



# Survival of potentially probiotic enterococci in the GI tract

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## INTRODUCTION

Many lactic acid bacteria (LAB) are being used as probiotics and the interest in the characterization of new strains is increasing. *Enterococci* are LAB that are important in environmental, food and clinical microbiology (Franz *et al.*, 2003). They are part of the natural microflora of several traditional cheeses, where they play an important role in the development of suitable organoleptic characteristics throughout ripening (Giraffa, G., 2002). Due to their good technological properties, members of *Enterococcus* have been proposed as to have potential for probiotic applications. However, their use remains controversial because of their infection-causing potential. Although enterococci are common in various foods, their safety record sets high demands for the selection of strains used in food applications.

The aim of the present study was to investigate the survival of two enterococcal strains (*Enterococcus faecium* 32 and *Enterococcus durans* 37), isolated from a traditional Portuguese cheese, in the GI-tract.

## MATERIALS AND METHODS

The selection criteria of the strains included safety issues: antibiotic susceptibility (12 antibiotics), virulence factors (cytolysin, adhesins); stability and adherence issues: resistance to bile and low pH, stability in milk and yoghurt and adhesion to the Caco-2 cell line.

### Strain Selection

- > Identification
- > Antibiotic susceptibility
- > Virulence factors
- > Resistance to bile and low pH
- > Adhesion to Caco-2 cell line

### Technological properties

- > Growth in a fermentation medium (GEM)
- > Stability in milk and yoghurt

### Survival in the GI-tract



#### "in vivo" study

Both strains were added, separately, to yoghurt, which was consumed by four healthy adults (A, B, C, D) for 5 days (150g of each yoghurt per day). Faecal samples were taken before, at the end and one week after the feeding period. The detection of the enterococci was performed culturing the samples on selective media, followed by RAPD-PCR.



*E. faecium* 32  
*E. durans* 37



300g /day  
(10<sup>7</sup> cfu/ml)

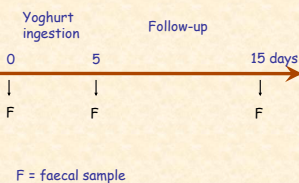
Faecal samples treated in anaerobic chamber



Culturing on selective media



2 morphotypes  
Identification by RAPD-PCR



## RESULTS AND DISCUSSION

The percentage of adherence to Caco-2 cell line was similar for both strains (ca.13%). Both strains exhibited good survival in the yoghurt, and proved to be resistant to bile and low pH.

Neither of the strains was detected in the faeces before the ingestion period (baseline) - see Fig 1.

*Enterococcus faecium* 32 and *E. durans* 37 were detected during the ingestion period (see Fig. 2), in all volunteers, although *E. faecium* 32 counts were 10 times higher than those of *E. durans* 37.

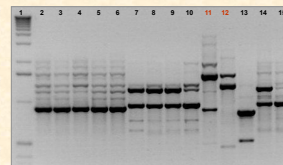


Fig. 1 - RAPD-PCR fingerprints of isolates obtained from baseline faecal sample of volunteer A. Lane 1: Ladder, Lanes 2-10: isolates from Slanetz & Bartley medium, Lane 11: *E. durans* 37, Lane 12: *E. faecium* 32, Lanes 13-15: isolates from Bile esculin azide medium

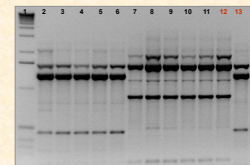


Fig. 2 - RAPD-PCR fingerprints of isolates obtained from faecal sample of volunteer A, during ingestion of yoghurt containing *Enterococcus*. Lane 1: Ladder, Lanes 2-11: isolates from Slanetz & Bartley medium, Lane 12: *E. durans* 37, Lane 13: *E. faecium* 32

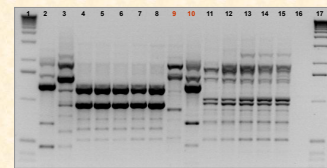


Fig. 3 - RAPD-PCR fingerprints of isolates obtained from faecal sample of volunteer A, 10 d after the ingestion (Follow-up). Lanes 1 and 17: Ladder, Lanes 2-8: isolates from Slanetz & Bartley medium, Lane 9: *E. durans* 37, Lane 10: *E. faecium* 32, Lanes 11-16: isolates from Bile esculin azide medium, Lane 17: negative control

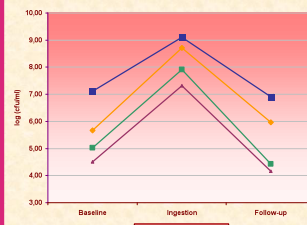


Fig. 4 - Viable counts (log cfu/ml) for volunteers A, B, C and D before (Baseline), during (Ingestion) and 10 d after the consumption of yoghurt (Follow-up)

The total viable numbers of enterococci increased during the consumption period by 3.0 log-cycles in individual A (see Fig. 4). The corresponding figures for individuals B, C and D were 2.9, 2.8 and 2.0, respectively. The numbers decreased to the baseline level during the follow-up of one week (Fig. 4). *Enterococcus faecium* 32 showed to be the prevalent strain during the consumption period in all volunteers, while *E. durans* 37 survived moderately through the GI-tract.

## CONCLUSION

- ❖ Two enterococci strains were chosen for the GI-tract survival study, since they had desirable properties of probiotics and no risk-related characteristics
- ❖ The strains were technologically feasible and showed excellent stability in milk and yoghurt
- ❖ The strains survived passage through the GI-tract and comprised a predominant part of faecal *Enterococcus* population during the ingestion of the yoghurt

## ACKNOWLEDGEMENTS

Financial support for the first author was provided by a Ph.D. fellowship (SFRH/BD/7000/2001), granted by the Portuguese government through program PRAXIS XXI (Fundação para a Ciência e Tecnologia, Portugal).

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