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ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (1): 13-22, 2007
ISSN 1028-8880
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# Cloning and Transcriptional Analysis of *groES* and *groEL* in Ethanol-producing Bacterium *Zymomonas mobilis* TISTR 548

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**Abstract:** Heat and ethanol had an affect not only on growth and cell viability of an obligatorily fermentative Gram-negative bacterium *Zymomonas mobilis*, but also on protein synthesis. Analysis by SDS-polyacrylamide gel electrophoresis revealed pronounced increasing of two dominant proteins designated as *groES* and *groEL*. Molecular cloning of the gene encoding *groES* and *groEL* was performed by PCR technique using specific primers synthesized based on the *Z. mobilis groESL* gene. Sequencing analysis of 2179 bp led to the detection of two open reading frames encoded for 95 and 549 amino acids, respectively. The deduced amino acid sequence of the *Z. mobilis groES* and *groEL* shows a high degree of identity with other. The strongly conserved carboxyl-terminus Gly-Gly-Met motif and two small segments, which appear more conserved between ethanol-producing organisms, were found, suggesting that their may be related to stability of protein under heat or ethanol stress. Induction of *groES* and *groEL* occurs in response to heat and ethanol, but not to salt stress.

**Key words:** *Zymomonas mobilis*, *groES*, *groEL*, ethanol production

## INTRODUCTION

The inhibitory effect of ethanol during fermentation is complex and is the main reason for slow and incomplete enological fermentation in many organisms. In *Saccharomyces cerevisiae* and *Escherichia coli*, for example, ethanol retards the specific growth rate, their viability and their fermentative rate (Jones and Greenfield, 1985; Ansanay-Galeote *et al*., 2001). Ethanol also modifies plasma membrane fluidity, stimulates the activity of plasma membrane $H^+$-ATPase and inhibits glucose transport (Salmon *et al*., 1993; Alexandre *et al*., 1993, 1994; Piper *et al*., 1994). Furthermore, ethanol also induces an increase in the proportion of acidic phospholipids and 18:1 fatty acid at the expense of 16:0 and a decrease in the lipid-to-protein ratio in *E. coli* (Ingram, 1977; Carey and Ingram, 1983). Recently, ethanol has been shown to have an effect identical to that of heat in stimulating the synthesis of specific proteins known as heat shock proteins (HSPs) or stress proteins (Michel and Starka, 1986; An *et al*., 1991; Michel, 1993). These evolutionarily proteins were originally characterized on the basic of their strong induction by heat shock, however they are also induced by a variety of stresses other than heat including ethanol, nalidixic acid, UV irradiation and viral infection (Craig, 1986).

*Zymomonas mobilis* is an obligatorily fermentative gram-negative bacterium that converts glucose, fructose and sucrose to ethanol and $CO_2$ via the Entner-Doudoroff (ED) pathway (Rogers *et al*., 1982; Sprenger, 1996). Unlike *S. cerevisiae* and *E. coli*, *Z. mobilis* is capable of growth in the presence of up to 10% ethanol and of fermentation in media with up to 25% glucose. Thus, *Z. mobilis* may have evolved specialized features to allow survival under these environmental stresses. Previous studies have reported that heat and ethanol induce the synthesis of stress proteins in *Z. mobilis*. This biological change has been proposed as being adaptive for growth and survival of this organism under environmental stresses. The first stress gene cloned and sequenced from *Z. mobilis* encoded alcohol dehydrogenase II (*adhB*), the most abundant alcohol dehydrogenase in this organism, which serves as a stress protein and a primary function in central metabolism (An *et al*., 1991). The promoter region for this gene shared homology with other stress promoters of *E. coli*. Recently, several stress-responsive proteins provisionally identified as *dnaJ* and *dnaK* in two-dimensional PAGE of cytoplasmic extracts from *Z. mobilis* have been cloned and sequenced. Furthermore, the cloning and expression in *E. coli* of the *Z. mobilis dnaK* gene have been performed (Arfman *et al*., 1992; Michel,

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1993). In this study we present some evidences for the existence of a typical stress response induced by heat and ethanol in ethanol-producing bacterium *Z. mobilis* TISTR 548. We report here that both heat and ethanol stresses have affected not only on bacterial growth and cell viability but also on gene expression. The synthesis of several stress-responsive genes was remarkably increased, of which at least two identified as *groES* and *groEL* were cloned and sequenced. Expression analysis of these genes under different stresses including ethanol, heat and salt stresses by RT-PCR was also demonstrated.

## MATERIALS AND METHODS

**Strains, plasmids and culture conditions:** *Z. mobilis* TISTR 548 (The culture collection of the Thailand Institute of Scientific and Technological Research, Bangkok) was used in this study. The culture was grown at 30°C in the YPG medium containing (per liter): 3 g of malt extract, 3 g of yeast extract, 5 g of peptone and 20 g of glucose (Michel *et al*., 1985). Solid medium was prepared for culture maintenance by adding 2% agar. *E. coli* strain DH5α and pGEM T-easy vector were used for all cloning procedures.

**Stress induction and cell viability:** An overnight culture was grown at 30°C in the YPG medium described previously. Ethanol or heat stress was provoked according to the following procedures. Ethanol stress; cells were cultivated to an optical density (OD 550 nm) of 0.8, ethanol was added to a final concentration of 3.5, 7, 10.5 and 14% (v/v): heat stress; at an optical density of 0.8 cultures were shifted from 30°C to 35, 40, 45 and 50°C. The time of the shift was set as zero and samples were taken at the time indicated in the relevant figure legends. Viability of cell was measured by plating the cell on solid YPG medium after stress treatments. Cell viability was estimated by counting colonies after 72 h incubation at 30°C.

**Protein extraction and SDS-polyacrylamide gel electrophoresis:** Ethanol- and heat-shocked cultures were harvested, washed and suspended in 10 mM Tris-HCl (pH 7.0) buffer. Proteins were extracted by sonicating cell suspension in Bioruptor (Cosmo Bio, USA) for 10 min at 50% pulser duty cycle and output power 5. The extracts were centrifuged at 12,000 g for 2 min. The supernatant fractions were removed and lyophilized. Protein concentration of the cell free extracts was measured using Lowry reagent. For electrophoresis, approximately 20 μg of protein sample was heated at 100°C for 5 min and separated by SDS electrophoresis on 12% acrylamide gel with constant voltage of 50 V. After electrophoresis, proteins separated on the gel were visualized using Coomassie Brilliant Blue R250 and fixed in 10% ethanol. For sequencing of the N-terminal amino acids, proteins separated in the gel were electroblotted onto an Immobilon-P transfer membrane (Millipore, USA) and stained with ponceau S. The stained protein of interest was excised and the N-terminal amino acid sequences were determined by using a model PPSQ-21A protein sequencer (Shimadzu, Japan).

**DNA manipulations, cloning and sequencing:** Genomic DNA of *Z. mobilis* was extracted using CTAB-DNA precipitation method (Del Sal *et al*., 1989). The genomic DNA was quantified spectrophotometrically. All restriction enzymes, calf intestine phosphatase, T4 DNA ligase and *Taq* DNA polymerase were used as recommended by the manufacturer (Takara, Japan). Agarose gel electrophoresis and colony hybridization were performed following standard protocols (Sambrook and Russell, 2001).

The *Z. mobilis groES* and *groEL* genes were generated by PCR using primers designed based on the *groESL* sequence of *Z. mobilis* (GenBank Accession number: L11654). The forward ZM1 (5'-TTCGTCCGCTACATGAT-3') and reverse ZM2 (5'-CTTCATGATGAGGAGGT-3') were used to amplify the *groES* gene and the forward ZM3 (5'-GATATTCTTGCCGATGC-3') and reverse ZM4 (5'- CGACAGATAGCCGCGAT-3') were used to amplify the *groEL* gene. For 100 μL of PCR reaction, 50 ng of *Z. mobilis* genomic DNA was used. Thermal cycling conditions were initial denaturation at 94°C for 3 min followed by 30 cycles at 94°C for 1 min, 58°C for 2 min and 72°C for 5 min and final extension at 72°C for 10 min using Ex. *Taq* DNA polymerase (Takara, Japan). The PCR products of approximately 250 and 540 bp for *groES* and *groEL*, respectively, were purified using QIAquick PCR purification kit (QIAGEN, Germany). The purified PCR product was cloned into pGEM T-easy vector and was transformed into *E. coli* DH5α using the Rapid DNA ligation and transformation kit (Fermentas). Sequencing of the *groES* and *groEL* region of *Z. mobilis* was performed by the dideoxynucleotide chain termination method with a SQ3000 nucleotide sequencer (Hitachi Electronics Engineering, Tokyo, Japan). The complete *groES* and *groEL* of *Z. mobilis* was PCR amplify by forward ZM5 (5'-AGGTGAAGATATGAAT-3') and reverse ZM6 (5'-GCGTAATTCAACCCATC-3') and cloned into pGEM T-easy vector. The sequence of the *groES* and *groEL* and deduced amino acid sequence were analyzed

using GENETYX (Software Development, Tokyo, Japan). Homology searching was performed using FASTA and BLAST program in the GenBank and DDBJ databases.

**RT-PCR analysis:** Reverse transcription-PCR (RT-PCR) was carried out with 0.1 μg of total RNA, prepared as described previously (Aiba *et al*., 1981) and the mRNA Selective PCR kit (Takara Shuzo, Japan). The primers for *groES* region were the forward ZM1 and reverse ZM2 and *groEL* region were the forward ZM3 and reverse ZM4. The RT-PCR products were then electrophoresed on a 0.9% agarose gel and after staining with ethidium bromide, the relative amounts of the products were densitometrically estimated by using a Bio-Rad molecular imager. As a control, 10 μg samples of total RNA were subjected to agarose gel electrophoresis (1.2% agarose) and stained with ethidium bromide. Linearity of the amplification was observed at least up to the 23nd cycle. The experiment was repeated at least twice. Under our conditions, the RNA-selective RT-PCR was able to specifically detect mRNA because no band was observed when reverse transcriptase was omitted.

## RESULTS AND DISCUSSION

**Effect of ethanol and heat stress on growth:** Growth of *Z. mobilis* as measured by optical density was largely decreased when initial concentration of ethanol in fermentation medium or incubation temperature was increased (Fig. 1A and B). More than 50% of growth was decreased when concentration of ethanol or incubation temperature higher than 7% and 35°C, respectively. In addition, no growth was observed when ethanol was added to a final concentration of 10.5 and 14% as well as when temperature was shifted from 30 to 45°C. The results suggest that both heat and ethanol had an effect on growth of *Z. mobilis*, like that observed in other organisms such as *S. cerevisiae* (Jones and Greenfield, 1985) and *Bacillus subtilis* (Li and Wong, 1992). The present results also similar to those of Carey and Ingram (1983) who observed decreased in growth rate of *Z. mobilis* when grown in medium containing ethanol higher than 1.04 M. One possible explanation for the inhibitory effect of heat and ethanol on growth of *Z. mobilis* is that these stresses decreased the effectiveness of the plasma membrane as a semipermeable barrier, allowing leakage of cofactors and coenzymes necessary for the activity of enzymes involved in central metabolism particularly the glycolysis and alcohol production pathway (Osman and Ingram, 1985).

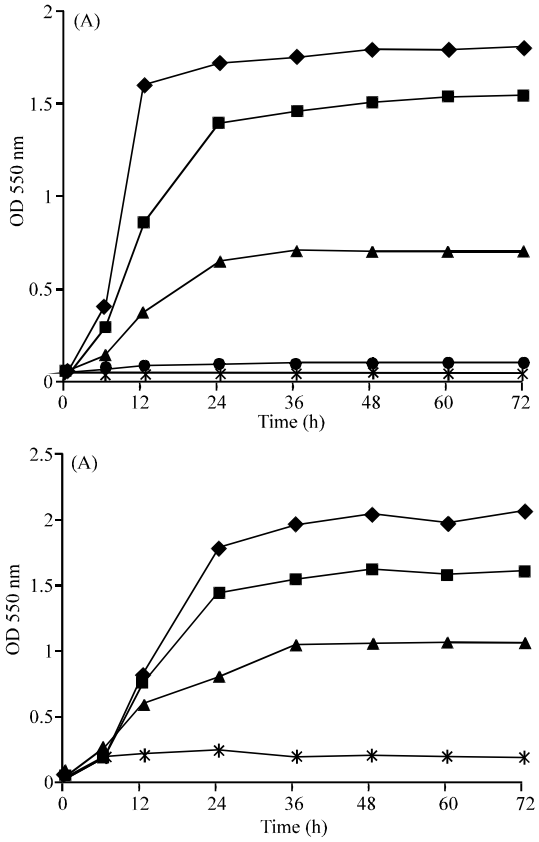


Fig. 1: Effect of ethanol or heat stress on growth of *Z. mobilis* TISTR548. Cells were grown in YPG medium and exposed to ethanol or heat stress. A, each stress of 3.5, 7.0, 10.5 and 14% ethanol at an initial concentration is represented by squares (■), triangles (▲), circles (●) and crosses (✱), respectively. Control of 0% ethanol at an initial concentration is shown by diamond (◆). B, each stress at 35, 40 and 45°C is represented by squares (■), triangles (▲) and crosses (✱), respectively. Control at 30°C is shown by diamonds (◆)

**Effect of heat and ethanol stress on cell viability:** Viability of *Z. mobilis* was slightly decreased when cells were incubated at 40°C for 1 h or addition of 7% ethanol. However, higher temperatures or ethanol concentrations caused dramatically decreased viability, resulting in only 81% at 45°C, 22% at 50°C and 59% at 10.5% and 10% at 14% ethanol, respectively (Table 1).

As expected, the lethality of exposure to stressful heat and ethanol levels was greatly reduced when cells were exposed to preheat treatment or sublethal ethanol. When cells were shifted from 30 to 45°C for 30 min and then transferred to 50°C for 30 min before plating, 67% of cells remained viable as compared to control cells without

Table 1: Viability of *Z. mobilis* TISTR548 under heat or ethanol stress

| Experimental treatment[a] | % of control |
|---|---|
| *Heat treatment* | |
| 30°C | 100 |
| 30°C → 40°C | 96 |
| 30°C → 45°C | 81 |
| 30°C → 50°C | 22 |
| *Ethanol treatment* (%) | |
| 0 | 100 |
| 7.0 | 93 |
| 10.5 | 59 |
| 14.0 | 10 |
| *Pretreatment* | |
| 30°C → 45°C (30 min) → 50°C (30 min) | 67 |
| 30°C (30 min, 7.0% ethanol) → 50°C (30 min) | 60 |
| 30°C (30 min, 7.0% ethanol) → 14.0% ethanol (30 min) | 43 |

[a]Treatment at each condition is for 1 h unless otherwise stated

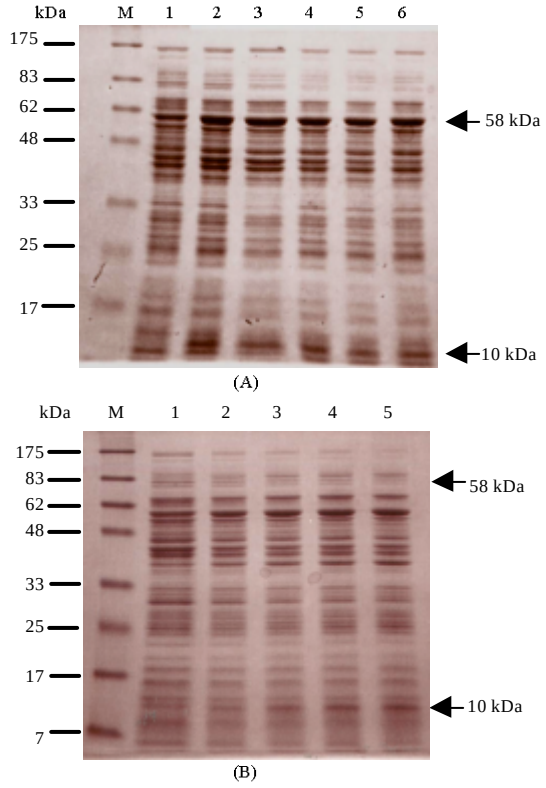


Fig. 2: SDS-polyacrylamide gel electrophoresis analysis of protein extracted from *Z. mobilis* TISTR548 after heat- or ethanol-stressed. A, proteins were extracted from cells incubated at 30°C (lane 1) and after exposed to heat at 45°C for 2 (lane 2), 4 (lane 3), 6 (lane 4), 8 (lane 5) and 10 h (lane 6). B, proteins were extracted from cells grown in YPG without addition of ethanol (lane 1) and in YPG with 10.5% ethanol and incubated for 2 (lane 2), 4 (lane 3), 6 (lane 4) and 8 h (lane 5). Positions of molecular mass markers (lane M) are indicated on the left and arrows indicate proteins of 58 and 10 kDa

pretreatment. Likewise, 60% of cells shifted to 50°C for 30 min retained viability when they were preincubated at 30°C for 30 min in YPG medium containing 7% ethanol. Moreover, 43% of cells subjected to 7% ethanol for 30 min prior to cultivate in YPG medium containing 14% ethanol retained viable. These results clearly indicate that heat and ethanol pretreatment increases tolerance of *Z. mobilis* to stressful heat and ethanol levels, which may be related to biological functions of some heat shock proteins synthesized during preexposure to a mild heat and ethanol shock.

**Effect of heat and ethanol on protein expression pattern:** It has been reported that induction of thermotolerance in many organisms such as *S. cerevisiae*, *B. subtilis* and *Neurospora crassa* is directly correlated with induction of specific set of HSPs (Plesofsky-Vig and Brambl, 1985). We therefore examine the effect of induced heat and ethanol tolerance on protein synthesis in *Z. mobilis*. Bacterial cells growing at 30°C in YPG medium were shifted to different high temperatures or cultivated in medium containing different levels of ethanol. Cells were collected at different times of exposure and proteins in the cellular extracts were separated by SDS-12% polyacrylamide gel. As shown in Fig. 2A and B, proteins, indicated by an arrow, with the apparent molecular weight of approximately 58 and 10 kDa, relative to protein standards, were remarkably induced in heat and ethanol-treated cells. These proteins were also detected as a faint signal particularly the 10 kDa polypeptide in non-treated cells growing at normal growth conditions (panel 1), indicating that the expression of stress proteins occurs not only in the heat or ethanol responses but also in the normal developmental process. The differential expression of the 58 and 10 kDa polypeptides in *Z. mobilis* suggests that these polypeptides are stress proteins play an important role in the cellular physiology and that they are involved in some essential functions in normal and shocked cells, like that observed in other organisms such as *Drosophila melanogaster* (Mason *et al*., 1984; Kurtz *et al*., 1986), *E. coli* (Fayet *et al*., 1989) and *S. cerevisiae* (Lindquist, 1984). In the figure, the protein patterns of heat and ethanol treated cells at different times of exposure to 45°C and 10.5%, respectively, are shown.

The N-terminal amino acid analysis of the 58 and 10 kDa polypeptides revealed distinct sequence, AAKDVKFSFD and MNFRPLLDDV, respectively. Homology search was performed and it was found that the sequences are identical to a region of polypeptides, the *groEL* and *groES* gene products, which are previously reported as the HSPs in *Z. mobilis* (Barbosa *et al*., 1994). Therefore, we designated 58 and 10 kDa polypeptides of *Z. mobilis* as GroEL and GroES, respectively.

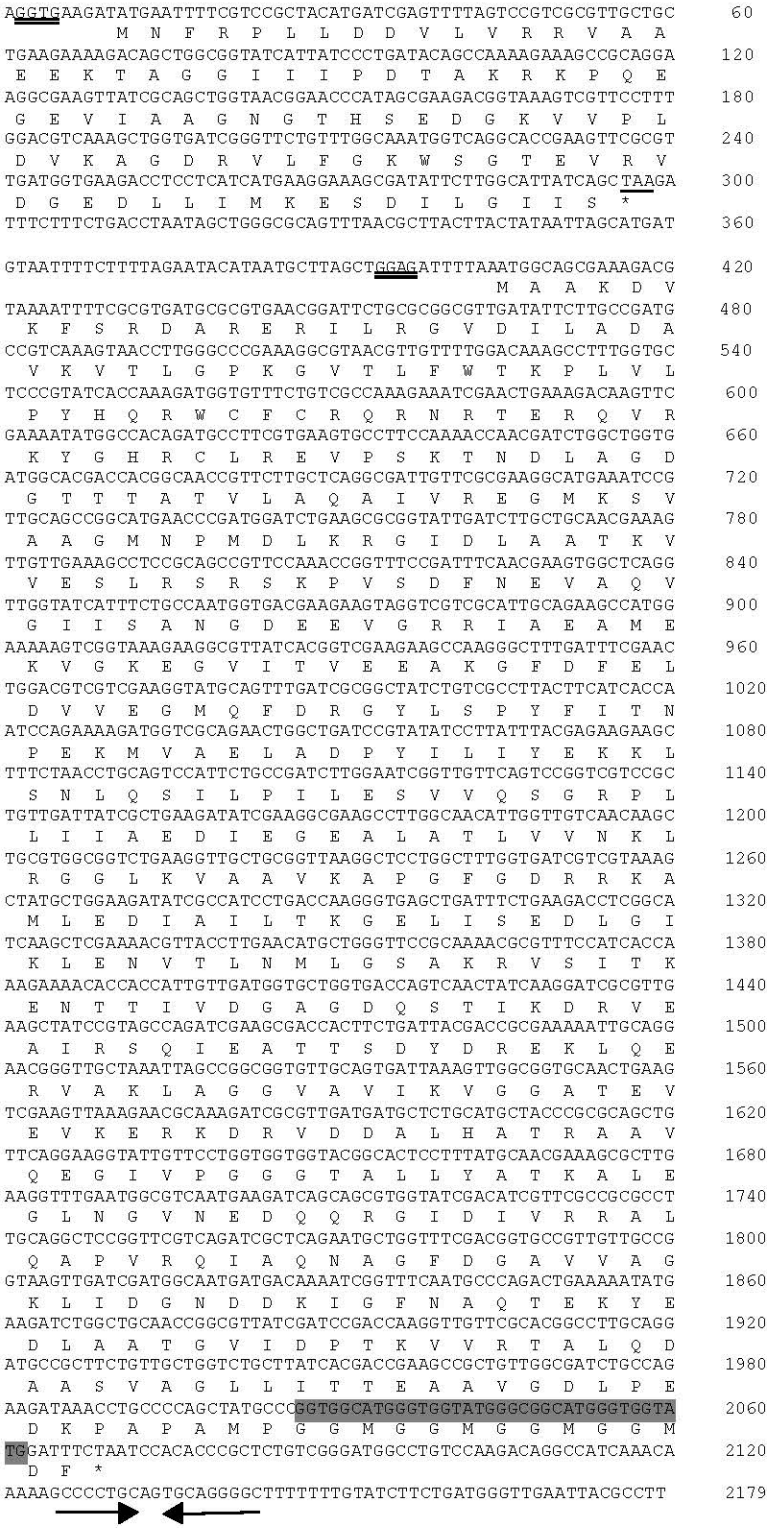


Fig. 3: Nucleotide sequences of the *Z. mobilis groES* and *groEL* gene and deduced amino acid sequence. Putative ribosome binding sites for *groES* and *groEL* are marked by double underlines. An asterisks mark the stop codon. The GGM motif is indicated by light shade. A palindromic sequence which may serve as a transcriptional terminator is marked with facing arrows

```
Zm   --------MNFRPLHDRVLVRRVAAEEKTAGGIIIPDTAKRKPQEGEVIAAGNGTHSEDG 52
Aa   -------MTKFRPLHDRVVVRRLEGEQKTAGGIIIPDTAQEKPMEGEVVAVGPGARNEQG 53
Bs   ---------MLKPLGDRVVIELVESEEKTASGIVLPDSAKEKPQEGKIVAAGSGRVLESG 51
Lp   ---------MLKPLGDRVIVEVVEEEEQTVGGIVLANNAKQKPQTGKVVAVGEGALTPEG 51
Sc   MSTLLKSAKSIVPLMDRVLVQRIKAQAKTASGLYLPEKNVEKLNQAEVVAVGPGFTDANG 60
                : ** ***::. :  : :*..*: :.:.  .*   .:::*.* *    .*

Zm   KVVPLDVKAGDRVLFGKWSGTEVRVDGED-LLIMKESDILGIIS-- 95
Aa   QIVALDVKAGDRVLFGKWSGTEVKIDGEE-LLIMKESDIMGVVTA- 97
Bs   ERVALEVKEGDRIIFSKYAGTEVKYEGTE-YLILRESDILAVIG-- 94
Lp   KRLPMAVKVGDTVLYDKYAGSEVKYEGQD-YLVLHEKDIKAIA--- 93
Sc   NKVVPQVKVGDQVLIPQFGGSTIKLGNDDEVILFRDAEILAKIAKD 106
     : :   ** ** ::  ::.*: ::  . :  ::::: :* .
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Fig. 4: Sequence alignments of *Z. mobilis groES* (Zm) *A. aceti groES* (Aa) *B. subtilis groES* (Bs) *L. paracasei groES* (Lp) and *S. cerevisiae hsp*10 (Sc). Amino acids residues identical and similar between each other are shown by asterisks and dots, respectively. Gaps introduced for alignment are indicated by a horizontal dash

**Cloning and sequence analysis of the *Z. mobilis groES* and *groEL* genes:** The *Z. mobilis groES* and *groEL* genes was cloned and sequenced as described in materials and methods. Sequencing analysis revealed the presence of two contiguous Open Reading Frames (ORFs) encoding the entire amino acid sequence of the *groES* and *groEL* gene product. The nucleotide sequence of these ORFs, its flanking region and the deduced amino acid sequence are shown in Fig. 3. The *groES* and *groEL* are present as a single copy in the genome of *Z. mobilis*, as demonstrated by Barbosa *et al.* (1994). The *groES* encodes a polypeptide of 95 amino acid residues with calculated relative molecular masses of 10, 290 Da. This polypeptide exhibits significant sequence identity to the GroES of *Acetobacter aceti* (71%) (Okamoto-Kainuma *et al.*, 2002), *B. subtilis* (53%) (Schmidt *et al.*, 1992), *Lactobacillus paracasei* (46%) (Desmond *et al.*, 2004) and hsp10 of *S. cerevisiae* (34%) (Hohfeld and Hartl, 1994) (Fig. 4). The *groEL* encodes a polypeptide of 549 amino acid residues with calculated relative molecular masses of 58,428 Da. The start codon of *groEL* is 106 nucleotides downstream from the stop codons of *groES*. The *groEL* contains a canonical ribosomal-binding site with a GGAG Shine-Dalgarno region while that for *groES* is less conserved (Barbosa *et al.*, 1994). The strongly conserved carboxyl-terminus Gly-Gly-Met (GGM) motif as seen in GroEL proteins of various species was also present in the *Z. mobilis* GroEL protein. Its biological function remains unclear, however, it is appealing to speculate from the GroEL protein encoded by thermophilic bacterium PS3 that the number of GGM motifs at the C-terminus of GroEL will prove to be correlated with the thermostability of the protein (Schon and Schumann, 1993). The encoded protein sequences for *Z. mobilis groEL* exhibits significant sequence identity to the GroEL of *A. aceti* (70%) (Okamoto-Kainuma *et al.*, 2002), *B. subtilis* (58%) (Schmidt *et al.*, 1992), *L. paracasei* (56%) (Desmond *et al.*, 2004) and hsp60 of *S. cerevisiae* (52%) (Johnson *et al.*, 1989) (Fig. 5). Two small segments in the C-terminus in which more conservation of sequence is evident between *Z. mobilis* and *S. cerevisiae* were found, suggesting that its may be related to stress responses in high ethanol environment (Barbosa *et al.*, 1994).

**Analysis of the *groES* and *groEL* expression:** The expression of *Z. mobilis groES* and *groEL* was investigated by RT-PCR using total RNA prepared either from 3 hours after the cells had been transferred to heat shock at 37, 40 and 45°C or to ethanol shock at 3.5, 7.0, 10.5 and 14.0% or to salt stress at 1.5, 3.0 and 6.0% NaCl as a template. The RT-PCR experiments showed that *groES* and *groEL* expression was about three to sixfold increased in heat and ethanol treated cells, as compared to non-treated cells (Fig. 6A and B). These results suggest that *groES* and *groEL* expression is relatively low in non-stressed cells, but increase rapidly in cell upon heat and ethanol stressed. The present results also revealed that induction of the *groES* and *groEL* is regulated at the level of transcription. This can be accomplished by increased synthesis of mRNA as proposed by Schon and Schumann (1993). In some certain organisms, such as *Enterococcus faecalis* (Laport *et al.*, 2004), the *groES* and *groEL* had been shown to highly express under salt stress conditions. This led us to further investigate the expression of *groES* and *groEL* under salt stress. As we found in the present study, there was no significant difference in the expression of *groES* and *groEL* mRNA between stressed and non-stressed cells

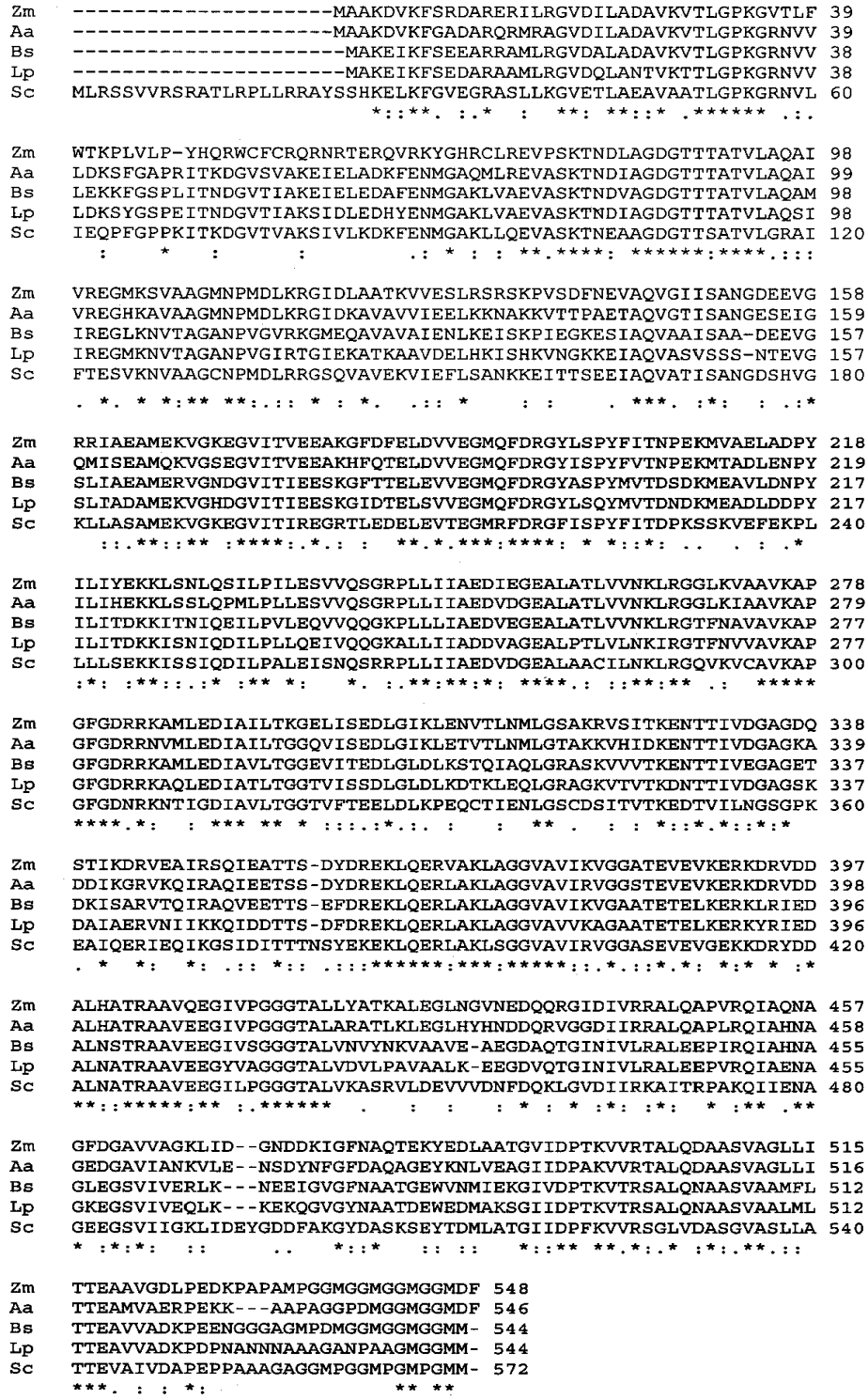


Fig. 5: Sequence alignments of *Z. mobilis groEL* (Zm) *A. aceti groEL* (Aa) *B. subtilis groEL* (Bs) *L. paracasei groEL* (Lp) and *S. cerevisiae hsp*60 (Sc). Amino acids residues identical and similar between each other are shown by asterisks and dots, respectively. Gaps introduced for alignment are indicated by a horizontal dash

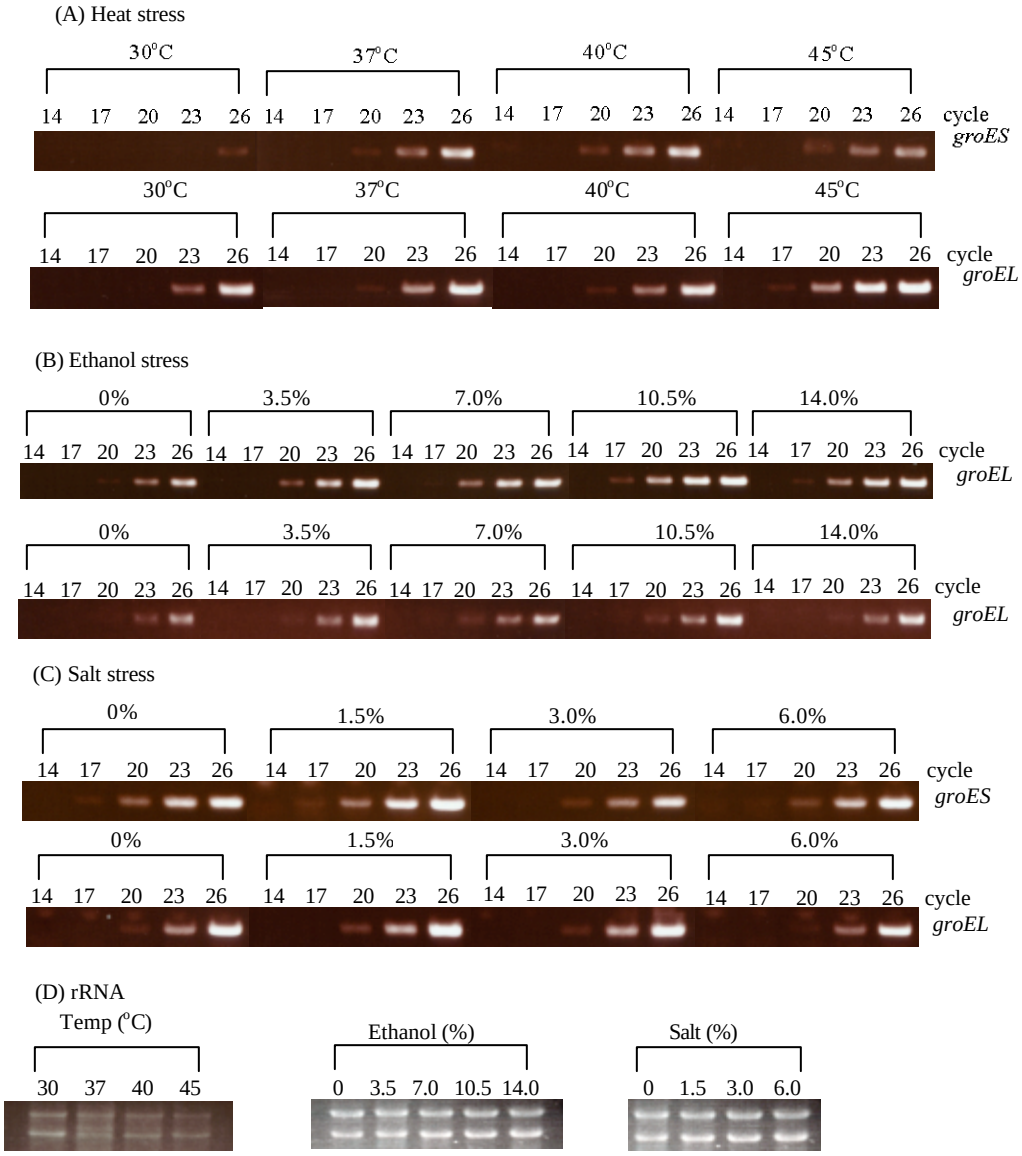


Fig. 6: RT-PCR analysis of the *groES* and *groEL* expression under heat, ethanol and salt stresses. The logarithmic phase of cells grown in YPG medium was exposed to heat (A), ethanol (B) and salt stress (C) for 3 hours and total RNAs were then prepared and subjected to RT-PCR analysis with primers specific for *groES* and *groEL* as described in Materials and Methods. The numbers above the lanes represent cycles of PCR. (D) rRNA extracted from heat-, ethanol- and salt-treated cells was used as a control. Total RNAs (10 μg) in panels A to C were used

under the experimental conditions followed in this assay (Fig. 6C). These results suggest that the expression of *Z. mobilis groES* and *groEL* is not correlated to salt stress response, likes that observed for *E. coli* (Gross *et al*., 1990) and *B. subtilis* (Volker *et al*., 1992).

Proteins induced by heat as well as ethanol shock have been shown to play an essential role in bacterial physiology under heat or ethanol stresses. In *E. coli*, for example, the heat shock proteins *groES* and *groEL* are well known for their roles in protein folding as molecular

chaperones, promoting the assembly, disassembly or translocation of other proteins (Weissman *et al*., 1995, 1996). Furthermore, the *groES* and *groEL* genes products in particular are essential for *E. coli* growth at all temperature (Fayet *et al*., 1989). In *B. stearothermophilus*, the *groES* and *groEL* genes had function homology to known GroE proteins, promoting growth of bacteria at high temperature and propagation of phage lambda (Schon and Schumann, 1993). With respect to physiological function of the *Z. mobilis groES* and *groEL*, it is not clear at present. The direct involvement of these genes as molecular chaperones could be elucidated by gene disruption experiments.

The present study clearly demonstrated that heat and ethanol stresses had an affect not only on growth and cell viability but also on protein synthesis. The stress of *Z. mobilis* by heat and ethanol increased the synthesis of several polypeptides, of which at least two, designated as GroES and GroEL, were heat shock proteins. The physiological significance of these proteins in the stress response is still unknown, but there is reason to believe that they are involved in the mechanism of tolerance of *Z. mobilis* and probably of other cells to heat and ethanol. Further experiments are needed to determine the mechanism of transcription regulation and cellular location of the *Z. mobilis groES* and *groEL*.

## ACKNOWLEDGMENTS

This study was done by collaboration in a core university program between Yamaguchi University and Kasetsart University, which was supported by the Scientific Cooperation Program agreed to by the Japan Society for the Promotion of Science (JSPS) and the National Research Council of Thailand (NRCT). The authors would like to thank the Energy Conservation Promotion Fund for a grant to K.S.

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