



TOLERANCE OF ENTEROCOCCI TO BILE SALTS

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INTRODUCTION

Enterococci are lactic acid bacteria (LAB) of great importance in foods. They might also be considered as probiotics, which are typically associated with the human gastrointestinal tract. Before a probiotic can benefit human health, it must fulfill several criteria: it must survive passage through the upper gastrointestinal tract and thus arrive viable at its site of action; and it must be able to function in the gut environment (Saarela *et al.*, 2000).

As dietary adjuncts, probiotics contact with such compounds as bile salts, the presence of which is a common growth-limiting factor in the gut. Bile tolerance is an 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)

The purpose of this study was to assess the survival of 73 *Enterococcus* strains isolated from dairy products, to bile salts.

MATERIALS AND METHODS



A total of 73 enterococci were isolated from various dairy products (milk, whey, cheese curd and cheese).

A total of 60 colonies were isolated from Terrincho Cheese – 15 colonies were taken from 4 producers (from cheese at 30 d of ripening) and 45 from another producer (from cheeses at various stages of ripening: 0, 1, 4, 7, 14, 30 and 60 d).



Growth in M17 agar

Assays using liquid medium



Growth in MRS broth medium
37°C / 18-22 h



Inoculation into MRS broth and MRSO broth (containing 0.3% Oxgall), and incubation at 37°C / 24 h

Assays using agar plates

Each bacterium was streaked onto MRS agar plates with 0.3% bile salts; the agar pH was adjusted to 5.0, 6.0 and 7.0

Incubation at 37°C and examination for growth daily up to 4 d



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

RESULTS

The isolates were previously identified as *E. gallinarum* (1%), as *E. faecalis* (3%), as *E. casseliflavus* (11%), as *E. faecium* (37%) and as *E. durans* (48%) (Pimentel *et al.*, 2003).

A - Culture into MRS agar plates

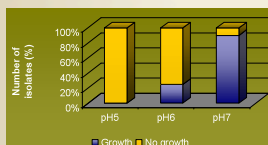


Fig. 1 – Total number of isolates (%) grown on MRS agar plates with 0.3% bile salts, at different pHs

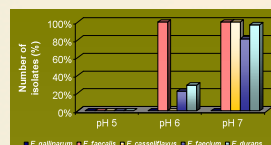


Fig. 2 – Number of isolates (%) of each species of *Enterococcus*

B - Culture into MRS broth

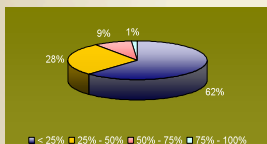


Fig. 3 – Percentage of inhibition of the total number of isolates, after 8 h of exposure to the effect of bile salts

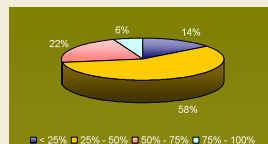


Fig. 4 – Percentage of inhibition of the total number of isolates after 24 h of exposure to the effect of bile salts

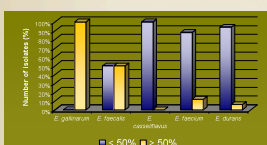


Fig. 5 – Number of isolates of each *Enterococcus* species, which revealed a percentage of inhibition <50% and >50% by 8 h of exposure to the effect of bile salts

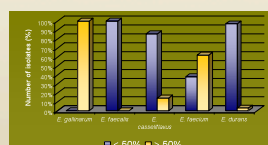


Fig. 6 – Number of isolates of each *Enterococcus* species, which revealed a percentage of inhibition <50% and >50% after 24 h of exposure to the effect of bile salts

CONCLUSIONS

- No isolate grew at pH 5 on MRS agar plates with 0.3% bile salts (Fig. 1 and 2).
- Only 25% of the isolates grew at pH 6, corresponding to 100% of the *E. faecalis* isolates, 22% of *E. faecium* isolates and 29% of *E. durans* isolates (Fig. 1 and 2).
- The great majority of the isolates grew at pH 7, except for only 10% (Fig. 1 and 2).
- After 8 h of exposure to bile salts in liquid medium, 62% of isolates showed an inhibition of less than 25%, which indicates that a great part of the isolates are quite tolerant to bile salts (Fig. 3).
- When exposed to bile salts for 24 h, the isolates have a higher inhibition (58% have an inhibition of 25%–50% (Fig. 4).
- Isolates from different producers of cheese (with the same time of ripening – 30 d) have a percentage of inhibition lesser than 50%, except for one isolate identified as *E. gallinarum* which revealed a percentage of inhibition higher than 50%, both at 8 and 24 h of incubation (Fig. 5 and 6).
- Great part of the isolates identified as *E. durans* have shown to be tolerant to bile salts (94% of isolates showed an inhibition below 50% after 8 h, and after 24 h, 97% of isolates exhibited similar inhibition) – Fig. 5 and 6
- On the other hand, 62% of *E. faecium* isolates had a percentage of inhibition higher than 50% after 24 h (Fig. 6).

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