Hylin-a1: a new weapon peptide against antibiotic-resistant bacteria

A. Chianese[1,2], C. Zannella[1,2], R. Giugliano[1], F. Foglia[1,2], R. Della Marca[1], A. Ambrosino[1,2], MV. Morone[1], F. Palma[1], E. Panico[2], A. Monti[3], N. Doti[3], G. Franci[4], A. De Filippis[1], M. Galdiero[1,2].

1 Department of Experimental Medicine, University of Campania Luigi Vanvitelli, 80138 Naples, Italy.
2 Microbiology and Virology Unit, University hospital of Campania Luigi Vanvitelli, 80138 Naples, Italy
3 Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), and CIRPEB, Centro Interuniversitario di Ricerca sui peptidi Bioattivi, 80131 Naples, Italy
4 Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, 84081 Baronissi, Italy

Background. In recent years, the resistance of pathogenic microorganisms to common antimicrobial agents is representing a severe public health problem. Moderate and wise use of antimicrobials and prevention of infections are the most effective methods for decreasing the spread and development of resistance. Therefore, the World Health Organization (WHO) intensified searching for new agents capable of fighting emerging bacteria. Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs), play a crucial role in innate immunity, representing one of the first barriers against external attack. In the present study, we evaluated the antibacterial activity of the Hylin-a1 peptide, derived from the frog skin of *Heleioporus albopunctatus*, against several strains of Gram-positive bacteria, e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus mutans*, and clinical isolates).

Methods. Peptide toxicity was evaluated on human keratinocytes cells (HaCaT) by the metabolic 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT). Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and time-killing assays were performed to deepen how the peptide exerted its action on bacterial cells. The antibacterial activity was evaluated following the broth microdilution method outlined by the National Committee on Clinical Laboratory Standards (NCCLS) using sterile 96-well microliter plates. Peptide concentrations were selected based on cytotoxicity calculated by MTT assay (50-0.39 µM). The antibacterial power was evaluated against Gram-positive bacteria, in detail *S. aureus* ATCC, *S. epidermidis* ATCC, *E. faecalis* ATCC, *S. mutans* ATCC. The antibacterial activity of Hylin-a1 was tested against clinical strains isolated from Microbiology Laboratory of the University of Campania "Luigi Vanvitelli".

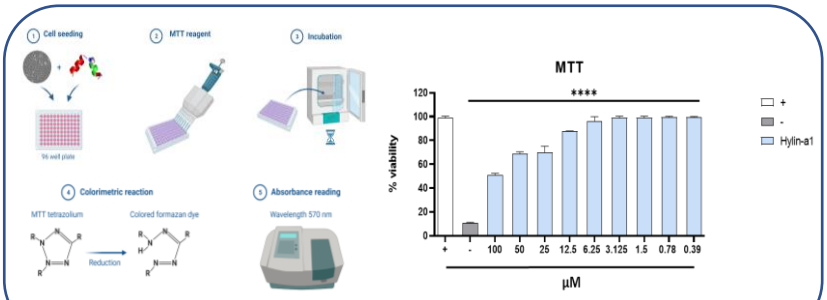


Figura 1: Evaluation of toxicity of Hylin-a1 on HaCaT cells

Results.
The peptide showed no detected toxicity except at high concentrations, identifying a 50% cytotoxic concentration (CC_{50}) at 50 µM. Hylin-a1 exerted a very strong antibacterial effect against *S. aureus* and its clinical isolates. In detail, the peptide inhibited bacterial infectivity at the MIC concentration of 6.25 µM. On the other hand, Hylin-a1 also interfered with the activity of other Gram-positive bacteria with MIC values ranging from 25 to 6.25 µM.

Antibacterial activity

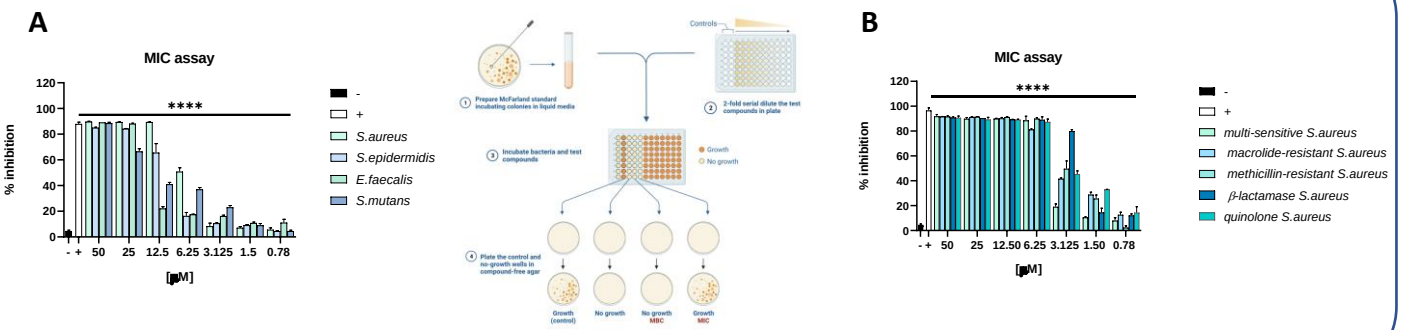
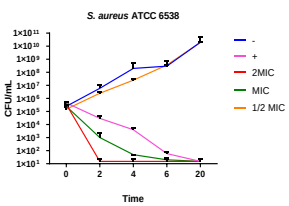
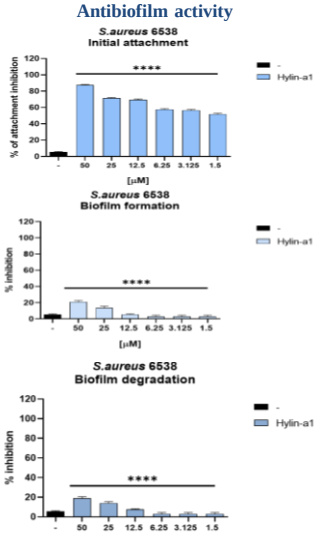


Figure 2: Antibacterial activity against gram-positive bacteria

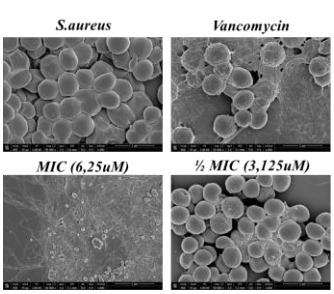
Killing kinetics of Hylin-a1 against *S. aureus* strains



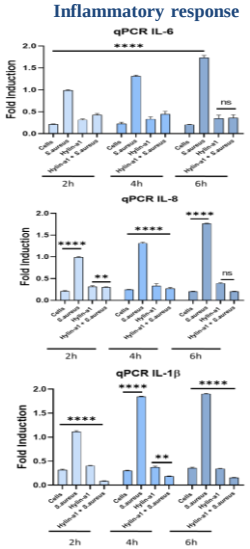
Antibiofilm activity



SEM analysis



Inflammatory response



Conclusions. Altogether, our results indicated Hylin-a1 as a peptide with potential therapeutic effects against a wide variety of human pathogenic bacteria.