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Magnesium Ions Improve Growth and Ethanol Production of *Zymomonas mobilis* under Heat or Ethanol Stress

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Abstract: Like other ethanologenic organisms, *Zymomonas mobilis* showed a significant decrease in cell viability and fermentation capability when the initial ethanol concentration or temperature was elevated. The cell mass and ethanol production were largely decreased at more than 35°C or in the presence of higher than 7% (by vol) ethanol at an initial concentration. Neither growth nor ethanol production was observed at more than 40°C or at higher than 14% (by vol) ethanol. The supplementation of magnesium (10-20 mM) dramatically improved the negative effects by these stresses. Magnesium also increased in fermentation ability and repressed the synthesis of stress proteins under such conditions. These results clearly demonstrated the usefulness of magnesium for ethanol fermentation in *Z. mobilis* under heat or ethanol stress.

Key words: Ethanol, *Zymomonas mobilis*, magnesium ions, fermentation

INTRODUCTION

The Gram-negative ethanologenic bacterium, *Z. mobilis*, utilizes the Entner-Doudoroff (ED) (2-keto-3-deoxy-6-phosphogluconate, KDPG) pathway for sugar catabolism and produces ethanol and carbon dioxide as dominant fermentation products (Swings and DeLey, 1977; Sprenger, 1996). This bacterium may be mostly dependent on the substrate-level phosphorylation for energy acquisition and obtains about one mole of ATP per mole of glucose utilized via the ED pathway. As a consequence, it shows less biomass production than yeast and thus more efficient conversion to fermentation products. This organism appears to maintain a high glucose flux through the ED pathway to compensate its low ATP yield (Barnell *et al.*, 1990). Unlike *Saccharomyces* sp. which are capable of oxidative and fermentative growth, the pyruvate-to-ethanol pathway is indispensable for NADH oxidation in *Z. mobilis*. The ethanologenic pathway in *Z. mobilis* consists of a single pyruvate decarboxylase (PDC) and two isozymes of alcohol dehydrogenase (ADH). These three enzymes and all of the glycolytic enzymes comprise 30-50% of the total soluble proteins in *Z. mobilis* (Vega *et al.*, 2000).

During ethanol fermentation, *Z. mobilis* may encounter various environmental stresses which adversely affect the ability of cells to perform efficient and consistent conversion of sugars to ethanol. The major chemical and physical stresses experienced by the organism are ethanol toxicity and temperature stress. Ethanol and heat stresses retard the growth rate, viability (Michel and Starka, 1986) and fermentation ability of the cells (Osman and Ingram, 1985). They also modify plasma membrane fluidity (Carey and Ingram, 1983; Moreau *et al.*, 1997) and trigger specific stress responses (Michel and Starka, 1986; An *et al.*, 1991; Barbosa *et al.*, 1994). In addition, ethanol and heat stresses can also cause disruption of cellular ionic homeostasis, leading to a reduction of metabolic activity and eventually cell death.

Magnesium is an important divalent cation in metabolic processes and physiological functions, including cell growth, cell division and enzyme activity in yeast (Walker, 1994). Magnesium ions decrease protons and especially, anion permeability of the plasmalemma by interacting with membrane phospholipids, stabilizing the membrane bilayer. The protective effect of magnesium in response to toxic levels of ethanol in *S. carlsbergensis* (Petrov and Okorokov, 1990) has suggested that it plays

a crucial role in the cellular protection and recovery from the stress. Several reports have also demonstrated that magnesium ions are implicated in the amelioration of the detrimental effects of stress in *S. cerevisiae* (Dombek and Ingram, 1986; Blackwell *et al.*, 1997; Birch and Walker, 2000). However, in respect to the ethanologenic *Z. mobilis*, studies on such a protective effect of magnesium ions have hardly been reported.

Here, we examined the effect of magnesium ions on growth and ethanol production under heat or ethanol stress in *Z. mobilis*. Our results indicate that magnesium acts as a stress protectant and improves the fermentation performance under such stresses.

MATERIALS AND METHODS

Bacterium and growth conditions: *Z. mobilis* TISTR 548 (the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok) was grown in YPG (0.3% yeast extract, 0.5% peptone, 3% glucose) medium (Michel *et al.*, 1985). When necessary, sugar stock solution autoclaved separately was added. Media were inoculated with 10% of a preculture at mid-log phase. For ethanol-stress experiments, absolute ethanol was added to sterile medium. Cells were grown anaerobically at 30°C in 500 mL⁻¹ Erlenmeyer flask filled with 400 mL⁻¹ of medium and the flask was equipped with an air-lock (Taherzadeh *et al.*, 1997). Cultures were maintained on medium solidified with 2% agar at 4°C before use.

Cell growth and viability: Under stress conditions as described below, cell growth (cell mass) was monitored by measuring optical density at 550 nm with a Shimadzu UV-1601 spectrophotometer; an optical density at 550 nm (OD₅₅₀) of 0.8 corresponded to 4×10⁸ cells mL⁻¹ or 0.180 mg of dry cell mass per mL. Cell viability was estimated by measuring Colony Forming Units (CFU) or viable cell staining as described below. For measurement of CFU, at appropriate time intervals, a part of culture was diluted and spread on YPG plates. After 72 h of incubation at 30°C, the colony number on the plate was counted.

Ethanol stress: To examine the effect of different concentrations of ethanol on growth and ethanol production of *Z. mobilis*, ethanol at a final concentration of 0, 3.5, 7.0, 10.5 or 14.0% (by vol) was added into growth media and culture was incubated at 30°C. The protective effect of magnesium against ethanol stress was tested according to the method described by Birch and Walker

(2000). Briefly, the culture was exposed to 10.5% ethanol at 30°C in the presence of 10 or 20 mM magnesium. Control cultures were performed in parallel with and without ethanol.

Heat stress: Cultures were exposed to heat shock by upshifting incubation temperatures from 30°C to 35, 40 or 45°C. To test the effect of magnesium, the culture was exposed to heat shock at 40°C in the presence of 10 or 20 mM magnesium. Control cultures were performed at 30°C and 40°C without magnesium.

Morphological observation: Cells were grown in YPG medium and subjected to stresses as described above. Cells were collected at different time intervals, centrifuged and resuspended in 50 mM potassium phosphate buffer (pH 6.5). They were stained with fluorescence staining solutions of Calcein-AM, Hoechst 33342 and PI (Dojin, Japan) at a final concentration as recommended by manufacturer. After incubation at room temperature for 15 min, morphology of the stained cells was observed under a Nikon E600 microscope with fluorescence capability (Nikon, Tokyo, Japan). Photographs were taken with a charge-coupled device camera with an exposure time of 10 ms and printed by using a CP710A printer (Mitsubishi, Tokyo, Japan).

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.

Chemical analyses: Ethanol was analyzed by gas chromatography (Shimadzu, Model 14B; Japan, equipped with a Parapak Q column) using isopropanol as an internal standard. A flame ionization detector and integrator were used for detection and quantitative determination, respectively. Total reducing sugars were measured using dinitrosalicylic acid (DNS) (Miller, 1959).

All experiments were performed in quadruplet and repeated twice. The data are means of the results of the experiments.

RESULTS AND DISCUSSION

Effect of ethanol or heat stress on cell growth: In order to examine the effect of ethanol or heat stress on *Z. mobilis* growth, 10% of the mid-log phase cells grown at 30°C was added into 400 mL⁻¹ YPG medium and subjected to ethanol or heat stress as described in materials and methods. The results found that increase in concentration of ethanol supplemented and in temperature caused significant decrease in bacterial cell mass. Cell density was largely decreased when the initial ethanol concentration in the fermentation medium or incubation temperature were higher than 7% or 35°C, respectively. At 14% ethanol of the initial concentration and at 45°C, almost no growth was observed (data not shown). These results suggest that both stresses give a drastic effect on growth of *Z. mobilis* as in other organisms such as *S. cerevisiae* (Jones and Greenfield, 1985) and *Bacillus subtilis* (Li and Wong, 1992). The results are also consistent with those of Carey and Ingram (1983) in which *Z. mobilis* showed a significant reduction in growth rate in the presence of ethanol higher than 1.04 M. Ethanol or heat stress may inhibit RNA and protein synthesis and increase membrane leakage in *Z. mobilis* as demonstrated (Osman and Ingram, 1985) and thereby cause the reduction in its growth and cell mass.

Effect of ethanol or heat stress on ethanol production:

The effect of ethanol and heat on the conversion rate of sugar to ethanol was tested. The addition of ethanol or the elevation of incubation temperature also showed an immediate effect on fermentation ability of *Z. mobilis* as shown in Fig. 1. As ethanol concentration or temperature increases, progressive decrease in ethanol yield was observed. Initial concentrations of 3.5, 7 and 10.5% ethanol in the fermentation medium resulted in about 30, 60 and 90% reduction in ethanol yield, respectively. No ethanol was produced when the initial concentration of ethanol was 14%. With respect to temperature, no significant influence of elevated incubation temperature was seen at 35°C compared to that at 30°C. At 40°C, however, a pronounced decreased in ethanol production was observed, about 80% reduction in ethanol yield and at 45°C, no ethanol was produced. These results clearly demonstrate that ethanol and heat stresses caused inhibition of fermentation in *Z. mobilis*. One possible explanation that has been proposed for such detrimental effects is that high ethanol concentration or temperature

decreases the effectiveness of the plasma membrane as a semipermeable barrier, allowing leakage of essential cofactors and coenzymes required for the activity of enzymes involved in glucose catabolism and alcohol production (Osman and Ingram, 1985).

Effect of magnesium on cell viability and morphology under ethanol or heat stress conditions:

The cellular responses to ethanol or heat stress appears to be varied, including many aspects of cellular metabolism and physiology, of which the most obvious and irreversible one is cell death. We thus examined the impact of these stresses on cell viability. The heat stress treatment was performed by upshifting the temperature from 30 to 40°C in YPG medium containing 3% glucose and the ethanol stress treatment was done by the addition of ethanol

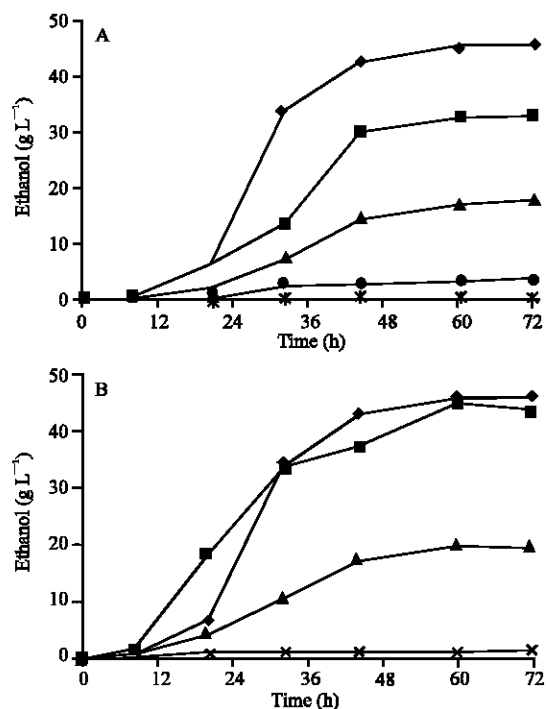


Fig. 1: Effect of ethanol or heat stress on ethanol production of *Z. mobilis*. Cells were grown in YPG medium containing 16% glucose and exposed to ethanol or heat stress. A, each stress of 3.5, 7.0, 10.5 and 14% ethanol at an initial concentration is represented by squares (■), triangles (▲), circles (●) and crosses (x), respectively. Control of 0% ethanol at an initial concentration is shown by diamonds (◆). B, each stress at 35, 40 and 45°C is represented by squares (■), triangles (▲) and crosses (x), respectively. Control at 30°C is shown by diamonds (◆)

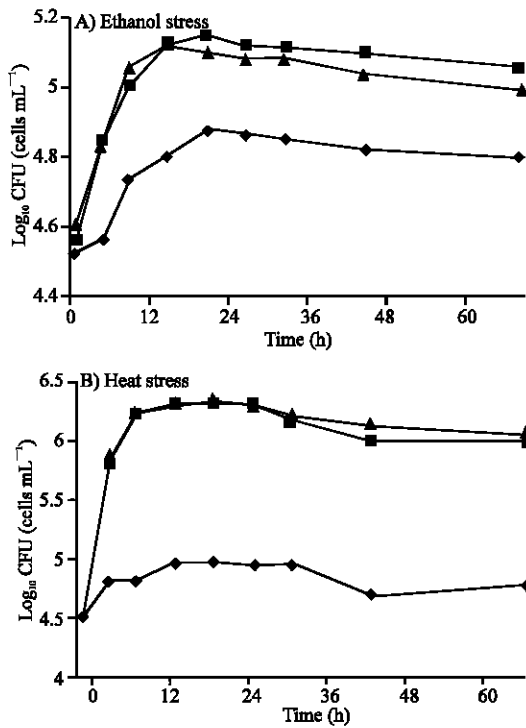


Fig. 2: Protective effect of magnesium on viability of ethanol- or heat-stressed cells in *Z. mobilis*. Cells were grown in YPG medium containing 16% glucose. Cells were exposed to ethanol stress (A), 10.5% ethanol at the initial concentration, or to heat stress (B) at 40°C. Magnesium was added at a final concentration of 0, 10, or 20 mM (diamonds (♦), squares (■) and triangles (▲), respectively) at 0 h

at a final concentration of 10.5% into YPG medium containing 3% glucose, followed by incubation at 30°C. The number of viable cells was determined by staining with Calcein-AM solution and cell morphology was observed after staining as described in material and methods. Table 1 summarized the viability of cells under heat or ethanol stress for 6 or 12 h. The heat or ethanol stress caused significantly decrease in cell viability to be 60% at 6 h and 51% at 12 h and 56% at 6 h and 23% at 12 h, respectively.

In yeast, magnesium has been shown to reduce the mortality effect of exposure to heat or ethanol shock, maintaining viability levels over 50% (Birch and Walker, 2000). Therefore, the impact of magnesium supplementation on viability of *Z. mobilis* under stresses was examined. Elevation of magnesium concentration in the medium to 10 and 20 mM significantly increased cell viability compared to the control without magnesium supplement, indicating resistance to the subsequent heat

Table 1: Effect of magnesium on viability of *Z. mobilis* under ethanol or heat stress

Treatment conditions	Survival (% of control) ^a	
	6 h	12 h
Heat treatment		
30°C (control)	100.00±10.18	100.00±7.77
30°C - 40°C	60.10±6.31	51.37±9.19
30°C - 40°C+10 mM Mg	100.00±9.86	94.10±6.87
30°C - 40°C+20 mM Mg	99.38±6.19	97.55±4.95
Ethanol treatment		
0% (control)	100.00±10.18	100.00±7.77
10.5%	56.23±2.39	23.42±7.01
10.5%+10 mM Mg	83.61±6.72	49.96±4.96
10.5%+20 mM Mg	73.90±4.65	44.59±10.29

^a±SD

or ethanol treatment. More than 90% of cells retained viability even after 12 h heat treatment and more than 70 or 40% of cells remained viable for 6- or 12 h ethanol treatment, respectively, in the presence of magnesium (Table 1). The long-term protection of cells from stresses by magnesium is also evident from Fig. 2, which shows improvement in survival of cells at both 10 and 20 mM Mg. These results suggest that magnesium exerts a protective role for *Z. mobilis* cells toward heat or ethanol stress, enabling cells to remain viable at high levels for relatively long periods.

Surprisingly, the addition of magnesium caused cells to be large and long in 12 h incubation under heat stress at 40°C, but did not under ethanol stress at 10.5% (Fig. 3). Several studies have been reported that osmotic pressure, ionic stress, increased phosphate levels, calcium ions or chloride ions gave rise to filamentous cells in *Z. mobilis* (Fein *et al.*, 1984; Stevnsborg and Lawford, 1986; Vriesekoop *et al.*, 2002). There is, however, no report regarding such influence of magnesium on cell size. Further study to clarify this phenomenon is therefore necessary.

Effect of magnesium on ethanol production under ethanol or heat stress:

It is evident from Table 1 and Fig. 2 and 3 that magnesium restored viability of cells under stress conditions tested. Osman and Ingram (1985) also demonstrated that the inhibition of fermentation by increased ethanol concentrations in *Z. mobilis* was primarily due to ethanol-induced leakage, particularly of magnesium. We thus examined the influence of magnesium supplementation on fermentation ability of *Z. mobilis* (Fig. 4). The addition of magnesium at 10 or 20 mM increased in glucose consumption rate (data not shown) and in ethanol production rate. After 32 h of incubation, magnesium-supplemented cultures showed 7- and 4-fold (20 and 10 mM magnesium, respectively) higher ethanol yield under ethanol stress and almost 5-fold higher ethanol yield under heat stress than those of the

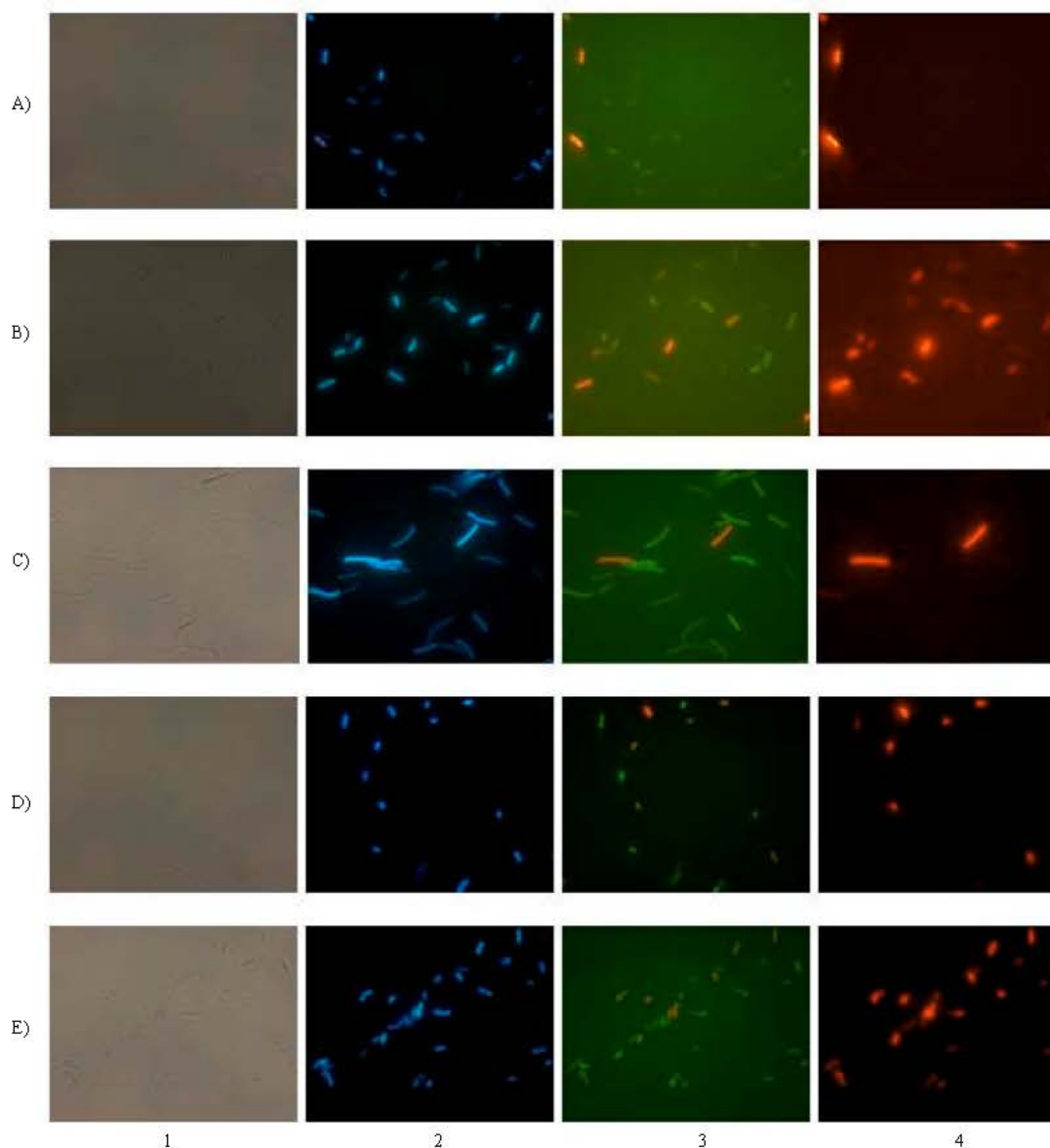


Fig. 3: Morphology of the *Z. mobilis* cells under heat and ethanol stressed at 6 h after stress treatments. Cells were grown in YPG medium and incubated at 30°C (A), or subjected to heat stress at 40°C (B), or to heat stress at 40°C in the presence of 10 mM Mg (C), or to ethanol stress at 10.5% (D), or to ethanol stress at 10.5% in the presence of 10 mM Mg (E). Cells without staining (1) and stained with Hoechst (2), Calsein AM (3) and PI (4) staining solution are presented

control cells. These results demonstrate that magnesium reduced the sensitivity in fermentation ability to ethanol or heat. It may be possible that the decline in fermentation ability under ethanol or heat stress is related in part to a magnesium deficiency. Indeed, a variety of enzymes in ED pathway require magnesium as a cofactor, including

glucokinase, glucose-6-phosphate dehydrogenase, phosphoglycerate kinase and enolase. The leakage of magnesium ions, therefore, may result in reduction in fermentation ability as demonstrated (Millar *et al.*, 1982; Osman and Ingram, 1985; Dombek and Ingram, 1986; Birch and Walker, 2000).

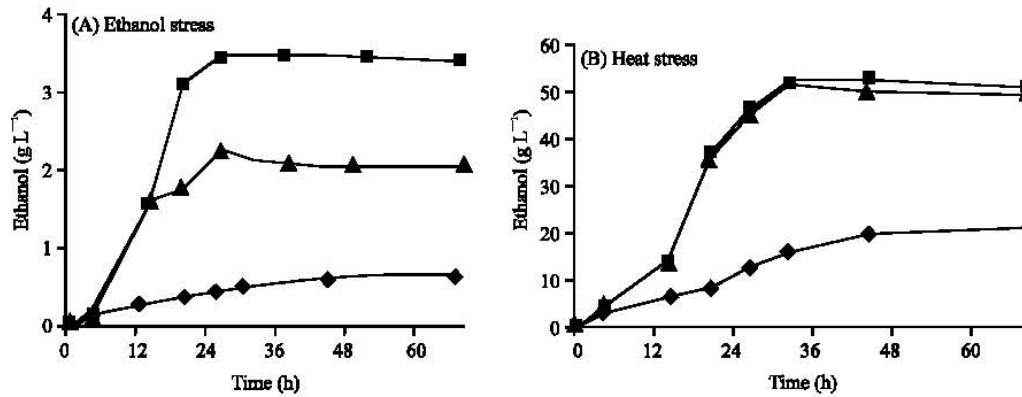


Fig. 4: Effect of magnesium on ethanol production of *Z. mobilis*. Cells were grown in YPG medium containing 16% glucose. Cells were exposed to ethanol stress (A), 14% ethanol at the initial concentration, or to heat stress (B) at 40°C. Magnesium was added at a final concentration of 0, 10, or 20 mM (diamonds (◆), squares(■) and triangles (▲), respectively) at 0 h

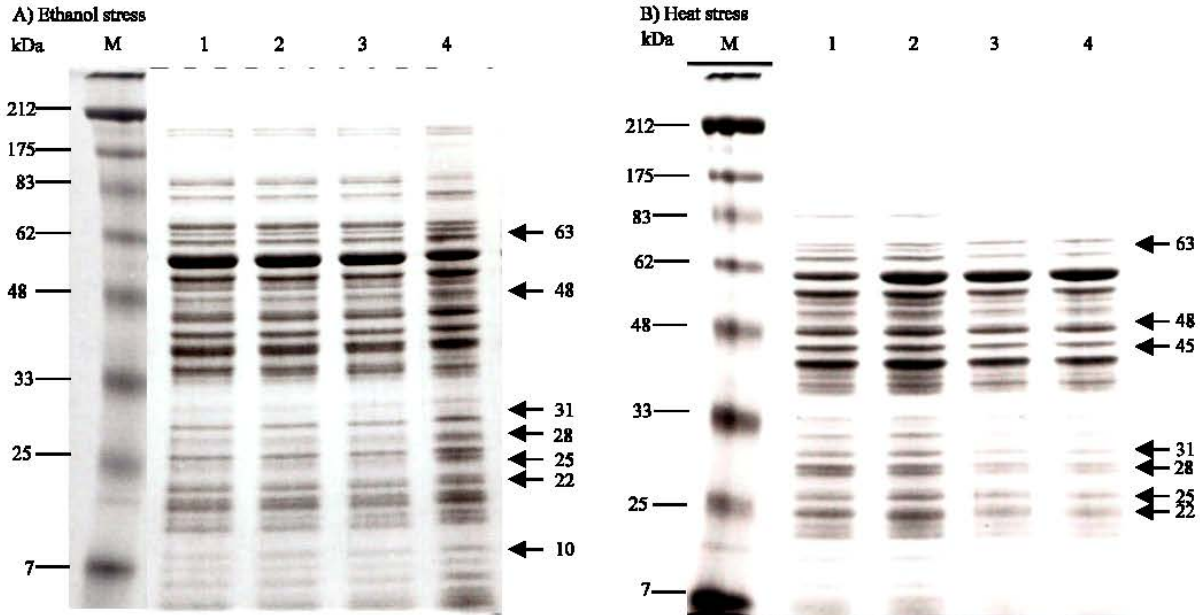


Fig. 5: Effect of elevated magnesium concentration on biosynthesis of stress proteins in *Z. mobilis*. A, proteins were extracted from cells grown at 30°C (1), cells ethanol stressed with 10.5% at 30°C in 10 mM Mg (2), cells ethanol stressed with 10.5% at 30°C in 20 mM Mg (3) and cells ethanol stressed with 10.5% at 30°C (4). B, proteins were extracted from cells grown at 30°C (1), cells heat shocked at 40°C (2), cells heat shocked at 40°C in 10 mM Mg (3) and cells heat shocked at 40°C in 20 mM mg (4). Positions of molecular mass markers (M) are indicated on the left and arrows indicate stress proteins

Effect of magnesium on stress protein synthesis under ethanol or heat stress: Activation of stress response to protect stressed cells is a transient molecular response culminating in differential gene expression and the production of stress-responsive proteins. The effect of altered magnesium concentration on production of stress proteins in *Z. mobilis* is shown in Fig. 5. After a 3 h ethanol shock at 10.5%, stress proteins were visible at: 63, 48, 31 kDa and some lower molecular weights (Fig. 5A),

compared to their absence in control cultures (at 30°C without ethanol). These results compare favorably with those of An *et al.* (1991) who observed the induction of stress proteins at 63, 48, 31 and 26 kDa in *Z. mobilis* cells exposed to ethanol. The addition of magnesium at 10 or 20 mM reduced the synthesis of most of these stress proteins (Fig. 5A), suggesting that magnesium protects cells from ethanol stress, thus negating the requirement for stress protein synthesis.

Similarly, exposure to 40°C caused the formation of stress proteins as part of a stress response. These heat shocked proteins were visible at: 63, 48, 45, 31 kDa and some lower molecular weights (Fig. 5B). Reduction of most of these heat shock proteins synthesis was exhibited in heat-shocked cells on addition of magnesium at 10 and 20 mM. Elevation of magnesium appears to negate the requirement for stress protein synthesis in response to elevated temperatures suggesting that magnesium can take over the role of heat shock proteins in conferring thermotolerance as demonstrated by Birch and Walker (2000).

CONCLUSIONS

Magnesium constitutes a major portion of the cellular cations and the various roles of it in cellular processes have been demonstrated. Most of magnesium occurs as a bound form in structures such as ribosome and the cell envelope. The free cation concentration, however, may play a more direct role in regulating overall cellular metabolism, cell division and membrane integrity (Walker and Duffus, 1980; Hu *et al.*, 2003). Many of the enzymes that function in DNA replication, transcription and translation require magnesium for their activities. In fermentation pathways, magnesium is also a crucial cofactor and nucleotide counterion in many reactions. Magnesium is typically maintained at the millimolar level in cells and would be one of the limiting factors under stress conditions. With regard to heat or ethanol stress, present results suggest that increasing magnesium availability may partially counteract stress responses by acting in a protective manner. Specifically, magnesium exerts protective effects on stressed cells resulting in reduction in cell mortality and repression of stress protein biosynthesis. On the contrary, a complete understanding of the biochemical basis for the decline in growth, cell viability and fermentation ability in *Z. mobilis* under stress condition may require identification of the factors responsible for the termination of exponential growth and determination of the associated physiological and enzymatic changes.

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REFERENCES

- An, H., R.K. Scopes, M. Rodriguez, K.F. Keshav and L.O. Ingram, 1991. Gel electrophoretic analysis of *Zymomonas mobilis* glycolytic and fermentative enzymes: identification of alcohol dehydrogenase II as a stress protein. *J. Bacteriol.*, 173: 5975-5982.
- Barbosa, M. de F.S., L.P. Yomano and L.O. Ingram, 1994. Cloning, sequencing and expression of stress genes from the ethanol-producing bacterium *Zymomonas mobilis*: The *groESL* operon. *Gene*, 94: 51-57.
- Barnell, W.O., K.C. Yi and T. Conway, 1990. Sequence and genetic organization of a *Zymomonas mobilis* gene cluster that encodes several enzymes of glucose metabolism. *J. Bacteriol.*, 172: 7227-7240.
- Birch, R.M. and G.M. Walker, 2000. Influence of magnesium ions on heat shock and ethanol stress responses of *Saccharomyces cerevisiae*. *Enzyme Microb. Technol.*, 26: 678-687.
- Blackwell, K.J., J.M. Tobin and S.V. Avery, 1997. Magnesium uptake and toxicity in magnesium-supplemented and unsupplemented *Saccharomyces cerevisiae*. *Applied Microbiol. Biotechnol.*, 47: 180-184.
- Carey, V.C. and L.O. Ingram, 1983. Lipid composition of *Zymomonas mobilis*: Effects of ethanol and glucose. *J. Bacteriol.*, 154: 1291-1300.
- Dombek, K.M. and L.O. Ingram, 1986. Magnesium limitation and its role in apparent toxicity of ethanol during yeast fermentation. *Applied Environ. Microbiol.*, 52: 975-981.
- Fein, J.E., D.L. Barber, R.C. Charley, T.J. Beveridge and H.G. Lawford, 1984. Effect of commercial feedstocks on growth and morphology of *Zymomonas mobilis*. *Biotechnol. Lett.*, 6: 123-128.
- Hu, C.K., F.W. Bai and L.J. An, 2003. Enhancing ethanol tolerance of a self-flocculating fusant of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* by Mg²⁺ via reduction in plasma membrane permeability. *Biotechnol. Lett.*, 25: 1191-1194.
- Jones, R.P. and P.F. Greenfield, 1985. Replicative inactivation and metabolic inhibition in yeast ethanol fermentations. *Biotechnol. Lett.*, 7: 223-228.
- Li, M. and S.L. Won, 1992. Cloning and characterization of the *groESL* operon from *Bacillus subtilis*. *J. Bacteriol.*, 174: 3981-3992.

- Michel, G.P.F., T. Azoulay and J. Starka, 1985. Ethanol effect on the membrane protein pattern of *Zymomonas mobilis*. Ann. Inst. Pasteur Microbiol., 136: 173-179.
- Michel, G.P.F. and J. Starka, 1986. Effect of ethanol and heat stresses on the protein pattern of *Zymomonas mobilis*. J. Bacteriol., 165: 1040-1042.
- Michel, G.P.F. and J. Starka, 1987. Preferential synthesis of stress proteins in stationary *Zymomonas mobilis* cells. FEMS Microbiol. Lett., 43: 361-365.
- Millar, D.G., K. Griffiths-Smith, E. Algar and R.K. Scopes, 1982. Activity and stability of glycolytic enzymes in the presence of ethanol. Biotechnol. Lett., 9: 601-606.
- Miller, G.C., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem., 31: 426-428.
- Moreau, R.A., M.J. Powell, W.F. Fett and B.D. Whitaker, 1997. The effect of ethanol and oxygen on the growth of *Zymomonas mobilis* and the levels of hopanoids and other membrane lipids. Curr. Microbiol., 35: 124-128.
- Osman, Y.A. and L.O. Ingram, 1985. Mechanism of ethanol inhibition of fermentation in *Zymomonas mobilis* CP4. J. Bacteriol., 164: 173-180.
- Petrov, V.V. and L.A. Okorokov, 1990. Increase of the anion and proton permeability of *Saccharomyces carlsbergensis* plasmalemma by n-alcohols as a possible cause of its de-energization. Yeast, 6: 311-318.
- Sprenger, G.A., 1996. Carbohydrate metabolism in *Zymomonas mobilis*: A catabolic highway with some scenic routes. FEMS Microbiol. Lett., 145: 301-307.
- Stevnsborg, N and H.G. Lawford, 1986. Effect of calcium on the performance and morphology of *Zymomonas mobilis* ATCC 29191 in continuous ethanol fermentations. Biotechnol. Lett., 8: 181-186.
- Swings, J. and J. DeLey, 1977. The biology of *Zymomonas mobilis*. Bacteriol. Rev., 41: 1-46.
- Taherzadeh, M.J., R. Eklund, L. Gustafsson, C. Niklasson and G. Liden, 1997. Characterization and fermentation of dilute-acid hydrolyzates from wood. Ind. Eng. Chem. Res., 36: 4659-4665.
- Vega, M.I., M. de R. Cuenca, E. Rodriguez and D. Callieri, 2000. Identification of heptadecanoic and C19 cyclopropane fatty acids in the lipid fraction of *Zymomonas mobilis*. Curr. Microbiol., 41: 305-306.
- Vrieskoop, F., M. Rasmusson and N.B. Pamment, 2002. Respective effects of sodium and chloride ions on filament formation and growth and ethanol production in *Zymomonas mobilis* fermentations. Lett. Applied. Microbiol., 35: 27-31.
- Walker, G.M. and J.H. Duffus, 1980. Magnesium ions and the control of the cell cycle in yeast. J. Cell. Sci., 42: 329-356.
- Walker, G.M., 1994. The roles of magnesium in biotechnology. Crit. Rev. Biotechnol., 14: 311-354.