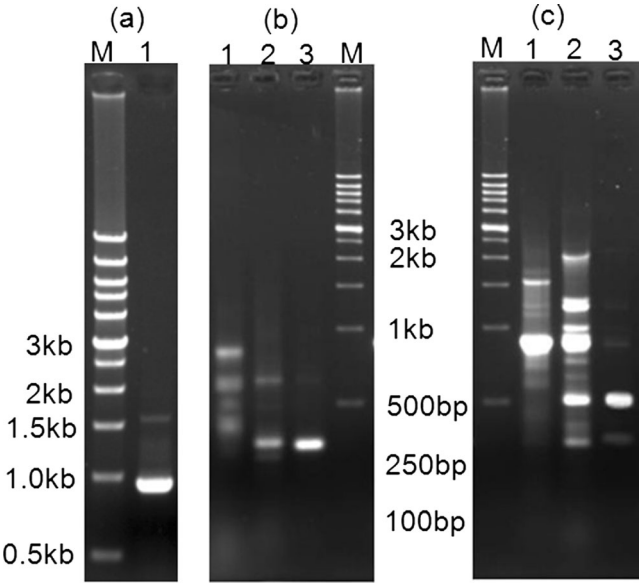


Fig. 2 P*glyA* gene fragment and its flanking gene fragments. (**a**) A part of the full-long *AmglyA* gene (P*glyA*, 936 bp) was amplified using degenerate primers DP-F and DP-R. (**b**) The left flanking sequence (335 bp) was amplified through TAIL-PCR technology. (c) The right flanking sequence (613 bp) was amplified through TAIL-PCR technology

Expression, purification, and characterization of *Am*SHMT

*Am*SHMT was expressed, purified, and its molecular mass was determined by SDS-PAGE analysis (Fig. 1b). SHMT showed optimal activity at pH 7.8, and retained over 75 % of the maximal activity between pH 7.0 and 8.0. Furthermore,

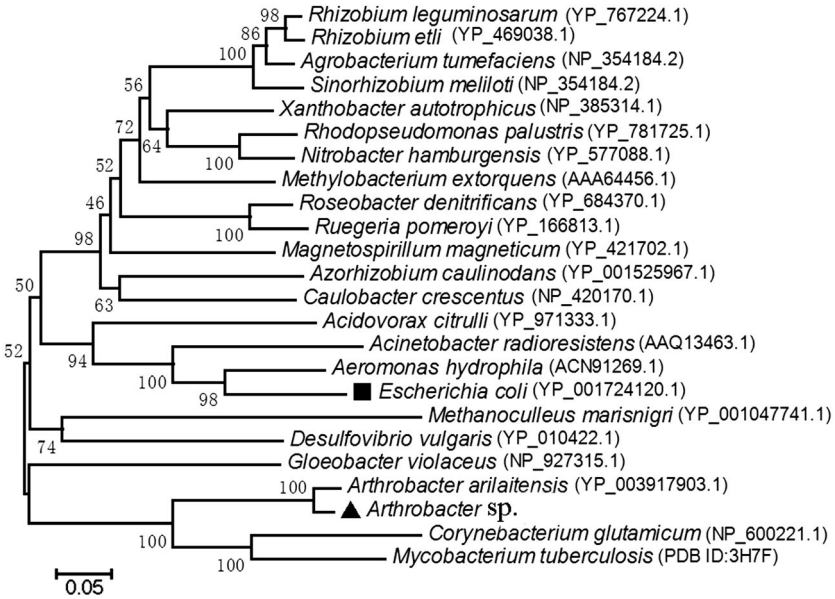


Fig. 3 Phylogenetic analysis of *Am*SHMT. The phylogenetic tree of *Am*SHMT was constructed using the neighbor-joining method (MEGA 5.05). Except for *Am*SHMT, the other SHMT sequences were obtained from GenBank and PDB (http://www.rcsb.org/pdb/home/home.do). The numbers at node indicate the bootstrap percentages of 1,000 resamples. The units at the bottom of the tree indicate the number of substitution events

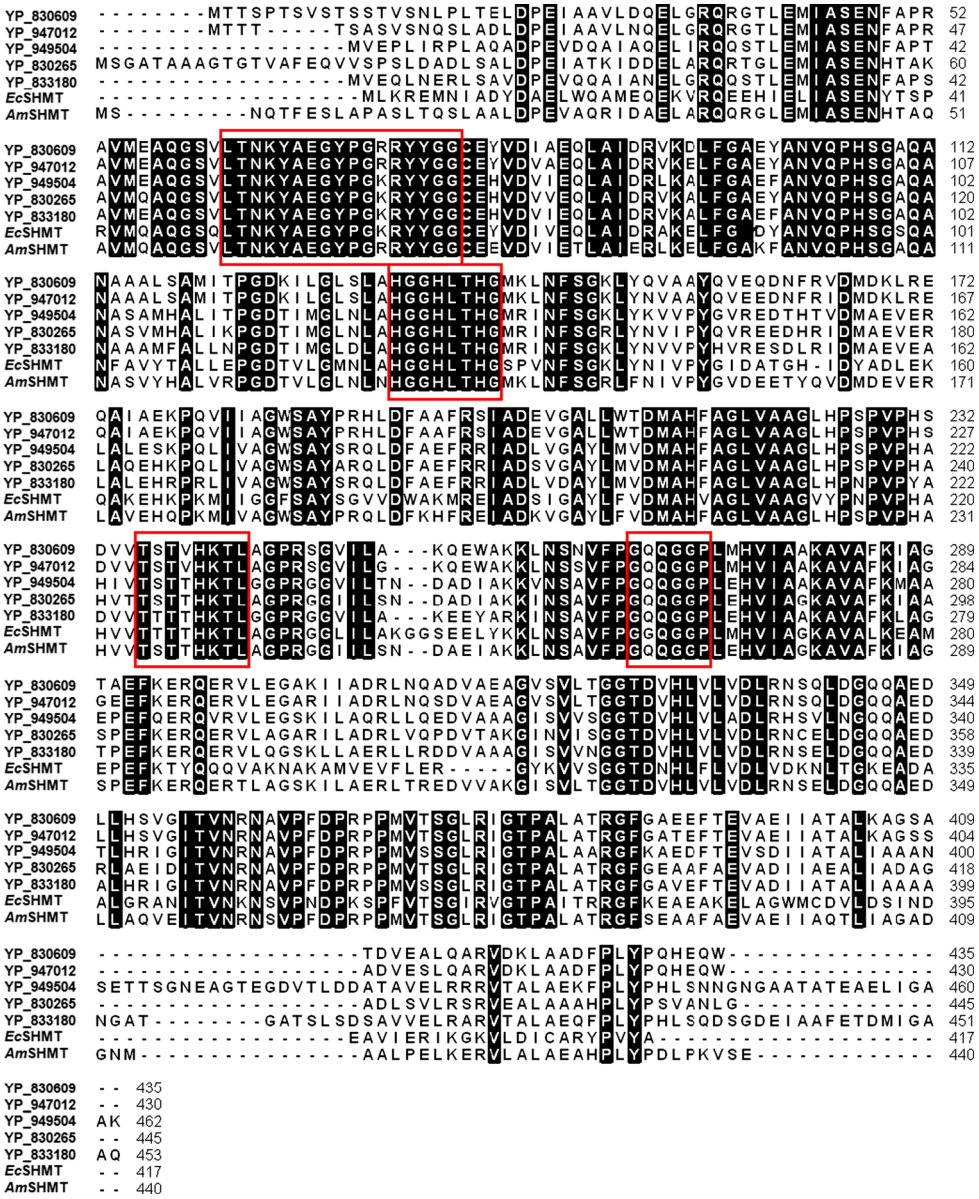


Fig. 4 Multiple sequence alignment of SHMTs. The sequences in the boxes are conserved amino acid residues

the enzyme was sensitive to low pH, displaying less than 20 % of its maximal activity at pH 6.5 and nearly no activity below pH 5.8 (Fig. 5a). Without any stabilizer, the purified SHMT was apparently stable under weakly alkaline conditions (pH 7–7.4), retaining over 85 % of the maximal activity after 3 h at 45 °C. However, it was unstable under acidic conditions or in strongly alkaline environment, especially when the pH was over 9.0 (Fig. 5c).

The maximal activity of SHMT was observed at 45 °C (Fig. 5b). However, SHMT retained over 50 % of its maximal activity after 3 h incubation under pH 7.8 (Fig. 5c), and decreased rapidly in activity at temperatures over 40 °C.

The effects of metal ions and chemical reagents on SHMT are shown in Table 3, indicating that the activity of the enzyme was weakly enhanced by Mg^{2+}, Ca^{2+}, Pb^{2+} and EDTA, but was strongly inhibited by Hg^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+} and Fe^{2+}. In addition, SDS and CTAB also inhibited SHMT activity, and K^{+}, Na^{+} and NH_4^{+} showed no appreciable impact on the SHMT activity.

In terms of activity, the *Am*SHMT (287.9 units/mg) was 2.75-fold higher than the *Ec*SHMT (104.7 units/mg) under standard assay conditions.

Production of L-serine by resting cells reaction

After 48 h incubation at 28 °C as described in section of the resting cells reaction system, 1.8±0.3 mg/ml L-serine was obtained by RP-HPLC analysis.

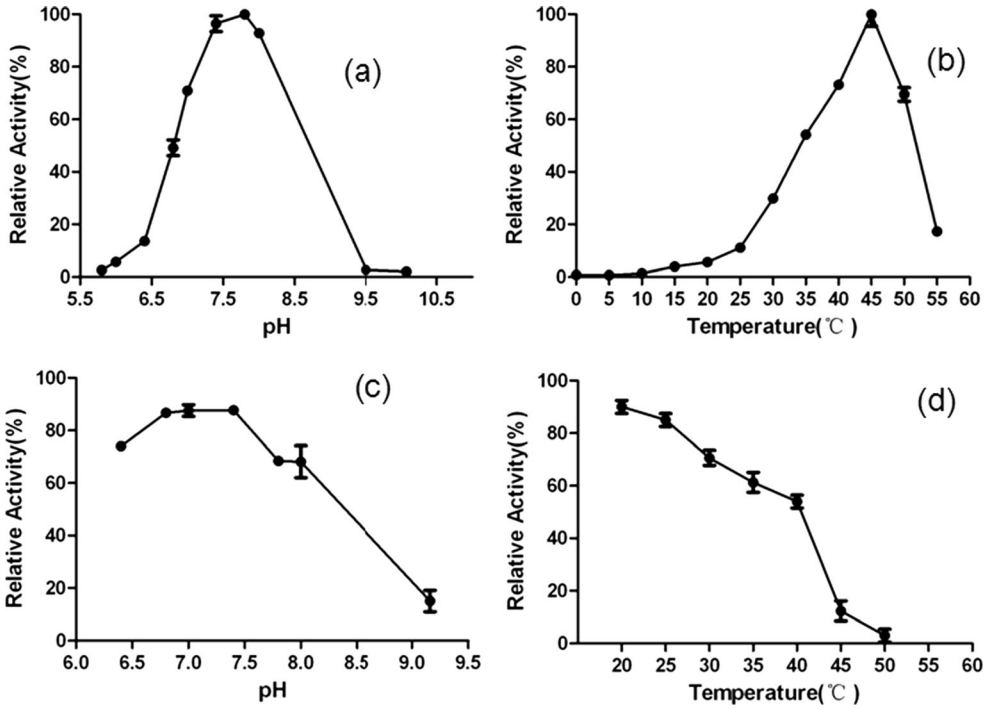


Fig. 5 Effects of temperature and pH on the activity. (**a**) Effect of pH on the activity of *Am*SHMT. Assays were conducted in buffers over a pH range from 5.8 to 9.5 at 30 °C for 1 h, under standard conditions. The maximal activity was taken as 100 %. (**b**) Effect of temperature on the activity of *Am*SHMT. Assays were conducted at the optimal pH 7.8, under standard conditions. The maximal activity was taken as 100 %. (**c**) Effect of pH on the stability of *Am*SHMT. At the optimal temperature 45 °C, the purified enzyme was pre-treated at a different pH for 3 h. Then assays were conducted under standard conditions and the enzyme activity without pre-treatment was taken as 100 %. (**d**) Effect of temperature on the stability of *Am*SHMT. At the optimal pH 7.8, the purified enzyme was pre-treated at a different temperature for 3 h. Then assays were conducted under standard conditions and the enzyme activity without pre-treatment was taken as 100 %

Production of L-serine by enzymatic reaction

Using the enzymatic reaction system described in the methods, L-serine was detected by RP-HPLC analysis 24 h later (Figs. 6 and 7a). The L-serine concentration at the 12th hour was calculated to be 93.6 mM in the pET-15b-*AmglyA* system, but 71.1 mM in the pET-15b-*EcglyA* system, indicating that the former was 70.3 % in the molecular conversion rate, which was 1.32-fold higher than the latter (53.4 %).

Discussion

In this study, we obtained *Arthrobacter* sp., a methanol-using strain with high SHMT activity, which was first reported in 1972 as a new species excreting L-glutamic acid (Nand and Rao 1972). The *glyA* gene was cloned by TAIL-PCR, an efficient PCR strategy, using AD1-10 primers separately. However, only AD8 and AD10 primers were better suited for PCR amplification, and all the other AD primers often led to non-targeted, dispersive or small fractional products, probably due to codon usage and the cycling conditions. In any case, choosing more AD primers is helpful for TAIL-PCR amplification.

SHMT, a member of the α-family of PLP-dependent enzymes (Mehta and Christen 2000), exists as a dimer in *Psychromonas ingrahamii* (Siglioccolo et al. 2010) and is ubiquitous for generating one-carbon fragments for the synthesis of nucleotides, methionine, thymidylate, and choline (Appaji Rao et al. 2003). This enzyme is also useful in the synthesis of serine using glycine and formaldehyde. Therefore, studying the enzymatic properties of SHMT can provide guidance for the industrial production of L-serine.

Using the resting cells reaction system, 1.8±0.3 mg/ml L-serine was produced. The reasons for the low yield might be that the metabolic pathways in vivo are so complex that L-serine can be quickly degraded and converted to other substances. For instance, even the *Methylobacterium* sp. strain MN43, which has the highest glycine conversion rate ever reported, can degrade 32 g/l L-serine in 2 days (Hagishita et al. 1996).

From Fig. 5a, it can be seen that the two engineering bacteria produced little L-serine during the first 6 h, but both of them could produce L-serine rapidly 6 h later, especially engineering bacterium pET-15b-*AmglyA*. This was probably because the coenzyme (PLP and THFA) and substrate were binding to SHMT during this time, but 6 h later when the binding process was completed, the conversion reaction would proceed rapidly and more L-serine

Table 3 Effects of metal ions and chemical reagents on the activity of purified SHMT*

Metal ion and chemical reagent	Concentration (mM)	Relative activity (%)
None	0	100 %
Hg^{2+}	1	– [a]
Co^{2+}	1	65.0±0.2 [b]
K^{+}	1	99.4±0.1
Na^{+}	1	98.7±0.4
NH_4^{+}	1	99.5±0.7
Mg^{2+}	1	101.4±0.6
Ca^{2+}	1	103.7±0.4
Cu^{2+}	1	48.9±0.8
Zn^{2+}	1	83.1±0.1
Mn^{2+}	1	63.1±0.4
Fe^{2+}	1	68.3±0.1
Pb^{2+}	1	101.9±0.3
EDTA	1 %	102.1±0.3
CTAB	0.03 %	71.5±0.5
SDS	0.1 %	–[a]

*The data are the average of three replicates

[a] Unmeasured data

[b] Relative activity ± the standard deviation

All assays were performed in the standard conditions and the activity measured without additional reagents and ions was taken as 100 %

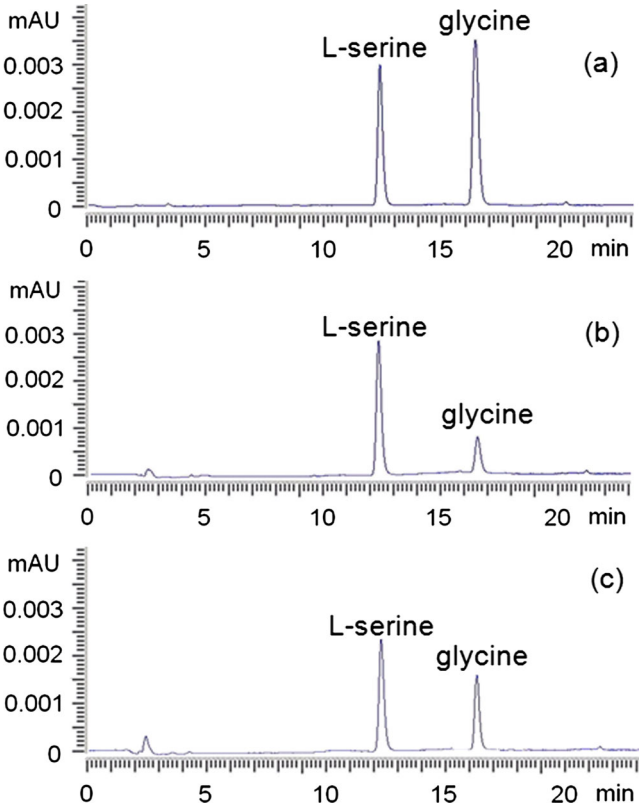
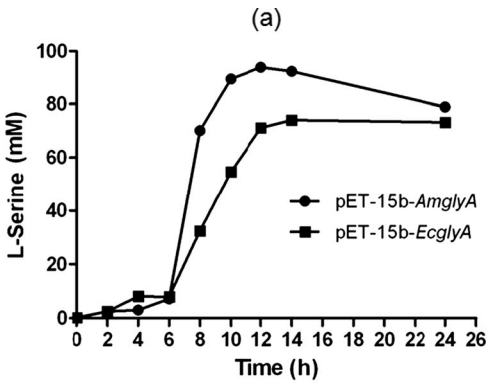


Fig. 6 HPLC detection of the L-serine in the enzymatic reaction system. (**a**) Determination of glycine and L-serine standards; (**b**) Determination of L-serine synthesis by pET-15b-*AmglyA* in the enzymatic reaction system at the 12th hour; (**c**) Determination of L-serine synthesis by pET-15b-*EcglyA* in the enzymatic reaction system at the 12th hour

could be produced during 6–12 h. At the 14th hour, the concentration of L-serine significantly decreased in the pET-15b-*AmglyA* reaction system, while that of the pET-15b-*EcglyA* reaction system showed a steady increase, resulting in a significant descrease in L-serine conversion in both systems, which could be attributed to the possibility that the coenzymes were almost entirely consumed in the former reaction system; thus, the enzymatic reaction would be favourably performed in the reverse reaction direction to generate glycine and formaldehyde. At the 24th hour, the L-serine concentration showed a significant decrease in the pET-15b-*AmglyA* reaction system, but little variation in the pET-15b-*EcglyA* system compared with that at the 14th hour. The possible reason was that 14 h later when the THFA and PLP had been almost completely consumed, *Am*SHMT still retained a little activity, and thus the enzymatic conversion sequentially proceeded in the reverse



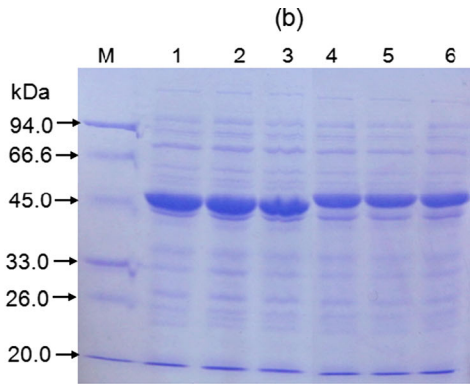


Fig. 7 HPLC and SDS-PAGE analysis of the induced *Am*SHMT and *Ec*SHMT. (**a**) HPLC analysis of the L-serine concentration in the enzymatic reaction system during 24 h. (**b**) SDS-PAGE analysis of the induced *Am*SHMT and *Ec*SHMT. Engineering bacteria pET-15b-*AmglyA* and pET-15b-*EcglyA* were treated in the same conditions. Lanes 1, 2, and 3 indicate the three replicates of induced *Ec*SHMT from engineering bacteria pET-15b-*Ec*glyA; lanes 4, 5, and 6 indicate the three replicates of induced *Am*SHMT from engineering bacteria pET-15b-*AmglyA*

reaction direction. In the pET-15b-*EcglyA* reaction system, the *Ec*SHMT almost completely lost activity and the reverse reaction was blocked, so that at the 24th hour, the L-serine concentration almost remained constant.

The above analyses indicated that the pET-15b-*AmglyA* engineering bacterium has more potential for industrial applications, especially at the 12th hour, when the L-serine concentration reached the maximum value, because its faster conversion rate was conducive to time saving and cost reducing. However, the present work cannot meet the requirements of industrial applications and much work needs to done to further improve the L-serine yield.

The two engineering bacteria were treated under the same conditions, and interestingly, *Am*SHMT was found to be lower than *E*cSHMT in the expression level (Fig. 5b), which might be caused by codon usage bias or other reasons. However, even under such conditions, pET-15b-*AmglyA* was still higher than pET-15b-*EcglyA* in the conversion rate during 12 h. So in the next study, the codons of *Am*SHMT should be optimized to improve its expression in *E. coli* (Carbone et al. 2003). Many published reports provide helpful information on how to improve the yield. With the triparental mating method, a new recombinant strain MB202 was screened, whose SHMT activity was approximately 3.5-fold higher than that of the parent strain, and L-serine output was 4.4-fold higher (Shen et al. 2010). *SdaA*-encoded L-serine dehydratase has been demonstrated to be involved in L-serine degradation (Netzer et al. 2004). With the presence of L-serine dehydratase in *Arthrobacter globiformis* SK-200, 80 % of the L-serine in the medium was consumed in 2 days (Tani et al. 1978). When *glyA* expression was reduced and L-serine dehydratase activity was missing, L-serine production increased up to 9.04 mg/ml (Peters-Wendisch et al. 2005). By using error-prone PCR, DNA shuffling and high throughput screening, a mutant with approximately eightfold higher enzyme activity and 41-fold higher enzyme productivity than the wild-type parent was obtained (Zuo et al. 2007).

Conclusions

The SHMT in the present study is a novel one from *Arthrobacter* sp., which was stable in weakly alkali conditions, showing optimal activity at pH 7.8 and 45 °C, and a 2.75-fold higher activity than the corresponding enzyme from *E. coli*. The molecular conversion rate of L-serine in the pET-15b-*AmglyA* system was 70.3 % and 1.32-fold higher than that in the pET-15b-*EcglyA* system (53.4 %). Based on these characteristics, this strain and its enzyme have the potential for further research and industrial use.

Acknowledgments This work was supported by grants from China National Natural Sciences Foundation (No. 31270162) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Conflict of interests The authors claim that they have no competing interests. And there are not any non-financial competing interests.

References

Amedeo P, Habu Y, Afsar K, Scheid OM, Paszkowski J (2000) Disruption of the plant gene MOM releases transcriptional silencing of methylated genes. Nature 405:203–206

Appaji Rao N, Ambili M, Jala V, Subramanya H, Savithri H (2003) Structure-function relationship in serine hydroxymethyltransferase. Biochim Biophys Acta 1647:24–29

Barra D, Martini F, Angelaccio S, Bossa F, Gavilanes F, Peterson D, Bullis B, Schirch L (1983) Sequence homology between prokaryotic and eukaryotic forms of serine hydroxymethyltransferase. Biochem Biophys Res Commun 116:1007–1012

Blakley RL (1955) The interconversion of serine and glycine: participation of pyridoxal phosphate. Biochem J 61:315–323

Byrne PC, Sanders P, Snell K (1992) Nucleotide sequence and expression of a cDNA encoding rabbit liver cytosolic serine hydroxymethyltransferase. Biochem J 286:117–123

Carbone A, Zinovyev A, Kepes F (2003) Codon adaptation index as a measure of dominating codon bias. Bioinformatics 19:2005–2015

Das S, Lyla P, Khan SA (2006) Marine microbial diversity and ecology: importance and future perspectives. Curr Sci 90:1325–1335

Emelyanov A, Gao Y, Naqvi NI, Parinov S (2006) Trans-kingdom transposition of the maize dissociation element. Genetics 174:1095–1104

Garrow T, Brenner A, Whitehead V, Chen XN, Duncan R, Korenberg J, Shane B (1993) Cloning of human cDNAs encoding mitochondrial and cytosolic serine hydroxymethyltransferases and chromosomal localization. J Biol Chem 268:11910–11916

Hagishita T, Yoshida T, Izumi Y, Mitsunaga T (1996) Efficient L-serine production from methanol and glycine by resting cells of *Methylobacterium* sp. strain MN43. Biosci Biotechnol Biochem 60:1604–1607

Hamilton B, Hsiao H, Swann W, Anderson D, Delente J (1985) Manufacture of L-amino acids with bioreactors. Trends Biotechnol 3:64–68

Hong MC, Wu ML, Chang MC (1999) Cloning and complete nucleotide sequence of *Acinetobacter radioresistens* CMC-1 *AglyA* gene encoding serine hydroxymethyltransferase. FEMS Microbiol Lett 170:413–418

Hsiao H, Walter J, Anderson D, Hamilton B (1988) Enzymatic production of amino acids. Biotechnol Genet Eng Rev 6:179–219

Izumi Y, Yoshida T, Miyazaki SS, Mitsunaga T, Ohshiro T, Shimao M, Miyata A, Tanabe T (1993) L-Serine production by a methylotroph and its related enzymes. Appl Microbiol Biotechnol 39:427–432

Jiang W, Xia B, Liu Z (2013) A serine hydroxymethyltransferase from marine bacterium *Shewanella algae*: Isolation, purification, characterization and l-serine production. Microbiol Res 168:477–484

Jones C, Priest D (1976) Metabolism of glutamine and ammonia in rat liver: the effects of N-acetylglutamate and phosphate. Arch Biochem Biophys 174:305–311

Li C, Hong Y, Shao Z, Lin L, Huang X, Liu P, Wu G, Meng X, Liu Z (2009) Novel alkali-stable, cellulase-free xylanase from deep-sea *Kocuria* sp. Mn22. J Microbiol Biotechnol 19:873–880

Lin L, Meng X, Liu P, Hong Y, Wu G, Huang X, Li C, Dong J, Xiao L, Liu Z (2009) Improved catalytic efficiency of endo-β-1,4-glucanase from *Bacillus subtilis* BME-15 by directed evolution. Appl Microbiol Biotechnol 82:671–679

Liu YG, Huang N (1998) Efficient amplification of insert end sequences from bacterial artificial chromosome clones by thermal asymmetric interlaced PCR. Plant Mol Biol Report 16:175–175

Liu YG, Whittier RF (1995) Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. Genomics 25:674–681

Mehta PK, Christen P (2000) The molecular evolution of pyridoxal-5′-phosphate-dependent enzymes. Adv Enzymol Relat Areas Mol Biol 74:129–184

Miyata A, Yoshida T, Yamaguchi K, Yokoyama C, Tanabe T, Toh H, Mitsunaga T, Izumi Y (1993) Molecular cloning and expression of the gene for serine hydroxymethyltransferase from an obligate methylotroph *Hyphomicrobium methylovorum* GM2. Eur J Biochem 212:745–750

Nand K, Rao D (1972) *Arthrobacter mysorens*-a new species excreting L-glutamic acid. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg 127:324–331

Netzer R, Peters-Wendisch P, Eggeling L, Sahm H (2004) Cometabolism of a nongrowth substrate: L-serine utilization by *Corynebacterium glutamicum*. Appl Environ Microbiol 70:7148–7155

Peters-Wendisch P, Stolz M, Etterich H, Kennerknecht N, Sahm H, Eggeling L (2005) Metabolic engineering of *Corynebacterium glutamicum* for L-serine production. Appl Environ Microbiol 71: 7139–7144

Rao DN, Appaji Rao N (1982) Purification and regulatory properties of mung bean (Vigna radiata L.) serine hydroxymethyltransferase. Plant Physiol 69:11–18

Schirch V, Hopkins S, Villar E, Angelaccio S (1985) Serine hydroxymethyltransferase from *Escherichia coli*: purification and properties. J Bacteriol 163:1–7

Shen P, Chao H, Jiang C, Long Z, Wang C, Wu B (2010) Enhancing production of L-serine by increasing the *glyA* Gene expression in *Methylobacterium* sp MB200. Appl Biochem Biotechnol 160:740–750

Siglioccolo A, Bossa F, Pascarella S (2010) Structural adaptation of serine hydroxymethyltransferase to low temperatures. Int J Biol Macromol 46:37–46

Sun J (2000) Study on synthesis of L-serine catalyzed by SHMT recombinant bacteria. Dissertation, China Pharmaceutical University

Sun J, Wu W, Chen Z (2000) Synthesis of coenzyme THFA and determination of its relative activity. Pharm Biotechnol 7:38–41

Tani Y, Kanagawa T, Hanpongkittikun A, Ogata K, Yamada H (1978) Production of L-Serine by a methanol-utilizing bacterium, *Arthrobacter globiformis* SK-200. Agric Biol Chem 42:2275–2279

Ulevitch RJ, Kallen RG (1977) Studies of the reactions of lamb liver serine hydroxymethylase with L-phenylalanine: kinetic isotope effects upon quinonoid intermediate formation. Biochemistry 16: 5350–5354

Usha R, Savithri HS, Appaji Rao N (1994) The primary structure of sheep liver cytosolic serine hydroxymethyltransferase and an analysis of the evolutionary relationships among serine hydroxymethyltransferases. Biochim et Biophys Acta (BBA)-Protein Struct Mol Enzymol 1204:75–83

Vidal L, Calveras J, Clapes P, Ferrer P, Caminal G (2005) Recombinant production of serine hydroxymethyl transferase from *Streptococcus thermophilus* and its preliminary evaluation as a biocatalyst. Appl Microbiol Biotechnol 68:489–497

Zhao M, Ma Y, Dai L, Zhang D, Li J, Yuan W, Li Y, Zhou H (2012) A high-performance liquid chromatographic method for simultaneous determination of 21 free amino acids in Tea. Food Anal Methods 6: 69–75

Zuo Z-Y, Zheng Z-L, Liu Z-G, Yi Q-M, Zou G-L (2007) Cloning, DNA shuffling and expression of serine hydroxymethyltransferase gene from *Escherichia col*i strain AB90054. Enzym Microb Technol 40: 569–577