Heterologous expression of *Oenococcus oeni* sHSP20 confers temperature stress tolerance in *Escherichia coli*

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Abstract

Small heat shock proteins (sHSPs) are heat shock proteins sized 12–43 kDa that can protect proteins from denaturation, particularly under high temperature; sHSPs thus increase the heat tolerance capability of an organisms enabling survival in adverse climates. sHSP20 is overexpressed in *Oenococcus oeni* in response to low temperatures. However, we found that overexpression of sHSP20 in *Escherichia coli* BL21 increased the microbial survival ratio at 50 °C by almost 2 h. Adding sHSP20 to the glutamate dehydrogenase solution significantly increased the stability of the enzyme at high temperature (especially at 60–70 °C), low pH values (especially below 6.0), and high concentration of metal ions of Ga²⁺, Zn²⁺, Mn²⁺, and Fe³⁺. Notably, the coexpression of sHSP20 significantly enhanced soluble expression of laccase from *Phomopsis sp. XP-8* (CCTCCM209291) in *E. coli* without codon optimization, as well as the activity and heat stability of the expressed enzyme. In addition to the chaperone activity of sHSP20 in the gene containing host in vivo and the enzyme heat stability in vitro, our study indicated the capability of coexpression of sHSP20 to increase the efficiency of prokaryotic expression of fungal genes and the activity of expressed enzymes.

Keywords sHSP20 · Cell viability · Prokaryotic expression · Enzyme stability and activity · Coexpression

Highlights • Overexpression of sHSP20 in *Escherichia coli* can increase the heat tolerance of cells.

- Presence of sHSP20 increases enzyme stability under adverse environments.
- Coexpression of sHSP20 can increase the prokaryotic expression of fungal gene.
- Coexpression of sHSP20 is helpful to increase the expressed enzyme activity.

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Abbreviations

ACD	α -crystal domain
<i>E. coli</i>	<i>Escherichia coli</i>
GDH	glutamate dehydrogenase
HSP	Heat shock proteins
IPTG	isopropyl-b-D-thiogalactopyranoside
MLF	malolactic fermentation
<i>O. oeni</i>	<i>Oenococcus oeni</i>
SD	standard deviation
sHSP	small heat shock proteins

Introduction

Heat shock proteins (HSPs) are widespread conservative proteins that play a role as molecule chaperones during stress in organisms. HSPs can be divided into five families: HSP 100, HSP 90, HSP 70, HSP 60, and sHSP that differ in molecular size, structure, and function. sHSPs are the least studied, but offer significant value for the prevention of proteins from aberrant folding and aggregation, while promoting efficient protein folding, especially under stress conditions (Hartl et al. 2011; Hilton et al. 2013).

Most sHSPs are strongly induced by a variety of stresses and function constitutively in multiple cell types in animals, plants, and microorganisms, such as *Escherichia coli*, yeast, and the ancient mycobacterium tuberculosis bacteria (Bagneris et al. 2009; Basha et al. 2012; Guzzo 2012; Hanazono et al. 2013; Hasanuzzaman et al. 2013; Chen and Zhang 2015). Many sHSPs have molecular chaperone activities in vitro and in vivo. Some sHSPs can rapidly accumulate after exposure to heat shock (Nahomi et al. 2015). Expression of sHSP genes can also be induced by other abiotic stresses. Apart from the salinity (Muthusamy et al. 2017), low temperature and drought (Sarkar et al. 2009; Basha et al. 2012) and other abiotic stresses such as extreme pH, insecticides, and heavy metal can also induce sHSPs (Chen and Zhang 2015).

sHSPs vary greatly in structure and have even larger differences between different genera within the same family. In structure, sHSPs are a family with a molecular weight range from 12 to 43 kDa, where most range between 14 and 27 kDa. Most of them have the same points and are highly conservative in some part of the structure, such as the ACD, the N-terminal arm, and the C-terminal extension structure. The ACD contains a consensus sequence of about 90 amino acid residues, similar to the vertebrate eye lens α -crystalline proteins—ACD that is flanked by divergent sequences of nearly 55 amino acids with an N-terminal arm and a flexible C-terminal extension sequence, which is generally smaller than 20 residues (Brady et al. 1997;

Stamler et al. 2005; Basha et al. 2012). The stoichiometric amounts of alpha A and alpha B suppress the thermally induced aggregation of various enzymes (Andley et al. 2014; Banerjee et al. 2015). The alpha-crystalline functions as a molecular chaperone responsible for protein folding, assembly, translocation, and degradation in many normal cellular processes.

Heterologous expression of sHSP confer abiotic stress tolerance was also reported in insect, e.g., *Cydia pomonella* (Yang et al. 2017), as well as *Arabidopsis thaliana*, rice, tomato, potato, and tobacco (Wang et al. 2017b). A functional sHSP *PpHsp16.4* was found to be essential for the recovery of *Physcomitrella patens* under heat, salt, and osmotic stresses (Muthusamy et al. 2017; Ruibal et al. 2013). Expression of *CsHSP17.2* from *Camellia ainensis* demonstrated an increased tolerance of *E. coli* to heat stress (Wang et al. 2017a). Recently, coexpression of chaperones from *Pyrococcus furiosus* was found to enhance the soluble expression of the recombinant hyperthermophilic α -amylase in *E. coli* (Peng et al. 2016).

sHSP20 is an sHSP with a molecular weight of 20 kDa that was previously discovered in *O. oeni* and plays an important role in the improvement of wine quality and flavor (Cecconi et al. 2009; Zhang and Lovitt 2005). In contrast to most extensively studied sHSPs, sHSP20 was discovered under cold shock, instead of heat shock, and showed a different molecular weight from all reported HSPs (Fan 2012). Information on the properties of sHSP20 is very limited in the literature. And, the potential of sHSP20 in promoting gene expression in vivo and protecting enzyme stability and activity in vitro has not been explored before.

NADP⁺-dependent glutamate dehydrogenase (NADP⁺-GDH) is the first step in ammonia assimilation pathway in *Saccharomyces cerevisiae* and the knowledge of its regulation is the key for many biotechnological purposes such as single cell protein production. A novel glutamate dehydrogenase (GDH) with higher alcohol and amino acid activity was isolated from *Geotrichum candidum* (Zhu et al. 2017). However, it was easy to be inactive under conditions of high temperature, pH, and heavy metals.

Therefore, the expressed sHSP20 was used together with the GDH from *G. candidum* to test the capability of sHSP20 in helping GDH to confer the abiotic stress tolerance in vitro. Overexpression sHSP20 in *E. coli* was also carried out and tested its influence on the heat tolerance of *E. coli* in vivo, and the enzyme activity and on the tolerance of GDH under heat shock, pH, and metal ions in vitro. Coexpression of sHSP20 and fungal laccase in *E. coli* was also conducted to test the capability of sHSP20 to increase the activity and stability and the soluble expression of fungal enzymes without codon optimization.

Material and methods

Genes, plasmids, and *E. coli*

sHSP20 from *O. oeni* (GenBank accession no. OLQ44579.1) and GDH (GenBank accession no. KJ442577.1) was used in the study.

E. coli BL21 and pETDuet-1 were used as host and plasmid for gene expression throughout the study. Tables 1 and 2 list all nucleotide sequences of primers and plasmids used in the study.

pET28as-sHSP20 was constructed by amplifying sHSP20 from *Oenococcus oeni* genomes using primers P-SF (BamH I), P-SR (HindIII), and cloning the resulting product into the BamH I/HindIII sites of pET28a-sumo.

pETDuet-sHSP20 was constructed by amplifying sHSP20 from the plasmid pET28as-sHSP20 using primers P-SF (BamH I), P-SR (HindIII), and cloning the resulting product into the BamH I/HindIII sites of pETDuet-1.

pETD-sHSP20-laccase was constructed by amplifying laccase from *Phomopsis* sp. XP-8 using primers P-LF (NdeI), P_LR (BlnI) and cloning the resulting product into the NdeI/BlnI sites of pETD-sHSP20.

pETDuet-laccase was constructed by amplifying laccase from pET28as-laccase using primers P-LF (BamH I), P_LR (HindIII), and cloning the resulting product into the BamH I/HindIII sites of pETDuet-1.

pETD-laccase-sHSP20 was constructed by amplifying sHSP20 from pET28as-sHSP20 using primers P-SF (NdeI), P_SR (BlnI) and cloning the resulting product into the NdeI/BlnI sites of pETD-laccase.

Gene expression, SDS-PAGE detection, and purification of expressed proteins

Total DNA was isolated from *Oenococcus oeni* using Bacteria Genomic DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. sHSP20 was amplified using the primers P-SF (BamH I) and P-SR (HindIII). The products

were cloned into the pEASY-T1 Simple vector and sequenced. The sHSP20 fragment was ligated into a pET-28 as vector digested with the same enzymes (Wang et al. 2017a, b). The pET28as-sHSP20 recombinant plasmid and the empty vector were used to transform *E. coli* BL21 (DE3) cells.

Pre-cultivation was conducted at 37 °C and 180 rpm overnight in LB medium and then transferred into fresh LB medium with a starting OD₆₀₀ of 0.1 until the OD₆₀₀ reached 0.6, corresponding to the inoculation size about 1%. Induction of the expression of recombinant genes was performed using IPTG at a final concentration of 0.5 mM and 4 h at 30 °C.

Bacteria were harvested 4 h after IPTG induction via centrifugation (10,000×g, 10 min, 4 °C). After disruption via sonification and subsequent centrifugation, the supernatant containing expressed proteins was mixed with 4× SDS loading buffer. After heating the sample in a boiling water bath for 5 min and centrifugation at 12000×g for 2 min, the supernatant was loaded onto a preparative 15% polyacrylamide gel (pH 8.8) with a 5% stacking gel (pH 6.8). The loaded protein material was electrophoresed on the SDS-PAGE using Tris-glycine running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.7) and a mighty-small vertical electrophoresis unit (Bio-Rad). The completed SDS-PAGE gel was stained (Coomassie Blue R-250 1.0 g, 450 mL methanol, 100 mL acetate, and 450 mL deionized water) for 15 min and destained for 45 min.

His-tagged recombinant proteins were purified using Ni-affinity chromatography, according to the manufacturer's instructions (GE Healthcare) (Dąbrowski and Kiær Åhring 2003).

Determination of enzyme activity

The activity of GDH was measured using the method previously reported by Turano et al. (1996). GDH activity was determined in the presence of 100 mM NH₄Cl or 50 mM (NH₄)₂SO₄, 13 mM α-ketoglutarate, 0.25 mM NADH, and 1 mM CaCl₂, in 100 mM Tris-HCl, pH 8.0, and the increase in A₃₄₀ was recorded for 1 min.

Table 1 Nucleotide sequences of primers

Oligonucleotides	Sequences, 5'-3'	Relevance
P-SF (BamH I)	<u>GGATCC</u> ATGGCAAATGAATTAA	Subcloning of sHSP20
P-SR (HindIII)	<u>AAGCTT</u> TATTGGATTTC	Subcloning of sHSP20
P-LF (NdeI)	<u>CATATG</u> ATGAAGCTGTCATTCG	Subcloning of laccase
P_LR (BlnI)	<u>CCTAGG</u> TCAGATTCCTCGAATCGTC	Subcloning of laccase
P-LF (BamH I)	<u>GGATCC</u> ATGAAGCTGTCATTCG	Subcloning of laccase
P_LR (HindIII)	<u>AAGCTT</u> TCAGATTCCTCGAATC	Subcloning of laccase
P-SF (NdeI)	<u>CATATG</u> ATGGCAAATGAATT	Subcloning of sHSP20
P_SR (BlnI)	<u>CCTAGG</u> TATTGGATTTCATATGAG	Subcloning of sHSP20

Bold and underlined letters are restriction enzyme cut sites

Table 2 Plasmids used in the study

Plasmids	Description	Source of reference
pETDuet-1	Double T7 promoter, Amp ^R	Novagen
pET28a-sumo	pET28a with sumo label, Kana ^R	
pETD-sHSP20	pETDuet-1 carrying sHSP20	This study
pETD-lac1	pETDuet-1 carrying laccase in BamH I/HindIII	This study
pETD-lac2	pETDuet-1 carrying laccase in NdeI/BlnI	This study
pETD-sHSP20-laccase	pETDuet-1 carrying sHSP20 and laccase	This study
pETD-laccase-sHSP20	pETDuet-1 carrying laccase and sHSP20	This study
pET28as-sHSP20	pET28a-sumo carrying sHSP20	This study

Experimental design

Influence of sHSP20 overexpression on the cell viability of *E. coli*

E. coli BL21 (DE3) cells containing pET28as-sHSP20 or pET28a-sumo plasmids (without any inserted cDNA sequence, as the control of gene expression) were separately cultured at 37 °C in LB medium supplemented with 100 mg/ml ampicillin. Up to an OD₆₀₀ of 0.3–0.4, induction of gene expression was conducted using IPTG, at a final concentration of 0.5 mM for 3 h. After that, all cells were subjected to a heat shock under 50 °C for 1 h. A similar treatment at 37 °C was conducted as temperature control.

All treated cells were inoculated at different dilutions in agar LB medium and cultivated overnight at 37 °C to test the survival ratio of each treatment according to the number of colonies derived from heat-treated cells and the 37 °C-treated cells. Each treatment was conducted in triple and the means were reported.

E. coli BL21 (DE3) cells containing pET28a-sumo-sHSP20 and pET28a-sumo plasmids were also cultured in the auto-induction protein expression system medium supplemented with 100 mg/ml ampicillin to investigate the influence of gene expression on the growth curves of *E. coli* at 37 °C. When cell growth reached a plateau, they were subjected to high-temperature treatment (50 °C) for different periods to test the influence of sHSP20 expression on the survival ratio of *E. coli* cells, which was detected using a spectrophotometer at 600 nm and expressed at the OD₆₀₀.

Influence of the sHSP20 protein on the activity and stability of GDH in vitro

GDH from *Geotrichum candidum* (CCTCC AF2012005) was expressed by *E. coli* BL21 and purified using Ni-affinity chromatography. Activity of the purified GDH was measured using NADP as substrate. In the deamination reaction, GDH activity was determined in the presence of 35 mM glutamate, 0.25 mM NAD, and 1 mM CaCl₂, in 100 mM Tris-HCl, pH 8.0, and the increase in A340 was recorded for 1 min. One unit of GDH

activity was defined as the reduction or oxidation of 1 μmol of coenzyme (NAD/NADP or NADH/NADPH, respectively) per minute at 30 °C. To determine cofactor specificity, NADP(H) replaced NAD(H) at the same concentrations as described above (Fontaine et al. 2013). Each measurement was conducted for three independent times and the results were expressed as the mean value ± standard errors of triples.

The enzyme solution was separately kept together with (or without) purified sHSP20 for 10 min at 25, 40, 50, 60, and 70 °C in the assays of temperature stability, pH 4, 5, 6, 7, 8, 9, 10, and 11 for the assays of pH stability, and different concentrations of Ca²⁺ (Cl⁻), Zn²⁺ (Cl⁻), Mn²⁺ (Cl⁻), and Fe³⁺ (Cl⁻) in the metal ion stability analysis.

Influence of sHSP20 coexpression on the soluble expression and the activity of fungal laccase from *Phanerochaete* sp. XP-8

The soluble expression of fungal laccase was tested using the SDS-PAGE method as described above. The volumetric activities of laccases were analyzed at 25 °C. The volumetric activities of laccases were determined using the ABTS assay. Briefly, the assay was performed with 0.5 mM ABTS in 0.1 M NaOAc, pH 5.0. The oxidation of ABTS was photometrically followed at 420 nm (Ricklefs et al. 2016).

Statistical analysis

Values are given as mean values and standard deviation of triplicate experiments. Significant *t* tests were carried out to compare the values of each sample and the control using Graphpad Prime 6 (GraphPad Software, San Diego, CA, USA). The difference was considered statically significant if *P* < 0.05.

Results

Heterologous expression of sHSP20 increased the thermotolerance of the *E. coli* host

Similar to the results reported for other sHSPs, expression of sHSP20 significantly enhanced the viability of *E. coli* cells

under high temperatures (Fig. 1a). After treatment at 50 °C for 1 h, the survival rate of *E. coli* without sHSP20 decreased to 45%, compared to 90% of that containing sHSP20, illustrating the capability of sHSP20 expression in increasing thromboresistance capability of cells in vivo.

As shown in Fig. 1b, the expression of sHSP20 slightly increased the growth rate of *E. coli* at the early stage and significantly at the later stage of the logarithmic phase, especially resulting in a much higher value of the total amount of cells at the end of culture. In addition, Fig. 1c indicated that the *E. coli* cells containing sHSP20 produced more protein (including sHSP20 under high temperature) compared to cells without sHSP20.

Purification of sHSP20 expressed in *E. coli* cells

SDS-PAGE analysis showed that sHSP20 was overexpressed in recombinant *E. coli* with plasmid pET28as-sHSP20 (Fig. 2). Initially, washing the column with the buffer containing 50 mM imidazole removed a large amount of non-specific protein and precipitated material. sHSP20 was gradually eluted by increasing the concentration of imidazole to 400 mM.

sHSP20 increase enzyme activity under adverse conditions in vitro

The capability of sHSP20 to influence enzyme activity in vitro was measured by detecting the GDH enzyme activity in both absence and presence of the sHSP20 protein. The purified sHSP20 protein was added to the enzymatic reaction system at different concentrations. As a result, the addition of sHSP20 significantly inhibited the decrease of enzyme activity at temperatures above 60 °C and higher sHSP20 concentration resulted in a little higher increase of GDH activity (Fig. 3a). This concentration-dependent effect indicated that sHSP20 had chaperone-like activity. Furthermore, the protecting effect was more significant at higher than at lower temperatures. After treatment at 60 °C for 10 min, the sHSP20-assistant GDH activity still remained at 75%, while that without sHSP20 addition was only 30%. Furthermore, after treatment at 70 °C for 10 min, the activity of GDH was completely lost with the absence of sHSP20, but still remained at about 50% at the presence of sHSP20 (Fig. 3b).

In addition to the increase of heat resistance, the addition of sHSP20 also showed significant protection of GDH activity under acidic conditions, especially pH below 7, but not under alkalinity stress at pH above 8, although pH 8 was the optimal pH for GDH. The results indicated that the addition of sHSP20 would have the potential to expand the application of GDH under more stress conditions, especially those with high temperatures and low pH values (Fig. 3c).

Fig. 1 Heterologous expression of sHSP20 increased the viability of *E. coli* under high temperatures. **a** The survival ratio of BL21 cells with or without over-expressing sHSP20 after heat treatment at the indicated temperatures. The error bars represent the SD of three independent experiments. Triple asterisks indicate the difference is significant at $P < 0.001$. **b** *E. coli* cell proliferation curve. After 7 h, the cells were cultured at 50 °C. **c** SDS-PAGE analysis indicates the proteins present in the total lysates fractions at 50 °C for different periods. (+), the sHSP20-containing cells; (-), the cells without sHSP20. 12, 5, and 0.5 h indicate the cells heated at 50 °C for 12, 5, and 0.5 h, respectively

Fig. 2 >SDS-PAGE analysis on the purification of recombinant sHSP20. The sHSP20 were purified using Ni-affinity chromatography. Lane 1, proteins in the total lysates; lane 2, soluble fractions; lane 3, purified sHSP20

Without the presence of sHSP20, the activity of GDH gradually decreased and was even completely lost with the increase of metal ions concentration. However, the presence of sHSP20 could significantly prevent the decrease of GDH activity at high metal ion concentrations (Fig. 4). Such an effect varied with the types and concentrations of metal ions.

Fig. 3 Relative activity of GDH under different conditions. This indicated that the effect of sHSP20 on the protection of GDH activity varied with sHSP20 contents (a), temperature (b), and pH (c). The GDH activity was measured after treatments at 60 °C for different periods (a), at different temperature for 2 h later (b), and at different pH values for 10 min (c), respectively. Triple asterisks indicate the difference is significant at $P < 0.001$

It is illustrated that the presence of sHSP20 could significantly protect the GDH activity under high metal ion concentration stress.

Effect of sHSP20 coexpression on the soluble expression and activity of fungal laccase in *E. coli*

As indicated in Fig. 5, the coexpression of sHSP20 could significantly increase the soluble expression of laccase from *Phomopsis sp.* XP-8 in *E. coli* without codon optimization and the activity of expressed enzyme protein. As shown in Fig. 5, the coexpression of sHSP20 significantly increased the activity of expressed laccase (Fig. 5b) and the soluble expression of the fungal laccase, implying the application of sHSP20 in improving the soluble expression of fungal enzymes in *E. coli*. More importantly, activity of the expressed fungal laccase was also significantly increased under high temperatures with a relatively high activity in a wide range of high temperatures from 45 to 75 °C, overcoming the shortcoming of low activity under lower and higher temperatures (Fig. 5c).

Discussion

sHSP have been widely studied due to their ability to confer to cells under stressful conditions. These proteins play critical roles in bacteria and the cell physiology is severely altered by sHSP gene deletion. (Wang et al. 2017a). This study showed that the

Fig. 4 Relative GDH activities in the conditions with different metal ions and their concentrations of Ca^{2+} (a), Zn^{2+} (b), Mn^{2+} (c), Fe^{3+} (d) respectively. Double asterisks and triple asterisks indicate the difference is significant at $P < 0.01$ and $P < 0.001$, respectively

expression of sHSP20 from *O. oeni* significantly increased the cell growth and viability of *E. coli* cells.

Numerous studies have previously demonstrated that sHSPs are regulated by various abiotic stresses (Chen and Zhang 2015; Muthusamy et al. 2017). The expression of the *Primulas* HSP gene *PfHSP17.1* was induced by heat, cold, salt, PEG-induced drought stress, and oxidative stress (Zhang et al. 2013). The three *Camellia sinensis* *shsp* genes *CsHSP17.7*, *CsHSP18.1*, and *CsHSP21.8* have been reported to be triggered by cold and heat stress (Wang et al. 2017b). For microorganism, α -crystallin-type sHSP15.8 and sHSP16 were induced by prolonged high-temperature treatment (Glatz et al. 2016). sHSP20 used in this study was previously found in *O. oeni* under the induction of cold (Zhang et al. 2012).

O. oeni is a lactic acid bacterium that grows in wine and performs MLF that occurs subsequent to the alcoholic fermentation by yeasts and plays a critical role for wine quality (Margalef-Catala et al. 2016). It has been reported that *O. oeni* can produce a large amount of sHSPs *Lo18* (Margalef-Catala et al. 2016), particularly upon ethanol shock (Maitre et al. 2014). The molecular weight of sHSP20 is different from all currently reported sHSPs, including sHSP *Lo18*. Therefore, it is reasonable to assume that it possesses properties that differ from other sHSPs. As what is expected, sHSP20 from *O. oeni* showed the capability to prevent the decrease of GDH activity under high temperature, low pH, and high concentration of metal ions when it was added in the enzymatic reaction system.

Several recent studies have been conducted in the expression of *sHSP* genes in *E. coli*. Expression of a

Medicago sativa mitochondrial *HSP23* in *E. coli* was found to protect *E. coli* under salinity and arsenic stresses (Lee et al. 2012). Overexpression of a *Rosa chinensis* *RcHSP17.8* enhanced *E. coli* viability when the cells were exposed to heat and cold stresses (Jiang et al. 2009). When *CpHSP40* from *Cydia pomonella* was overexpressed in *Escherichia coli* BL21 (DE3), the cells harboring recombinant pET-28a (+)-*CpHSP40* plasmid showed significant temperature tolerance (Yang et al. 2016). Similarly, the *E. coli* cells with sHSP20 were more viable under heat stress than that without sHSP20, implying the improvement of thermotolerance in vivo.

More important, coexpression of sHSP20 could increase the soluble expression of fungal laccase and improve its activity and resistance to high temperature without codon optimization. Laccases are a family of copper-containing oxidases with wide applications in bioremediation textile, pulp, paper, and food industries (Yang et al. 2017), biosensors, biofuel cells, medical diagnostics tool, as bioremediation agents to clean up herbicides, pesticides, and certain explosives in soil (Shraddha et al. 2011). However, there is no efficient way to synthesize this enzyme at large scale, except for using some plants and fungus that secrete laccase. Due to their specific structure, fungal and plant laccases have not been successfully expressed in *E. coli*. This study found that the coexpression of sHSP20 could significantly increase the soluble expression of fungal laccase in *E. coli* and the enzymatic activity of expressed laccase. This might be because the presence of sHSP20 may promote the proper folding of proteins. The

Fig. 5 Effect of coexpression of sHSP20 on the soluble expression of fungal laccase in *E. coli* without codon optimization. **a** SDS-PAGE analysis of the proteins in *E. coli* recombined with different plasmids, lane (-), pETDuet-laccase; lane (+), pETDuet-sHSP20-laccase. **b** Activity of expressed laccases by the recombined *E. coli* with different plasmids. **c**

Activity of solely expressed laccase and coexpressed laccase with sHSP20 under different temperatures. Double asterisks and triple asterisks indicate the difference is significant at $P < 0.01$ and $P < 0.001$, respectively

new finding reported in this study is that the expression of sHSPs increased the enzymatic activity at high temperatures, indicating a new way for the application of sHSP.

In addition, the influence of high temperature on the overexpression of sHSP20 was focused in this study due to two reasons. Firstly, in our preliminary experiments, the overexpression of sHSP20 in *E. coli* resulted in significant promotion of heat resistance of the host cells, but not significant influence at low temperature. Secondly, the increase of thermostability and the activity of enzymes is normally more valuable at high temperatures which provides higher enzymatic reaction rate than low temperatures. Therefore, this study focused on the properties of sHSP20 under high temperatures, although it is overexpressed in *Oenococcus oeni* in response to low temperatures.

Conclusion

The study showed the expression of sHSP20 in *E. coli* BL21 could increase the microbial survival ratio under heat shocks. Presence of sHSP20 in the GDH reaction solution was helpful to increase the stability of the enzyme at high temperatures, low pH values, and high concentration of metal ions. More important, the coexpression of sHSP20 could enhance the soluble expression of fungal laccase in *E. coli* without codon optimization, as well as the activity and heat stability of the expressed enzyme. Overall, besides of the chaperone activity in the gene containing host in vivo, sHSP20 was also helpful for the enzyme heat stability in vitro. What's more significant, the study discovered the

capability of coexpression of sHSP20 to increase the efficiency of prokaryotic expression of fungal genes and the activity of expressed enzymes.

Author contributions Li Y performed most of the experiments and data analysis and wrote the manuscript. Xu X constructed the plasmids used in the study and performed several experiments. Qu R helped to do some experiments. Zhang G provided the genes in the study. Riaz rajoka MS helped to make some language revision. Shao D and Jiang C helped to arrange the experimental places and instruments. Shi J supported and designed the study and revised the article.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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