PROBIOTIC PROPERTIES OF ENTEROCOCCI – SURVIVAL IN SIMULATED GASTRIC JUICE AND WITH BILE SALTS

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INTRODUCTION

Enterococci are lactic acid bacteria, which can be employed as adjuncts in food to provide a wide variety of benefits (Chou and Weimer, 1999). Provided they are viable by the time of consumption, and do exhibit a beneficial effect upon the health of the host (via improvement of properties of the indigenous gut microflora of the consumer), they can be claimed as probiotics (Franz *et al.*, 1999; Sandholm *et al.*, 2002). They may positively contribute to the microbial balance in the intestine because of their ability to survive such adverse conditions as the acidic pH of gastric juice in the stomach, as well as the bile salts therein.

Cellular stress begins in the stomach, which bears a pH as low as 1.5. As such, acid lactic bacteria used as probiotic adjuncts should have the ability to resist the digestion process in the stomach, as well as the stressful conditions that are present there. After the bacteria have passed the stomach, they enter the upper intestinal tract where bile is secreted into the gut (Chou and Weimer, 1999). Bile tolerance is another important characteristic of probiotic bacteria since this enables them to survive, to grow and to perform their beneficial action in the small intestine (Taranto *et al.*, 1996).

A total of 73 strains of enterococci, isolated from Terrincho cheese - *Enterococcus faecium* (45.2% of all isolates), *Enterococcus durans* (39.7%) and *Enterococcus faecalis* (2.74%) were used in this work.

The main purpose of this study was to assess the survival of specific species of enterococci (following previous identification), when exposed to simulated gastric juice and bile salts.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Growth in M17 agar

Cultures were grown in MRS broth medium at 37ºC for 18-22h

Survival in simulated gastric juice

1% inoculum of each culture was added to test tubes containing prewarmed (37ºC) simulated gastric juice (0.08M HCl, containing 0.02% NaCl) at pH 1.5 (Sun *et al.*, 2000)

Control: cultures were added to 0.05 M sodium phosphate buffer, pH 7.0

Incubation at 37ºC

Sampling

0 min | 30 min | 60 min | 120 min

Samples of 1 ml were serially diluted in peptone water

20 µl were spotted onto M17 agar

Incubation at 37ºC

Viable counts (in cfu/ml)

Tolerance to bile salts

Cultures were inoculated (1%) into MRS broth and MRSO broth (MRS containing 0.3% Oxgall)

Incubation at 37ºC

Determination of optical density at 560nm ($O.D._{560}$) every hour for the first 8h and by 24h (Taranto *et al.*, 1996)

Experimental values were plotted against incubation time

Determination of time required to increase the $O.D._{560}$ by 0.3 units (via interpolation)

RESULTS

A screening of acid- and bile salt-tolerant enterococci isolated from dairy products was carried out in order to select the most resistant strains, as only those that satisfy both requirements may be candidates for actual probiotic strains. When placed in simulated gastric juice, the cultures were immediately exposed to the extreme low pH that parallels the conditions prevailing in the human stomach. Nevertheless, it should be noted that pH conditions were extremely low (pH = 1.5), which are very seldom observed under physiological conditions, especially after ingestion of a meal.

The results showed that the 73 strains of *Enterococcus* were very different in their resistance when exposed to acidic conditions (see Fig. 1).

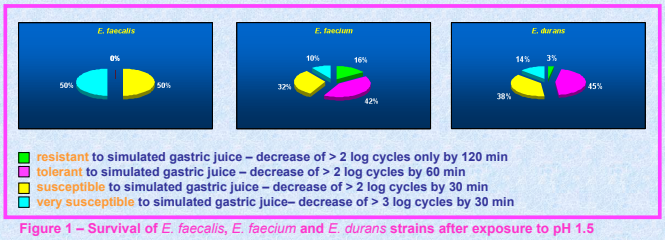

Figure 1 – Survival of *E. faecalis*, *E. faecium* and *E. durans* strains after exposure to pH 1.5

The resistance pattern is not characteristic of the species since within the same species of *Enterococcus* it is possible to observe different behaviours.

The strains of *E. faecalis* showed certain susceptibility to acid. Most strains of *E. faecium* and *E. durans* were tolerant to acidic conditions, and only a few *E. faecium* (16%) and *E. durans* (3%) were resistant to simulated gastric juice.

The bile salt tolerance was evaluated by comparing the growth of the cultures in the two culture media (MRS and MRSO broths). The ability to develop tolerance toward treatment with bile salts was expressed by a growth delay index (D – defined as the difference, in min, between the time required for the culture to reach $O.D._{560}$ = 0.3 in MRSO and the corresponding time in MRS). The lower the value of D, the higher the bile tolerance.

Significant variations in growth delay (D) were observed when comparing the growth rate of the enterococcal strains in MRS broth to that in MRSO broth (see Table 1).

Table 1: Range of values reported for the effect of bile salts on the growth of *Enterococcus* species – Time (min) needed to reach $O.D._{560}$ = 0.3

Strains	MRSO	MRS	D
E. faecalis 3	196	170	26
E. faecalis 1	218	181	37
E. faecium 27	146	144	2
E. faecium 17	134	129	5
E. faecium 16	135	129	6
E. faecium 23	142	127	15
E. faecium 18	240	220	20
E. faecium 12	121	95	26
E. faecium 19	243	205	38
E. faecium 20	186	147	39
E. durans 25	189	185	4
E. durans 30	189	179	10
E. durans 15	146	132	14
E. durans 11	146	125	21
E. durans 14	144	116	28
E. durans 22	192	161	31
E. durans 21	187	149	38

For all strains, the time required to reach $O.D._{560}$ = 0.3 was always higher when the cultures were grown in MRSO. Some strains showed to be very resistant to 0.3% bile salts, with low growth delays, whereas other strains exhibited high values for D. The most resistant strains shown in Table 1 (note that only strains for which different values were observed are listed) are those for which the delay of growth is lower than 15 min - *E. faecium* 27, 17 and 16; *E. durans* 25, 15 and 11.

- The ability of *Enterococcus* to survive in the gastrointestinal tract varies considerably between different strains of the same species.
- Only those strains which proved to be simultaneously resistant (or tolerant) to simulated gastric juice and bile salts can be candidates to probiotic strains.

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