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Determination of Heat Resistance of Exponential Phase Enterococcal Cells

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Abstract: The heat resistance of exponential phase cells of environmental barley isolates ***Enterococcus faecium*** BAR_1 and a hospital isolate ***Enterococcus faecalis*** MI_2 grown at 37 or 45 was determined at 55, 60 and 62.5°C for 30 min. From the survival curves, the decimal reduction times (D-values) were determined. Cells grown at 45°C showed higher D-values. It was determined that the exponential phase cells grown at 45 were more resistant to heat than the heat resistance of cells grown at 37°C. The cells of both isolates were found resistant to heat treatment at 55, 60 and 62.5 as compared with the control in which cells were grown at 37°C and treated at 62.5°C.

Key words: Heat resistance, isolates, exponential, heat

Introduction

Bacterial thermo tolerance is a major issue in food safety because numerous foods and feeds are thermally processed to limit human exposure to pathogens (Bunning ***et al.***, 1992 and Ahmad ***et al.***, 2002). The enterococci are of great interest due to their characteristics of being the most thermo resistant among the non-sporulating eubacteria (Perez ***et al.***, 1982). Heat resistance of enterococci depends on several factors such as age of cultures, external pH and the composition of suspending medium (Perez ***et al.***, 1982). Enterococci can grow in media that contain 6.5% NaCl, 40% bile, or 0.1% methylene blue. They can survive in media at 60°C for 30 min and can grow at pH 9.6 and at 10-45°C (Kaye, 1982). Their relative resistance to adverse conditions such as temperature is advantageous when determining the sanitary history of moderately heated, frozen, salted or other foods and drinks in which other coliforms might not have survived (Thian and Hartman, 1981). Enterococci are predominantly inhabitants of the gastrointestinal tract of man and animals (Knudtson and Hartman, 1992; Aguirre and Collins, 1993) and also commonly occur on vegetables and plant material (Aguirre and Collins, 1993). The enterococci are widely distributed in foods, they decompose lactose to lactic acid and grow in a wide range of temperatures (Perez ***et al.***, 1982). The enterococci have been implicated in the spoilage of food products and may be etiologic agents of food poisoning (Gordon and Ahmad, 1991).

Hence proper care must be taken to avoid excessive numbers of enterococci in processed food products.

The heat resistance of microorganisms is usually expressed as decimal reduction times (D-values). This is the time (in minutes) required to reduce the microbial population by 90% (Gordon and Ahmad, 1991).

The aim of this work was to determine and compare heat resistance values for an environmental isolate ***E. faecium*** BAR_1 and hospital isolate ***E. faecalis*** MI_2, when grown at optimum and above optimum temperature.

Materials and Methods

Microorganisms and Culture Conditions

E. faecium BAR_1 was isolated form barley seeds while ***E. faecalis*** MI_2 was obtained from University College Hospital, U.K. Both isolates were identified by API 20 STREP kits. The stock cultures were maintained in Microbank cryovials, containing cryopreservatives and stored at -70°C. Working cultures were grown on Brain Heart Infusion (BH1, Oxide CM 375 agar) (37°C for 24 h) and stored in refrigerator and sub-cultured every week. Fresh sub cultures were used in each experiment.

Determination of Heat Resistance

Cultures for heat resistance determinations were grown overnight in Brain Heart Infusion (Oxoid CM 225) broth at 37 or 45°C. Samples of 0.1 ml. of these cultures were added to 25 ml. Maximum Recovery Diluent's (Oxoid CM 733) in 100 ml. conical flasks and placed at 55, 60 and 62.5°C in shaking water baths. At 5 min intervals upto 30 min, 1 ml samples were removed and diluted into 9 ml. MRD at room temperature and then further diluted in MRD by factors of 10 upto 4 dilutions. This involved spotting duplicate 20 µl samples from each dilution on the well dried BHI agar plates. After allowing the spots (7 $plate^{-1}$) to dry, the plates were incubated at 37°C for 24 h.

The colonies from each spot were counted and the viable count calculated as follows:

$$\text{Viable count} = \text{number of colonies} \times 1/\text{Dilution}$$

The log of the number of survivors was plotted against time of heat treatment.

Survival curves and Determination of the D-values

For each temperature, a survival curve was obtained by plotting the logarithm of the number of survivors against time at a specific temperature. The D-values was calculated as the time taken at a particular temperature for a 1 log fall in viable count.

Results

In this study the heat tolerance of *E. faecium* BAR_1 and *E. faecalis* MI_2 was determined. It was found that when the cells of both isolates were grown at 37°C and exposed to heat treatment at 55, 60 and 62.5°C, both isolate survived at 55 and 60°C for half an hour. At 62.5°C *E .faecium* BAR_1 survived for 15 min and *E. faecalis* MI_2 survived for 10 min only. At 62.5°C the environmental isolate was resistant to heat treatment as compared with the hospital isolate. The results of these experiments were shown in Fig. 1 and Fig. 2. The D-values from these experiments are shown in Table 1. When both isolates were grown to exponential phase at 45 and heat tolerance was determined at 55, 60 and 62.5°C in order to compare the heat tolerance of

Table 1: D-Values (min) for heat treatment exponential phase *Enterococcus* stains grown at 37°C

D-values (min)			
Strains	55°C	60°C	62.5°C
E. faecium BAR1	No killing	6.5	3.0
E. faecalis MI2	>30.0	6.0	2.5

Table 2: D-Values (minutes) for heat treatment exponential phase *Enterococcus* stains grown at 45°C

D-values (min)				
Strains	55°C	60°C	62.5°C	Control
E. faecium BAR1	No killing	14.0	7.75	3.0
E. faecalis MI2	No killing	>30.0	9.75	2.75

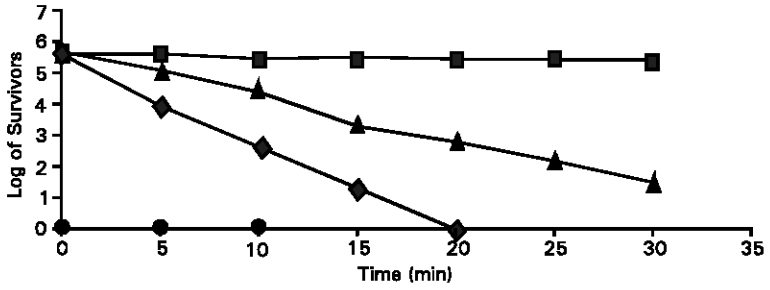

Fig. 1: Heat tolerance of barely isolate *E. faecium* BAR_1 exponentially grown cells at 37°C in BH1 broth

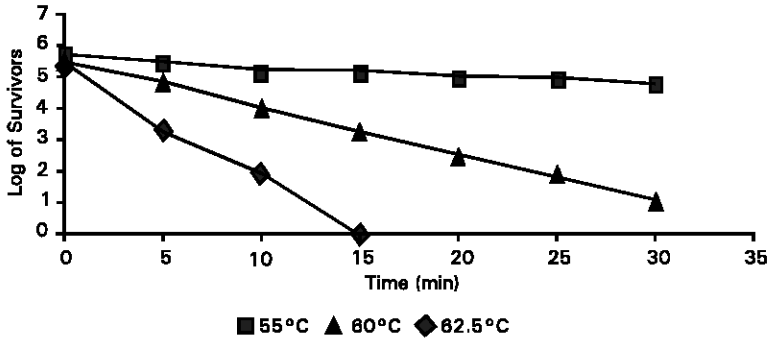

Fig. 2: Heat tolerance of hospital isolate *E. faecalis* MI_2 exponentially grown cells at 37°C in BH1 broth

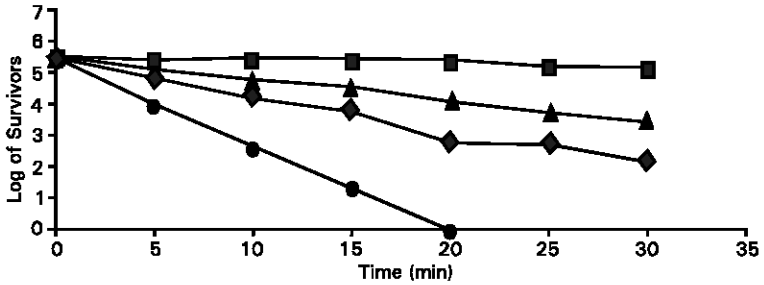

Fig 3: Heat tolerance of barely isolate *E. faecium* BAR_1 exponentially grown cells at 45°C in BH1 broth

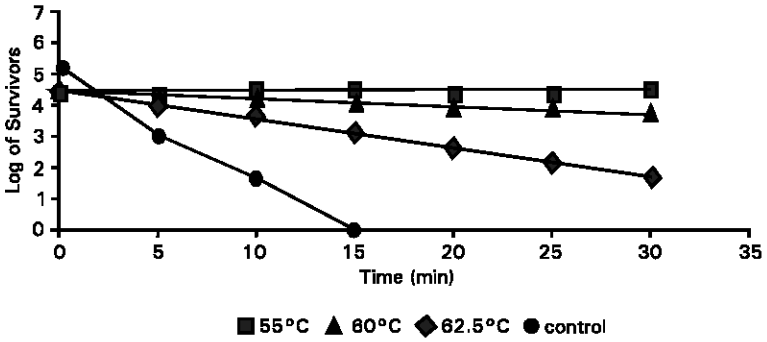

Fig. 4: Heat tolerance of hospital isolate *E. faecalis* MI_2 exponentially grown cells at 45°C in BH1 broth

exponential phase cells grown at optimum temperature. It was found that the exponential phase cells grown at 45°C were more resistant to heat treatment than the cells grown at 37°C.

The cells of both isolates were found resistant to heat treatment at 55, 60 and 62.5°C as compared with the control experiment in which cells were grown at 37 and

treated at 62.5°C . The results of these experiments were shown in Fig. 3 and Fig. 4. The D-values from these experiments are shown in Table 2.

Discussion

The two ***Enterococcus*** isolates ***E. faecium*** BAR_1 and ***E. faecalis*** MI_2 were grown at 37 or 45°C to exponential phase. Therefore, the heat tolerance of exponential phase cells was measured at 55, 60 and 62.5. Exponential cells of ***E. faecium*** BAR_1 and ***E. faecalis*** MI_2 grown at 37 survived at 55 and 60°C for half an hour. This study confirms that enterococci can survive a heat treatment at 60°C for 30 min. At 62.5°C cells of ***E. faecium*** BAR_1 survived for 15 min (D-value = 3 min.) and cells of ***E. faecalis*** MI_2 survived for 10 min (D-value = 2.5 min.) The environmental isolate ***E. faecium*** BAR_1 was more resistant to heat treatment than the hospital isolate ***E. faecalis*** MI_2. The D-values are reported in Table 1. The same isolates were grown to exponential phase at 45°C. Exponential cells grown at 45°C were more resistant to heat than the cells grown at 37°C. The cells grown at 45 survived at 55, 60 and 62.5°C for half an h. The D-values are reported in Table 2. It was confirmed that, when cells are grown above the optimum growth temperature they become more resistant to heat treatment than the cells grown at optimum temperature.

Heat treatments which induce thermo-tolerance also cause the synthesis of heat shock proteins (Webber, 1982 and Ahmad ***et al.***, 2002). Lindquist (1986) reported that there was a relationship between heat shock protein induction and the acquisition of thermo-tolerance. It was also reported that the inhibition of protein synthesis leads to an inhibition of thermo-tolerance. In certain cells the acquisition of heat resistance requires the induction of one or more heat shock proteins. These cells require protein synthesis for the induction of thermo-tolerance (Smith and Yaffe, 1991). Mackey and Derrick (1990) demonstrated that the levels of heat shock proteins did not correlate with the degree of thermo-tolerance. There is also a lack of correlation between the heat shock protein levels and acquired thermo-tolerance in ***L. monocytogenes***. This suggests an additional role of heat shock proteins in cell physiology. These proteins are also present in unstressed cells and some of them are necessary for cell viability at all temperatures (Jorgensen ***et al.***, 1996). Heat tolerance of ***E. faecium*** DP2181 isolated from frankfurters was determined at 55, 63 and 68°C in BH1 broth and ***E. faecium*** DP2181 showed increased thermal resistance (Gordon and Ahmad, 1991). Mangus ***et al.*** (1988) studied the heat resistance of ***enterococci. E. faecium*** P-1A and ***E. faecium*** E-20 were found more resistant to heat treatment than ***E. faecalis***. P-2A. ***L. monocytogenes*** grown at 30 was not more resistant to heat than the cells grown at 10°C. There was no significant difference in the death rate of cells at 55°C (Patchett ***et al.***, 1996). Cells of ***Listeria*** grown at 5 to 10°C above the optimum growth temperature are more heat resistant than the cells grown at room temperature (Jorgensen ***et al.***, 1996).

The heat tolerance of bacteria may be affected many factors such as temperature of the medium in which bacteria are grown before heating, age of the bacterial culture and rate of heating temperature (Sörquist, 1994).

It was concluded from the results of the present study that when cells are grown above the optimum growth temperature they become more resistant to heat treatment than cells grown at optimum temperature.

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