

Identification, enzymatic spoilage potential, and adhesion ability of *Pseudomonas* spp. isolated from the dairy production chain

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Pseudomonas spp. are among the most common spoilage bacteria in refrigerated dairy products. They can produce heat-resistant extracellular enzymes that degrade milk components, constituting the main cause of spoilage in milk and dairy products. Furthermore, the high capacity of these bacteria to adhere and form biofilms on surfaces contributes to their persistence in the dairy environment. In this study, *Pseudomonas* spp. were isolated from different sources in dairy production chain, subsequently identified, and characterized regarding their proteolytic and lipolytic activities and ability to adhere to polystyrene at two temperatures. A total of 157 *Pseudomonas* spp. were isolated from samples of water (n = 38), milk (n = 71), surface (n = 22), and Minas Frescal Cheese (n = 26) collected from 10 dairies and identified by checking a genus-specific PCR using 16S rDNA gene. Furthermore, their proteolytic and lipolytic activities were evaluated using 2% skimmed milk agar and tributyrin agar, respectively. These isolates were characterized in terms of their ability to adhere to polystyrene both at 30 °C for 48 h and at 4 °C for 168 h, using 1% skimmed milk culture medium, simulating the presence of milk residues on an inadequately sanitized surface. Only 20.4% (n = 32) of the isolates exhibited the ability to adhere to polystyrene, as well as protease and lipase activities, regardless of the incubation conditions. Most isolates with these characteristics (n = 19, 59.4%) were obtained from raw milk. However, a greater number of isolates (n = 71, 45.2%) adhered to polystyrene and exhibited protease and lipase activities when incubated at 30 °C for 48 h compared to at 4 °C for 168 h (n = 37, 23.6%). Regarding biomass quantification, 34.4% (n = 11) of the isolates exhibited an increase in biomass adhered to polystyrene at 4 °C, highlighting the impact of thermal stress on adhesion of *Pseudomonas* spp. and, consequently, their persistence in dairy environment. These results demonstrate variability in the potential for enzymatic spoilage and adhesion ability among the *Pseudomonas* spp. evaluated. The expression of these characteristics can be influenced by the incubation temperature, depending on the isolate. Although few, isolates that exhibit all these characteristics, regardless of the incubation temperature, were isolated from the four sources. Our findings can contribute to the understanding of the diversity of dairy spoilage *Pseudomonas* species and their sources of contamination, helping to develop effective elimination strategies and thus extending shelf life of dairy products.

Key words: Biofilm; Cheese; Proteases; Lipases.

Identificação, potencial de deterioração enzimática e capacidade de adesão de *Pseudomonas* spp. isolados da cadeia produtiva de lácteos

Pseudomonas spp. formam biofilmes em superfícies incorretamente higienizadas e produzem enzimas hidrolíticas que deterioram lácteos. Neste estudo, 157 *Pseudomonas* spp. foram isoladas em laticínios, identificadas, avaliadas quanto às atividades enzimáticas e adesão ao poliestireno em duas temperaturas. A maioria aderiu e produziu enzimas a 30 °C, comparado com 4 °C. Alguns isolados (n = 32, 20,4%) aderiram e produziram enzimas nas duas temperaturas, sendo a maioria (n = 19, 59,4%) provenientes de leite cru. Os resultados revelam a diversidade bacteriana e de suas fontes de contaminação, enfatizando a importância de se desenvolver estratégias eficazes de eliminação, visando preservar produtos lácteos.

Palavras-chave: Biofilme, queijo, proteases, lipases