Expression, purification and function of cysteine desulfurase from *Sulfobacillus acidophilus* TPY isolated from deep-sea hydrothermal vent

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Abstract The cysteine desulfurase (SufS) gene of *Sulfobacillus acidophilus* TPY, a Gram-positive bacterium isolated from deep-sea hydrothermal vent, was cloned and over-expressed in *E. coli* BL21. The recombinant SufS protein was purified by one-step affinity chromatography. The TPY SufS contained a well conserved motif RXGHHCA as found in that of other microorganisms, suggesting that it belonged to group II of cysteine desulfurase family. The recombinant TPY SufS could catalyze the conversion of L-cysteine to L-alanine and produce persulfide, and the enzyme activity was 95 μmol of sulfur ion per minute. The growth of *E. coli* BL21 was promoted by over-expressing TPY SufS in vivo or by directly adding recombinant TPY SufS in the medium ($4.3\text{--}4.5 \times 10^8$ cells/mL vs. $3.2\text{--}3.5 \times 10^8$ cells/mL). Furthermore, the highest cell density of *E. coli* BL21 when the TPY SufS was over-expressed was about 3.5 times that of the control groups in the presence of sodium thiosulfate. These results

indicate that the SUF system as the only assembly system of iron–sulfur clusters not only has significant roles in survival of *S. acidophilus* TPY, but also might be important for combating with high content of sulfide.

Keywords Iron–sulfur clusters · Cysteine desulfurase · *Sulfobacillus acidophilus* · Growth · Function

Introduction

Iron–sulfur clusters (Fe–S clusters) are one of the most ancient and versatile inorganic cofactors due to their intrinsic chemical properties in biology (Py and Barras 2010; Blanc et al. 2015). They are ubiquitous in almost all living organisms, including microbes, plants, and mammals (Mettert and Kiley 2015; Freibert et al. 2017). Proteins containing Fe–S clusters play important roles in a wide variety of biological processes, including respiration, nitrogen fixation, photosynthesis, DNA replication and repair, gene regulation and RNA modification (Mettert and Kiley 2015). Biological assembly of the Fe–S clusters and their insertion into apo-proteins are mediated by four multicomponent systems in microorganisms, termed nitrogen fixation (NIF), iron–sulfur cluster (ISC), sulfur mobilization (SUF) and cysteine desulfurase (CSD) systems (Ayala-Castro et al. 2008; Mettert and Kiley 2015). Generally, Gram-negative bacteria contain three or four of these systems, for example *Dickeya dadantii* and *E. coli* (Roche et al. 2013), whereas most Gram-positive bacteria and archaea only own SUF system, and even some only contain a part of the SUF system (Hidese et al. 2011; Roche et al. 2013).

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To date, researches on microbial Fe–S cluster assembly and functions of cysteine desulfurase are based primarily on *E. coli*, *Azotobacter vinelandii*, *Saccharomyces cerevisiae*, *Salmonella enterica*, *Acidithiobacillus ferrooxidans*, etc. (Zeng et al. 2007; Py and Barras 2010; Tanaka et al. 2016). Little is known about the roles of cysteine desulfurase in Gram-positive bacteria, in particular for SUF system (Py and Barras 2010; Rybníček et al. 2014). Furthermore, the studies on Fe–S cluster assembly of marine microorganisms from extreme environments are far less. We isolated a Gram-positive acidophilic bacterium (*S. acidophilus* TPY) from a deep-sea hydrothermal vent located in the Pacific Ocean, which can grow at 30–65 °C and has an optimal growth temperature of 45–50 °C (Li et al. 2011). The acidophilic bacteria play key roles in biogeochemical processes, especially for sulfur and iron, which attracts more and more attention (Baker-Austin and Dopson 2007; Hua et al. 2015). Species of genus *Sulfobacillus* own surprisingly different metabolic systems, including carbon, sulfur, iron, nitrogen, hydrogen, etc. (Justice et al. 2014). The genome of *S. acidophilus* TPY contains a *suf* operon which is the only assembly system of Fe–S clusters (Li et al. 2011). The information on Fe–S cluster assembly and the functions of cysteine desulfurase of *S. acidophilus* TPY may give useful insights into how bacteria respond and adapt to extreme environments, for example deep-sea hydrothermal vent.

In this study, the SufS gene of *S. acidophilus* TPY was cloned and successfully expressed in *E. coli*. After that, the recombinant TPY SufS protein was purified by one-step affinity chromatography. Finally, the TPY SufS activity and its functions in promoting growth of *E. coli* in vivo and in vitro were investigated in the absence and the presence of sodium thiosulfate.

Methods and materials

Bacterial strains and plasmids

DH5 α competent cells and *E. coli* BL21 competent cells were from Thermo Fisher Scientific (Waltham, MA, USA) and were preserved in –80 °C until use. Bacterial strains and plasmids used in this study were as described by our previous studies (Zhang et al. 2013; Zhou et al. 2016).

Gene cloning, expression and purification of the SufS of *S. acidophilus* TPY

The genomic DNA of *S. acidophilus* TPY was extracted using TIANamp Bacteria DNA kit (Tiangen Biotech Co. Ltd., Beijing, China) in accordance with the manufacturer's instructions. The gene was amplified by PCR using primers

that contain six continuous histidine codons in the forward primer. The sequence of forward primer was GCTCTA-GAATGACACGGACTTTAGTGG, containing an *Xba*I site. The sequence of the reverse primer was CGAGCTCTCAGCGAAAGTACCTCTGC, containing a *Sac*I site. The PCR amplification program was initial 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 56 °C, 60 s at 72 °C, and a final cycle of 72 °C for 10 min. The PCR products were checked by 1.5% agarose gel electrophoresis. Then, the resulting PCR products were purified using a QIAquick-spin PCR purification kit (Qia-gen, Hilden, Germany), double digested by *Xba*I and *Sac*I, and ligated into pET-His expression vector. The following steps, including expression and purification of recombinant protein, were as described by Zeng et al. (2007).

Sequence analyses

Amino acid sequences for multiple alignment were obtained from NCBI database. Multiple sequence alignments were generated using the CLUSTALW software with default parameters and visualized by GENEDOC program. A neighbor joining phylogenetic tree was constructed using MEGA version 6.0 with bootstrap values determined by 1000 replicates. Catalytic residue, chemical binding site, and conserved domain of SufS were analyzed according to BLAST results based on NCBI database and UNIPROT database.

Effects of the TPY SufS in vivo and in vitro on growth of *E. coli* in the absence or the presence of sodium thiosulfate

In order to investigate whether the TPY SufS can promote growth of *E. coli* BL21, the variation in cell density of *E. coli* BL21 was monitored when the TPY SufS was over-expressed in these cells, or recombinant TPY SufS was directly added into medium. The experiments were carried out in 250-mL shake flasks containing 100 mL LB medium (Amp⁺) in the absence or the presence of sodium thiosulfate (6%, w/v). And the *E. coli* BL21 cells with pET-His-SufS were inoculated into shake flasks. The shake flasks were incubated in a rotary shaker at 170 rpm and 16 °C. Samples were withdrawn at regular intervals to measure optical density (OD) at 600 nm with a spectrophotometer. Cell density was calculated according to the previously prepared standard curve. In addition, the prepared inoculum was also diluted and spread on LB agar (Amp⁺), and then was incubated at 16 °C to monitor the growth of *E. coli* BL21. The growth of *E. coli* BL21 cells transformed with pET-His acted as control experiment. All experiments were carried out in triplicate.

The over-expressed TPY SufS amounts determined by western blotting

The equal mass of crude extracts of cells obtained by sonication were analyzed by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Immunodetection was performed with antibodies raised against Sufs-His in mouse and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG by ECL Western blotting detection reagents (GE Healthcare). Procedures for western blotting were as described in our previous report (Zhang et al. 2013). All experiments were performed at least in triplicate.

Enzyme activity assays

The activity of SufS from *S. acidophilus* TPY was determined by measuring the rate of production of sulfide. The reaction mixtures contained 50 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 1 mM L-cysteine, 5 mM dithiothreitol, 0.25 mM pyridoxal 5'-phosphate (PLP), and appropriate enzyme. Sulfide concentration was determined as described by Zheng et al. (1993). The sulfide concentration was calculated based on a Na₂S standard curve. Control assays were also carried out by replacing enzyme sample or L-cysteine with distilled water. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 nmol sulfur ion as described by Zheng et al. (1993).

Results

Cloning of the SufS gene from *S. acidophilus* TPY

The full length of SufS gene was successfully amplified from genomic DNA of TPY strain and cloned into pET-His expression vector. The ligated product was then transformed into competent cells of *E. coli* DH5 α . The identified positive colonies were selected for sequencing. The results indicate that full length of the gene contained 1221 nucleotides, encoding a protein of 407 amino acids. BLAST shows that the putative TPY SufS protein had a highest similarity of 70% to SufS in *S. thermosulfidooxidans* and *Ferropasma* sp. Type II as shown in Fig. 1. Extended sequence alignment shows that the amino acid sequence contained a conserved domain of cysteine desulfurases and a well conserved motif RXGHHCA as highlighted by rectangle in Fig. 2.

Fig. 1 Phylogenetic tree based on amino acid sequences of the SufS proteins. A neighbor joining phylogenetic tree was constructed using MEGA version 6.0 with bootstrap values determined by 1000 replicates

Expression and purification of SufS from *S. acidophilus* TPY

The heterologous expression of TPY SufS in *E. coli* BL21 was carried out at different temperature and IPTG concentration, and the recombinant TPY SufS proteins were obtained at all tested conditions. It was found that the optimal condition was at temperature 16 °C with IPTG 0.1 mM (data not shown). Nickel metal-affinity resin column was used for single-step purification of His-tagged TPY SufS proteins. The recombinant proteins were dialyzed immediately after purification as described by Zeng et al. (2007). The purified TPY SufS was further checked by SDS-PAGE. As shown in Fig. 3, the protein bands were single and molecular mass of His-tagged TPY SufS protein was about 45 kDa which was in agreement with the theoretical size.

Effects of TPY SufS over-expression on growth of *E. coli* BL21

It can be seen from Fig. S1 that the colony size of cells over-expressed with the recombinant TPY SufS on LB plates was almost twice larger than that of cells transformed with pET-His. In order to further investigate this scenario, variation in cell density of *E. coli* was monitored in liquid LB medium when the TPY SufS was over-expressed in these cells. As can be seen from Fig. 4a, growth rates of *E. coli* BL21 increased significantly after about 2 h of inoculation in both experiments. As time progressed, the growth rate of *E. coli* BL21 without over-expression of TPY SufS showed an obviously downward trend after 5.5 h. However, there was no obvious change in growth rate at that point in time when recombinant TPY SufS was over-expressed in vivo. Furthermore, cell density was up to 4.5×10^8 cells/mL in the experiment of over-expression of recombinant TPY SufS at the end of the run, while it only was about 3.2×10^8 cells/mL in the control experiment. Enzyme activity assays show that the recombinant

Fig. 2 Amino acid sequence alignment of several SufS and SufS-like proteins. (The conserved motif RXGHCA is highlighted by rectangle. Black stars indicate the positions of the Schiff base-forming Lys of the PLP-containing active site and the catalytic cysteine.)

Fig. 3 SDS-PAGE analysis of purified cysteine desulfurase (SufS) over-expressed in *E. coli* BL21. **a** Lane1, marker (kDa); lane2, crude extract of supernatant of cells transformed with pET-His; lane3, crude extract of cells transformed with pET-His-SufS; lane4, crude extract of pellets after lysing cells transformed with pET-His-SufS; lane5, crude extract of supernatant after lysing cells transformed with pET-His-SufS. **b** Lane1, marker (kDa); lane2, purified TPY SufS

TPY SufS activity was 95 μM of sulfur ion per minute and the activity was lower than 0.84 μM in the control groups (data not shown).

We also determined the recombinant TPY SufS amounts of over-expressed cells at 4th h and 6th h by western blotting analysis (Fig. S2). The results indicate that the amounts of the recombinant TPY SufS increased by about 37% from 4th h to 6th h in accordance with high growth rate as mentioned above. Furthermore, we also investigated effects of recombinant TPY SufS in vitro on growth of *E. coli* BL21 by directly adding purified TPY SufS in liquid LB medium. The experiments with adding actin protein and nothing acted as control experiments as shown in Fig. 4b. The results show that there was no difference in growth between the treatment groups and the control groups in the first 4 h. However, growth rate of *E. coli* with addition of recombinant TPY SufS increased significantly after 4th h, resulting in a highest biomass compared with the control experiments at the end of the run.

Fig. 4 Effects of recombinant TPY SufS on the growth of *E. coli* BL21 in the absence and the presence of sodium thiosulfate. **a** The variation of cell density of *E. coli* BL21 cells in which recombinant TPY SufS was over-expressed in vivo; **b** the variations of cell density of *E. coli* BL21 when directly adding purified TPY SufS (520 ng/mL) and actin protein (520 ng/mL) into medium; **c** the variation of cell density of *E. coli* BL21 with over-expression of recombinant TPY SufS in the presence of sodium thiosulfate of 6% (w/v)

Effects of over-expressed recombinant TPY SufS on growth of *E. coli* BL21 in the presence of sodium thiosulfate

Figure 4c shows the growth of *E. coli* BL21 cells into which pET-His-SufS or pET-His was transformed, respectively, in the presence of sodium thiosulfate (6%, w/v). As can be seen, cell density in the control experiment (the cells were transformed with pET-His) was at very low level (about 2×10^7 cells/mL) during the whole process. The growth rate of *E. coli* BL21 in the control experiment was almost zero after about 4 h. On the contrary, the *E. coli* BL21 cells transformed with pET-His-SufS grew well in the presence of sodium thiosulfate of 6% (w/v). The growth rate of *E. coli* BL21 was obviously higher than that in the control experiment, and the highest cell density was up to 7×10^7 cells/mL at 7th h (Fig. 4c). After that, the cell density no longer changed obviously.

Discussion

SUF system is one of multicomponent systems for assembling Fe–S clusters and inserting them into apo-form of Fe–S proteins (Ayala-Castro et al. 2008; Mettert and Kiley 2015). These Fe–S proteins are indispensable for living organisms (Metttert and Kiley 2015; Freibert et al. 2017). SufS is one of the most important components of the SUF system, which can catalyze the conversion of L-cysteine to L-alanine and then provides sulfur for Fe–S cluster assembly (Smith et al. 2001; Roche et al. 2013; Yamanaka et al. 2013; Tanaka et al. 2016). Although the putative SufS of *S. acidophilus* TPY shares considerable sequence similarity with that in *S. thermosulfidooxidans* and *Ferroplasma* sp. Type II (Fig. 1), their SufS genes have not been cloned and over-expressed, and the functions are still not very clear. SufS from *S. acidophilus* TPY owns a particularly high degree of conservation including the catalytic residue (Cys-364), PLP-binding site (Lys-226) and binding pocket of cofactor (Fig. 2) as found in that of other microorganisms (Fujii et al. 2000; Loiseau et al. 2003; Boyd et al. 2014; Fernández et al. 2016). The conserved motif RXGHHCA suggests that the cysteine desulfurase from *S. acidophilus* TPY belongs to group II of cysteine desulfurases (SufS) (Ayala-Castro et al. 2008). The harvested cells of *E. coli* BL21 and the recombinant purified TPY SufS proteins both showed obviously yellow color due to the presence of the PLP, which is in agreement with other recombinant cysteine desulfurases reported in the previous studies such as SufS and IscS (Loiseau et al. 2003; Zeng et al. 2007). The previous studies also indicated that molecular mass of His-tagged SufS protein is about 45 kDa

(Loiseau et al. 2003; Selbach et al. 2010). Enzyme activity assays show that the recombinant SufS from *S. acidophilus* TPY can catalyze the conversion of L-cysteine to L-alanine and produce sulfur as indicated by Zheng et al. (1993).

Currently, although we have a better understanding on assembly mechanisms of cysteine desulfurase systems (Johnson et al. 2005; Lill 2009; Py and Barras 2010; Shepard et al. 2011; Hidese et al. 2014; Mettert and Kiley 2015), their effects on physiological and biochemical properties of microorganisms have not been thoroughly investigated, especially for SUF system (Py and Barras 2010; Rybniker et al. 2014). In this study, we investigated effects of the recombinant SufS derived from *S. acidophilus* TPY on growth of *E. coli* BL21. As shown in Fig. 4a, over-expression of recombinant SufS derived from *S. acidophilus* TPY can significantly promote growth of *E. coli* BL21. Previous reports indicated that the deletion of IscS in *E. coli* led to an overall decrease in the activity of a number of Fe–S proteins and significant growth defect phenotypes (2- to 50-fold decrease) (Schwartz et al. 2000; Mihara et al. 2008). Furthermore, it has been proved that these phenotypes could be rescued by over-expressing heterologous NifS and SufS (Takahashi and Tokumoto 2002; Johnson et al. 2005; Rybniker et al. 2014). It is, therefore, no surprise that over-expression of heterologous SufS derived from *S. acidophilus* TPY, a Gram-positive acidophilic bacterium isolated from deep-sea hydrothermal vent, can significantly promote growth of *E. coli* BL21.

To further investigate whether recombinant SufS derived from *S. acidophilus* TPY can promote growth of *E. coli* BL21, we also investigated effects of recombinant TPY - SufS in vitro on growth of *E. coli* BL21 by directly adding purified TPY SufS in liquid LB medium. It also suggests that recombinant TPY SufS in vitro was beneficial to growth of *E. coli* BL21 and the final biomass was highest in all experiments (Fig. 4b). As indicated by previous studies, over-expression of cysteine desulfurase can increase the yield of recombinant Fe–S proteins (Mihara and Esaki 2002; Takahashi and Tokumoto 2002). Thus, it is reasonable to believe that heterologous SufS can promote growth of *E. coli* BL21 not only in vivo but also in vitro.

As can be seen from Fig. 4c, the growth of *E. coli* BL21 was significantly inhibited in the presence of sodium thiosulfate in the control experiment, while the growth was restored by over-expressing recombinant TPY SufS in vivo. It is believed that functions of the ISC system of *E. coli* BL21 were suppressed under sodium thiosulfate stress, and its SUF system could not completely complement the functions of ISC system (Fig. 3 shows that the SufS of *E. coli* BL21 itself was also expressed in this study), although the SUF system is believed to be involved in adverse stresses for example oxidative stress (Ayala-Castro et al. 2008; Hidese et al. 2011; Ezraty et al. 2013).

As indicated by Ezraty et al. (2013), higher than normal level expression of SUF system could mature some Fe–S proteins during certain stresses, which was helpful to enhance adaptation ability and resistance of *E. coli*. It is also suggested that over-expression of the recombinant SufS derived from *S. acidophilus* TPY can restore growth defect of *E. coli* BL21 under sodium thiosulfate stress in this study, although they have a distant genetic relationship and different survival environment, which is also in agreement with opinions of Johnson et al. (2005) and Takahashi and Tokumoto (2002).

In conclusion, *S. acidophilus* TPY inhabits at very adverse environment which is rich in sulfur, sulfide, and metal ions (Michard et al. 1984; Jean-Baptiste and Fouquet 1996; Zeng et al. 2010; Breier et al. 2012). The SUF system is believed to be strongly induced in response to a variety of adverse environments (Ayala-Castro et al. 2008; Mettert and Kiley 2015). Therefore, the SUF system, the only assembly system of Fe–S clusters in *S. acidophilus* TPY, not only has significant roles in survival of *S. acidophilus* TPY, but also might be important for combating with adverse environmental stresses, although this is only verified by over-expressing SufS in *E. coli* under sodium thiosulfate stress, and also needs further and extensive studies. The results might be helpful to better understand evolutionary adaptations made by bacteria in response to adverse environments.

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Author contributions XC conceived and designed the research. QL and YW performed the experiments. YW and HZ analyzed the results and drafted the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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