

**TITLE:** HYICIN 4244: EVALUATION OF ITS POTENTIAL BIOTECHNOLOGICAL APPLICATION AS FOOD BIOPRESERVATIVE AND ANTIMICROBIAL AGENT

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**ABSTRACT:**

Hyicin 4244 is a bacteriocin related to subtilisin A and produced by *Staphylococcus hyicus* 4244, a strain isolated from milk of cow with bovine mastitis. This bacteriocin showed a broad spectrum of activity, inhibiting strains of different genera and species, including bacterial strains of food importance, such as *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus sporothermodurans*, and of clinical relevance, such as *Staphylococcus aureus* resistente à meticilina (MRSA), *Enterococcus* resistente à vancomicina (VRE), *Neisseria gonorrhoeae* and *Neisseria meningitidis*. In order to test the potential application of hyicin 4244 as a food biopreservative, a crude preparation of this bacteriocin were added to skimmed milk samples previously inoculated with either *L. monocytogenes* or *S. aureus*. Hyicin 4244 was able to inhibit the growth of both food pathogens in TSB medium and also in skimmed milk for 24 h at 37°C. Furthermore, hyicin 4244 proved to be effective in inhibiting the biofilm formation by strong biofilm producer strains isolated from nosocomial infections and bovine mastitis, even when the bacteriocin was pre-adsorbed to an abiotic surface. Hyicin 4244 reduced significantly the number of CFU and the biofilm formation by two strong biofilm-forming Staphylococcal strains involved in human infections and bovine mastitis. Cell viability analysis performed by using SYTO 9/propidium iodide showed that hyicin 4244 (256 AU/mL) treatment caused a reduction (approximately 50%) of the viable cell counts in both staphylococcal pre-formed biofilms, when compared to the controls subjected to no bacteriocin treatment. The effectiveness of hyicin 4244 against biofilm formation by both strains was also confirmed by scanning electron microscopy. Therefore, hyicin 4244 proved not only to prevent biofilm formation by planktonic cells but also to penetrate the biofilm matrix *in vitro*, exerting bactericidal activity against staphylococcal sessile cells. These data have shown the potential biotechnological applications of hyicin 4244 as a food biopreservative and as an antimicrobial agent for either prevention and/or treatment of human and animal biofilm-related infections caused by different staphylococcal species.

**Keywords:** *Staphylococcus hyicus*; bacteriocin; hyicin 4244; biotechnological application; biofilm-related infections, food biopreservative.