Ruminococcin C VS Nisin : natural antimicrobial peptides against biofilms formation

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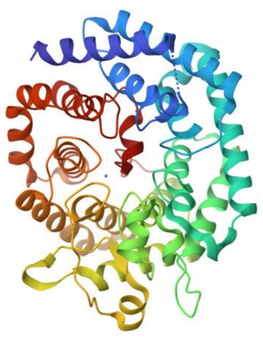
The use of antimicrobial peptides (AMPs) covalently grafted on surfaces has been recognized in recent years as a promising strategy to fight against biofilms formation. However, after grafting, the understanding of AMPs-bacteria interactions is still debated in the literature. In this study, **Ruminococcin C (RumC)** and **Nisin (Nis)** were covalently immobilized on silicon and gold surfaces to study and compare their bactericidal activity against two bacterial species : *Staphylococcus aureus* (Gram+ bacterium) and *Escherichia coli* (Gram- bacterium).

NISIN



Nisin 3D structure
34 AA

Lantibiotic, cyclic AMP.

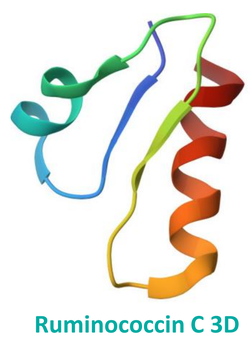
Produced by ***Lactococcus lactis***

5 thioether rings : Ala 3 - Ala 6, **Ala** 11 - Abu 7, **Ala** 19 - Abu 13, **Ala** 26 -Abu 23, **Ala** 28 - Abu 24

Mechanism of action : binds lipid II, blocks cell wall synthesis, and forms pores that cause leakage and rapid cell death → **rapid effect**.

Antimicrobial spectrum : broadly active against **Gram-positive bacteria,** limited or no activity against **Gram-negatives**.

RUMINOCOCCIN C



Ruminococcin C 3D structure
44 AA

Sactipeptide, double-hairpin structure.

Produced by ***Ruminococcus gnavus* E1,** from human gut symbiot.

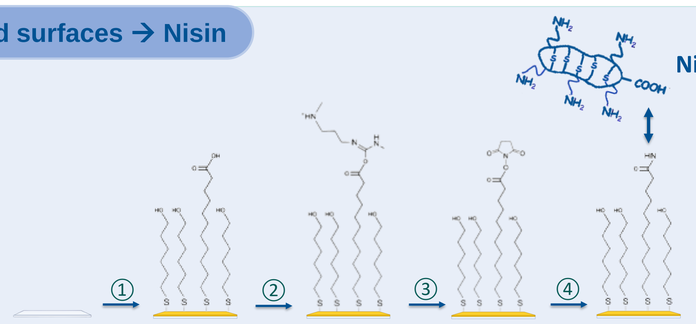
4 sulfur-to-α-carbon thioether bridges : **Cys** 3 - Asn 16, **Cys** 5 – Ala 12, **Cys** 22 - Lys 42, **Cys** 26 - Arg 34

Mechanism of action : binds lipid II and inhibits cell wall synthesis and essential biosynthetic pathways (DNA, RNA, ATP) but does **not** form pores → **slow effect**.

Antimicrobial spectrum : effective against several **Gram-positive** pathogens, small activity against some **Gram-negative species.**

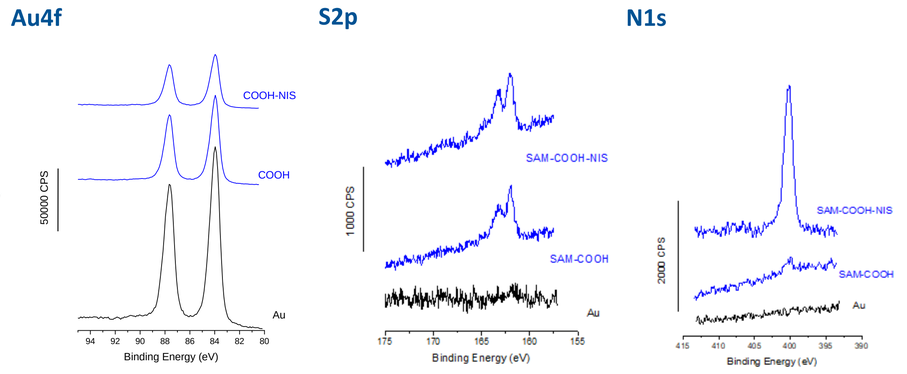
GRAFTING STRATEGY

Gold surfaces → Nisin

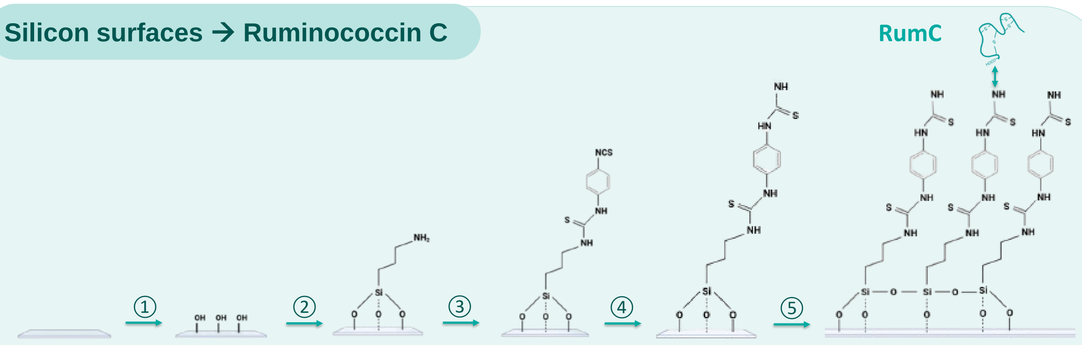


Cathodic deposition of gold layer on glass ① before creation of a thiols Self-Assembled Monolayer (SAM) ②, then activated thanks to carboxylic function ③ before Nisin molecule grafting ④.

XPS

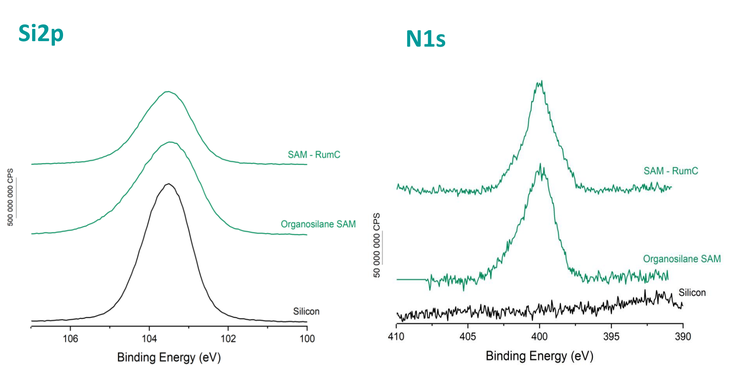


Silicon surfaces → Ruminococcin C



Plasma activation of silicon surface ① before creation of an organosilane Self-Assembled Monolayer (SAM) ②.
Grafting of a thiocyanate spacer ③ before Ruminococcin molecule grafting ④.

XPS



Quantification
(equivalent thicknesses)

$n\text{-}S_{COOH}$ = 5,6 /nm²

$n\text{-}Nis_{S/COOH}$ = 0,55 /nm²

$n\text{-}APTES_{Si}$ = 33 /nm²

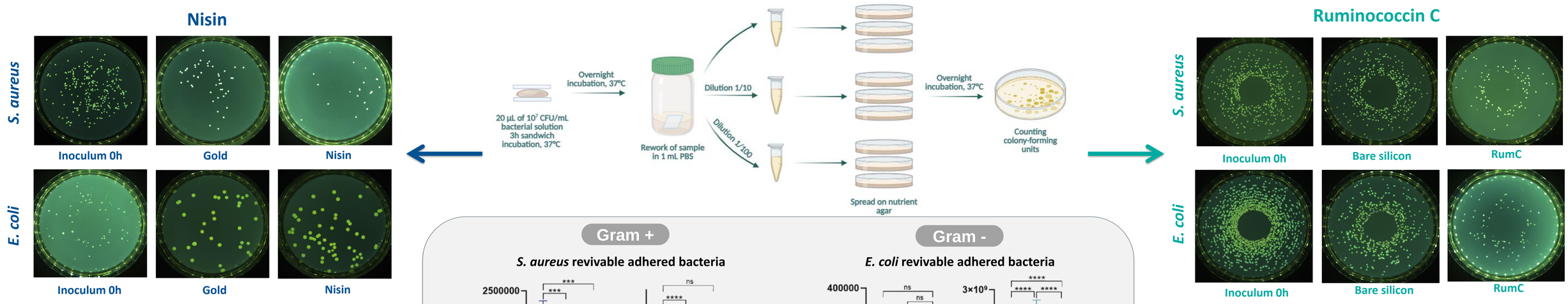
$n\text{-}RumC_{APTES}$ = 1,9 /nm²

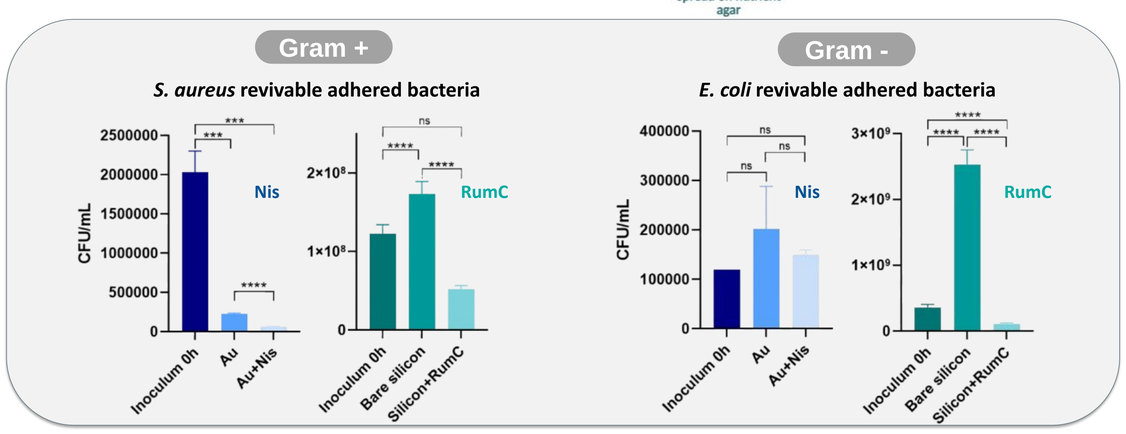
Large rate of **APTES** molecules on silicones :

- high roughness of the silicone surface (breast prothesis)
- **APTES** forms a **dense layer** on the surface, unlike **thiols**, which are **ordered**

4 x more RumC on surfaces

MICROBIOLOGICAL ANTIBACTERIAL TESTS





Nisin	% Killing/inoculum	% Killing/ref. surface (i.e. gold surface)
Staphylococcus aureus	96,8	72,2
Escherichia coli	< 0	23,2

RumC	% Killing/inoculum	% Killing/ref. surface (*i.e.* bare silicon)
Staphylococcus aureus	73,6	76,9
Escherichia coli	83,2	89,78

CONCLUSION & PROSPECTS

Both AMPs **effective against *S. aureus* bacteria** on surfaces, as expected. Concerning **Gram-negative bacterium**, **Nisin** has **no real activity against *E. coli***, as expected, but **RumC** is **effective against *E. coli***. RumC seems to be a better antibacterial peptide to use to prevent biofilm formation.

Next, antimicrobial activity would be done on **titanium surfaces** (to mimic implants surfaces) and a serie of experiments would be performed with **mammalian cells** to evaluate the **cytotoxicity** of the AMPs, on silicon and titanium surfaces. Moreover, bacterial concentration used in this study is 10^7 CFU/mL, far away from reality. Indeed, it could be interesting to determine the **bactericidal effect at lower concentration**, miming a more realistic concentration in case of infection (about 10^3 CFU/mL).
Finally, as the mechanisms of action of the two AMPs are different, it would be interesting to evaluate the antibacterial activity over time (from hours to several days).

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