

**TITLE:** Biofilm production and invasion of mammary epithelial cells by *Enterococcus faecalis* and *Enterococcus mundtii* isolated from bovine subclinical mastitis

**AUTHORS:** RODRIGUES, D.S., SANTOS, G.S., SOUSA, K.S., CRISTOFORÊTO, M.C., SILVA, R.S.F, OLIVEIRA, J.S.S., MIRANDA, P.S.D, BARBOSA, V.N., PIMENTEL, B.A.S, NAGAO, P.E.

**INSTITUTION:** UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO, RJ (RUA SÃO FRANCISCO XAVIER, 524, PAVILHÃO HAROLDO LISBOA CUNHA, INSTITUTO DE BIOLOGIA ROBERTO ALCANTARA GOMES, DEPARTAMENTO DE BIOLOGIA CELULAR, 5º ANDAR, SALA 501B, CEP 20550-013, RIO DE JANEIRO – RJ, BRAZIL).

**ABSTRACT:**

Udder inflammations (mastitis) are the most frequent and cost-generating illness of dairy cows all over the world. Many different microorganisms can infect the mammary gland. Enterococci are one of the environmental causative agents of mastitis. These opportunistic bacteria are a part of normal physiological gut flora in humans and animals. There are no sufficient data from Brazil about the occurrence of *Enterococcus* spp. in mastitic cow's milk or about their susceptibility to antimicrobials, biofilm formation and interaction with bovine mammary epithelial cells. This investigation was undertaken to evaluate the occurrence of enterococci in cows with subclinical mastitis and to assess their antimicrobial resistance, genetic diversity, biofilm formation and the ability to interact with bovine mammary epithelial cells (MAC-T). The bacteria were identified using biochemical tests, MALDI TOF-MS and PCR multiplex (*tuf*, *sodA* and *ddl* genes) for identification of enterococci spp. A total number of 11 enterococci spp. were identified. Data showed that *Enterococcus faecalis* (*ddl<sub>E. faecalis</sub>*) was the predominant species (73%), followed by *E. mundtii* (*sodA<sub>E. mundtii</sub>*; 27%). Antimicrobial susceptibility tests showed that the highest level of resistance was observed with tetracycline (82%), erythromycin (72%) and quinupristin-dalfopristin (63%). The presence of the *vanA*, *vanB* and *vanC1/2* genes were not detected. Biofilm formation was performed using crystal violet staining at different times (6, 24 and 48h). Results demonstrated that all strains were biofilm producers in microplates. However, *E. mundtii* presented lower biofilm production when compared with *E. faecalis*. The biofilm formation was higher in bovine milk than in TSB medium after 24h incubation. PFGE analysis discerned 3 clones. For interaction assays with MAC-T, 3 strains were selected according to the different PFGE-types. All strains were able to adhere and invade MAC-T. These results indicated that biofilm formation by *Enterococcus*, especially in bovine milk might contribute in the pathogenesis during intramammary infection favoring the epithelial adhesion and invasion.

**Keywords:** Bacteria-host cell interaction; Biofilm production; Bovine mastitis; *Enterococcus*; MAC-T.

**Development Agencies:** CNPq, Capes.