Antibiotic resistance of Enterococcus strains isolated from rabbit faeces and molecular characterization of the plasmid pCM1 harboured by Enterococcus faecium 8G

Raquel Linaje, Manuel Zúñiga, Gaspar Pérez-Martínez
Laboratory of Lactic Acid Bacteria and Probiotics
Department of Biotechnology, I.A.T.A. (C.S.I.C.), Spain

Introduction

Rabbits are monogastric herbivorous, which are widely used for research, purpose and studies. Digestive disorders are the main causes of mortality in commercial rabbit farms. These disorders have been linked to the administration of antibiotics in animal feeding and antibiotics may constitute a substitute alternative. In previous studies, the facultative anaerobic Enterococcus species were characterized as the sessile nature of Enterococcus strains isolated from rabbit faeces. Enterococcus were the anaerobic facultative bacteria isolated from rabbit faeces samples although certain strains of E. faecium and E. faecalis have been associated with food poisoning and foodborne infections. SeveralEnterococcus and enterococci bacteria isolated from different cultures of different cheeses and milk fermented products. Furthermore, probiotic properties have been claimed for certain enterococci strains.

The main concern for the use of enterococcal strains is their potential role as vehicles for the dissemination of antibiotic resistance genes among bacteria, particularly in the enterococcal species which are resistant to multiple antibiotics within the gut microbiota of rabbits, which is a concern for public health.

Conjugal transfer of antibiotic resistant markers from E. faecium 8G to Lactococcus lactis LM2301

Resistance to Co. Enterococcus species are known to transfer resistance from E. faecium 8G to L. lactis LM2301, however at a slower frequency (1.10^-4 transfers/cell)

Southern analysis confirmed the presence of genes, Cm, Sm and Tc in L. lactis LM2301. Both enterococci were analyzed by PCR amplification of enterococcal resistance genes. The resistance genes were detected in both enterococcal species by using the restriction enzyme Dde I.

Resistance genes are harbor by a plasmid

Electrophoretic analysis of DNA from Enterococcus faecium 8G and Lactococcus lactis LM2301 revealed the presence of two large plasmids (2.5-3 Mb) and one small (0.5 Mb). Resistance genes were detected in both enterococcal species by using the restriction enzyme Dde I.

Partial sequencing of pCM1 and comparative analysis

Regions of pCM1 adjacent genes, Sm and Tc were sequenced by inverse PCR (323 and 341 bp, respectively). Analysis of the region around Tc and Sm reveals a composite structure. An 8155-bp element separates two distinct regions. Region 1 is gene Sm and downstream sequence, which is homologous to conjugative transposon of P1-like excision, regulating the expression of Sm and Tc resistance genes. Region 2 was identified as a plasmid replicon.

Conclusion

Resistances to tetracycline, and to a lesser extent erythromycin, are common among Enterococcus strains isolated from rabbit faeces. Enterococcus carries a large collection of integron, which encodes resistance to a broad range of antibiotics. Enterococcus strains isolated from rabbit faeces were found to harbor integrons.

Phylogenetic analysis of genes carried by the plasmid pCM1

Phylogenetic analysis of enterococcal strains isolated from rabbit faeces was performed by using clonal analysis. The analysis revealed that all enterococcal strains isolated from rabbit faeces belong to the Enterococcus faecium 8G. Finally, the results of this study suggest that enterococcal strains isolated from rabbits may be potential vehicles for the dissemination of antibiotic resistance genes among bacteria.