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Determination of the Heat Shock Response in *Enterococcus faecium* and *E. faecalis*

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ABSTRACT

The heat tolerance of heat shocked cells of *Enterococcus faecium* BAR1 and *E. faecalis* MI2 was determined. The heat tolerance (55, 60 and 62.5°C, 3 min) of log phase cells of *E. faecium* BAR1 and *E. faecalis* MI2 grown at 37°C was enhanced by exposing cells to heat shock at 50°C for 15 min. From the survival curves, the D-values were determined. It was determined that the heat shocked cells of *E. faecium* BAR1 were more resistant to heat treatment than the heat shocked cells of *E. faecalis* MI2. The heat shocked cells of *E. faecium* BAR1 survived at 62.5°C for half an hour while the heat shocked cells of *E. faecalis* MI2 survived at 62.5°C for 20 min only. The heat shocked cells of both isolates were found resistant to heat treatment as compared with the control experiment in which the log phase cells grown at 37°C were treated at 62.5°C.

Key Words: Enterococcus sp.; Heat shock

INTRODUCTION

Exposure of cells and organisms to an abrupt increase in temperature triggers the induction of a phenomenon known as heat shock response. This response, which appears to be universally present from microbes to mammals (Yura *et al*., 1993). It is a rapid and transient accumulation of heat shock proteins, which ensure survival during the stress period and protect the cells against heat damage and allow a rapid resumption of normal cellular activities during the recovery period (Burdon, 1986). The organisms were found to respond to sudden increases in temperature by synthesizing a small set of proteins called the heat shock proteins (Parsell & Lindquist, 1993). The heat shock response is highly conserved (Parsell & Lindquist, 1993). It has been indicated that during heat shock, abnormal proteins rapidly accumulate and these abnormal proteins may trigger the heat shock response. One heat shock protein is ubiquitin. In normal cells, ubiquitin is associated in the ATP-mediated proteolysis of unstable or abnormal cellular proteins in eukaryotes. The *lon* protease appears to play the same role in degrading unstable and abnormal proteins in an ATP-dependent manner in prokaryotes. During heat shock, both of these proteins are synthesized increasingly (Nagao *et al*., 1990).

Enterococci are Gram-positive bacteria. They are inhabitant of intestinal tract of humans and most animals. They are present in numerous foods. These microorganisms are major indicator of the hygienic quality of food, milk and drinking water in which they are subjected to numerous stress like temperature and pH shifts (Boutibonnes *et al*., 1993). Enterococci can survive in media at 60°C for 30 min and can grow at pH 9.6 and at 10-45°C (Kaye, 1982). The purpose of this work was to determine the heat tolerance of heat shocked cells of *E. faecium* BAR1 and *E. faecalis* MI2 conferred by the heat shock response.

MATERIALS AND METHODS

Microorganisms. *E. faecium* BAR1 was isolated form barley seeds provided by Dr. David G. Smith Department of Biology University College London U.K., while *E. faecalis* MI2 as obtained from Microbiology laboratory University College Hospital U.K. Both isolates were identified by API20 STREP kits. The stock cultures were maintained in Microbank cryovials and stored at –70°C.

Preparation of cultures. Cultures required for experimental work were grown on Brain Heat Infusion agar (BHI, Oxoid CM 375) at 37°C for 24 h and stored in refrigerator and subcultured every week. Fresh subcultures were used in each experiment. Both isolates were grown in Brain Heart Infusion broth (BHI, Oxoid CM 225) at 37°C for overnight. One mL of overnight culture was diluted into fresh BHI broth and incubated with shaking at 37°C for one and half hour to two hours till their optical density reached between 0.2 to 0.3. Optical density of the cultures in the BHI broth was measured with Hilger photoelectric colorimeter.

Determination of heat tolerance of heat shocked cells. One mL of log phase cells of each strain was diluted into 25 mL fresh BHI broth already placed at 50°C. The log phase cells were heat shocked at 50°C for 15 min. The 100 µL samples of heat shocked cells were transferred to 25 mL Maximum Recovery Diluent (MRD, Oxoid CM733) maintained at 55, 60 and 62.5°C for 30 min in shaking incubators. At 5 min intervals one mL cells were transferred to 9 mL MRD and further diluted in MRD by factors of 10 upto six dilutions.

Enumeration of survivors. At appropriate intervals, 20 µL samples were taken from each dilution and spotted on the BHI agar. The plates were examined after incubation at 37°C for 24 h.

D-value determination. D-values were determined by plotting the $\log_{10}$ of the number of survivors against time at a specific temperature for a 1 log fall in viable count.

RESULTS AND DISCUSSION

The heat tolerance of heat shocked cells of barley isolated *E. faecium* BAR1 and hospital isolate *E. faecalis* MI2 was investigated at 50, 60 and 62.5°C. Both isolates were grown to log phase in BHI broth at 37°C. The long phase cells were heat shocked at 50°C for 15 min. It was determined that the heat shocked cells of both isolates were more resistant to heat treatment than control experiment in which the log phase cells grown at 37°C were treated at 62.5°C. It was also found that the heat shocked cells of the *E. faecium* BAR1 were more resistant to heat than the heat shocked cells *of E. faecalis* MI2. The heat shocked cells of *E. faecium* BAR1 survived at 55, 60 and 62.5°C for half an hour (Fig. 1), while the heat shocked cells of *E. faecalis* MI2 survived at 55 and 60°C for half an hour and at 62.5°C for 20 min only (Fig. 2). At 62.5°C *E. faecium* BAR1 was found to be more resistant to heat (D-value = 25 min) than *E. faecalis* MI2 (D-value = 5 min). The D-values for these experiments are reported in Table I.

Table I. D-values (minutes) for heat treated cells of *Enterococcus* strains heat shocked at 50°C for 15 min.

Strains	55 °C	60 °C	62.5 °C	Control
E. faecium BAR1	> 30.0	>30.0	25.0	2.5
E. faecalis MI2	> 30.0	>30.0	5.0	1.75

E. faecium is able to survive the mild heat processing of curd meats and predominate in the spoilage microflora of meat products. It can grow readily at room temperatures (Bell & DeLacy, 1984). *E. faecalis* can grow at temperatures ranging from 5 to 51°C. It was found to survive exposure to temperatures in the range of 52°C to 59°C for many hours but at 65°C cells die within seconds. The heat treatment at 60 or 62.5°C for 30 min of log phase cells of *E. faecalis* grown at 37°C was found to be enhanced by exposure of cells to prior heat shock at 45°C or 50°C for 30 min (Boutibonnes *et al*., 1993). In another study, the heat tolerance of *E. faecium* and *E. faecalis* was reported. The isolates of *E. faecium* were found to survived at 65°C for 20 min and the cells of *E. faecalis* survived at 65°C for 10 min (Kearns *et al*., 1995). These studies showed that the *E. faecium* is more heat tolerant than the *E. faecalis* that is proved in this study in which the heat shocked cells of *E. faecium* were found more resistant to heat than the *E. faecalis*. It was reported that the some isolates of *E. faecium* are able to survive the British Standard for heat disinfection (80°C for 1 min) of bedpans (Kearns *et al*., 1995).

Heat shock response protects the bacterial cells against lethal temperatures and enhances their survival at high temperature (Neidhardt & Van Bogelen, 1987). It was shown that the heat shock response of *E. faecalis* is similar to heat shock response that occurs in other prokaryotes (Boutibonnes *et al*., 1993). Cells of *Bacillus subtilis* if exposed to 48°C for 30 min can survive the lethal temperature of 52°C. Heat shock response enhances the survival at high temperature (Völker *et al*., 1992). Exposure of cells to a sudden increase in temperature induces the heat

Fig. 1. Heat shock response of barely isolate *E. faecium* BAR1. Cells were heat shocked at 50°C for 15 min. Heat tolerance was determined at 55, 60 and 62.5°C for half an hour

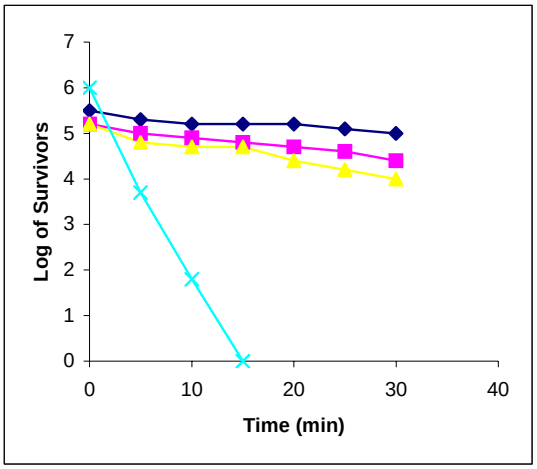
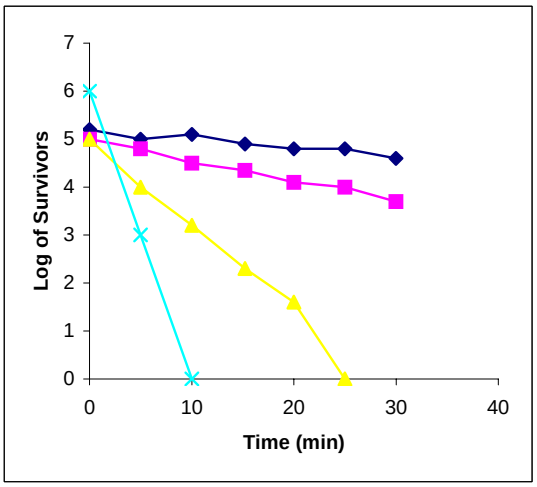


Fig. 2. Heat shock response of hospital isolate *E. faecalis* MI2. Cells were heat shocked at 50°C for 15 min. Heat tolerance was determined at 55, 60 and 62.5°C for half an hour



shock response. This response has been observed in all living cells and is characterized by enhanced synthesis of heat shock protein (Blondin *et al*., 1993). Cells exposed to mild stress conditions are better able to tolerate lethal stress conditions (Ang *et al*., 1991). It has been reported that *Lactobacillus bulgaricus* shows more heat resistance if cells were exposed to 10°C above the optimum growth temperature before exposure to lethal temperatures (Teixeira *et al*., 1994). It was demonstrated that when *Listeria monocytogenes* cells grown at 4°C were heat socked at 46 °C for 30 min and treated at 58°C, an increased heat resistance was seen (Jorgensen *et al*., 1996). It was shown that the heat shock is responsible for an increase in the heat resistance of cells in the log phase (Teixeira *et al*., 1994).

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