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Effect of pH of the Medium During Growth on Heat Tolerance of *Enterococcus faecium* and *Enterococcus faecalis*

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Abstract: Effect of pH of the growth medium on heat tolerance was investigated in *Enterococcus faecium* BAR1 and *Enterococcus faecalis* MI2. The heat tolerance was determined at 55, 60 and 62.5°C for half an hour. Cells of both isolates were grown to exponential phase at pH 5 or 7.4. Cells grown at 37°C at pH 5.0 were found tolerant to heat treatment as compared with cells grown at pH 7.4. Cells did not differ in their tolerance to heat at 55°C but at 60 and 62.5°C, cells of *E. faecium* BAR1 were found more heat tolerant than the cells of *E. faecalis* MI2. The acidic pH of the growth medium had affected the susceptibility of *E. faecium* BAR1 and *E. faecalis* MI2 on heat tolerance. The cells grown at acidic pH showed greater heat tolerance that the cells grown at neutral pH.

Key words: *Enterococcus faecium*, *Enterococcus faecalis*, heat tolerant, acidic pH

Introduction

Enterococci are part of the normal gastrointestinal flora, they are recognized as important causes of endogenous and exogenous nosocomial infections (Lewis and Zervos, 1990). They are a frequent cause of a wide variety of infections in humans (Jett *et al.*, 1994). Enterococci can grow at pH 9.6 and at 10-45 °C (Kaye, 1982). The capacities of microorganisms to adapt to new environments can be important in a variety of situations (Belli and Marquis, 1991). For example, *Streptococcus mutans* not only has constitutive acid tolerance but is also able to develop an adaptive acid tolerance during prolonged growth at low pH (Belli and Marquis, 1991).

It has been reported that pH is the most important factor affecting bacterial growth (Pimentel *et al.*, 1994). Low pH is an important stress condition which is faced by many pathogenic bacteria (Karem *et al.*, 1994). When food and water-borne pathogenic microorganisms are ingested they are exposed to an acid environment in the stomach and small intestine (Karem *et al.*, 1994). Bacteria associated with infections of the animal body usually adapt to acid environments. For example, *Salmonella typhimurium* can survive at pH 3. *S. typhimurium* can survive dramatic acid stresses: it would be expected that this organism possesses an adaptive acid tolerance mechanism (Foster and Hall, 1990). *E. coli* can adapt to both acid and alkaline growth conditions (Belli and Marquis, 1991). Exposure to mild acid, at pH 5.0-6.0 brings about increased acid tolerance in *S. typhimurium* (Foster, 1991) and *E. coli* (Rowbury *et al.*, 1992). Exposure of *E. coli* to acid pH leads to habituation and the habituated organisms can survive levels of lethal acidity as compared to non-habituated ones (Goodson and Rowbury, 1989). *Enterococcus hirae* also shows an adaptive acid tolerance. The constitutive acid tolerance of *E. hirae* is similar to *S. mutans* stains. However enterococci has greater capacity to adapt to acid environments than the streptococci (Belli and Marquis, 1991). The acid tolerance response requires protein synthesis and it seems to be a specific defense mechanism for acid (Foster and Hall, 1990). The acidity produced by lactic acid bacteria is a important factor in food safety. In mixed cultures acidifying activity and acid tolerance may result to the presence of the more acid resistant strains (Rodriguez and Manca de Nadra, 1995). In this study we compared the effect of the pH of the medium during growth on heat tolerance of an environmental isolate *E. faecium* BAR1 with that of a hospital isolate *E. faecalis* MI2.

Materials and Methods

Organisms and growth conditions: *E. faecium* BAR1 was isolated form malted barley seeds provided by Dr. David G. Smith Department of Biology University College London U.K. In order to isolate barley strain, 50 grams of malted barely seeds were suspended in 50ml Maximum Recovery Diluent (MRD, Oxoid CM 733), shaken thoroughly and left for one hour, then shaken thoroughly and 0.1 ml sample spread on Slanetz and Bartley medium (Oxoid, CM 377). The plates were incubated at 37 °C for two days. After two days barely strain was isolated. *E. faecalis* MI2 was obtained from Microbiology laboratory University College Hospital U.K. Both isolates were identified by API 20 STREP kits. Stock cultures were maintained in Microbank cryovials and stored at -70 °C. Subcultures required for experimental work were kept in refrigerator. Both isolates were grown in Brain Heat Infusion (BHI, Oxoid CM 225) broth at 5.0 and 7.4 pH. The pH of the BHI broth was adjusted by the addition off HCl and NaOH to required values. Cells grown overnight in BHI broth of 5.0 and 7.4 pH were diluted respectively into fresh BHI broth of 5.0 and 7.4 pH and grown to exponential phase for a period of one and half to two hours at 37 °C under shaking conditions.

Determination of heat tolerance: Cultures for heat tolerance determinations were grown in (BHI) broth at 5.0 and 7.4 pH. Samples of 100 μl of these cultures were added to 25 ml MRD placed at 55, 60 and 62.5 °C for half an hour. Samples (1000 μl) were taken at 5 minutes intervals and diluted into 9 ml MRD at room temperature and then further diluted in MRD by factors of 10 upto 6 dilutions. At appropriate intervals 20 μl samples were removed from each dilution and spotted on Brain Heart Infusion (BHI, Oxoid CM 375) agar plates. The plates were incubated at 37 °C for 24 h. The colonies from each spot were counted. The D-values were determined by plotting the $\log_{10}$ of the number of survivors against time at a specific temperature. The D-value is the time taken at a specific temperature for a 1 log fall in viable count.

Results and Discussion

In these experiments it was found that the cells of both isolates grown at acidic pH were more resistant to heat as compared with the control experiments in which the exponentially grown cells at neutral pH (7.4) were treated at 62.5 °C. In this study it was determined that the environmental barley isolate *E. faecium* BAR1 grown at pH 5.0 showed more tolerance to heat as compared to

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Table 1: D-values (minutes) for *E. faecium* BAR1 and *E.* faecalis MI2 grown at pH 5.0 and pH 7.4. Heat tolerance was determined at 55, 60 and 62.5°C for half an hour (pH=5)

	D-values (min.)			
	Temperature (°C)			
Strains	50	60	62.5	Control
E. faecium BAR1	>30.0	21.0	8.5	2.5
E. faecalis MI2	>30.0	9.0	3.5	1.75

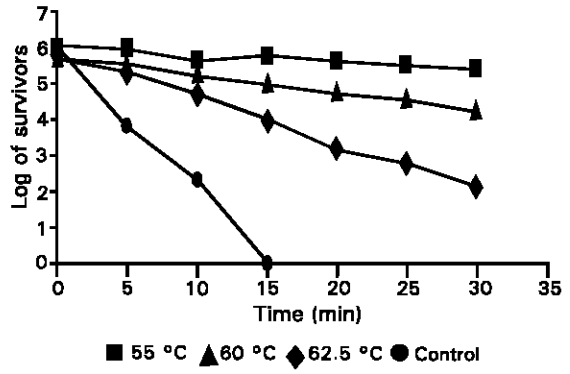

Fig. 1: Heat tolerance of a barley isolate *E. faecium* BAR1 grown at pH 5.0 in BHI broth. Heat tolerance was determined at 55, 60 and 62.5 °C for half an hour. In control experiment exponentially grown cells at 37 °C were treated at 62.5°C.

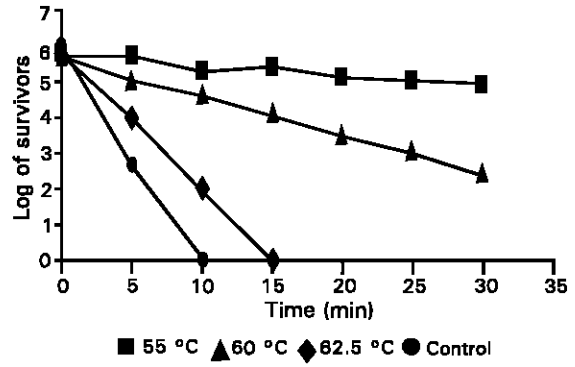

Fig. 2: Heat tolerance of hospital isolate *E. faecalis* MI2 grown at pH 5.0 in BHI broth. Heat tolerance was determined at 55, 60 and 62.5 °C for half an hour. In control experiment exponentially grown cells at 37°C were treated at 62.5°C.

the hospital isolate *E. faecalis* MI2.

E. faecium BAR1 survived at 55, 60 and 62.5 °C for half an hour (Fig. 1). At 62.5 °C cells showed more tolerance to heat (D-value=8.5 min) as compared with the control experiment in which cells survived for 10 min only (D-value=2.5 min) (Table 1). Cells of hospital isolate *E. faecalis* MI2 survived at 55 and 60 °C for half an hour, but at 62.5 °C they survived for 10 min only (D-value=3.5 min) (Fig. 2). At 55 °C both isolate, showed similar results, but at 60 and 62.5 °C *E. faecium* BAR1 sowed more heat tolerance than the *E. faecalis* MI2.

It is advantageous for bacteria to adapt to acid environments in order to avoid the lethal effects of acidification. *S. mutans* and *E. hirae* are able to adapt to acid environments in continuous culture at minimum pH values for growth (Belli and Marquis, 1991). Bowden and Hamilton (1989) have found that *S. mutans* can survive progressive acidification but is inactivated by rapid acidification, presumably because rapid acidification does not allow for adaptation. The response to low pH environment has been studied in *S. typhimurium*. It can survive pH 3.8 for prolonged period (Foster, 1991). The ability of pathogenic bacteria to withstand acidic conditions is an important factor in the pathogenesis of these organisms (Foster and Hall, 1990). The exposure of cells to acid pH leads to habituation and the habituated cells are able to survive at lethal pH (Goodson and Rowbury, 1989). *Aeromonas hydrophila* can adapt to survive severe acidic pH. Acid tolerance response requires prior exposure to a relatively mild pH 5.0 for 20 min before challenge at lower pH 3.5. The adaptation requires protein synthesis and these proteins play an important role in protecting the cells at low pH (Karem *et al.*, 1994). It has been reported that when *E. coli* cells were shifted from neutral pH to alkaline pH, they become more sensitive to acid. The transfer of cultures from 7.0 to 9.0 pH for 15 minutes induced acid sensitivity. These cells could not survived at 3.0 and 3.5 pH (Rowbury *et al.*, 1993).

Acid tolerance either activates the pre-existing protecting system of cells or induces acid tolerance response proteins which protect cells by following different mechanism:

- These proteins enable the cells to maintain the internal pH.
- Chaperonin proteins protect the cells from acid denaturation or damage.
- DNA binding proteins also play a role in acid tolerance response (Karem *et al.*, 1994).

It has been reported that the heat tolerance of the acid shocked cells depends on the temperature at which the cells were grown and the temperature at which cells were acid shocked. In the case of *Listeria monocytogenes* the cells subjected to pH 2.5 acid shock at 30 °C were more resistant to heat than the cells grown at 10 °C. It was also demonstrated that the cells grown at a slow growth rate were found to be more tolerant to acid shock than the cells grown at a fast growth rate (Patchett *et al.*, 1996). It was shown that the susceptibility of the *L. monocytogenes* to acid shock conditions is affected not only by the temperature at which the cells are grown but also affected by the growth rate (Patchett *et al.*, 1996).

It was determined that the pH of the growth medium can affect the heat tolerance. The enterococci can adapt acidic growth conditions. The cells grown at acidic pH were found more tolerant to heat than the cells grown at neutral pH.

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References

Belli, W.A. and R.E. Marquis, 1991. Adaptation of *Streptococcus mutans* and *Enterococcus hirae* to acid stress in continuous culture. Appl. Environ. Microbiol., 57: 1134-1138.

Bowden, G.H.W. and I.R. Hamilton, 1989. Competition between *Streptococcus mutans* and *Lactobacillus casei* in mixed continuous culture. Oral Microbiol. Immunol., 4: 57-64.

Foster, J.W., 1991. *Salmonella* shock proteins are required for the adaptive acid tolerance response. J. Bacteriol., 175: 6896-6902.

Foster, J.W. and H.K. Hall, 1990. Adaptive acidification tolerance response of *Salmonella typtimurium*. J. Bacteriol., 172: 771-778.

Goodson, M. and R.J. Rowbury, 1989. Habituation of normal lethal acidity by prior growth of *Escherichia coli* at a sublethal acid pH value. Lett. Appl. Microbiol., 8: 77-79.

Jett, B.D.M.M. Huycke and M.S. Gilmore, 1994. Virulence of enterococci. Clin. Microbiol. Rev., 7: 462-478.

Kaye, D.M.D., 1982. Enterococci: Biologic and epidemiologic characteristics and in vitro susceptibility. Archives of Internal Medicine, 142: 2006-2009.

Karem, K.L., J.W. Foster and A.K. Bej, 1994. Adaptive acid tolerance response in *Aeromonas hydrophila*. Microbiol., 140: 1731-1736.

Lewis, C.M. and M.J. Zervos, 1990. Clinical manifestations of enterococcal infection. European J. Clini. Microbiol. Infec. Dis., 9: 111-117.

Patchett, R.A., N. Watson, P.S. Fernandez and R.G. Kroll, 1996. The effect of temperature and growth rate on the susceptibility of *Listeria monocytogenes* to environmental stress conditions. Lett. Appl. Microbiol., 22: 121-124.

Pimentel, M.P., M.H. Silva, I. Cortes and A.M. Faia, 1994. Growth and metabolism of sugar and acids of *Leuconostoc oenos* under different conditions of temperature and pH. J. Appl. Bacteriol., 76: 42-48.

Rodriguez, A.V. and M.C. Manca de Nadra, 1995). Effect of pH and hydrogen peroxide produced by *Lactobacillus hilgadii* on *Pediococcus pentosaceus* growth. FEMS Microbiol. Lett., 128: 59-62.

Rowbury, R.J., M. Goodson and A.D. Wallace, 1992. The PhoE porin and transmission of the chemical stimulus for induction of acid resistance (acid habituation) in *Escherichia coli*. J. Appl. Bacteriol., 72: 233-243.

Rowbury, R.J., M. Goodson and T.J. Humphrey, 1993. Novel acid sensitivity induced in *Escherichia coli* at alkaline pH. Lett. Appl. Microbiol., 16: 223-227.