Title: BIOFILMS FORMATION *IN VITRO* BY PSYCHROTROPHIC BACTERIA ISOLATED FROM BUFFALOS RAW MILK REFRIGERATED

Authors: BOGO, M.[1]; FRAZZON, A. P.[1]; MOTTA, A. S.[1]

Institution: [1] UFRGS – Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas e da Saúde, Departamento de Microbiologia, Imunologia e Parasitologia (Rua Sarmento Leite, n° 500, CEP 90050-170, Bairro Farroupilha, Porto Alegre/RS, Brasil.

Abstract:

Psychrotrophic bacteria pose a negative impact on the dairy industry. These microorganisms thrive at low temperatures and possess the ability to form biofilms, which may hinder the microbiological quality of dairy products. Thus, this study aimed to evaluate the formation of biofilms by 21 psychrotrophic bacteria isolated from raw milk from buffalo cooled. The formation of biofilms was performed in 96-well microplates by inoculation of 20 uL of a suspension of each isolate (1.5 x 10^8UFC / ml) in 180µL Tryptone Soy Broth (TSB). The plates were incubated at 7 ° C for 72 hours and at 30 ° C and 37 ° C for 24 hours. Thereafter, the TSB medium was aspirated and the wells washed with 0.85% saline. The adhered cells were fixed with methanol PA, stained with crystal violet solution (5%), washed in water and the remaining dye was re-solubilized in ethyl alcohol (95%). The experiment was conducted in octoplicata. As a negative control, sterile TSB medium was used and as positive control was used a strain of *Staphylococcus epidermidis* ATCC 35984. The reading of the optical density (OD) of each well was performed in microplate reader (λ = 450 nm). The cutoff (OD) was defined as the mean optical density (OD) added three standard deviations of the negative control. Biofilms were classified as weak when ODC <OD ≤ (2 x ODC), when moderate (2 x ODC) <OD ≤ (4 x ODC), when strong (4 x ODC) <OD and negative when OD <ODC. Of the 21 bacterial isolates analyzed in this study, 16 (76.19%) were biofilm formers to 7 ° C. Of these 16 isolates, 2 (9.52%) were classified as moderate forming biofilms and 14 (66.6%) as weak. At 30 ° C, 20 isolates (95.23%) were biofilm formers. Of these, 3 isolates (14.28%) were termed moderate and 17 (80.95%) were weak biofilm formers. For the temperature of 37 ° C, 17 (80.95%) were biofilm-forming weak. No isolate was determined as strong biofilm-forming in the evaluated conditions. These results highlight the importance of psychotropic bacteria *in vitro* biofilm-forming; and this behavior can be displayed in the dairy industry. The importance of implementing the appropriate sanitization process is intended to avoid the accommodation of microorganisms on the contact surfaces with dairy products.

Keywords: contamination, dairy products, psychrotrophic bacteria, biofilm.

Development agency: CNPq