Curr Microbiol (2013) 67:601–608
DOI 10.1007/s00284-013-0409-3

Response of Heat-Shock Protein (*HSP*) Genes to Temperature and Salinity Stress in the Antarctic Psychrotrophic Bacterium *Psychrobacter* sp. G

Shuai Che · Weizhi Song · Xuezheng Lin

Received: 16 December 2012 / Accepted: 22 May 2013 / Published online: 20 June 2013

Abstract Temperature and salinity fluctuations are two of the most important factors affecting the growth of polar bacteria. In an attempt to better understand the function of heat-shock proteins (HSPs) in the adaptive mechanisms of the Antarctic psychrotrophic bacterium *Psychrobacter* sp. G to such conditions, genes *Hsp*845, *Hsp*2538, *Hsp*2666, and *Hsp*2667 were cloned on the basis of the draft genome. The expression characteristics of these HSP genes under different stress conditions were analyzed by the qRT-PCR method. Expression of *Hsp*845 and *Hsp*2667 was inhibited significantly by low temperature (0 and 10 °C, respectively). There was no difference of expression when *Hsp*2538 and *Hsp*2666 were exposed to 0 °C but the expression of *Hsp*2666 was inhibited when exposed to 10 °C. Expression of *Hsp*2538 and *Hsp*2667 was not sensitive but expression of *Hsp*845 and *Hsp*2666 was increased at low salinity (0 and 15, respectively). Expression of the four HSP genes was enhanced at high salinity (90 and 120) and at high temperature independent of salinity. By contrast, low temperature had no significant effect independent of salinity.

S. Che (✉) · W. Song · X. Lin
The First Institute of Oceanography, SOA, Qingdao 266061, China
e-mail: roy5513@163.com

X. Lin
e-mail: linxz@fio.org.cn

S. Che · W. Song · X. Lin
Key Lab of Marine Bioactive Substances, SOA, Qingdao 266061, China

Introduction

Antarctic ecosystems are characterized by extreme cold and poor nutrient availability. The ice in the Southern Ocean is dominated by strong gradients of salinity and drastic fluctuations of temperature, space, and light [23]. Temperature and salinity are two of the most important factors affecting polar bacteria, which have evolved mechanisms ranging from the molecular level to the whole cell level to survive under these conditions [19, 23]. The Antarctic bacterium *Psychrobacter* sp. G was extracted from a surface (depth 0–20 cm) sample of seawater off southwestern King George Island, one of the South Shetland Islands, and maintained in our laboratory.

Systems for the synthesis of proteins have a vital role in the acclimation process [15]. Many organisms, including bacteria, respond to a sudden temperature increase by the synthesis of heat-shock proteins (HSPs) [6]. HSPs exist in a wide range of organisms and are highly conserved. HSPs are classified on the basis of molecular mass, amino acid sequence and function into seven families; HSP110/HSP100, HSP90, HSP70, HSP60, HSP40, small HSPs (sHSP), and HSP10 [5]. As molecular chaperones, HSPs interact with other proteins and minimize the probability that these other proteins will interact inappropriately with one another. HSPs can recognize and bind to other proteins that are in non-native conformations because of protein-denaturing stress or because the constituent peptides have been localized to an inappropriate cellular compartment or have not been fully synthesized, folded or assembled [3]. Although several HSPs are generally induced by heat stress, earlier studies revealed that other sourced of stress, such as salinity [8], UV-B light [15], changes in pH or the presence of ethanol, could induce a heat-shock response [7].

Although heat-shock response is well documented at the molecular level in mesophilic microorganisms, including *Escherichia coli* [17], *Chaetomium brasiliense* [18], and *Lactobacillus plantarum* [21], relatively few studies have been done with cold-adapted microorganisms. Moreover, most of the earlier studies focused on the response to only one stress factor, such as temperature. In fact, organisms in nature seldom undergo only one source of stress at a time [3]. The mechanism of HSPs in response to salinity and combinations of multiple stresses are not well understood. We obtained four HSP genes, *Hsp*845, *Hsp*2538, *Hsp*2666, and *Hsp*2667 on the basis of the draft genome of *Psychrobacter* sp. G (unpublished data). The expression characteristics of the four genes under different stress conditions, (temperature, salinity and combinations of temperature and salinity) were investigated by real time quantitative PCR (qRT-PCR).

Materials and Methods

Bacteria, Medium and Growth Conditions

The Antarctic psychrotrophic bacterium *Psychrobacter* sp. G was isolated from seawater and maintained in our laboratory [13]. *Escherichia coli* DH5α (D9057, TaKaRa, Dalian, China) and vector pMD18-T (D101A, TaKaRa, Dalian, China) were used in gene cloning.

Psychrobacter sp. G was cultured at 20 °C with rotation at 150 rpm [20] in medium consisting of 5 g of tryptone, 1 g of yeast extract, and 14 g of NaCl in 1 L of seawater [20]. The composition of the medium used for salinity stress treatment was as described [20]. Seawater (salinity ~31) was taken from the coast at Qingdao (Shandong, China).

Cloning of the Four HSP Genes

The genomic DNA of *Psychrobacter* sp. G was extracted using a Genomic DNA Prep Kit (DP302, Tiangen Biotech, Beijing, China) following the manufacturer's instructions. To obtain the full sequences of the four HSP genes (Table 1), the draft genome of *Psychrobacter* sp. G was used to design primers by the Primer Premier 5.0 program. The four gene fragments were amplified by PCR programed for 35 cycles (*Hsp*845, 95 °C for 1 min, 42 °C for 30 s and 72 °C for 1 min; *Hsp*2538, 95 °C for 1 min, 42 °C for 30 s and 72 °C for 40 s; *Hsp*2666, 95 °C for 1 min, 42 °C for 30 s and 72 °C for 30 s; *Hsp*2667, 95 °C for 1 min, 45 °C for 30 s and 72 °C for 2 min). The fragments were excised, purified with an agarose gel DNA fragment recovery kit (DP209-2, Tiangen Biotech, Beijing, China), and subcloned into vector pMD-18T and sequenced (Sunny Biotechnology, Shanghai, China).

The nucleotide sequences were compared and aligned with the nucleotide sequence database in Genbank (http://www.ncbi.nlm.nih.gov) using the basic local alignment search tool (BLAST). The theoretical isoelectric point (p*I*) and molecular mass of the deduced amino acid sequences were analyzed with the Expert Protein Analysis System (http://www.expasy.org/).

The nucleotide and deduced amino acid sequences of genes *Hsp*845, *Hsp*2538, *Hsp*2666, and *Hsp*2667 were submitted to GenBank with accession numbers JQ806357, JQ806358, JQ806359, and JQ806360, respectively.

The phylogenetic tree was constructed using the program Mega 4.0 [11], and the reliability of the tree was analyzed using bootstrap probabilities.

Stress Treatment

Psychrobacter sp. G was initially cultured at its optimal growth temperature (20 °C) and salinity (45). When the absorbance of the culture at 600 nm (A_{600}) reached 0.5, the stress treatments were applied as follows. (1) Temperature stress: Cultures were kept at different temperatures (0, 10, 30 °C) for different lengths of time (2, 6, 12 h). (2) Salinity stress: After the temperature stress, cultures were centrifuged at 8,000×*g* for 5 min at 20 °C. The pellets were suspended thoroughly with the same volume of medium to a final salinity of 0, 15, 90, or 120 and then kept at 20 °C for 2, 6, or 12 h, respectively. (3) Combined stress: Cells were cultured under the following conditions: a final salinity of 15 at 0 or 30 °C and a final salinity of 90 at 0 or 30 °C, respectively. A strain cultured continuously under optimal conditions was used as the control for qRT-PCR analysis.

Selection of Reference Genes

Genes encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH), methyltransferase (MT), uridine monophosphate synthetase (RU) or 16S rRNA were used as the reference

Table 1 Primers used to obtain the full sequences of the four HSP genes

Gene	PCR primers (5′–3′)	
*Hsp*845	F: TGACGCTATTAAATTATGAC	R: CCTACTTACTACTGACCGA
*Hsp*2538	F: TATGAGGTTTTGATGAGTAA	R: TTAAGATGAGGCGATATT
*Hsp*2666	F: TTTTGAAGATAGGATGTT	R: GTATGAGAATGCAATTAA
*Hsp*2667	F: GGAGTAATTTAACATGGCA	R: AATCGCTTTGTAGCAGAA

housekeeping genes [1, 2]. Primers of the candidate reference genes were designed by the Primer Premier 5.0 program and are given in Table 2. Total RNA was extracted using the RNAiso Plus Kit (D9108D, TaKaRa, Dalian, China) according to the manufacturer's manual. The purity of prepared RNA was judged by the A_{260}/A_{280} ratio. The PrimeScript RT Reagent Kit (DRR037A, TaKaRa, Dalian, China) was used for the reverse transcription of RNA into cDNA. The cDNA for qRT-PCR was prepared using 500 ng of total RNA with the primers given in Table 2. The average expression stability of the four candidate reference genes under different temperature and salinity stresses was analyzed by Norm Finder and the gene with the minimum average expression stability value was selected as the Ref. [26].

Expression Analysis of the Four HSP Genes

The SYBR PrimeScriptTM RT-PCR Kit (DRR041A, TaKaRa, Dalian, China) was used for qRT-PCR analysis using primers targeted against sequences of the four HSP genes. The GAPDH, MT, RU, and 16S rRNA genes of *Psychrobacter* sp. G are given in Table 2. qRT-PCR was done with the Stratagene Mx3000P qPCR System for 40 cycles (95 °C for 1 min, 45 °C for 30 s and 72 °C for 20 s). All reactions were done in triplicate. Quantification of mRNA was based on C_t (threshold cycle) values. The C_t values of the four HSP genes were normalized using the C_t value corresponding to the selected reference gene and the efficiency of each qRT-PCR was calculated. Data analysis was done with the comparative C_t ($2^{-\Delta\Delta Ct}$) method [16]. Data obtained from qRT-PCR analysis were subjected to analysis of variance to identify significant differences between mean values among treatments. All statistical analysis was done with SPSS 13.0 (SPSS, Chicago, IL, USA). The level of statistically significant difference was set at $P < 0.05$ and highly significant difference was set at $P < 0.01$.

Results

According to earlier studies, the growth temperature range of *Psychrobacter* sp. G is 0–35 °C and the salinity range is 0–160. The optimal growth temperature and salinity of *Psychrobacter* sp. G are 20 °C and 45, respectively, according to Song et al. [20]. Under such conditions, the specific growth rate (SGR) of the strain is 0.18 and 0.2 h^{-1}, respectively.

Cloning and Bioinformatics Analysis of the Four HSP Genes

The open reading frame (ORF) of the *Hsp*845 gene is 615 bp long and encodes a protein consisting of 204 amino acid residues with a molecular mass of 22.7 kDa and a calculated p*I* of 4.54. The BLAST program analysis showed that the deduced amino acid sequence of Hsp845 shares homology with the amino acid sequences of other known cytosolic GrpEs. GrpE belongs to the HSP family; its synthesis is induced transiently upon a temperature upshift under the control of the heat-shock transcription factor σ^{32} [28, 31].

The ORF of the *Hsp*2538 gene is 441 bp long and encodes a protein consisting of 146 amino acid residues with a molecular mass of 16.8 kDa and a calculated p*I* of 10.0. Hsp2538 shares homology with the amino acid sequences of other known Hsp15.

The *Hsp*2666 and *Hsp*2667 ORFs are 291 and 1,650 bp long, respectively, and encode proteins consisting of 96 and 549 amino acid residues with a molecular mass of 10.3 and 57.9 kDa and a calculated p*I* of 4.87 and 4.65, respectively. *Hsp*2666 and *Hsp*2667 share homology with amino acid sequences of other known GroES and GroEL, respectively.

Amino Acid Sequence Analysis of Hsp845, Hsp2538, Hsp2666, and Hsp2667

Representative bacterial HSPs were selected to construct a phylogenetic tree using the program Mega 4.0 (Fig. 1). Hsp845, Hsp2538, Hsp2666, and Hsp2667 are clustered closely with the HSPs of *Psychrobacter arcticus* 273-4. Phylogenetic analysis showed that *Hsp*2667 has a relatively closer relationship with *Hsp*2666.

Table 2 Primers used in qRT-PCR analysis

Gene	qRT-PCR primers (5′–3′)	
*Hsp*845	Q-F: TGGTGGATAACTTGGA	Q-R: ACCGACCGCTTCAT
*Hsp*2538	Q-F: TATTGGGTTTGTTGTCT	Q-R: AGTCGTTGTTGAAGCA
*Hsp*2666	Q-F: TTGCTTCCTGGTTCA	Q-R: ATAATCAATAGTTCTTCG
*Hsp*2667	Q-F: GCTTCAAGAGCGTATG	Q-R: GCCACCAGGGACAA
GAPDH	Q-F: AGTCAGGCACATTTAGCG	Q-R: GGCATAGCCCCATTCATT
16S rRNA	Q-F:AGTCGTTGGGTCCCTTGA	Q-R:ATCGAATTAAACCACATGCTC
MT	Q-F:CTTCGGTAAATGAGCGTT	Q-R: AAAGTAGGCAAAGAGCAG
RU	Q-F: ACGCTTTTCATTTTCAGA	Q-R: CGTATGGGACTTGGTTCA

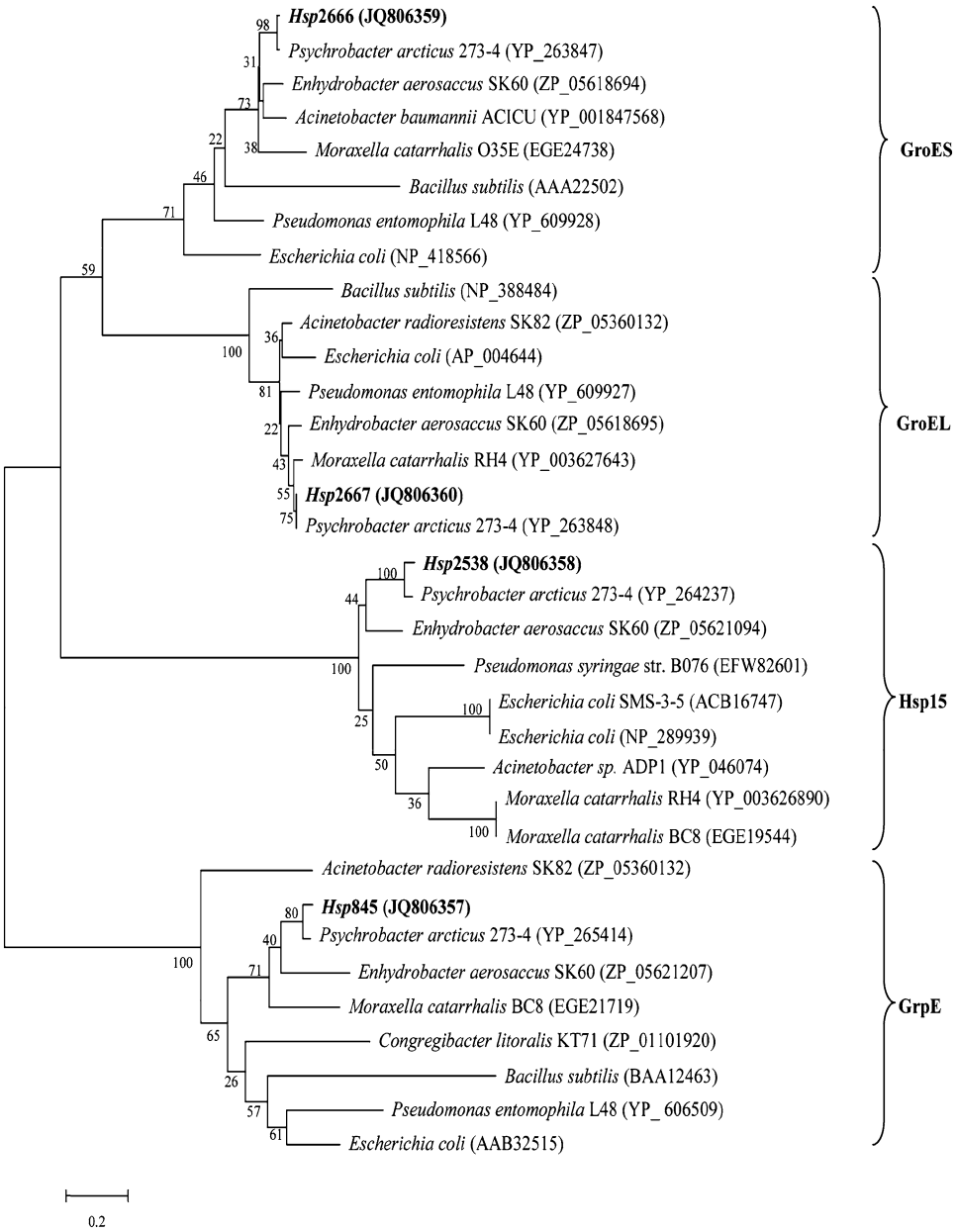

Fig. 1 Phylogenic tree of *Hsp*2666, *Hsp*2667, *Hsp*2538, and *Hsp*845 constructed using the program Meg 4.0. The *numbers* at each branch indicate the percentage of times a node was supported in 1,000 bootstrap pseudo replications by neighbor-joining. Accession numbers are shown in *parentheses*

Selection of the Four Candidate Reference Genes

GAPDH was selected as the reference gene on the basis of the minimum average expression stability under both temperature and salinity stress (Table 3).

Expression Characteristics of the Four HSP Genes in Response to Temperature Stress

The expression characteristics of the four HSP genes in response to temperature stress were different. The results showed that the expression of *Hsp*845 and *Hsp*2667 is inhibited significantly by low temperature (0 and 10 °C) but the expression of *Hsp*2538 is not sensitive to such treatments. The expression of *Hsp*2538 and *Hsp*2666 did not change significantly when exposed to 0 °C but the expression of *Hsp*2666 was inhibited when exposed to 10 °C. Expression of the four HSP genes was enhanced immediately after exposure to high temperature (30 °C) and then decreased gradually during the next 10 h. When exposed to 30 °C, the expression of *Hsp*845 reached a maximum after treatment for 2 h with a value of 11.7-fold greater compared to the control (Fig. 2).

Expression Characteristics of the Four HSP Genes in Response to Salinity Stress

Figure 3 shows the expression characteristics of the four HSP genes in response to salinity stress. The results

Table 3 Expression stability values of the candidate reference genes under different stress treatments calculated by Norm Finder

Name	Stability	
	Temperature stress	Salinity stress
GAPDH	0.287	0.405
16S rRNA	0.287	0.496
RU	1.299	0.567
MT	1.389	0.766
Best reference gene	GAPDH and 16SrRNA	GAPDH

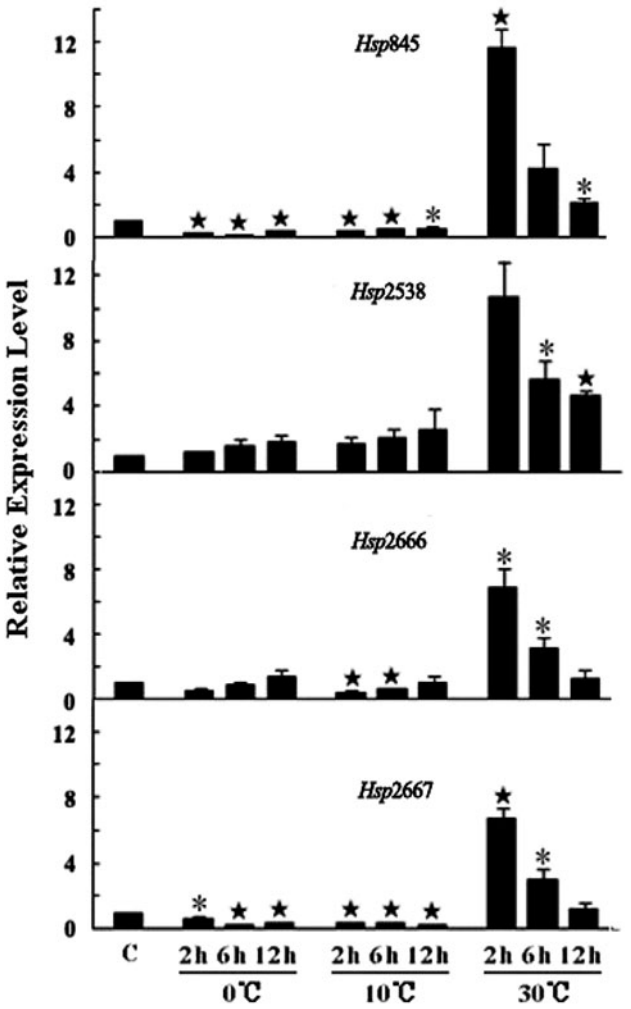

Fig. 2 qRT-PCR analysis of the four HSP genes in response to temperature stress. Standard *error bars* are shown: * $P < 0.05$; ★ $P < 0.01$

showed the expression of *Hsp*2538 and *Hsp*2667 was not sensitive to low salinity (0 and 15) but the expression of *Hsp*845 and *Hsp*2666 was enhanced by such treatment. Expression of the four HSP genes was enhanced by high salinity (90 and 120). It is noteworthy that the expression of *Hsp*845 and *Hsp*2666 was enhanced immediately after exposure to low salinity (0 and 15), and then decreased gradually during the next 10 h; by contrast, when they were exposed to high salinity (90 and 120), their expression increased gradually and reached a maximum after 12 h.

Expression Characteristics of the Four HSP Genes in Response to Combined Temperature and Salinity Stress

The results showed that the expression of *Hsp*845, *Hsp*2538, *Hsp*2666, and *Hsp*2667 was similar under

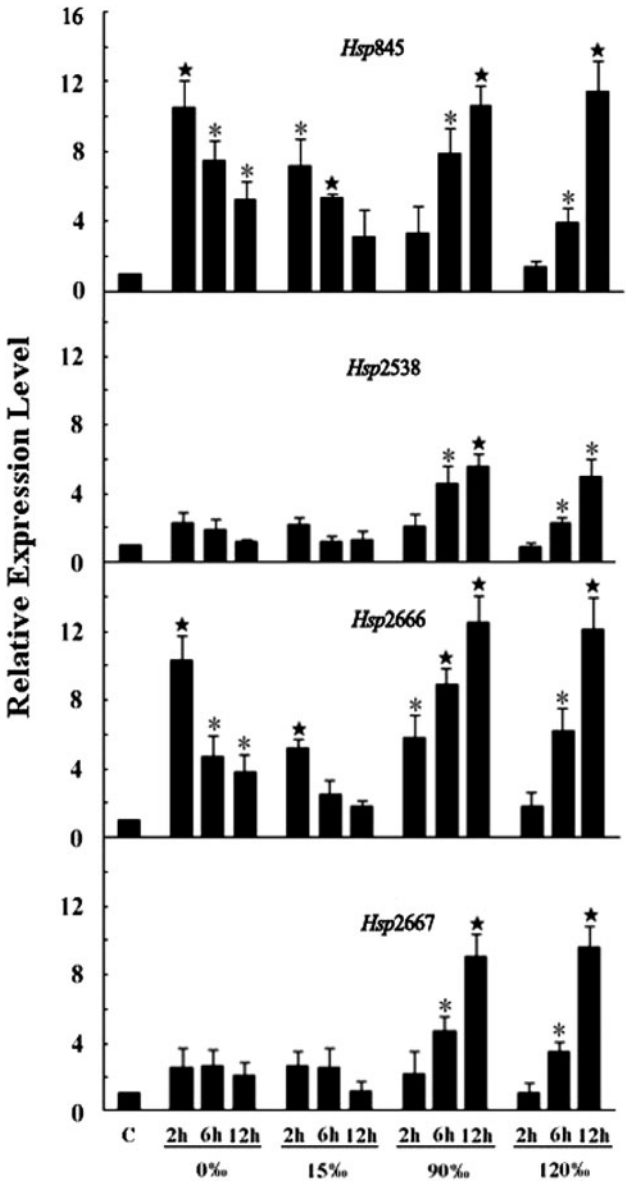

Fig. 3 qRT-PCR analysis of the four *HSP* genes in response to salinity stress. Standard *error bars* are shown: * $P < 0.05$; ★ $P < 0.01$

combined temperature and salinity stress; they were all enhanced by treatment at 30 °C with a salinity of 15 or 90. By contrast, treatment at 0 °C and a salinity of 15 or 90 had no significant effect (Fig. 4).

Discussion

HSPs have an important role in the response of cells to a sudden increase of external temperature. GrpE, a member of the HSP family, is transiently induced under various stress conditions. Earlier studies focused mainly on stress conditions including heat, ethanol, viral infection, and heavy metals [19, 27]. The role of GrpE in *E. coli* is to modulate the functions of the DnaK chaperone. This auxiliary role of GrpE must be very important, because mutations in either DnaK-DnaJ or GrpE genes exert similar global effects on the physiology of *E. coli* [28]. The DnaK-DnaJ-GrpE complex has an important role in autoregulation of the heat-shock response [4, 12, 22, 24]. Lipinska et al. [14] reported that GrpE messenger ribonucleic acid was induced rapidly and transiently in response to elevated temperatures. This could explain why the expression of *Hsp*845, which belongs to GrpE in *Psychrobacter* sp. G, increased immediately after exposure to 30 °C and reached a maximum (11.7-fold greater compared to the control) then decreased significantly during the next 10 h. The expression of *Hsp*845 was inhibited significantly at 0 °C and at 10 °C. Hsp15 was recognized as a well conserved and abundant HSP that binds nucleic acids [9, 10]. Korber et al. [10] and Yura et al. [31] reported Hsp15 was bound with high affinity specifically to the free 50S subunit of the ribosome, and it was more abundant under heat-shock conditions. The expression of *Hsp*2538 reached a maximum at 2 h when exposed to 30 °C (10.7-fold greater compared to the control) and then decreased significantly during the next 10 h and it was not sensitive to low temperature (0 or 10 °C). The GroEL-GroES chaperone complex is one of the most common HSPs that organisms synthesize in response to heat shock. GroEL can recognize many different non-native polypeptides in vitro and promote their folding to the native state. The action of GroEL requires the timely binding and hydrolysis of ATP, the binding of GroES and the binding and release of polypeptide. This is a highly synchronized system in which ATP acts both as energy source and allosteric effector, exerting positive cooperativity within one ring and negative cooperativity across rings [25, 29, 30]. The expression of *Hsp*2666 and *Hsp*2667 was highly synchronized after exposure to 30 °C, and reached maximum values of 6.9-fold (*Hsp*2666) and 6.7-fold (*Hsp*2667) greater compared to the control and then decreased significantly during the next 10 h. These results are in accord with those of earlier studies. Korber et al. [10] found that Hsp15 was more highly heat-inducible than nearly all of the well-studied HSPs, including GroEL and GroES, and our results agree with theirs.

In Antarctica, where *Psychrobacter* sp. G lives, sea ice freezes and thaws during different seasons, which affects the salinity of offshore seawater [15]. So, we investigated the effect of salinity on the expression of *Hsp*845, *Hsp*2538, *Hsp*2666, and *Hsp*2667. qRT-PCR analysis showed that both low (0 and 15) and high salinity (90 and 120) can enhance the expression of *Hsp*845 and *Hsp*2666

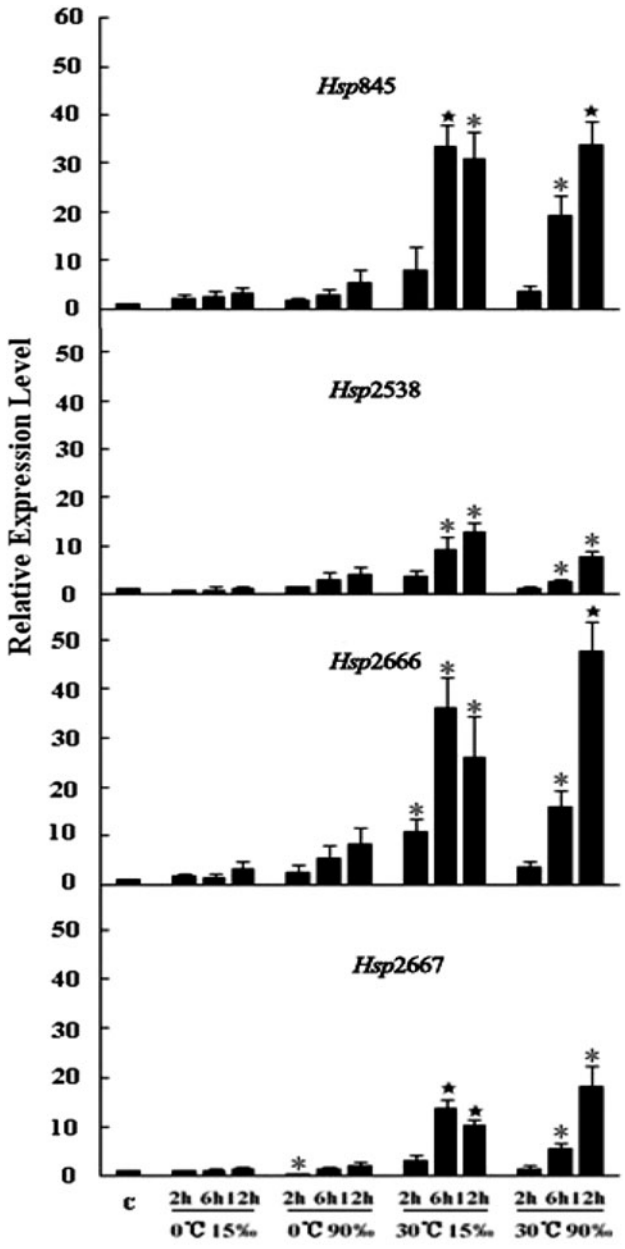

Fig. 4 qRT-PCR analysis of the four *HSP* genes in response to the cooperative stress of temperature and salinity. Standard *error bars* are shown: * $P < 0.05$; ★ $P < 0.01$

significantly. The expression of *Hsp*2538 and *Hsp*2667 was not sensitive to low salinity (0 or 15). The expression of *Hsp*2538 was enhanced significantly at a salinity of 90; after treatment for 12 h, its expression was ~5.5-fold greater compared to the control. The expression of *Hsp*2667 was enhanced significantly by high salinity (90 and 120) and a maximum was reached after 12 h. Kilstrup et al. [8] reported DnaK, GroEL, and GroES were induced during salinity stress, whereas Hsp15 in *Lactococcus lactis* was not sensitive to such conditions, similar to the results of this study. The functional contribution of HSPs to salinity stress adaptation is not clear; one possibility is that the HSPs are induced as a consequence of the accumulation of denatured proteins, resulting from a lowered water activity during plasmolysis of salinity-shocked cells [8].

In nature, *Psychrobacter* sp. G often faces more than one environmental stress factor simultaneously (e.g., freezing is often accompanied by salinity stress caused by a lack of unfrozen water) [23]. So, the combined effect of temperature and salinity on the expression of the four HSP genes was investigated. The expressions of *Hsp*845, *Hsp*2538, *Hsp*2666, and *Hsp*2667 showed similar characteristics under combined stress conditions; they were all enhanced by treatment at 30 °C with a salinity of 15 or 90. The other two treatments had no effect. So, we conclude expression of *Hsp*845, *Hsp*2538, *Hsp*2666, and *Hsp*2667 is more sensitive to high temperature than it is to different levels of salinity.

Acknowledgments This work was supported financially by the National Natural Science Foundation of China (41176174).

References

1. Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. Plant Physiol 139(1):5–17
2. Dheda K, Huggett JF, Bustin SA, Johnson MA, Rook G, Zumla A (2004) Validation of housekeeping genes for normalizing RNA expression in real-time PCR. Biotechniques 37(1):112–119
3. Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–282
4. Gamer J, Bujard H, Bukau B (1992) Physical interaction between heat shock proteins DnaK, DnaJ, and GrpE and the bacterial heat shock transcription factor σ^{32}. Cell 69(5):833–842
5. Gething MJ (1997) Guidebook to molecular chaperones and protein folding catalysts. Oxford University Press, Oxford
6. Jana B, Panja S, Saha S, Basu T (2009) Mechanism of protonophores-mediated induction of heat-shock response in *Escherichia coli*. BMC Microbiol 9:20
7. Jobin MP, Delmas F, Garmyn D, Diviès C, Guzzo J (1997) Molecular characterization of the gene encoding an 18-kilodalton small heat shock protein associated with the membrane of *Leuconostoc oenos*. Appl Environ Microbiol 63(2):609–614
8. Kilstrup M, Jacobsen S, Hammer K, Vogensen FK (1997) Induction of heat shock proteins DnaK, GroEL, and GroES by salt stress in *Lactococcus lactis*. Appl Environ Microbiol 63(5):1826–1837
9. Korber P, Zander T, Herschlag D, Bardwell JC (1999) A new heat shock protein that binds nucleic acids. J Biol Chem 274(1):249–256
10. Korber P, Stahl JM, Nierhaus KH, Bardwell JC (2000) Hsp15: a ribosome-associated heat shock protein. EMBO J 19(4):741–748
11. Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologistcentric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9(4):299–306
12. Liberek K, Georgopoulos C (1993) Autoregulation of the *Escherichia coli* heat shock response by the DnaK and DnaJ heat shock proteins. Proc Natl Acad Sci USA 90(23):11019–11023
13. Lin XZ, Cui SS, Xu GY, Wang S, Du N, Shen JH (2010) Cloning and heterologous expression of two cold-active lipases from the Antarctic bacterium *Psychrobacter* sp. G. Polar Res 29:421–429
14. Lipinska B, King J, Ang D, Georgopolous C (1988) Sequence analysis and transcriptional regulation of the *Escherichia coli* GrpE gene, encoding a heat shock protein. Nucleic Acids Res 16(15):7545–7562
15. Liu S, Zhang P, Cong B, Liu C, Lin X, Shen J, Huang X (2010) Molecular cloning and expression analysis of a cytosolic Hsp70 gene from Antarctic ice algae *Chlamydomonas* sp. ICE-L. Extremophiles 14(3):329–337
16. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(–Delta Delta C(T)) method. Methods 25(4):402–408
17. Neidhardt FC, VanBogelen RA (1987) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. In: Neidhardt FC (ed) Heat shock response. American Society for Microbiology, Washington DC, pp 1334–1345
18. Oberson J, Rawyler A, Brandle R, Canevascini G (1999) Analysis of the heat-shock response displayed by two *Chaetomium* species originating from different thermal environments. Fungal Genet Biol 26(3):178–189
19. Pearce DA (2008) Climate change and the microbiology of the Antarctic peninsula region. Sci Prog 91(2):203–217
20. Song WZ, Lin XZ, Huang XH (2012) Characterization and expression analysis of three old shock protein (CSP) genes under different stress conditions in the Antarctic bacterium *Psychrobacter* sp. G. Polar Biol 35:1515–1524
21. Spano G, Beneduce L, Perrotta C, Massa S (2005) Cloning and characterization of the Hsp 18.55 gene, a new member of the small heat shock gene family isolated from wine *Lactobacillus plantarum*. Res Microbiol 156(2):219–224
22. Straus D, Walter W, Gross CA (1990) DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of σ^{32}. Genes Dev 4(12A):2202–2209
23. Thomas DN, Dieckmann GS (2002) Antarctic sea ice–a habitat for extremophiles. Science 295(5555):641–644
24. Tilly K, McKittrick N, Zylicz M, Georgopoulos C (1983) The dnaK protein modulates the heat-shock response of *Escherichia coli*. Cell 34(2):641–646
25. Todd MJ, Viitanen PV, Lorimer GH (1994) Dynamics of the chaperonin ATPase cycle: implications for facilitated protein folding. Science 265(5172):659–666
26. Vandesompele J, Preter KD, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3(7):research 0034.1–0034.11
27. Wu B, Ang D, Snavely M, Georgopoulos C (1994) Isolation and characterization of point mutations in the *Escherichia coli* grpE heat shock gene. J Bacteriol 176(22):6965–6973

28. Wu B, Wawrzynow A, Zylicz M, Georgopoulos C (1996) Structure-function analysis of the *Escherichia coli* GrpE heat shock protein. EMBO J 15(18):4806–4816
29. Xu Z, Sigler PB (1998) GroEL/GroES: structure and function of a two-stroke folding machine. J Struct Biol 124(2–3):129–141
30. Yifrach O, Horovitz A (1995) Nested cooperativity in the ATPase activity of the oligomeric chaperonin GroEL. Biochemistry 34(16):5303–5308
31. Yura T, Nagai H, Mori H (1993) Regulation of the heat-shock response in bacteria. Annu Rev Microbiol 47:321–350