

Tolerance to sanitizers and influence of stress conditions on biofilm formation by *Staphylococcus gallinarum* isolated from sweetened condensed milk

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Sweetened condensed milk (SCM) is a product obtained through the partial dehydration of milk with added sugar. Osmotolerant *Staphylococcus* spp. can contaminate, grow, and spoil low-aw foods, including those containing high sugar. Bacteria belonging to this group can also form biofilms on food-processing surfaces. This study aimed to determine the tolerance of *Staphylococcus* spp., isolated from SCM to different sanitizers, evaluate the biofilm-forming ability of the isolates, and evaluate the influence of stress conditions on biofilm formation. We determined the tolerance of 13 strains of *Staphylococcus* spp. to sodium dichloroisocyanurate (Cl^{-free}), quaternary ammonium (QAC), and peracetic acid (PAA) by exposing planktonic cells (10⁷ CFU/ml) to concentrations ranging from 0 to 1,000 ppm for 15 minutes. The biofilm-forming ability of the isolates was assessed in 96-well microtiter plates using brain heart infusion broth (BHI) and an inoculum of 10⁶ CFU/ml, followed by incubation at 30 °C for 48 hours. Biofilm quantification was carried out by crystal violet assays. A strong biofilm-forming and QAC-tolerant isolate was cultured at both 20 °C and 30 °C for 48 hours in BHI under varying conditions of pH (5.5; 7.0), sucrose (0%, 5%, 10%, and 20%), and sublethal concentration of QAC (0; 1.96 ppm) to assess its ability to form a biofilm under these conditions. The tolerance of planktonic cells to sanitizers ranged from 1.95 to 7.81 ppm for Cl^{-free}, 3.91 to 31.25 ppm for PAA, and 7.81 to 31.25 ppm for QAC. Only one of the isolates could form biofilm and was identified as *Staphylococcus gallinarum* by matrix-time-of-flight laser-assisted desorption mass spectrometry (MALDI-TOF-MS) and confirmed by whole genome sequencing and comparative analysis. The sessile cells of *S. gallinarum* were more resistant to disinfection with QAC, supporting up to 16,000 ppm, compared to the planktonic cells, which supported only 32.5 ppm. The presence of 5% and 10% sucrose in the culture medium favored biofilm formation only at 30 °C. Inversely, 20% sucrose did not influence the formation of biofilms at 30 °C but reduced the formation of biofilms at 20 °C. Both acid pH and sublethal concentration of QAC negatively affected the biofilm formation regardless of the incubation temperature. Our data demonstrate variability in the tolerance to sanitizers *Staphylococcus* spp. isolated from condensed milk. In addition, sucrose can promote biofilm formation depending on temperature and sugar concentration, which can compromise the eradication of these bacteria in the SCM-processing industry.

Key words: *Staphylococcus* spp.; biofilm; quaternary ammonium; sucrose.

Tolerância a sanitizantes e influência de condições de estresse na formação de biofilme por *Staphylococcus gallinarum* isolado de leite condensado

O leite condensado (LC) é o produto resultante da desidratação parcial do leite adicionado de açúcar. *Staphylococcus* osmotolerantes podem contaminar, crescer e deteriorar alimentos com alto teor de açúcar. O objetivo deste estudo foi determinar a tolerância de *Staphylococcus* spp., isolados de LC a diferentes sanitizantes, avaliar a capacidade de formação de biofilme e avaliar a influência de condições de estresse na formação de biofilme. Os resultados demonstraram variabilidade na tolerância de *Staphylococcus* spp. isolados de LC aos sanitizantes. Além disso, a sacarose pode promover a formação de biofilme por essas bactérias dependendo da temperatura e concentração do açúcar.

Palavras-chave: *Staphylococcus* spp.; biofilme; quaternário de amônio; sacarose.

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