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Biofilm Formation among *Enterococcus* Species from Milk and Raw Milk Cheese

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Introduction

Nosocomial bacteria called *enterococci* have the ability to create biofilms, which increase their pathogenicity and drug resistance. While several genes involved in biofilm formation have been discovered, little is known about how these genes are distributed among *enterococci* at the genomic level or how they might be used to diagnose biofilm morphologies. Gram-positive bacteria called *enterococci* cause dangerous nosocomial infections such as endocarditis, bloodstream infections, and urinary tract infections [1]. The potential of *enterococci* to create biofilms—populations of cells that are permanently adhered to and encased in a variety of biotic agents and abiotic surfaces—is well documented. Biofilms, which seem to be populations of cells that are permanently attached to and enclosed in a range of biological agents and abiotic substrates in a hydrated matrix of exopolysaccharide components, proteins, polysaccharides, and nucleic acids [2]. Bacterial pathogenicity is increased by biofilms in a number of ways. Due to Enterococcus spp.'s high rate of survival, the food preparation procedure does not completely eliminate all microbial cells from the raw material, allowing bacteria to adhere to the catheter by adhesion, an early stage of biofilm development. These bacteria thus constitute a component of the residual microflora in final goods [3].

One of the most crucial characteristics of Enterococcus spp. pathogenicity is their capacity to create biofilms [4, 5]. A population of cells is encased in a hydrated matrix called biofilms, which are made up of exopolymeric materials, proteins, polysaccharides, and nucleic acids and linked to diverse biotic and abiotic surfaces. The biofilm structure fosters the transmission of mobile genetic elements across various bacterial species and provides an ideal environment for bacterial development [6]. The production of biofilms involves several proteins. One of Enterococcus spp.'s most important virulence traits is its capacity to produce biofilms. By defending against antimicrobial agents and facilitating host cell attachment, this capacity permits colonization of inert and biological surfaces. The capacity of *enterococci* to create biofilms is well documented. A hydrated matrix of exopolysaccharides, materials, enzymes, polymers, and nucleic acids surrounds these populations of cells, which are permanently bound to numerous biotic and abiotic surfaces [7]. The biofilm structure promotes the transfer of mobile genetic components between bacteria and offers an ideal habitat for development [6].

On stents and other artificial devices, *enterococci* frequently build biofilms that call for long-term antibiotic treatment if removal of the device is not an option. Nonetheless, there is debate concerning the processes behind the pathogenicity and biofilm development of urinary *enterococci*. The oral pathogen *E. faecalis* that forms biofilms has been linked to conditions such caries, endodontic infections, periodontitis, and peri-implantitis. Consequently, in individuals with enterococcal infection, biofilm elimination with antibiotic treatment is crucial. Bacterial biofilms exhibit a phenotype of antibiotic resistance and are challenging to remove. In order to cure infections brought on by bacterial biofilms, combined therapy is advised. Surprisingly, vancomycin reduces biofilm formation in robust biofilm-forming isolates when given at subminimal inhibitory doses the faecal streptococcal regulatory operon, which consists of the three genes *fsrA*, *fsrB*, and *fsrC*, regulates biofilm formation, which is crucial for quorum sensing quorum sensing, which controls microbial gene expression in reaction to increased cell population densities, is generally linked to biofilm development. The genetic variables discussed above that are involved in biofilm production and control in enterocytes as well as proteins known as autoinducers often drive this regulation, and no conclusive genotype-phenotype link has been demonstrated for these determinants. These genes interact via *Fsr* quorum signaling as well as communicate via peptide pheromones secreted by recipient cells to trigger a donor cell conjugation mechanism that facilitates the transfer of pheromone-responsive plasmids. Connections among certain of these genes and biofilm formation have also been discovered. Several of these plasmids include genes, such as plasmid-encoded aggregate agent genes, that control or encourage the production of biofilms.

Materials and Method

Collection and Identification of Enterococcal Strains: From the Industry and Food Microbiology Collection, 85 isolates belonging to the *Enterococcus* genus were used in the study. They were separated using accepted techniques using raw milk and cheese. Matrix-assisted laser desorption ionization and duration mass spectrometry (MALDI-TOF MS) (Biomerieux) was used to identify the strain in accordance with the manufacturer's instructions [7, 8]. Each individual morphotype strain chosen after bacterial strain isolation and physicochemical characterization was assessed qualitatively in acidic environments (2 -

4 pH) and at various bile salt concentrations (300 - 3000 mg/dl). Using MRS medium at various pH (4 - 8), temperature (4 - 55 °C), NaCl concentration (2000 - 6000 mg/dl), and hydrocarbon concentration (400 and 600), Congo red agar (CRA) assay, and manufacturing by microtiter plate assay, the physical properties of the positive way grown isolated bacteria were checked further.

The technique developed by Freeman et al. [9] was used to examine mucus production using the CRA test. Onto CRA plates were put brand-new 24-hour colonies. The plates were then kept at 37 °C for a further 24 hours. Based on the color of the colony, strains were categorized as slime producers or non-slime producers, with Bordeaux and Red falling into the latter category. In a spectrophotometric microplate, absorbance at 570 nm was measured. Wells containing just broth served as the negative control. The arithmetic mean of three replicates was used to calculate the optical density (OD) of each test strain. The quantity was contrasted to the negative control's (ODc) maximum standard error above the mean. The arithmetic mean of three replicates was used to calculate the OD of each test strain. The number was contrasted to the negative control's (ODc) maximum standard error above the mean [10]. These findings led to the classification of the isolates as either non-biofilm producers (OD ODc), light biofilm producers (ODc OD 2 ODc), medium biofilm producers (2 ODc OD 4 ODc), or powerful biofilm producers (4 ODc OD). In order to separate the amplified products using 1.5% agarose gel electrophoresis in 1x Tris-borate-EDTA (TBE) buffer spiked with 0.5 g/ml ethidium bromide and visualize the results, microbial genes and pathogenicity have been amplified by the method of polymerase chain reaction using published specific primers and conditions.

Results and Discussion

Total 85 isolates from raw milk and raw milk cheese were identified by the MALDI-TOF MS method as *E. faecalis* (71; 83.5%), *E. faecium* (10; 11.8%), *E. gallinarum* (3; 3.5%), and *E casseliflavus* (1; 1.2%). *E. faecalis* species predominates among the strains recovered from both sources. 36 (73.5%) milk isolates generated a robust biofilm. While just one isolate of *E. faecalis* (2%) produced a significant biofilm, none of the strains did [11, 12]. Half of *Enterococcus* spp. are unable to create a biofilm with 24.5% of raw milk isolates. Any of the cheese's isolates developed a moderate biofilm, while the isolates from unpasteurized milk and dairy products cheese, had slime-forming capacities of 21/42.9% and 20/55.6% according to the CRA technique. In CRA, a distinct pattern of mucus production was seen. This ability was 55.2% and 100% of isolates when contrasted to isolates from raw milk, respectively. Isolates of *E. casseliflavus* and *E. gallinarum* were found in raw milk. *Asa1*, another extremely prevalent gene discovered in raw milk isolates, was identified in 66.7% of *E. faecalis* strains and 50% of strains from other species. The least frequent genes in the strains from this source were *cylA*, *fsrB*, and *sprE*, with frequencies of 4.1%, 6.1%, and 2.4%, respectively [13]. Also, the relationship between the source of isolation and the investigated genes *ebpC*, *pil*, *srt*, and *sprE* was discovered while taking into account all *Enterococcus* spp. -source of isolation of all the isolation strain (raw milk vs cheeses prepared from unpasteurized milk). At the 0.05 threshold of significance. The outcomes further shown that there are no discernible variations in the presence of virulence genes between biofilm-producing and non-biofilm-producing isolates of *Enterococcus* spp (Table 1) [14].

Table 1: Association between the occurrences of virulence genes and biofilm formation ability of all *enterococci* isolates, both from milk and cheeses [14].

Virulence genes	*Enterococcus* species (n = 85)		
	Biofilm Producers (n = 55)	Non-Biofilm Producers (n = 30)	p-value
	n (%)	n (%)	
gelE	48 (87.3)	26 (86.7)	1.000
esp	19 (34.5)	9 (30.0)	0.810
asa1	36 (65.5)	21 (70.0)	0.810
cylA	2 (3.6)	0 (0.0)	0.537
agg	16 (29.1)	12 (40.0)	0.341
ebpA	36 (65.5)	23 (76.7)	0.332
ebpB	34 (61.8)	23 (76.7)	0.228
ebpC	30 (54.5)	22 (73.3)	0.107
pil	29 (52.7)	21 (70.0)	0.167
srt	31 (56.4)	22 (73.3)	0.162
fsrA	2 (3.6)	4 (13.3)	0.179
fsrB	7 (12.7)	4 (13.3)	1.000

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