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Nisin Tolerance of DnaK-overexpressing *Lactococcus lactis* Strains at 40°C

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ABSTRACT

The effect of different concentrations of nisin on DnaK-overproducing *Lactococcus lactis* strains while growing at 40°C was examined. The plasmid pNZ-8048 which has a nisin promoter was used as a vector for transformation and expression of heterologous or homologous DnaK into *L. lactis* NZ9000. The transformants were then induced with different concentration of nisin and allowed to grow in presence of nisin. It was found that nisin, the antimicrobial peptide used for induction of DnaK-overexpression, itself conferred a lower but significant stress to the strains. *Escherichia coli* DnaK ($DnaK_{Eco}$)-overexpressing cells showed better tolerance to nisin than *L. lactis* DnaK ($DnaK_{Lla}$) and *T. halophilus* DnaK ($DnaK_{Tha}$)-overexpressing cells. However, all DnaK-overexpressing strains showed better tolerance than the cells without DnaK overexpression. These findings suggest the possibility for a relationship of DnaK protein and tolerance to different antimicrobial peptides.

Key words: Nisin, stress, overexpression, tolerance, chaperone, DnaK

INTRODUCTION

L. lactis, a spherical-shaped gram-positive lactic acid bacterium, has been used for long time for industrial production of fermented dairy products such as milk, cheese and yogurt. Microbial biomass of Lactic Acid Bacteria (LAB) are also widely used in the food and pharmaceutical industries (Lee *et al.*, 2007). Because of the importance of lactic acid bacteria in food industry extensive research has been done on their metabolic pathway to increase its efficiency for fermentation. Recently, the bacterial group is getting much attention even in medical science (Anukam, 2007). Food supplemented with LAB have been shown to work against cancer development (Gursoy and Kinik, 2006). Some of their special characteristics such as faster growth, acidification and resistance to bacteriocin made them suitable for selection in the dairy industries (Van de Guchte *et al.*, 2002; Yateem *et al.*, 2008; Ali, 2011). On the other hands, these strains need to overcome the stress conditions encountered in the fermentation processes for better productivity.

Many efforts have been made to overcome these problems. With the recent developments of molecular genetics and proteomics, some genes are figured out to be involved in stress responses of LAB, other bacteria and mammalian cells (Abdullah-Al-Mahin *et al.*, 2010; Fiocco *et al.*, 2007; Prasad *et al.*, 2003; Walker *et al.*, 1999; Walter *et al.*, 2003; Kullen and Klaenhammer, 1999; Lentze and Narberhaus, 2005; Ahmed, 2006).

Hsp70s play a very important role for folding of newly synthesized proteins, refolding of misfolded proteins and transportation of proteins through biological membranes both under normal and stressed conditions (Mayer *et al.*, 2001). DnaK, a 70-kDa heat shock protein homolog in bacteria, transduces signals to other cellular factors when a shift of temperature increase occurs (Craig and Gross, 1991). The hsp70 genes are widely conserved in all organisms except some archaeal strains. However, the functional studies of this heat responsive gene were widely carried out with *Saccharomyces cerevisiae*, *E. coli* and *Bacillus subtilis* (Homuth *et al.*, 1997; Schulz *et al.*, 1995). DnaK functions in cooperation with DnaJ and GrpE and the complex of DnaK-DnaJ-GrpE plays a significant role in the refolding of thermally damaged proteins. However, the complex also assists in the folding of nascent protein chains under normal growth conditions (Bukau, 1993; Bukau and Walker, 1989, 1990; Cegielska and Georgopoulos, 1989). Liberek *et al.* (1991) reported that DnaK requires ATP for its activity *in vitro* and this ATPase activity is stimulated by its co-chaperone DnaJ and GrpE. Like other bacteria, *L. lactis* also exhibits a heat shock response in which molecular chaperones play key roles (Arnau *et al.*, 1996; Kilstrup *et al.*, 1997; Whitaker and Batt, 1991). Previously, overproductions of GroEL/ES and small Hsps have been reported to improve the stress tolerance of lactic acid bacteria (Desmond *et al.*, 2002, 2004). We reported earlier that both heterologous and homologous expression of DnaK in *L. lactis* improves heat, salt, ethanol and acid (low pH)-stress. We also found that nisin, the antimicrobial peptide used for induction of DnaK-overexpression also conferred stress both at normal physiological conditions and high temperature (Abdullah-Al-Mahin *et al.*, 2010). Therefore in this report, we aimed to investigate more in detail the effect of DnaK-overexpression in *L. lactis* strains to nisin when growing at 40°C.

MATERIALS AND METHODS

Bacterial strains and media: *L. lactis* subsp. *cremoris* NZ9000 and *E. coli* JM109 were used throughout the study. *L. lactis* NZ9000 was grown in GM17 medium (M17 broth supplemented with 0.5% glucose) at 30°C, unless stated otherwise. *E. coli* JM109 was grown aerobically in Luria-Bertani (LB) broth at 37°C, unless stated otherwise. *T. halophilus* JCM5888 was grown in MRS medium (Oxoid, Hampshire, UK) containing 1 M NaCl at 30°C. The medium was adjusted to pH 7.5 before sterilization. In order to facilitate clonal selection, 5 μg mL^{-1} chloramphenicol was added to the media.

Construction of DnaK-expressing *L. lactis*: Chromosomal DNA was isolated from *E. coli* JM109, *L. lactis* NZ9000 and *T. halophilus* JCM5888 using combination of two methods as described earlier (Berns and Thomas, 1965; Marmur, 1961). The $DnaK_{Eco}$ gene was amplified from *E. coli* chromosome using primers 5'-CCCCTATTAGGATCCCACAACCACATGATGACCGAATATAT-3' and 5'-GTCAGTATAATTACCCGTTTATAGAGCTCTTATTT-3'. The *Bam*HI and *Sac*I sites were simultaneously inserted into the amplified $DnaK_{Eco}$ gene. A *Bam*HI restriction endonuclease site was inserted into the plasmid pNZ8048 (De Ruyter *et al.*, 1996) by inverse PCR using primers 5'-

CTAGAGAGCTCAAGCTTTCTTTGAACCAAA-3' and 5'-TTTTGTGGATCCTTTCGAACGAAATC-3'. The $DnaK_{Lla}$ was amplified from *L. lactis* NZ9000 using primers 5'-ATATTGACCGCCATGGCTTTAAACTATTC-3' and 5'- ACTGACGAAACGATGAGCTCTTTTTAAA-3', The *Nco*I and *Sac*I sites were simultaneously inserted into the amplified $DnaK_{Lla}$ gene. The $DnaK_{Tha}$ gene was amplified from *T. halophilus* JCM5888 chromosome using primers 5'-AGATCAATATCATGAGTAAGATAATTGGTATTGACT-3' and 5'-ATTTCCCAAATAGAGCTCTTATTGATTATCGTT-3'. The *Pag*I and *Sac*I sites were simultaneously inserted into the amplified $DnaK_{Tha}$ gene. PCR was performed with KOD plus Dna polymerase (Toyobo, Osaka, Japan). The amplified PCR products were purified with the QIAquick PCR Purification Kit (Qiagenne West Sussex, United Kingdom). All the amplified DnaK genes and the plasmid were digested with their respective restriction enzymes. For insertion of $DnaK_{Lla}$ and $DnaK_{Tha}$ the plasmid pNZ8048 was enzymatically digested with *Nco*I and *Sac*I restriction enzymes. Enzymatically digested products were then ligated using Ligation High ver. 2 (Toyobo), according to the manufacturer's instructions. The resulting plasmids which contained $DnaK_{Eco}$ $DnaK_{Lla}$ and $DnaK_{Tha}$ were named as pNZ-EDnaK, pNZ-LDnaK and pNZ-TDnaK. All these three plasmids were then transformed into *L. lactis* NZ9000, according to the method developed by Holo and Nes (Holo and Nes, 1989) and the transformants were designated as NZ-EDnaK, NZ-LDnaK and NZ-TDnaK, respectively. An empty plasmid pNZ8048 were also transformed into *L. lactis* NZ9000 and the transformant was named as NZ-Vector which is used as the control strain throughout the study.

Overexpression of DnaK and investigation of stress tolerance: All the transformants were grown in GM17 medium (10 mL) containing 5 μg mL^{-1} chloramphenicol at 30°C. When optical density at 600 nm (OD_{600}) reached at 0.5-0.6, the expression of DnaK was induced with different concentrations of nisin (0, 0.05, 1.0, 10.0, 25.0 and 50 ng mL^{-1}) for overnight. Nisin solution was prepared with commercial nisin of *Streptococcus lactis* (Sigma). Aliquots of the nisin-induced cultures were then transferred to fresh GM17 medium (30 mL) containing 5 μg mL^{-1} chloramphenicol and the same concentrations of nisin that was used for induction to obtain an OD_{600} of 0.04-0.05. Bacterial strains were then allowed to grow at 40°C and growths were measured at OD_{600}. Bacterial strains that survived combined stresses of nisin and heat were detected using the same method. All the experiments were repeated at least three times to check the reproducibility of the results.

Confirmation of DnaK production by Western blotting: To check the production level of DnaK proteins after induction with different nisin concentration, overnight bacterial cultures (10 mL) were harvested by centrifuging at 6,000x g for 5 min at 4°C. Cell-free extracts were prepared from the pellets suspended in chilled 50 mM potassium phosphate buffer (pH 7.4) by using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) at 2,500 rpm for 1 min at 4°C; this process was repeated 5 times with 1 min intervals. The cell-free extracts were obtained by centrifuging at 2,000x g for 15 min to remove the cell debris and the supernatant that contained the soluble proteins was collected. The protein concentration in the supernatant was determined by using the Bradford assay kit (Nacalai Tesque, Kyoto, Japan). The soluble proteins (10 μg) were then subjected to separation on a 12% (w/v) SDS-PAGE gel. The proteins were transferred onto a polyvinylidene difluoride membrane. Immunoblotting and detection of DnaK proteins were performed as previously described using anti-*T. halophilus* DnaK antibody (Sugimoto *et al*., 2008).

RESULTS

Overexpression of DnaK in *L. lactis*: To check the production of DnaK upon nisin induction, cell lysates of all four transformants induced with different concentrate of nisin (0-50 ng mL^{-1} nisin) was used for Western blot analysis (Fig. 1a-c). We could detect *L. lactis* DnaK ($DnaK_{Lla}$) in all the transformants including control strain (NZ-Vector). An additional band was detected in NZ-EDnaK only in presence of 10 ng mL^{-1} nisin which had an equal mobility of that of DnaK from *E. coli* cell extract used as a control (Fig. 1b). $DnaK_{Eco}$ could be clearly distinguished from $DnaK_{Lla}$ in NZ-EDnaK based on the difference in their mobilities. Between the two protein bands obtained from the cell lysate of NZ-EDnaK, the upper band was identified as $DnaK_{Eco}$. It was also observed that the amount of heterologously produced $DnaK_{Eco}$ is very low compared to the NZ-LDnaK`s own $DnaK_{Lla}$. When NZ-LDnaK and NZ-TDnaK were induced with different nisin concentration overproduction of *L. lactis* DnaK ($DnaK_{Lla}$) and *T. halophilus* DnaK ($DnaK_{Tha}$) were detected in cell lysates of NZ-LDnaK and NZ-TDnaK, respectively (Fig. 1b). Although

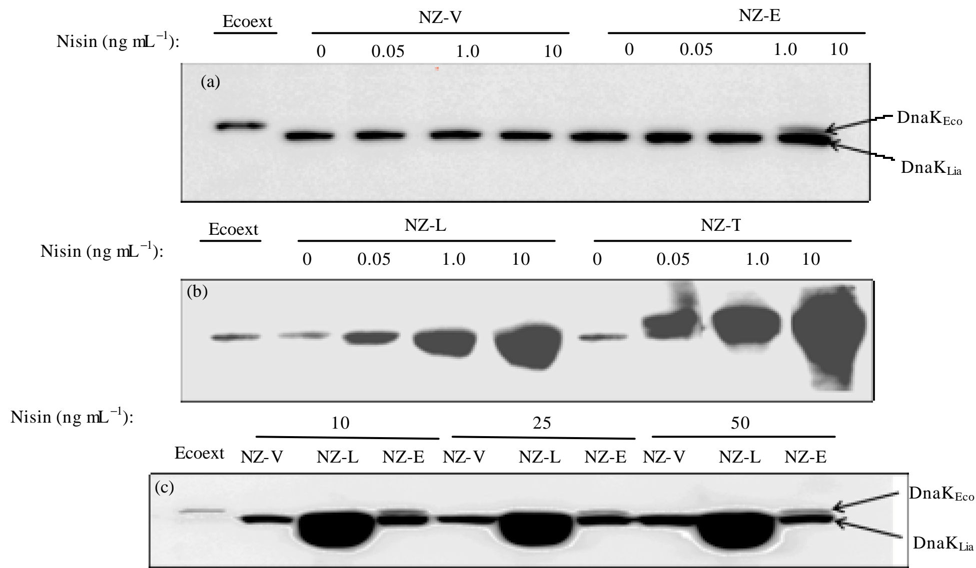

Fig. 1(a-c): DnaK production after nisn induction. Cell-free extracts of NZ-Vector (NZ-V), NZ-EDnaK (NZ-E), NZ-LDnaK (NZ-L) and NZ-TDnaK (NZ-T) prepared from cultures that were induced overnight with indicated concentration of nisin in GM17 liquid medium supplemented with 0.5% glucose at 30°C were separated using a 12% SDS gel for Western blotting. In cell free extracts production of DnaK was confirmed using DnaK polyclonal antibody raised against *T. halophilus* DnaK. (a) DnaK productions after induction with 0-10 ng mL^{-1} nisin were checked by western blot where a single blot was use for NZ-Vector and NZ-EDnaK. Here cell free extract of *E. coli* (Ecoext) was used as control for $DnaK_{Eco}$. (b) $DnaK_{Lla}$ and $DnaK_{Tha}$ production were checked in another blot in presence of 0-10 ng mL^{-1} nisin where same concentration of NZ-Vector cell free extract was used to compare the amount of these DnaKs. (c) DnaK productions in cell free extracts of NZ-Vector, NZ-EDnaK and NZ-LDnaK were compared in another blot after induction with 10-50 ng mL^{-1} nisin. Ecoext was used as control. Equal amounts of proteins were used in all lanes

Table 1: Generation time and maximum growth yields of *L. lactis* strains in presence of different concentration of nisin GM17 media at 40°C

Nisin conc. (ng mL^{-1})	Strains	Generation times (min)[a]	Generation time relative to that of NZ-vector	Maximum OD_{600}	Maximum OD_{600} to that of NZ-vector
0	NZ-Vector	36.15	1.00	1.29	1.00
	NZ-EDnaK	36.67	1.01	1.36	1.05
	NZ-LDnaK	34.20	0.95	1.33	1.03
	NZ-TDnaK	35.02	0.97	1.33	1.03
0.05	NZ-Vector	35.36	1.00	1.31	1.00
	NZ-EDnaK	36.83	1.04	1.35	1.03
	NZ-LDnaK	35.24	1.00	1.29	0.98
	NZ-TDnaK	36.19	1.02	1.28	0.98
1.0	NZ-Vector	35.53	1.00	1.29	1.00
	NZ-EDnaK	36.42	1.03	1.35	1.05
	NZ-LDnaK	35.43	1.00	1.28	0.99
	NZ-TDnaK	39.39	1.11	1.11	0.86
10.0	NZ-Vector	ND[b]	-[c]	0.16	1.00
	NZ-EDnaK	37.08	-	1.25	7.81
	NZ-LDnaK	146.06	-	0.76	4.75
	NZ-TDnaK	231.59	-	0.71	4.44

[a]Generation time was determined in the exponential growth phase. [b]ND: Not detected (since the cells were not in exponential phase). [c]: Uncalculated

chromosomal- and plasmid-borne DnaK could not be separated in NZ-LDnaK and NZ-TDnaK the increased band intensity confirmed the overproduction of $DnaK_{Lla}$ and $DnaK_{Tha}$ in cell lysates of NZ-LDnaK and NZ-TDnaK, respectively. Figure 1 clearly shows that although 0.05 ng mL^{-1} nisin was quite enough to induce DnaK production in NZ-LDnaK and NZ-TDnaK (Fig. 1b) production of *E. coli* DnaK was induced only after induction with 10 ng mL^{-1} nisin (Fig. 1a). To check whether increased nisin induce further production of DnaK in the studied strains cell lysates of the strains after induction with 25 and 50 ng mL^{-1} nisin were compared with the cell lysates induced with 10 ng mL^{-1} nisin. It was then detected that nisin concentration more than 10 ng mL^{-1} did not induce more DnaK production in any of the strains of NZ-Vector, NZ-LDnaK, NZ-EDnaK (Fig. 1c) and NZ-TDnaK (Data not shown).

Tolerance to nisin stress by DnaK-overexpression at 40°C: While comparing the nisin stress tolerance of all heterologous or homologous DnaK-producing cells at 40°C, no growth difference of the strains in absence of nisin (Fig. 2a) and no visible growth of the strains in presence of 25 and 50 ng mL^{-1} nisin (Fig. 2e, f) clearly indicates a stress effect of this antimicrobial agent in presence of 40°C temperature. In presence of 0.05 ng mL^{-1} nisin, there were no significant difference in growth pattern (Fig. 2b), generation time and maximum OD_{600} (Table 1) although in this concentration of nisin NZ-LDnaK and NZ-TDnaK over-produced $DnaK_{Lla}$ and $DnaK_{Tha}$ (Fig. 1b). Again, in presence of 1 ng mL^{-1} nisin, the generation time and maximum OD_{600} of NZ-Vector, NZ-EDnaK and NZ-LDnaK were also not significantly different. However, $DnaK_{Tha}$ producing cells showed a little higher generation time (1.11-fold) and lower maximum OD (0.86-fold) than the corresponding values of NZ-Vector (Table 1). A clear difference in stress tolerance by the studied strains was visible in presence of nisin concentration of 10 ng mL^{-1} only (Fig. 2c). At 10 ng mL^{-1} nisin concentration, the concentration which is sufficient for the DnaK-overproduction in all

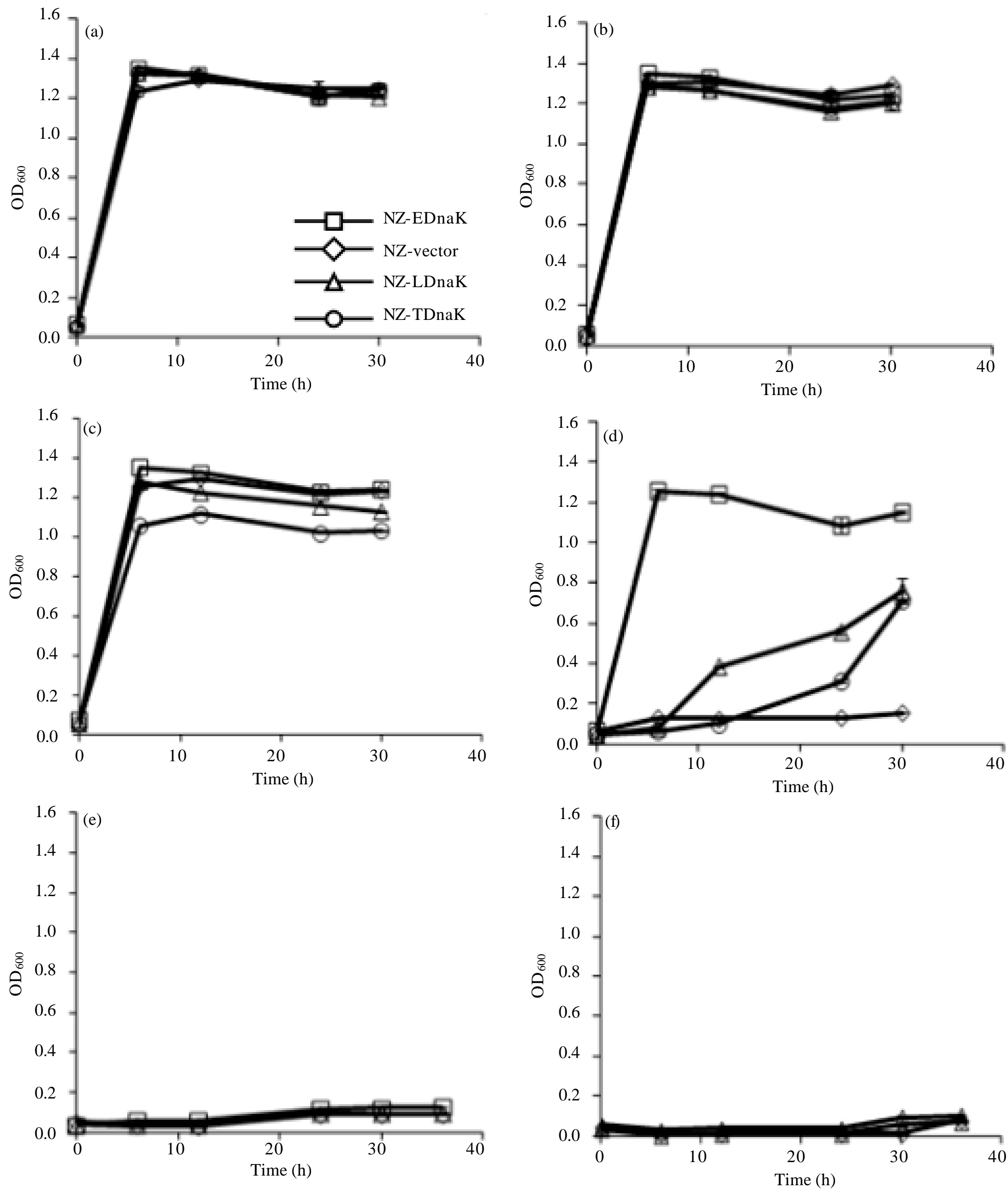

Fig. 2(a-f): Effect of nisin on growth at 40°C. NZ-Vector, NZ-EDnaK, NZ-LDnaK and NZ-TDnaK were grown in GM17 medium containing 0.5% glucose as the sole carbon source containing without pH control. Nisin concentrations of (a) 0 ng mL^{-1}, (b) 0.05 ng mL^{-1}, (c) 1 ng mL^{-1}, (d) 10 ng mL^{-1}, (e) 25 ng mL^{-1} and (f) 50 ng mL^{-1} were used in the media and the periodic growth was measured at 600 nm. The values shown are the Means± standard errors (error bars) for three independent experiments

the strains, NZ-EDnaK showed maximum growth yields among the strains indicating highest tolerance to the combined effect of nisin and heat. Since NZ-Vector was not in log phase

generation time could not be compared. However, the maximum OD of NZ-EDnaK, NZ-LDnaK and NZ-TDnaK were 7.81, 4.75 and 4.44 fold higher than that of NZ-Vector (Table 1) suggesting the potency of overproduced DnaK to make the strains capable to grow even the conditions when NZ-Vector showed a very negligible growth.

DISCUSSION

Although recent developments in fermentation technology are capable of minimizing the stress conditions during fermentation, scientists are conducting research on stress-stable fermentation microorganisms and overexpression of molecular chaperone genes is now considered to be one of the most promising approaches to achieve that goal. We successfully overexpressed $DnaK_{Eco}$ in *L. lactis* which showed multiple stress tolerance and higher lactic acid production at high temperature (Abdullah-Al-Mahin *et al.*, 2010). However, the problem that was found is that nisin, the antimicrobial peptide used for DnaK overproduction, itself conferred stress to *L. lactis* strains (Abdullah-Al-Mahin *et al.*, 2010). Antimicrobial agents were also reported to effect on the production of lactic acid and other fermentation products by LAB (Abou Ayana *et al.*, 2011). Therefore, this study was aimed at evaluating the stress tolerance to nisin after expression of DnaK in lactic acid bacterium *L. lactis* NZ9000. Our study concluded with the finding that the expression of DnaK conferred increased tolerance to combined stress effect of heat and nisin to *L. lactis* NZ9000. The role of molecular chaperone in stress tolerance has already been reported by many researchers. Susin *et al.* (2006) reported the importance of DnaK/DnaJ for the survival of *Caulobacter crescentus* when exposed to heat stress. Previously, GroESL-overproducing *L. lactis* was reported to be more tolerant to heat (54°C for 30 min), salt (5 M NaCl for 1 h), or solvent (0.5% butanol) stress (Desmond *et al.*, 2004). Sugimoto *et al.* (2003) earlier reported the remarkable suppression of the 5% (0.86 M) NaCl-induced protein aggregates by the overproduction of *T. halophilus* DnaK in *E. coli*. Tomas *et al.* (2003) reported that the overexpression of *groESL* in *Clostridium acetobutylicum* resulted in a 38 and 30% increase in acetone and butanol production, respectively, relative to the plasmid control strain during pH-controlled glucose-fed batch acetone-butanol fermentation. The *groESL*-overexpressing strain also showed increased tolerance against butanol than plasmid-controlled strain. Involvement of heat shock proteins to protect plants against heat and salt stresses has also been reported (Essemine *et al.*, 2010; Mudgal *et al.*, 2010; Joseph and Jini, 2010). These findings with regard to improved stress tolerance efficiency due to the expression of chaperone genes were consistent with the findings of present study. Further, the most important finding of this study was the tolerance of all the DnaK-overproducing strains to combined effects of heat and nisin.

Nisin is known to effect on cell membrane of gram-positive bacteria having the target site of lipid II (Guder *et al.*, 2000; Sahl and Bierbaum, 1998). Increased sensitivity of heat stressed *B. cereus* and *L. lactis* to nisin was reported earlier (Beuchat *et al.*, 1997; Kalchayanand *et al.*, 1992, 1994). It was suggested that nisin prevented the repair of heat damaged membrane. In this study, combined action of heat and nisin (10 ng mL^{-1}) effected on the cell growth of NZ-Vector. Homologously or heterologously overproduced DnaK, especially $DnaK_{Eco}$ helped the strains to overcome the combined stresses.

Although $DnaK_{Lla}$ and $DnaK_{Tha}$ were produced higher than $DnaK_{Eco}$, more potentiality for combined stress tolerance was found by $DnaK_{Eco}$. These differences in DnaK overproduction ability can be explained by differences in codon usase (Abdullah-Al-Mahin *et al.*, 2010). Our findings also suggested that $DnaK_{Eco}$ was more efficient than $DnaK_{Lla}$ and $DnaK_{Tha}$ to overcome the stress at

early growth period (Fig. 1d). Difference in stress tolerance ability by different DnaK has already been reported earlier (Abdullah-Al-Mahin *et al.*, 2010; Sugimoto *et al.*, 2003). However, despite higher DnaK production the failure to confer better stress tolerance by NZ-LDnaK and NZ-TDnaK suggested the importance of functional potency rather than the level of production.

Finally, it can be conclude that both homologously or heterologously overproduced DnaK had the effect to rescue the growth inhibition due to nisin at high temperature. This finding could have important implication for exploring the scope for further study to find the relationship of DnaK/chaperones and tolerance to different antimicrobial peptides. Since, the target site of nisin is cell membrane, the tolerance to nisin thereby opens a scope to research about the role of DnaK in membrane stability against bacteriocins.

REFERENCES

Abdullah-Al-Mahin, S. Sugimoto, C. Higashi, S. Matsumoto and K. Sonomoto, 2010. Improvement of multiple-stress tolerance and lactic acid production in *Lactococcus lactis* NZ9000 under conditions of thermal stress by heterologous expression of *Escherichia coli* DnaK. Applied Environ. Microbiol., 76: 4277-4285.

Abou Ayana, I.A.A., A.A. Gamal El-Deen and M.A. El-Metwally, 2011. Behavior of certain lactic acid bacteria in the presence of pesticides residues. Int. J. Dairy Sci., 6: 44-57.

Ahmed, R.G., 2006. The relation between biological consequences and temperature on some non-mammalian species. Int. J. Zool. Res., 2: 136-149.

Ali, A.A., 2011. Isolation and identification of lactic acid bacteria isolated from traditional drinking yoghurt in Khartoum State, Sudan. Curr. Res. Bacteriol., 4: 16-22.

Anukam, K.C., 2007. Probiotic toxicity, any evidence? J. Pharmacol. Toxicol., 2: 590-598.

Arnau, J., K.I. Sorensen, K.E. Appel, F.K. Vogensen and K. Hammer, 1996. Analysis of heat shock gene expression in *Lactococcus lactis* MG1363. Microbiology, 142: 1685-1691.

Berns, K.I. and C.A. Thomas, 1965. Isolation of high molecular weight Dna from *Haemophilus influenzae*. J. Mol. Biol., 11: 476-490.

Beuchat, L.R., M.R. Clavero and C.B. Jaquette. 1997. Effects of nisin and temperature on survival, growth and enterotoxin production characteristics of psychrotrophic *Bacillus cereus* in beef gravy. Applied Environ. Microbiol., 63: 1953-1958.

Bukau, B. and G. Walker, 1990. Mutations altering heat shock specific subunit of RNA polymerase suppress major cellular defects of *E. coli* mutants lacking the DnaK chaperone. EMBO J., 9: 4027-4036.

Bukau, B. and G.C. Walker, 1989. Cellular defects caused by deletion of the *Escherichia coli* DnaK gene indicate roles for heat shock protein in normal metabolism. J. Bacteriol., 171: 2337-2346.

Bukau, B., 1993. Regulation of the *Escherichia coli* heat-shock response. Mol. Microbiol., 9: 671-680.

Cegielska, A. and C. Georgopoulos, 1989. Functional domains of the *Escherichia coli* DnaK heat shock protein as revealed by mutational analysis. J. Biol. Chem., 264: 21122-21130.

Craig, E. and C.A. Gross, 1991. Is hsp70 the cellular thermometer. Trends Biochem. Sci., 16: 135-140.

De Ruyter, P.G., O.P. Kuipers and W.M. de-Vos, 1996. Controlled gene expression systems for *Lactococcus lactis* with the food-grade inducer nisin. Applied Environ. Microbiol., 62: 3662-3667.

Desmond, C., R.P. Ross, E. O'Callagahan, G. Fitzegerald and C. Stanton, 2002. Improved survival of *Lactobacillus paracasei* NFBC 338 in spray dried powders containing gum acacia. J. Applied Microbiol., 93: 1003-1011.

Desmond, C., G.F. Fitzgerald, C. Stanton and R.P. Ross, 2004. Improved stress tolerance of GroESL-overproducing *Lactococcus lactis* and probiotic *Lactobacillus paracasei* NFBC338. Applied Environ. Microbiol., 70: 5929-5936.

Essemine, J., S. Ammar and S. Bouzid, 2010. Impact of heat stress on germination and growth in higher plants: Physiological, biochemical and molecular repercussions and mechanisms of defence. J. Biol. Sci., 10: 565-572.

Fiocco, D., V. Capozzi, P. Goffin, P. Hols and G. Spano, 2007. Improved adaptation to heat, cold and solvent tolerance in *Lactobacillus plantarum*. Applied Microbiol. Biotechnol., 77: 909-915.

Guder, A., I. Wiedemann and H.G. Sahl, 2000. Posttranslationally modified bacteriocins-the lantibiotics. Biopolymers, 55: 62-73.

Gursoy, O. and O. Kinik, 2006. Probiotics: A new popular option for cancer inhibition. Int. J. Dairy Sci., 1: 100-103.

Holo, H. and I.F. Nes, 1989. High-frequency transformation, by electroporation, of *Lactococcus lactis* subsp. *cremoris* grown with glycine in osmotically stabilized media. Applied Environ. Microbiol., 55: 3119-3123.

Homuth, G., S. Masuda, A. Mogk, Y. Kobayashi and W. Schumann, 1997. The DnaK operon of *Bacillus subtilis* is heptacistronic. J. Bacteriol., 179: 1153-1164.

Joseph, B. and D. Jini, 2010. Proteomic analysis of salinity stress-responsive proteins in plants. Asian J. Plant Sci., 9: 307-313.

Kalchayanand, N., M.B. Hanlin and B. Ray, 1992. Sublethal injury makes gram-negative and resistant gram-positive bacterial sensitive to the bacteriocins, pediocin AcH and nisin. Lett. Applied Microbiol., 15: 239-243.

Kalchayanand, N., T. Sikes, C.P. Dunne and B. Ray, 1994. Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. Applied Environ. Microbiol., 60: 4174-4177.

Kilstrup, M., S. Jacobsen, K. Hammer and F.K. Vogensen, 1997. Induction of heat shock proteins DnaK, GroEL and GroES by salt stress in *Lactococcus lactis*. Applied Environ. Microbiol., 63: 1826-1837.

Kullen, M.J. and T.R., Klaenhammer, 1999. Identification of the pH-inducible, proton-translocating F1Fo-ATPase (*atpBEFHAGDC*) operon of *Lactobacillus acidophilus* by differential display: gene structure, cloning and characterization. Mol. Microbiol., 33: 1152-1161.

Lee, B.B., H.J. Tham and E.S. Chan, 2007. Fed-batch fermentation of lactic acid bacteria to improve biomass production: A theoretical approach. J. Applied Sci., 7: 2211-2215.

Lentze, N. and F. Narberhaus, 2005. Small heat shock proteins OR: A subgroup of molecular chaperones. J. Biol. Sci., 5: 1-9.

Liberek, K., J. Marszalek, D. Ang, C. Georgopoulos and M. Zylicz, 1991. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc. Natl. Acad. Sci. USA., 88: 2874-2878.

Marmur, L.J., 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol., 3: 208-218.

Mayer, M.P., D. Brehmer, C.S. Gassler and B. Bukau, 2001. Hsp70 chaperone machines. Adv. Protein. Chem., 59: 1-44.

Mudgal, V., N. Madaan and A. Mudgal, 2010. Biochemical mechanisms of salt tolerance in plants: A review. Int. J. Bot., 6: 136-143.

Prasad, J., P. McJarrow and P. Gopal, 2003. Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. Applied Environ. Microbiol., 69: 917-925.

Sahl, H.G. and G. Bierbaum, 1998. Lantibiotics: Biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria. Ann. Rev. Microbiol., 52: 41-79.

Schulz, A., B. Tzschaschel and W. Schumann, 1995. Isolation and analysis of mutants of the DnaK operon of *Bacillus subtilis*. Mol. Microbiol., 15: 421-429.

Sugimoto, S., J. Nakayama, D. Fukuda, S. Sonezaki, M. Watanabe, A. Tosukhowong and K. Sonomoto, 2003. Effect of heterologous expression of molecular chaperone DnaK_from *Tetragenococcus halophilus* on salinity adaptation of *Escherichia coli*. J. Biosci. Bioeng., 96: 129-133.

Sugimoto, S., K. Saruwatari, C. Higashi, K. Tsuruno, S. Matsumoto, J. Nakayama and K. Sonomoto, 2008. *In vivo* and *in vitro* complementation study comparing the function of DnaK chaperone systems from halophilic lactic acid bacterium *Tetragenococcus halophilus* and *Escherichia coli*. Biosci. Biotechnol. Biochem., 72: 811-822.

Susin, M.F., R.L. Baldini, F. Gueiros and S.L. Gomes, 2006. GroES/GroEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in *Caulobacter crescentus*. J. Bacteriol., 188: 8044-8053.

Tomas, C.A., N.E. Welker and E.T. Papotasakis, 2003. Overexpression of *groESL* in *Clostridium acetobutylicum* resulted in increased solvent production and tolerance, prolonged metabolism and changes in cell's transcriptional program. Applied Environ. Microbiol., 69: 4951-4965.

Van de Guchte, M., P. Serror, C. Chervaux, T. Smokvina, S.D. Ehrlich and E. Maguin, 2002. Stress responses in lactic acid bacteria. Antonie van Leeuwenhoek, 82: 187-216.

Walker, D.C., H.S. Girgis and T.R. Klaenhammer, 1999. The groESL chaperone operon of *Lactobacillus johnsonii*. Applied Environ. Microbiol., 65: 3033-3041.

Walter, J., N.C.K. Heng, W.P. Hammes, D.M. Loach, G.W. Tannock and C. Hertel, 2003. Identification of *Lactobacillus reuteri* genes specifically induced in the mouse gastrointestinal tract. Applied Environ. Microbiol., 69: 2044-2051.

Whitaker, R.D. and C.A. Batt, 1991. Characterization of the heat shock response in *Lactococcus lactis* subsp. lactis. Applied Environ. Microbiol., 57: 1408-1412.

Yateem, A., M.T. Balba, T. Al-Surrayai, B. Al-Mutairi and R. Al-Daher, 2008. Isolation of lactic acid bacteria with probiotic potential from camel milk. Int. J. Dairy. Sci., 3: 194-199.