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Effect of Heat Treatment on Stationary Phase Cells of *Enterococcus faecium* and *E. faecalis*

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ABSTRACT

The heat tolerance of one-day old stationary phase cells of water isolate *Enterococcus faecium* MA1 and hospital isolate *E. faecalis* MI2 grown at 37 or 45°C was determined at 65, 67.5 and 70°C for 30 min. Cells of both isolates grown at 45°C were found resistant to heat treatment as compared with the cells grown at 37°C. It was found that water isolate *E. faecium* MA1 was more heat tolerant that the hospital isolates *E. faecalis* MI2. From the survival curves D-values were calculated. Water isolate *E. faecium* MA1 showed higher D-values in comparison to hospital isolate *E. faecalis* MI2.

Key Words: Heat; *Enterococcus faecium; Enterococcus faecalis*

INTRODUCTION

Enterococci are the most common aerobic, Gram-positive cocci found in the bowel flora of human and other animals. These organisms were originally classified as streptococci on the basis of their morphology and their reaction with Lancefield group D antisera. Recent DNA homology studies however, have demonstrated that members of the genus *Enterococcus* are closely related to each other but not to members of the genus *Streptococcus* (Schleifer & Kilpper-Bälz, 1984; Collin *et al.,* 1984). The heat resistance of the common food-borne pathogenic or spoilage microbes varies quite widely. For example, *Listeria monocytogenes* is more heat resistant than *Salmonella typhimurium*, whilst certain enterococci are more heat resistant than both and can be particularly troublesome in pasteurized meat products (Magnus *et al.,* 1986). The heat resistance of the enterococci has been known for a long time and this characteristic has been used to classify these microorganisms (Perez *et al.,* 1982). Heat resistance of enterococci depends on several factors such as age of cultures, external pH (White, 1953), and composition of the suspending medium (Perez *et al.,* 1982). The thermoduric nature of enterococci and the mechanism by which they suffer heat injury and subsequently recover have been investigated by several researchers (White, 1953; Clark *et al.,* 1968). Bacteria spend most of their lifetime in a starving or non-growing state because they are frequently faced with different adverse environmental conditions most typical in nature (Roszak & Colwell, 1987; Morita, 1988). It was described that the heat tolerance is related to growth phase. The stationary phase cells are naturally more resistant to heat than exponential phase cells (Steels *et al.,* 1994). Growth conditions may affect an organism's susceptibility to environmental stress (Patchett *et al.,* 1996). Magnus *et al.* (1988) studied the heat resistance of *Enterococcus faecium* and *E. faecalis*. It was demonstrated that these organisms showed great thermal resistance. Therefore, temperature becomes a critical factor for determining to what extent cells become thermotolerant (Jorgensen *et al.,* 1996). The purpose of this work was to determine and compare the thermotolerance in stationary phase cells of an environmental isolate *E. faecium* MA1 and hospital isolate *E. faecalis* MI2.

MATERIALS AND METHODS

Organisms and growth conditions. *E. faecium* MA1 was isolated from Thames water collected form Charing Cross U.K. and filtered 5 mL water sample through Millipore membranes induplicate. The membranes were placed face up on Slanetz and Bartley medium (Oxoid CM 377) and incubated at 37°C for two days. After two days, bright red colonies were grown on the Millipore membranes. A well separated colony was inoculated into test tube containing 5 mL Brain Heart Infusion (BH1, Oxoid CM 225) broth and incubated at 37°C for overnight growth. The overnight culture was streaked on Brain Heart Infusion (BHI, Oxoid CM 375) agar plates. The plates were incubated at 37°C for two days. The plates were examined after incubation at 37°C for 48 h. *E. faecalis* MI2 was obtained form Microbiology laboratory University College Hospital U.K. Both isolates were identified by API 20 STREP kits. A single colony of each strain grown on BH1 agar plate was inoculated into Microbank cryovials and stored at -70°C for long term storage.

Heat treatment of bacteria. To determine the heat tolerance of stationary phase cells of *E. faecium* MA1 and *E. faecalis* MI2, both isolates were grown overnight in BH1 broth at 37 or 45°C. One day old cultures were used to determine the heat tolerance. Samples of 0.1 mL of one-day old cultures were added to 100 mL. Maximum Recovery

Diluents (MRD, Oxoid CM 733) in 250 mL conical flasks placed at 65, 67.5 and 70°C in shaking incubators. At 5 min interval up to 30 min 1 mL samples were removed from the flasks and diluted into 9 mL MRD at room temperature and then further diluted by factors of 10 up to 6 dilutions. At appropriate intervals 0.1 mL samples were taken from each dilution and spread on BH1 agar plates. The plates were incubated at 37°C for 24 h. The plates were examined after 24 h. The colonies from each plate were counted. The log of number of survivors was plotted against time of heat treatment. From the survival curves the D-values were calculated as the time taken to reduce the viable population by 90%.

RESULTS AND DISCUSSION

The heat tolerance of one day old stationary phase cells of *E. faecium* MA1 and *E. faecalis* MI2 grown at 37 or 45°C was determined at 65, 67.5 and 70°C. It was found that stationary phase cells grown at 45°C were more resistant to heat than the cells grown at 37°C. Water isolate *E. faecium* MA1 was found more resistant to heat at 65, 67.5 and 70°C than the cells grown at 37°C and treated at 65°C as shown in Fig. 1. Cells of *E. faecium* MA1 survived at 65 and 67.5°C for half an hour (D-values=18.5 min. and 11.5 min) while at 70°C they survived for 20 min (D-value = 4.5 min). *E. faecium* MA1 isolate was found more heat tolerant as compared with the hospital isolate *E. faecalis* MI2. Cells of *E. faecalis* MI2 survived at 65°C for 20 min (D-value= 4 min) and at 67.5°C for 10 min (D-value=2.5 min) while at 70°C cells survived for five minutes (D-value=1.5 min). Cells of *E. faecalis* MI2 at 67.5°C and at 70°C were found sensitive to heat treatment as compared with cells grown at 37°C and treated at 65°C, these cells survived at 65°C for 15 min (D-value = 3.0 min). *E. faecalis* MI2 was found less heat tolerant as compared to the *E. faecium* MA1 as shown in Fig. 2. The D-values are reported (Table I).

Fig. 1. Heat tolerance of one-day old stationary phase cells of water isolate *E. faecium* MA1 grown at 45°C in BHI broth. Heat tolerance was determined at 65, 67.5 and 70°C for half an hour. In control experiment cells grown at 37°C in BHI broth were treated at 65°C

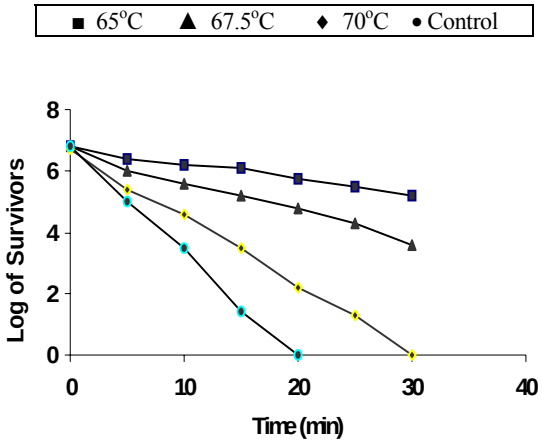

Fig. 2. Heat tolerance of one-day old stationary phase cells of hospital isolate *E. faecalis* M12 grown at 45°C in BHI broth. Heat tolerance was determined at 65, 67.5 and 70°C for half an hour. In control experiment cells grown at 37°C in BHI broth were treated at 65°C

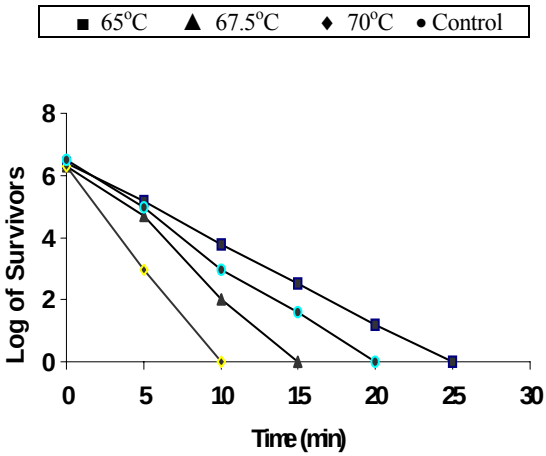

Table I. D-values (minutes) for heat treated stationary phase *Enterococcus* isolates grown at 45°C

Strains	D-values (min.)			Control
	65 °C	67.5 °C	70 °C	
E. faecium MA1	18.5	11.5	4.5	3.0
E. faecalis MI2	4.0	2.5	1.5	3.0

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

Magnus *et al.* (1988) and Kearns *et al*. (1995) reported that *E. faecium* strains are more heat resistant than *E. faecalis* strains. In this study it was determined that the *Enterococcus* isolates grown at 37°C survived at 65°C for 15 min. Kearns *et al*. (1995) reported that the stationary phase cells of enterococci are able to withstand at 65°C for 20 min. Enterococci are naturally present in food products at the time of thermal processing; they are usually in the stationary phase of growth. When stationary phase cells were heat treated they become heat resistant by virtue of their physiological state (Bell & DeLacy, 1984). The heat tolerance of stationary phase cells of *S. typhimurium* was enhanced if the cells were grown in nutritionally rich medium (Mackey & Derrick, 1990). Stationary phase cells

of *Saccharomyces cerevisiae* were reported more heat resistant than the exponential cells (Steels *et al.,* 1994). Some studies have reported the consistent appearance of a resistant tail for *E. faecium* strains at temperatures ranging from 62 to 70°C (Perez *et al.,* 1982; Bell & Delacy, 1984). This tailing phenomenon may be due to variation in thermal resistance of the cells at different stages of cell division (Bell & Delacy, 1984).

Overall, our results show that *E. faecium* MA1 is more thermotolerant than *E. faecalis* MI2. This work shows that the heat tolerance of enterococci affected not only by the temperature at which the organisms are grown but also is affected by the age of the bacterial culture and rate of heating temperature.

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