



# Identification and Characterization of the Small Heat Shock Protein Hsp20 from *Oenococcus oeni* SD-2a

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## Abstract

*Oenococcus oeni* can exert its function in hostile wine conditions during the malolactic fermentation process. Therefore, it is an important microbial resource for exploring resistance genes. Hsp20 is an important small heat shock protein from *O. oeni*. The conserved consensus motif “A-x-x-x-G-x-L” of Hsp20 announced its role as a member of the small heat shock protein family. The *hsp20* gene from *O. oeni* SD-2a was cloned to create the recombinant plasmid pTriEx-Hsp20. The recombinant plasmid was transformed into *Escherichia coli* BL21(DE3) competent cells, and the Hsp20 protein was induced by isopropyl-β-D-thiogalactoside (IPTG). The *hsp20* gene from *O. oeni* SD-2a was successfully expressed, and a 20-kDa fusion protein was identified by SDS-PAGE. The purified Hsp20 protein was obtained using Ni-affinity chromatography. Additionally, BL21(DE3)/Hsp20 and BL21(DE3)/Ctrl were treated at high temperatures of 42 and 52 °C, at pH values of 2.0–12.0, under oxidative shock with 0.1% (v/v) and 0.2% (v/v) H<sub>2</sub>O<sub>2</sub>, and under an osmotic shock of 430 and 860 mM NaCl to compare the effects of heterologous expression of the Hsp20 protein from *O. oeni* SD-2a for stress resistance. Notably, Hsp20 overexpression showed enhanced resistance than the control strain did when confronted with different elevated stress conditions. The results demonstrated heterologous expression of the *hsp20* gene from *O. oeni* SD-2a significantly improved the resistance of the host *E. coli* bacteria against stress conditions.

## Introduction

Malolactic fermentation (MLF) is an important biological process in making high-quality wine. The environmental conditions for MLF are highly unfavorable with low pH and high ethanol contents. Notably, *Oenococcus oeni*, an acidophilic and stress-tolerant bacterium, presents a predominance against atypical conditions therein when it is used as

a starter of MLF. The potential role of *O. oeni* is presumed to be dependent on the promotion of stress-related proteins.

Heat shock proteins are synthesized when organisms respond to environmental stress, function as molecular chaperones to safeguard proteins from denaturation and allow proteins to exert normal functions consistently. Heat shock proteins (Hsps) have generally been classified into five major families (Hsp100, Hsp90, Hsp70, Hsp60, and small Hsps (sHsps)) [1, 2]. sHsps are Hsps with molecular masses from 12 to 43 kDa, and the majority of these proteins are between 14 and 27 kDa [3].

Hsp20 belongs to the sHsps family, which possesses multiple biological functions. Hsp20 from *O. oeni* was previously discovered by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry (MS) analyses [4]. Although Hsp20 is expected to play a key role in protecting *O. oeni* from hostile environments, many fundamental questions concerning the exact function remain unanswered. Genetic tools for gene modification in *O. oeni* are deficient, and site-directed mutagenesis is presently not achievable because transformation efficiencies are lower than the frequency of recombination events [5, 6]. Nevertheless, *E. coli* is a universal host for heterologous

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protein production thanks to its well-characterized genome and a variety of mature tools available for genetic manipulation. Thus, the objective of the study was to dissect Hsp20 function from *O. oeni* SD-2a by the cloning, heterologous expression, purification, and survival analysis of strains overexpressing Hsp20 under a series of abiotic stresses, including heat, acidic, alkaline, oxidative, and osmotic stress conditions.

## Materials and Methods

### Bacteria Strains and Culture Medium

*O. oeni* SD-2a, which was isolated from Chinese wine [7], was used for *hsp20* gene isolation. *O. oeni* SD-2a was grown at 28 °C in acidic tomato broth (ATB) medium at pH 4.8. The ingredients of ATB are glucose (10 g/L), peptone (10 g/L), yeast extract (5 g/L), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.2 g/L), MnSO<sub>4</sub>·4H<sub>2</sub>O (0.05 g/L), Cysteine/HCl·H<sub>2</sub>O (0.5 g/L), and tomato juice (25%, v/v) [8]. *E. coli* DH5α and BL21(DE3), which was preserved in College of Life Sciences in Northwest A&F University, were grown in Luria–Bertani (LB) medium at 37 °C. LB medium is composed of tryptone (10 g/L), yeast extract (5 g/L), and NaCl (10 g/L) [9]. All the strains were stored at –80 °C in their proper broth supplemented with 25% (v/v) glycerol and recovered in LB broth prior to use.

### Bioinformatics Analyses of Hsp20 Sequence

Multiple alignments for similarity analysis were performed with the European Bioinformatics Institute (EBI) Clustal W [10] and visualized using Jalview [11]. “Conservation” and “Quality” are important alignment annotations. “Conservation” measures the amount of conservative physicochemical properties in each column. The higher score indicates that the column contains more amino acid substitutions of the same physicochemical class and columns with mutations where all properties are conserved are marked with a “+” (score of 10, indicating all properties are conserved). “Quality” measures the likelihood of observing the mutations in a column. A higher score indicates that there are few mutations or that most of the mutations observed are beneficial.

Multi-template modeling of Hsp20 was carried out using Modeller (<https://salilab.org/modeller/>). And the stability of the predicted protein model was evaluated sterically with PROCHECK [12].

### Cloning *hsp20* Gene from the *O. oeni* SD-2a Genome

The *O. oeni* genome was extracted using a Bacteria Genomic DNA Kit (TIANGEN, Beijing, China) according

to the manufacturer’s instructions. *Hsp20* was amplified using following primers: *hsp20*-His-F, 5'-tattttcagggatc-cgaattcATGGCAAATGAATTAATGGATAGAAA-3'; *hsp20*-His-R, 5'-atggtgatggtggtgctcgagTTGGATTTCATATGATGAGTTTGA-3'. PCR amplification was performed using the following protocol: initial denaturation at 98 °C for 3 min; 30 cycles of 98 °C for 10 s, 55 °C for 10 s, and 72 °C for 20 s; and final extension at 72 °C for 10 min. The band for the *hsp20* gene PCR product was detected by 1% agarose gel electrophoresis.

### Construction of the Recombination Cloning Vector

The target *hsp20* DNA fragment was purified using the TaKaRa Agarose Gel DNA Purification Kit Ver. 2.0 and inserted into the vector pTriEx1.1 digested with *EcoRI* and *XhoI* at 37 °C for 4 h. Using the ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China), homologous recombination was carried out. Consequently, the recombinant product was transformed into *E. coli* DH5α competent cells as described previously [13]. Transformants were selected on LB plates containing ampicillin (100 mg/mL), and individual clones were selected for plasmid extraction, enzyme digestion, and examination by 1% agarose gel electrophoresis.

### Recombinant Protein Expression and Solubility Detection of Hsp20

The recombinant pTriEx-Hsp20 plasmid was transformed into *E. coli* BL21(DE3) competent cells [14]. BL21(DE3)/pTriEx-Hsp20 individual clones were picked out and cultivated in liquid LB medium overnight at 37 °C with 220 rpm shaking and then transferred and inoculated in the fresh medium culture at 37 °C. When the OD<sub>600 nm</sub> of broth reached 0.8–1.0, isopropyl-β-D-thiogalactoside (IPTG) was added at a final concentration of 1 mM [15]. After 4 h of induction, strains were collected by centrifugation at 12,000 rpm for 10 min at 4 °C. The cell pellets were resuspended in 10 ml PBS (pH 7) and sonicated in an ice bath for 15 min. Subsequently, the sample was centrifuged at 12,000 rpm for 10 min. A sample of the intracellular soluble protein was taken from the supernatant. And a sample of the intracellular insoluble protein was taken by resuspending the remaining cell pellet in PBS (pH 7). In addition, cells that were not induced were collected after centrifugation, and the control group strain was resuspended with sterile ultrapure water. All the above-mentioned samples were mixed with an equal volume of 2× SDS loading buffer and analyzed by SDS-PAGE.

## Purification of Hsp20

The His-tagged recombinant proteins were purified using Ni-affinity chromatography [16]. The column was initially washed with buffer containing 10 mM imidazole to remove other proteins. Then, Hsp20 was gradually eluted with 250 mM imidazole. Afterwards, the obtained protein samples were examined by SDS-PAGE analysis.

## Comparative Growth Curve of *E. coli* at High and Optimum Temperatures

The pTriEx-Hsp20 recombinant plasmid and the empty vector were used to transform into *E. coli* BL21(DE3) competent cells and named “BL21(DE3)/Hsp20” and “BL21(DE3)/Ctrl,” respectively. Then, the two strains were induced with IPTG as described above and inoculated into LB liquid medium at 37 °C with 220 rpm shaking. Samples were taken at intervals of 1 h and measured at a wavelength of 600 nm. The data were collected to create a strain growth curve.

## Determination of Stress Resistance

### Bacterial Culture Preparation for Stress Treatment

Strains of BL21(DE3)/Hsp20 and BL21(DE3)/Ctrl were induced with IPTG as previously elucidated. The bacterial products were obtained for exposure to a series of environmental stressors, including heat, acidic, alkaline, oxidative, and osmotic stress [17, 18].

### Heat Shock

Aliquots of the bacterial cultures (BL21(DE3)/Hsp20, BL21(DE3)/Ctrl) were chosen to assess the thermal tolerance by thermostatic water baths at different temperatures (42 °C, 52 °C) for different lengths of times (30 min, 60 min). Bacteria strains that were cultured at 37 °C without heat shock were used as the control group. To achieve a comparable reduction of cell counts, the temperatures of 42 and 52 °C were chosen. Samples were collected by centrifugation after treatments and diluted with saline. Cultivable cells were counted on LB agar containing 100 mg/mL ampicillin.

### Acidic and Alkaline Shock

The induced bacteria cells were harvested by centrifugation. The pellets were used to assess acidic and alkaline tolerance, which were resuspended and mixed thoroughly in LB broth with altered pH ranges. The acidic and alkaline LB media were adjusted to pH values ranging from 2 to 12 with HCl and NaOH. The samples were incubated at 37 °C.

Samples were collected by centrifugation after 1-h intervals and diluted with saline. Cultivable cells were counted on LB agar containing 100 mg/mL ampicillin.

### Oxidative Shock

The strains treated with IPTG induction were used to estimate oxidative tolerance. Aliquots of the cells were pipetted, and a final concentration of 0.1% (v/v) or 0.2% (v/v) H<sub>2</sub>O<sub>2</sub> was added to the cell suspension and incubated at 37 °C with shaking for 5 or 10 min. Samples were collected by centrifugation after treatments and diluted with saline. Cultivable cells were counted on LB agar containing 100 mg/mL ampicillin.

### Osmotic Shock

The concentration of NaCl in LB medium is 172 mM. Therefore, the NaCl concentration was increased by 2.5 times to 430 mM and 5 times to 860 mM in this study to carry out osmotic tolerance. The osmotic tolerance test was performed in cells after IPTG induction. Aliquots of the bacterial cultures were gathered by centrifugation. Subsequently, the supernatants were discarded, and the strains were resuspended in LB medium containing NaCl additives with final concentrations of 172 mM, 430 mM, and 860 mM. These cultures were then incubated at 37 °C with shaking. Samples were collected by centrifugation after 12 h and diluted with saline. Cultivable cells were counted on LB agar containing 100 mg/mL ampicillin.

## Statistical analysis

Data analysis was conducted using statistical software (GraphPad Prism 6.0). *E. coli* strains had significant differences in their resistances to stress, and these differences were determined by using t-test. Significance was assessed at a significance level of  $P < 0.05$ .

## Results

### Sequence Alignment and Protein Homology Modeling of Hsp20

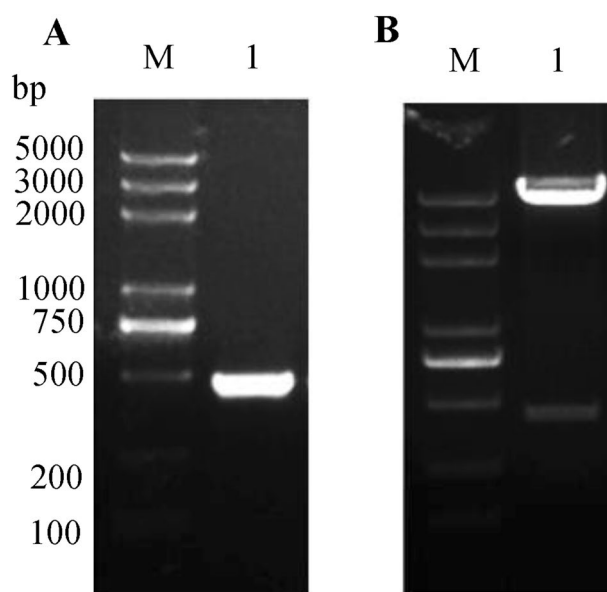
As is shown in Fig. S1, Hsp20 sequences were composed of a complete  $\alpha$ -crystallin domain flanked by a variable N-terminal region and a short C-terminal extension. Compared with N-terminal and C-terminal compositions, the crystalline domain located at amino acid residue sites M44–K136 was more conserved. Hsp20 of *O.oeni* SD-2a indicated moderate similarity among the  $\alpha$ -crystallin domains with other prokaryotes. And, 15 residues (corresponding to 47D, 50E,

61L, 62P, 63G, 92D, 108S, 109R, 114P, 123A, 125Y, 128G, 130L, 135P, and 136 K) were presented entirely conserved throughout all the strains.

The criteria for selecting template for a protein are sequence homology and integrity of amino acid residues as well as accuracy of the template protein structure. If the sequence identity of the two comparable amino acid sequences is higher than 30%, then they are proposed to have a similar three-dimensional structure of the proteins [19]. What's more, amino acid residues deficiency in the template should affect the modeling of its corresponding region of the target protein; thus, complete amino acid residues are preferred for modeling. Additionally, precision of the crystal structure of the template protein is preferably below 2.5 Å. Based on the considerations described above, Hsp20 protein sequence was used as a query and blast search was performed against the Protein Data Bank proteins (pdb) database. Possible protein templates from the alignment results with identity > 30% were selected out and subjected to Modeller for multi-template modeling and model optimizing. Secondary structure prediction of Hsp20 protein was carried out by PredictProtein, showing that the sequence composed 10.14% of helix (alpha spiral), 29.73% of strand (beta sheet), and 60.14% of loop (random coils). As is shown in Fig. S2A, the composition of protein structure of *O. oeni* Hsp20 was in accord with the prediction results of secondary structure of protein. And the Ramachandran plot shown in Fig. S2B also verified that the predicted protein conformation was reasonable. The phi/psi angles of 93.2% residues fell in the most favored regions, and 6.8% residues lied in the additional allowed regions. Ramachandran plot provided by the program PROCHECK assured very good confidence for the predicted protein.

### Cloning, Expression, and Purification of Hsp20

Total genomic DNA from *Oenococcus oeni* SD-2a was used for PCR amplification of the *hsp20* gene, and the evident PCR amplicon was observed at the expected length (447 bp), suggesting that the *hsp20* gene was efficiently amplified (Fig. 1a). The pTriEx-Hsp20 plasmid was extracted from *E. coli* DH5 $\alpha$  competent cells and confirmed by restriction enzyme digestion analysis. The *E. coli* DH5 $\alpha$  transformant in lane 1 was suggested to be correct (Fig. 1b). The plasmid pTriEx-Hsp20 was then transformed into *E. coli* BL21(DE3) competent cells, the strain was induced with IPTG, and the results of Hsp20 protein expression were shown in Fig. 2. The cells, the culture supernatants of the cells, the soluble fraction of the cytoplasm (ultrasonic supernatant), and the insoluble components (ultrasonic precipitation section) were processed and analyzed by SDS-PAGE. A significant electrophoretic band appeared at 18.4 kDa. Additionally, comparing bands 3 and 4, the majority of the target protein



**Fig. 1** Cloning of the *hsp20* gene. DL2000 DNA was used as the marker. **a** Product analysis of *hsp20*. Lane 1 showed that *hsp20* was amplified from the genomic DNA of *O. oeni* SD-2a. **b** Digest identification of pTriEx-Hsp20. Lane 1 showed that the recombinant plasmid was extracted from positive clones of *E. coli* DH5 $\alpha$  cells

existed in the supernatant, suggesting that the target Hsp20 protein existed in a soluble state. Moreover, Hsp20 was obtained with a high purity after purification by Ni-affinity chromatography (Fig. 3).

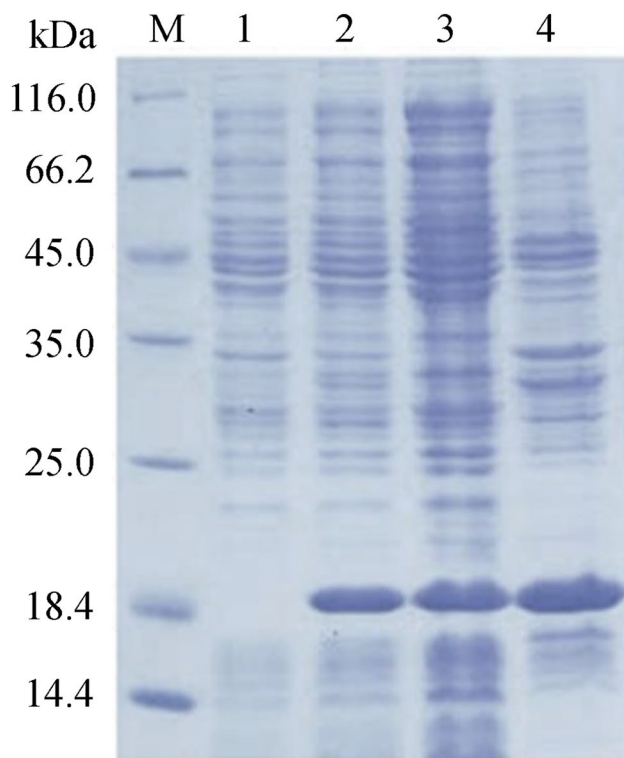
### Effect of Hsp20 Expression on the Growth of *E. coli* Cells

The growth status of recombinant *E. coli* with Hsp20 was more vigorous and robust than that of the control strain throughout the whole expression period. Compared with strain performance at 37 °C, both Hsp20 recombinant *E. coli* and control *E. coli* showed a decrease in bacterial number and growth rate and a longer lag phase under thermal condition. The longer lag phase time of the strains indicated that strains after shock might need more time to prepare for breeding. In terms of the performance of these two types of strains, a large discrepancy was reflected when the logarithmic phase started. These results could be attributed to the control strain being more sensitive to high temperature and suffered at 52 °C (Fig. 4).

### Stress Resistance of Hsp20 Recombinant *E. coli* Cells

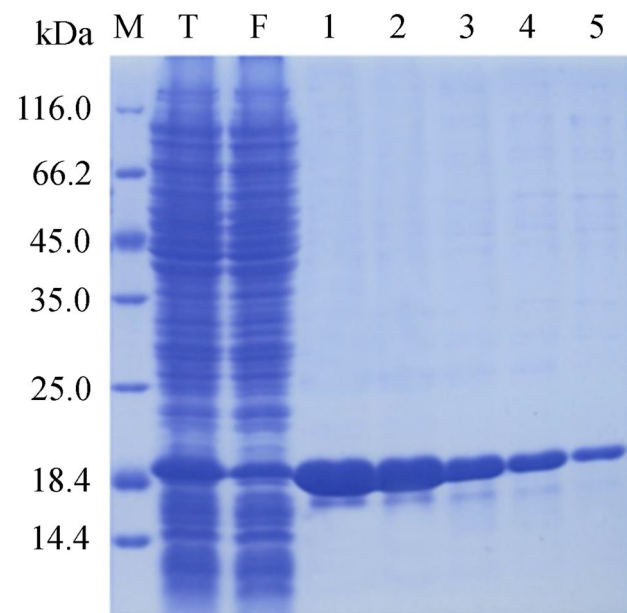
Strains cultured at 42 °C had fewer viable bacteria than strains cultured at optimum 37 °C. When the temperature rose to 52 °C, it was very difficult for both of the strains to survive and grow (Fig. 5a). Generally, the Hsp20





**Fig. 2** SDS-PAGE analysis of the solubility of Hsp20 expressed in *E. coli* BL21(DE3) competent cells. Unstained protein molecular weight marker was used. Lane 1, protein expression levels without inducing the bacteria. Lane 2, protein expression after inducing the bacteria. Lane 3, supernatant of the sonicated bacterial cells after induction. Lane 4, precipitate of the sonicated bacterial cells after induction

recombinant *E. coli* was less affected by thermal treatment than the control bacteria. Although the counts of the strains were tested in a wide range of pH values from 2 to 11, the bacterial strains could survive only in the pH range of 3–9 (Fig. 5b). Extreme acidic or alkaline environments were lethal for these strains. The recombinant strains containing Hsp20 performed better than the control bacteria when challenged under acidic and alkaline stress conditions, and the advantages of the Hsp20 expression strain were more obvious in stronger acidic and alkaline conditions. The effect of Hsp20 expression in *E. coli* host bacteria on its antioxidant capacity and osmotic tolerance was assessed with  $H_2O_2$  and NaCl, respectively. As seen in Fig. 5c, d, both bacteria showed a decrease in viable counts under  $H_2O_2$  and high NaCl conditions.  $H_2O_2$  shock was a large stress condition for the control *E. coli*, and the strain counts dramatically decreased even at a relatively slight shock of 0.1%  $H_2O_2$  for 5 min. Furthermore, the control strain was not present under the treatment of 0.2%  $H_2O_2$  for 10 min (Fig. 5c). Additionally, the number of Hsp20 recombinant strain counts was approximately twice that of the control strain when the NaCl concentration was 430 mM in LB medium. When the NaCl



**Fig. 3** SDS-PAGE analysis of the Hsp20 fusion protein purification. *M* protein molecular weight standard; *T* total protein; *F* flow-through from Ni-affinity chromatography; Lanes 1–5: 250 mM imidazole elution from Ni-affinity chromatography

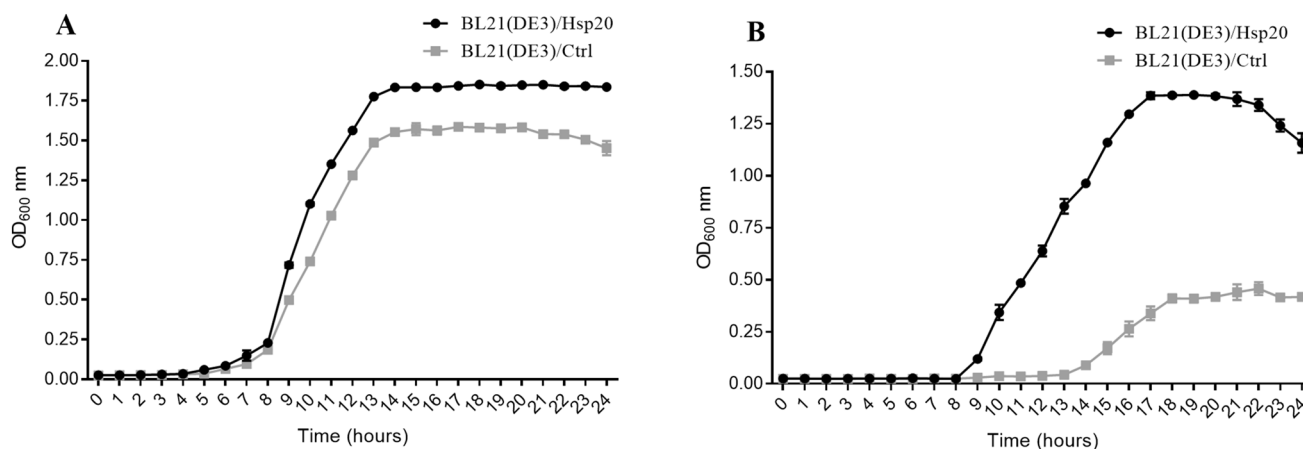
concentration was elevated to 860 mM, NaCl had a strong inhibitory effect on both strains, especially for the control strain (Fig. 5d).

## Discussion

Bioinformatics analysis of Hsp20 showed that the consensus motif A-x-x-x-G-x-L is conserved (Fig. S1). Moreover, the consensus motif A-x-x-x-G-x-L was observed to locate at the C-terminal end of the  $\alpha$ -crystallin domain, which is the most significant indicator of the small heat shock protein family [20, 21].

An analysis of bacterial growth kinetics showed that the behavior of the culture characteristics of the strains was affected by both Hsp20 expression and increased temperature. The recombinant Hsp20 *E. coli* grew better than the control strain, possibly because the expression of Hsp20 conferred the capability of the strains to withstand adverse stress conditions. Above all, the growth adaptability of recombinant Hsp20 *E. coli* to high temperatures was significantly higher than that of the control *E. coli* (Fig. 4).

To examine whether the Hsp20 recombinant *E. coli* performed better than the control strain under extreme conditions, these two types of strains were subject to a series of adverse treatments. Temporary high-temperature heat shock can significantly reduce the number of bacterial cells in these strains (Fig. 5a). The sensitive performance of both



**Fig. 4** Comparative growth curves of recombinant *E. coli* at optimum and thermal temperatures. **a** Growth performances of *E. coli* BL21(DE3)/Ctrl (gray squares) and BL21(DE3)/Hsp20 (black cir-

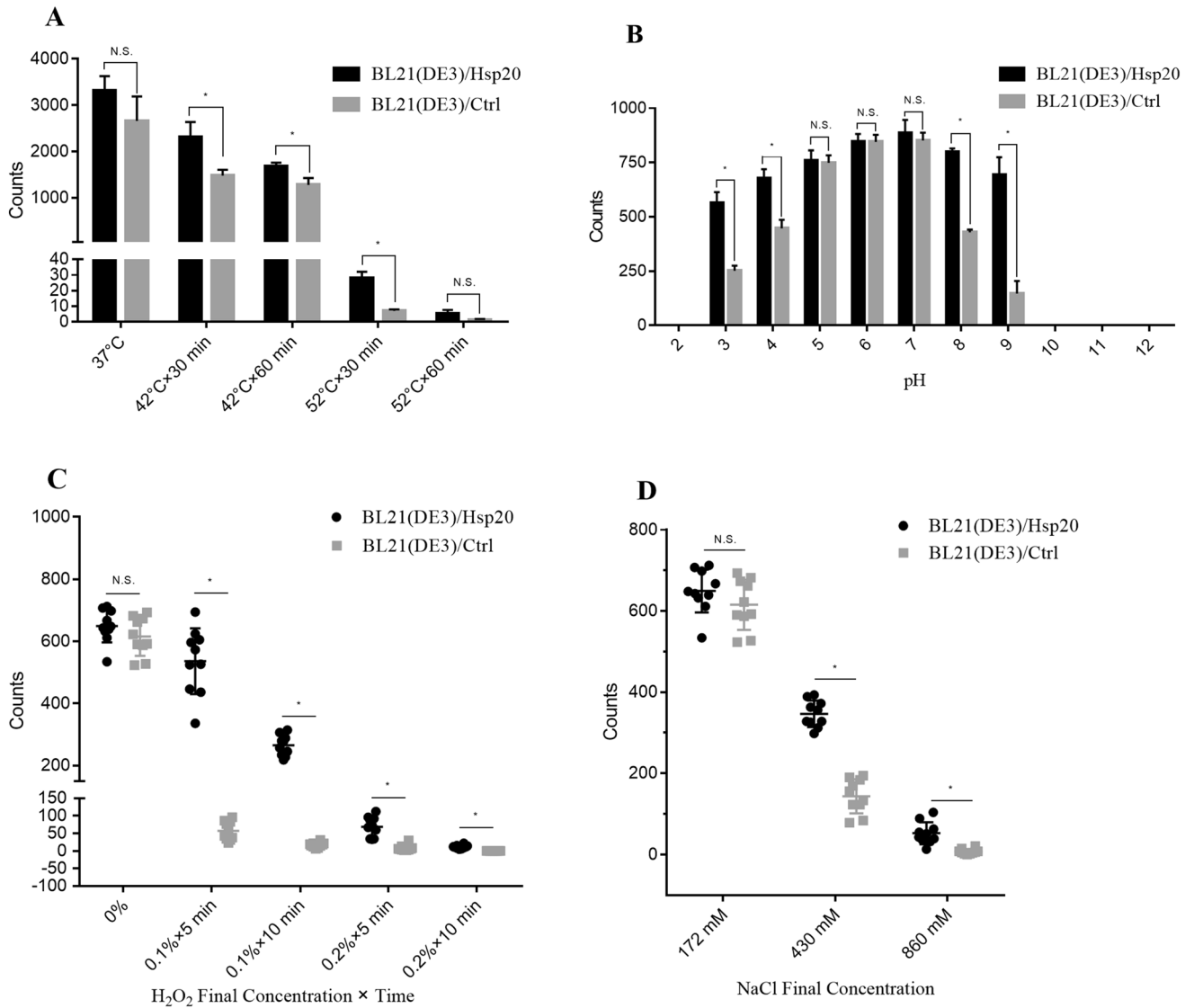
cles) under optimum growth temperature 37 °C. **b** Growth performances of BL21(DE3)/Ctrl (gray squares) and BL21(DE3)/Hsp20 (black circles) under a thermal temperature of 52 °C

strains revealed greater selectivity for viable counts due to heat shock. Moreover, when the exposure time was longer, or the temperature was higher, there were fewer viable bacteria. At high temperatures, the performance of these two strains was similar to previously studied strains, revealing that the thermotolerance of *E. coli* could be enhanced by the expression of various heterologous Hsps [22–25]. Hsp20 expression was found to be helpful for recombinant *E. coli* to resist both acidic and alkaline conditions in this study (Fig. 5b). Nevertheless, wine conditions often involve pH levels between 3.0 and 3.5, and mild acidic shock at an intermediate pH of 4.8 is known to induce an adaptive response during the shock [26]. Thus, Hsp20 might also participate in the mechanism of pH homeostasis of *O. oeni* to harsh wine conditions.

Moreover, Hsp20 from *O. oeni* could be important in explaining the endurance for cells to resist oxidation and osmotic pressures. Heterologous expression levels of Hsp20 in the recombinant strain were compared with the control strain after treatment with H<sub>2</sub>O<sub>2</sub> and NaCl. Intriguingly, in terms of the Hsp20 recombinant *E. coli*, the performance of each sample was quite different in the ten sets of experiments under 0.1% × 5 min H<sub>2</sub>O<sub>2</sub> stress. Therefore, the collected data of the strain counts were highly dispersed. However, with prolonged treatment time, under 0.1% × 10 min H<sub>2</sub>O<sub>2</sub> stress, the number of cells was significantly reduced (Fig. 5c). The analogous phenomenon also appeared in the data of the control strain. However, for the control strain,

this phenomenon was observed only if H<sub>2</sub>O<sub>2</sub> was added, which resulted in a significant and concentrated decrease in the cell number. Therefore, it was plausible that a state in which each Hsp20 recombinant strain battled with H<sub>2</sub>O<sub>2</sub> stress consequently led to individual differences. Broadly, when the H<sub>2</sub>O<sub>2</sub> concentration was higher and the treatment time was longer, the strains had a higher mortality rate form. Under the condition of 172 mM NaCl (the NaCl concentration of LB medium itself), the growth of the two bacteria was consistent with the number of colonies without the H<sub>2</sub>O<sub>2</sub> stress added. This result also proved that the condition with no stressor was optimum for bacteria. A high concentration of NaCl led to a central decline in the number of strain cells, which were at a faster rate and apparent in the control strain (Fig. 5d).

Above all, the expression of Hsp20 in *E. coli* BL21(DE3) competent cells improved the microbial survival both in the growth curve supervision under thermal conditions and stress tolerance examination under versatile stress conditions. Hsp20 is an important small heat shock protein in the wine-associated species *O. oeni*. There are various perplexing and adverse conditions that *O. oeni* may be encountered during MLF or wine storage. Although an efficient expression vector in *O. oeni* is difficult to obtain, the role that Hsp20 plays in *O. oeni* still needs to be investigated. Therefore, a useful overexpression construct or a gene knockdown preparation of the small shock protein in *O. oeni* is necessary in the future to clearly validate the action of Hsp20.



**Fig. 5** Resistance of *E. coli* strains to a series of adverse stresses. Black bars and marks indicate strains carrying the *hsp20* gene; gray bars indicate control strains. Asterisks indicate significant differences between the two types of strains (\* $P < 0.05$ ). The dilution gradient of the bacterial solution was  $10^{-5}$ . **a** Heat shock under different tempera-

tures (42 °C, 52 °C) for different lengths of times (30 min, 60 min); **b** pH gradient from acidic shock to alkaline shock; **c** Oxidative shock with a final concentration of 0.1% (v/v) or 0.2% (v/v)  $H_2O_2$  for 5 min or 10 min, respectively; **d** Osmotic shock with NaCl additives with a final concentration of 172 mM, 430 mM, and 860 mM

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**References**

- Guzzo J (2012) Biotechnical applications of small heat shock proteins from bacteria. *Int J Biochem Cell Biol* 44(10):1698–1705. <https://doi.org/10.1016/j.biocel.2012.06.007>

- Horváth I, Glatz A, Nakamoto H, Mishkind ML, Munnik T, Saidi Y, Goloubinoff P, Harwood JL, Vigh L (2012) Heat shock response in photosynthetic organisms: membrane and lipid connections. *Prog Lipid Res* 51(3):208–220. <https://doi.org/10.1016/j.plipres.2012.02.002>
- Narberhaus F (2002)  $\alpha$ -Crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. *Microbiol Mol Biol Rev* 66(1):64–93. <https://doi.org/10.1128/MMBR.66.1.64-93.2002>
- Cecconi D, Milli A, Rinalducci S, Zolla L, Zapparoli G (2010) Proteomic analysis of *Oenococcus oeni* freeze-dried culture to assess the importance of cell acclimation to conduct malolactic fermentation in wine. *Electrophoresis* 30(17):2988–2995. <https://doi.org/10.1002/elps.200900228>

5. Dicks LMT (1994) Transformation of *Leuconostoc oenos* by electroporation. *Biotechnol Tech* 8(12):901–904. <https://doi.org/10.1007/BF02447736>
6. Assad-García JS, Bonnin-Jusserand M, Garmyn D, Guzzo J, Alexandre H, Grandvalet C (2010) An improved protocol for electroporation of *Oenococcus oeni* ATCC BAA-1163 using ethanol as immediate membrane fluidizing agent. *Lett Appl Microbiol* 47(4):333–338. <https://doi.org/10.1111/j.1472-765X.2008.02435.x>
7. Li H, Zhang CH, Liu YL (2006) Species attribution and distinguishing strains of *Oenococcus oeni* isolated from Chinese wines. *World J Microbiol Biotechnol* 22(5):515–518. <https://doi.org/10.1007/s11274-005-9065-5>
8. Li H, Zhao WY, Wang H, Li ZC, Wang AL (2009) Influence of culture pH on freeze-drying viability of *Oenococcus oeni* and its relationship with fatty acid composition. *Food Bioprod Process* 87(C1):56–61. <https://doi.org/10.1016/j.fbp.2008.06.001>
9. Giuseppe B (2004) Lysogeny at mid-twentieth century: P1, P2, and other experimental systems. *J Bacteriol* 186(3):595–600. <https://doi.org/10.1128/JB.186.3.595-600.2004>
10. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and clustal X version 2.0. *Bioinformatics* 23(21):2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
11. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 25(9):1189. <https://doi.org/10.1093/bioinformatics/btp033>
12. Laskowski RA, Macarthur MW, Moss DS, Thornton JMJ (1993) PROCHECK: a program to check the stereochemical quality of protein structures. *J Appl Crystallogr* 26(2):283–291. <https://doi.org/10.1107/S0021889892009944>
13. Russell DW, Sambrook J (2001) *Molecular cloning: a laboratory manual*. Cold Spring Harbor. <https://doi.org/10.2307/1309366>
14. Chou CP (2007) Engineering cell physiology to enhance recombinant protein production in *Escherichia coli*. *Appl Microbiol Biotechnol* 76(3):521–532. <https://doi.org/10.1007/s00253-007-1039-0>
15. Hansen LH, Knudsen S, Sorensen SJ (1998) The effect of the *lacY* gene on the induction of IPTG inducible promoters, studied in *Escherichia coli* and *Pseudomonas fluorescens*. *Curr Microbiol* 36(6):341–347. <https://doi.org/10.1007/s002849900320>
16. Markwell J (2010) *Fundamental laboratory approaches for biochemistry and biotechnology*, 2nd edition. *Biochem Mol Biol Educ* 37(5):317–318. <https://doi.org/10.1002/bmb.20321>
17. Weidmann S, Maitre M, Laurent J, Coucheney F, Rieu A, Guzzo J (2016) Production of the small heat shock protein Lo18 from *Oenococcus oeni* in *Lactococcus lactis* improves its stress tolerance. *Int J Food Microbiol* 247:18–23. <https://doi.org/10.1016/j.ijfoodmicro.2016.06.005>
18. D'Angelo L, Cicotello J, Zago M, Guglielmotti D, Quiberoni A, Suarez V (2017) *Leuconostoc* strains isolated from dairy products: response against food stress conditions. *Food Microbiol* 66:28–39. <https://doi.org/10.1016/j.fm.2017.04.001>
19. Rodriguez R, Chinae G, Lopez N, Pons T, Vriend G (1998) Homology modeling, model and software evaluation: three related resources. *Bioinformatics* 14(6):523–528. <https://doi.org/10.1093/bioinformatics/14.6.523>
20. Caspers GJ, Leunissen JAM, Jong WW (1995) The expanding small heat-shock protein family, and structure predictions of the conserved “ $\alpha$ -crystallin domain”. *J Mol Evol* 40(3):238–248. <https://doi.org/10.1007/bf00163229>
21. Delmas F, Pierre F, Coucheney F, Divies C, Guzzo J (2001) Biochemical and physiological studies of the small heat shock protein Lo18 from the lactic acid bacterium *Oenococcus oeni*. *J Mol Microbiol Biotechnol* 3(4):601–610. <https://doi.org/10.1038/sj.jim.7000196>
22. Ronez F, Desroche N, Arbault P, Guzzo J (2012) Co-expression of the small heat shock protein, Lo18, with  $\beta$ -glucosidase in *Escherichia coli* improves solubilization and reveals various associations with overproduced heterologous protein, GroEL/ES. *Biotechnol Lett* 34(5):935–939. <https://doi.org/10.1007/s10529-012-0854-2>
23. Ezemaduka AN, Jiayu Y, Xiaodong S, Kaiming Z, Chang-Cheng Y, Xinmiao F, Zengyi C (2014) A small heat shock protein enables *Escherichia coli* to grow at a lethal temperature of 50 °C conceivably by maintaining cell envelope integrity. *J Bacteriol* 196(11):2004–2011. <https://doi.org/10.1128/JB.01473-14>
24. Singh H, Appukuttan D, Lim S (2014) Hsp20, a small heat shock protein of *Deinococcus radiodurans*, confers tolerance to hydrogen peroxide in *Escherichia coli*. *J Microbiol Biotechnol* 24(8):1118–1122. <https://doi.org/10.4014/jmb.1403.03006>
25. Li Y, Xu X, Qu R, Zhang G, Rajoka MSR, Shao D, Jiang C, Shi J (2018) Heterologous expression of *Oenococcus oeni* sHSP20 confers temperature stress tolerance in *Escherichia coli*. *Cell Stress Chaperones* 23(4):1–10. <https://doi.org/10.1007/s12192-018-0874-5>
26. Liu L, Zhao H, Peng S, Wang T, Su J, Liang Y, Li H, Wang H (2017) Transcriptomic analysis of *Oenococcus oeni* SD-2a response to acid shock by RNA-Seq. *Front Microbiol* 8:1586. <https://doi.org/10.3389/fmicb.2017.01586>

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