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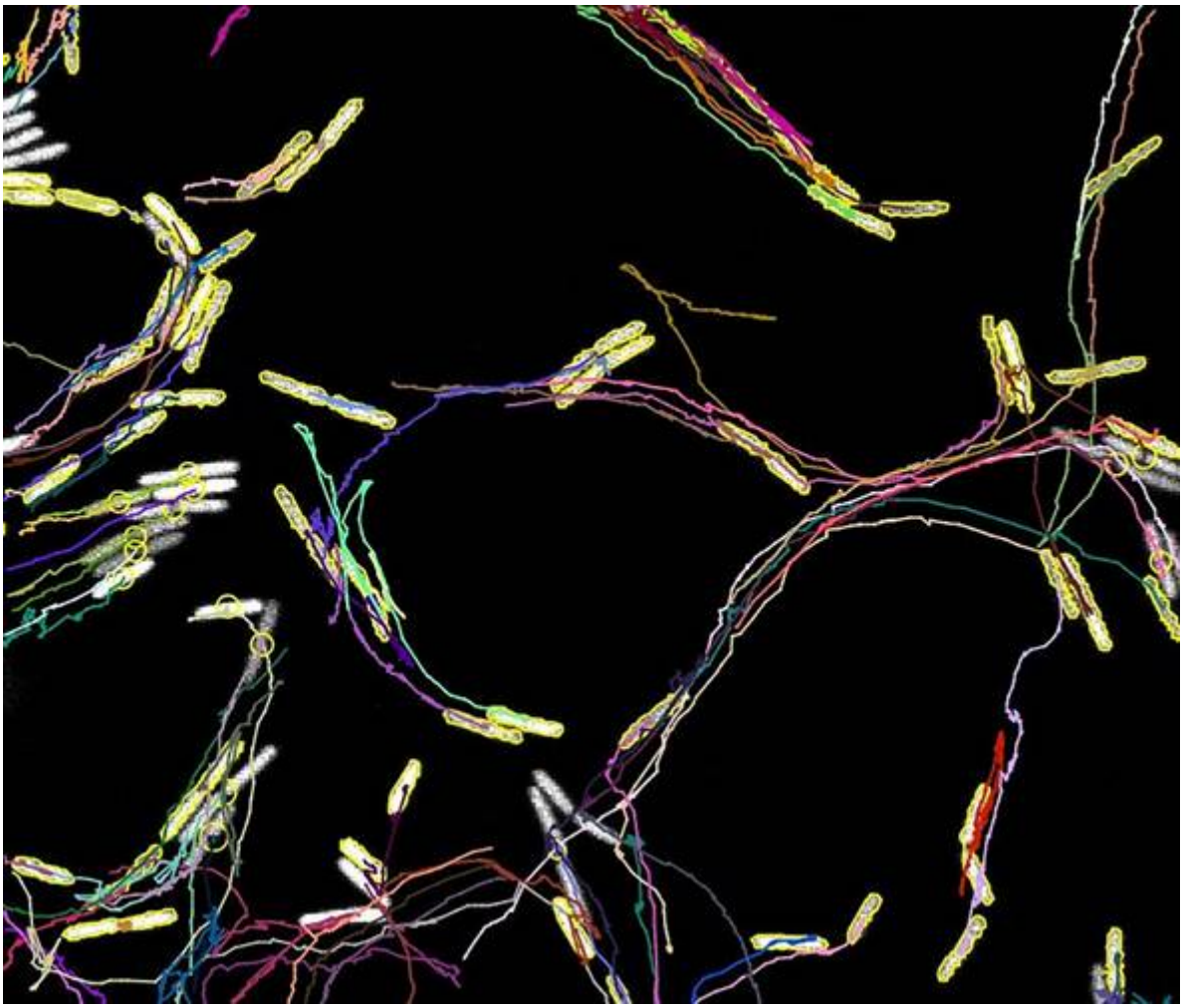

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CHEMICAL CHARACTERIZATION AND THERAPEUTIC POTENTIAL OF ACTIVE COMPOUNDS OBTAINED FROM THE COMMENSAL MICROBIOTA OF THE MAMMARY GLAND AGAINST *Staphylococcus aureus* BIOFILM

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Bovine mastitis (BM) is a multifactorial disease characterized by the inflammation of mammary tissue caused by microbial infections, leading to significant economic losses in the dairy industry. Despite rigorous control measures, the prevalence of BM remains high, highlighting the need for innovative therapeutic strategies. *Staphylococcus aureus* is the most common contagious pathogen in mastitis cases in Argentina and other dairy-producing countries. Its ability to form biofilms in the mammary gland is a key virulence factor, conferring resistance to antimicrobial agents and enabling the evasion of the host's immune response, contributing to the persistence of infection. The commensal microbiota of the mammary gland plays a critical role in the pathogenesis of mastitis and represents a promising source for developing alternative prophylactic or therapeutic products to conventional antimicrobials. This study aimed to characterize the chemical nature and therapeutic potential of antibiofilm compounds secreted by *Staphylococcus chromogenes* LN1 in preventing *S. aureus* colonization in the mammary gland. The LN1 strain, isolated from milk samples of healthy animals with no history of mastitis, produced a cell-free supernatant (CFS) with antibiofilm activity against *S. aureus*. The active compounds were purified through acetone precipitation (Pa-LN1) and dialysis (D-14). The antibiofilm activity against *S. aureus* strain V329, in vitro antioxidant capacity using DPPH• radical scavenging, Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR) analysis, and cytotoxicity in bovine mammary epithelial cells (MAC-T) using AlamarBlue and PicoGreen assays, were evaluated. Both Pa-LN1 and D-14 significantly reduced biofilm biomass starting at 0.125 mg/mL, achieving reductions between 50% and 65% at 2.5 mg/mL and 5 mg/mL respectively. D-14 demonstrated moderate antioxidant capacity (CD50 of 0.82 mg/mL) compared to Pa-LN1 (CD50 of 2.06 mg/mL). FTIR and ^{1}H-NMR analyses revealed no changes in band positions but did show variations in signal intensity. After 24 and 48 hours of treatment, Pa-LN1 resulted in nearly a 30% decrease in detectable DNA levels without significantly affecting the metabolic activity of MAC-T cells, while D-14 exhibited no adverse effects on these cells. Although both compounds were effective in reducing *S. aureus* biofilm, D-14 stands out for its lower cytotoxicity and moderate antioxidant capacity. Importantly, the use of compounds produced

by the mammary gland microbiota, such as those from *S. chromogenes* LN1, highlights a novel and promising approach for managing bovine mastitis. These findings suggest that utilizing the natural properties of commensal microorganisms could lead to safer and more effective prophylactic or therapeutic options, making D-14 a particularly promising candidate for future applications.

Palabras clave: bovine mastitis – biofilm - Staphylococcus aureus - comensal microbiota – bioactive compounds