

Genotypic characterization of *Enterococcus faecalis* and *Enterococcus faecium* strains and correlation with their origin and functional and safety properties

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In the framework of the research project “Enterococci in Food Fermentations. Functional and Safety Aspects” (FAIR-CT97-3078) about 400 enterococcal strains have been collected, biochemically characterized, identified at the species level, and deposited in the FAIR-E collection (centrally maintained at the BCCM/LMG Bacteria Collection). The species *Enterococcus faecalis* and *Enterococcus faecium* largely dominated the isolates whether they originated from food, animals or humans. The occurrence of both species in nosocomial infections has questioned their safety in (fermented) foods, where they contribute to the ripening and product flavour. This has led to numerous epidemiological studies dealing with the prevalence of vancomycin-resistant *E. faecium* in hospitals or their assumed transmission from animals to humans. The present work aimed to reveal the genomic relationships between vancomycin-resistant and susceptible *E. faecium* and *E. faecalis* strains from diverse sources and to correlate these data with available functional and safety properties in foods and probiotics.

E. faecium and *E. faecalis* strains were genotypically typed using pulsed-field gel electrophoresis (PFGE) of *Sma*I restriction patterns, random amplified polymorphic DNA (RAPD)-PCR and amplified fragment length polymorphism (AFLP). In both species, PFGE demonstrated that the majority of the strains originated from different clonal lineages. In *E. faecium*, two main genomic groups (I and II) were obtained in both RAPD-PCR and AFLP analyses. DNA-DNA hybridization values between representative strains of both groups demonstrated a mean DNA-DNA reassociation level of only 71%. The two groups could be further subdivided into four and three subclusters in RAPD-PCR and AFLP analyses, respectively, and a high correlation was seen between the subclusters generated by these two methods. Subclusters of group I were to some extent correlated with origin and pathogenicity of the strains. Host specificity was, however, not confirmed. In *E. faecalis*, a much higher homogeneity was observed among strains of the species in both RAPD-PCR and AFLP analyses. However, the congruency between groupings obtained by both approaches was very low. When correlating the typing results with origin and safety properties, no clusters with unique biochemical features could be delineated.

The main conclusion is that for both, *E. faecalis* and *E. faecium*, the presence of safety properties (e.g. antibiotic resistance and potential virulence factors) and functional properties (e.g. antimicrobial activity) is strain-specific. For *E. faecium*, however, the incidence of some of these features correlated well with the intraspecific grouping and may be useful information for the selection of strains for cultures in food or probiotic preparations.