TITLE: MULTISPECIES BIOFILM FORMED BY THE CONTAMINATING MICROBIOTA OF RAW MILK

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In the food industries, contaminating microorganisms may appear as adherent and form complex structures called biofilms. In general, biofilms are cells aggregates embedded by extracellular polymeric substances produced by the microorganisms able to adhere to a surface. A biofilm community may comprise a single and/or multiple species of bacteria, forming a single layer or three-dimensional arrangements. The microorganisms present in the biofilm are more resistant to the action of antimicrobial agents used in cleaning. This study aimed to evaluate the biofilms formed by the contaminating microbiota of raw milk, as well as its microbial diversity. Two samples of raw milk were used, one freshly milked with a low initial contamination of 10^4 Colony Forming Unit (CFU)/mL and one bulk tanks with contamination 10^6 CFU/mL. Stainless steel coupons were used for the biofilm formation at 7 °C $\pm$ 2 for 10 days, the milk being replaced every 48 hours. The biofilms were observed on the tenth day by laser confocal microscopy to determine the structure in its hydrated state and to visualize the cellular viability. The viable cells numbers were determined by spread plate method on Plate Count Agar (PCA). The biofilms formed by microbiota from freshly milked milk reached, at tenth day incubation, counts around 4.4 x 10^5 CFU/cm^2 and those from bulk tanks milk had a count of 1.6 x 10^6 CFU/cm^2. Compact biofilms were not visualized on coupons immersed in freshly milked milk by laser confocal microscopy, while in those immersed in bulk tanks milk, biofilms were observed with thicknesses around 18 μm and viable cells presented from the base to the apex. The diversity of the multispecies biofilm was analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) using universal primers for Bacteria. There was a similarity of 85% between the biofilms communities formed in the presence of the two milk samples used. However, in biofilms formed by the contaminating microbiota of freshly milked milk, a decrease in diversity was noted over days 6, 8 and 10, while in the biofilms formed by the contaminating microbiota of bulk tanks milk, the diversity increased. These results demonstrate that raw milk contaminants were capable of multi-species biofilm formation, and could become an important source of contamination for dairy products.

Keywords: multispecies biofilm, raw milk, contaminants, stainless steel.

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