

In vitro safety analysis of probiotic *Enterococcus* species isolated from dairy products and breast milk

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Enterococcus plays an important role in various food and feed industries. A number of *Enterococcus* species isolated from dairy products and breast milk are reported as probiotics. The most important criterion for these effective bacteria is their safety. Hence, before considering their use in probiotic supplements and or as starter cultures it is essential to evaluate their safety and confirm the absence of virulence traits including toxin production, antibiotic resistance etc. This study reports significant biochemical and molecular methods for assessing the virulence traits of *Enterococcus* species isolated from ewe milk, traditional cheese, poultry intestinal specimens, infant feces and mothers' milk. All isolates were identified to genus level by phenotypic characteristics and 16S rRNA sequencing. The selected isolates were screened for their probiotic properties by determining their acid and bile resistance, antibacterial activity against a number of pathogens, antibiotic resistance, hydrophobicity, aggregation and co-

aggregation properties. Phenotypic virulence parameters including lipase, DNase, hemolysis of red blood cells, lecithinase and coagulase production were determined. Virulence genes including *asa1*, *hyl*, *esp*, *agg*, *gelE*, *cylA*, *cylB*, *cylM*, *efaAfm*, *efaAfs* and vancomycin resistance genes including *vanA*, *vanH*, *vanR* and *vanY* were evaluated. Eight *Enterococcus* species were identified by 16S rRNA sequence. All isolates except *E. faecium* isolated from human milk were gelatinase positive. All isolates were alpha hemolytic and showed sensitivity to most often used antibiotics. Overall results indicated only the *Enterococcus* species isolated from breast milk to be lacking the tested virulence genes, while the other tested isolates harbored one or two of the virulence genes including vancomycin resistance genes. The results of the study calls for careful monitoring of the *Enterococcus* species to be used a probiotic adjunct in food or feed for animal or human use.