IPLA INSTITUTO DE PRODUCTOS LÁCTEOS DE ASTURIAS

25–28 MAY 2025 BRUSSELS PALACE OF THE ACADEMIES

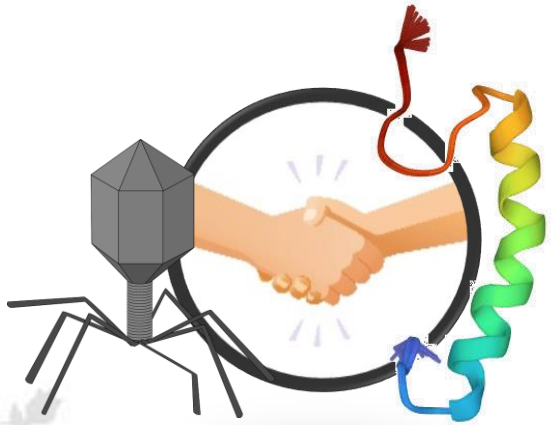
ONE HEALTH IN FOCUS

RASAB NACOMI
The Royal Academies for Science and the Arts of Belgium
National Committee of Microbiology

AS2AMR Advancing Solutions to Antimicrobial Resistance

2nd Bacteriocin International Conference BIC2025

The Bac-Bac network: bacteriocins and bacteriophages in food biotechnology



Beatriz Martínez, IPLA-CSIC

GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

CSIC Consejo Superior de Investigaciones Científicas

IPLA INSTITUTO DE PRODUCTOS LÁCTEOS DE ASTURIAS

CSIC Consejo Superior de Investigaciones Científicas

IPLA INSTITUTO DE PRODUCTOS LÁCTEOS DE ASTURIAS

ACKNOWLEDGMENTS

AIM: Understanding natural antimicrobials for biotechnological applications

PID2020-119697RB-I00

MINISTERIO DE CIENCIA E INNOVACIÓN

AGENCIA ESTATAL DE INVESTIGACIÓN

PID2023-149852OB-I00

MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

UNIÓN EUROPEA

FONDO EUROPEO DE DESARROLLO REGIONAL

"Una manera de hacer Europa"

AGENCIA ESTATAL DE INVESTIGACIÓN

IDE/2024/000721

Cofinanciado por la Unión Europea

SEKUENS

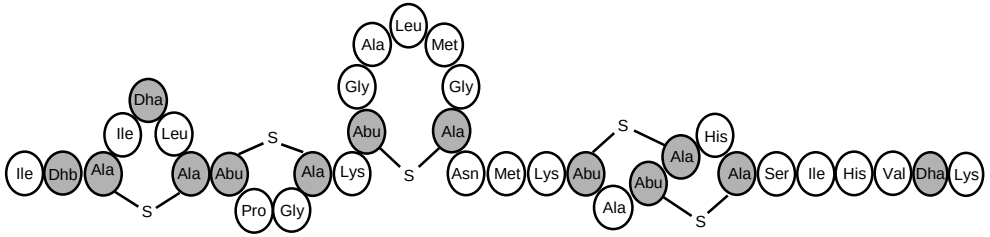
Agencia de Ciencia, Competitividad Empresarial e Innovación del Principado de Asturias

CSIC

Consejo Superior de Investigaciones Científicas

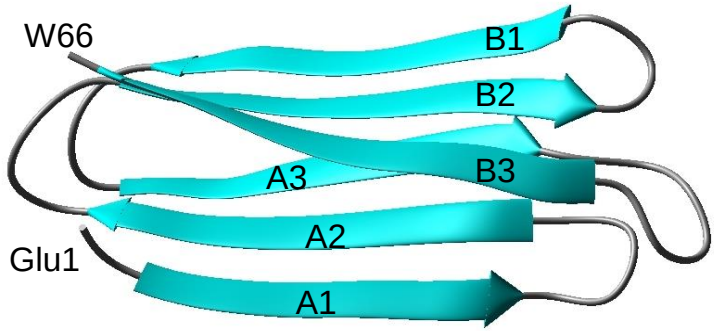
BACTERIOCINS

- Ribosomally-synthesized antimicrobial peptides produced by bacteria
  - Large structural and functional diversity



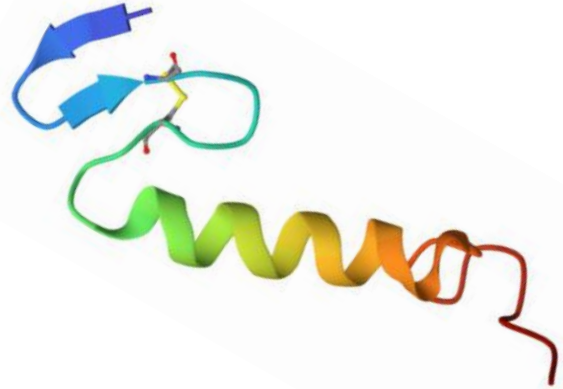
Nisin

- Lantibiotic
- Pore formation and inhibition of cell wall biosynthesis



Lcn972

- Class IId
- Inhibition of cell wall biosynthesis

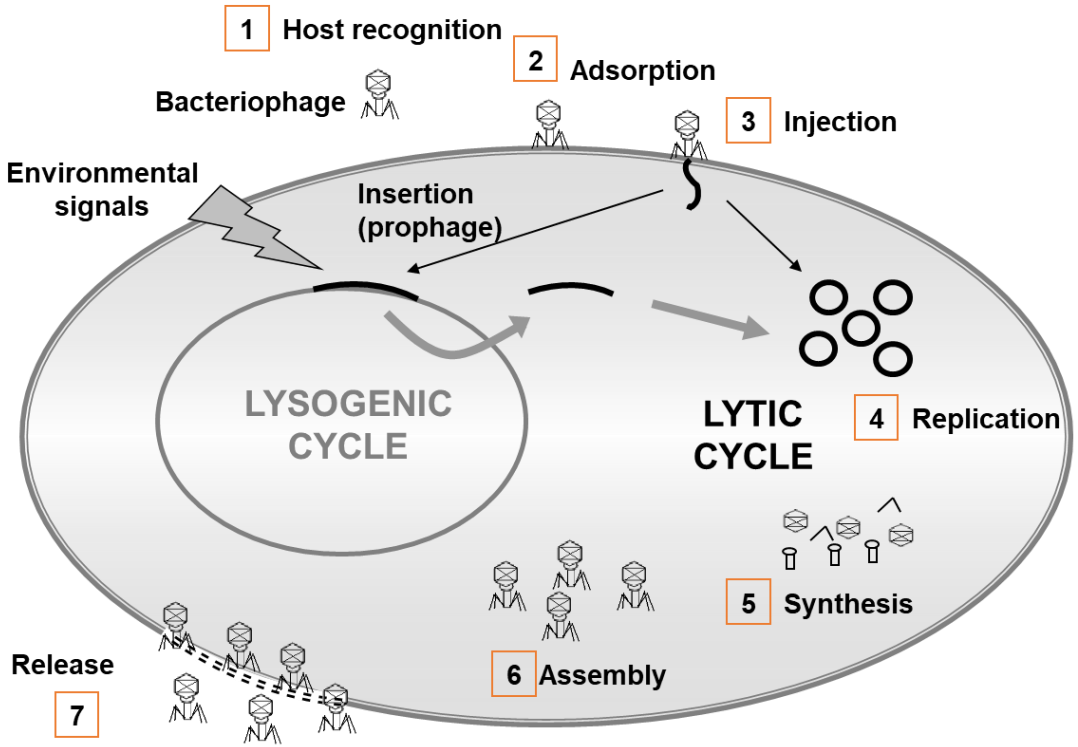


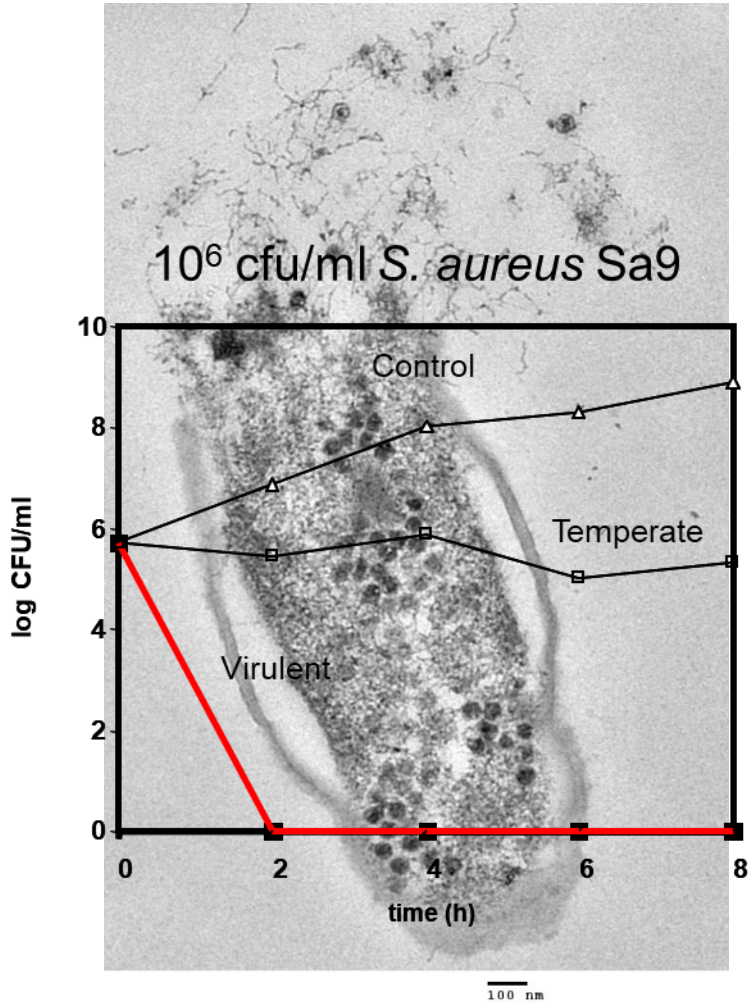
Pediocin-like

- Class IIa
- Man-PTS / Pore formation

BACTERIOPHAGES

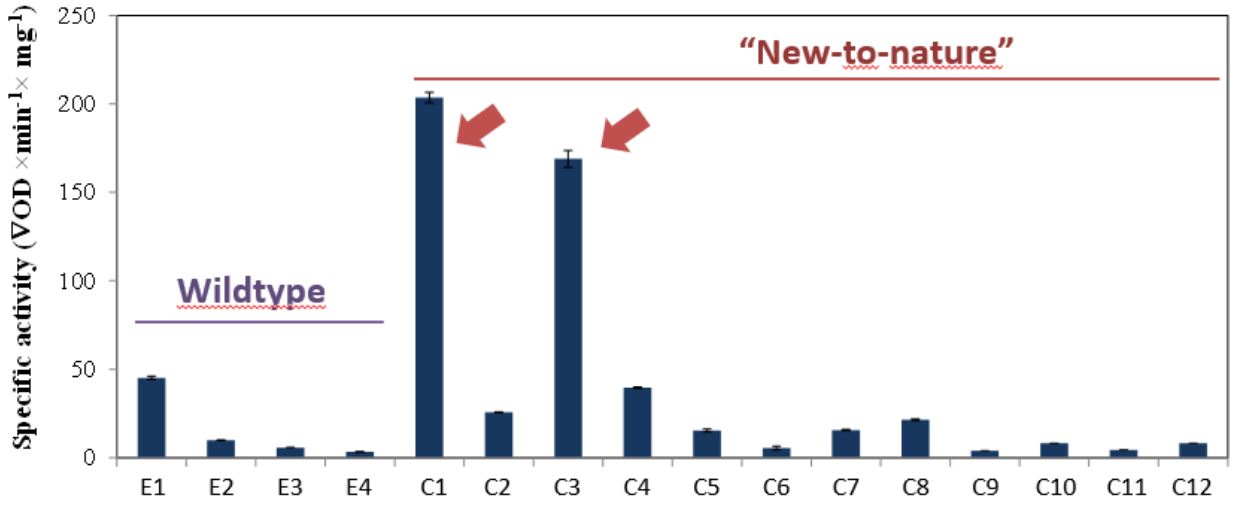
- Viruses of bacteria
  - Friends and enemies

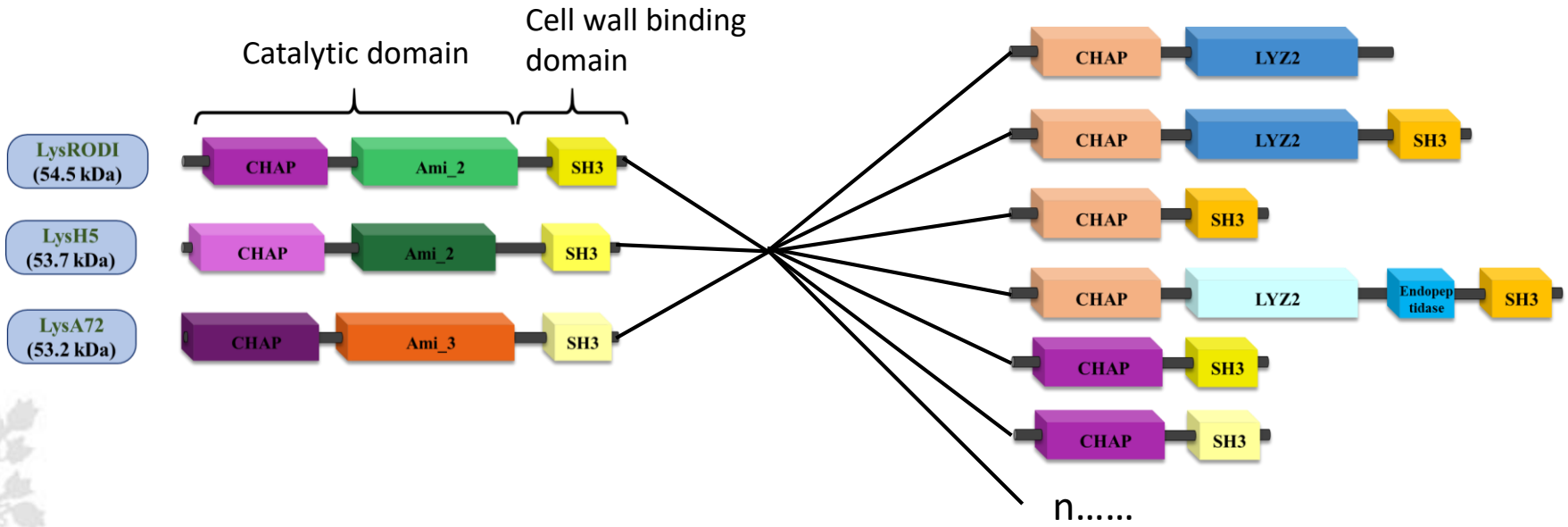




PHAGE ENDOLYSINS

- Peptidoglycan hydrolases
  - Modular enzymes



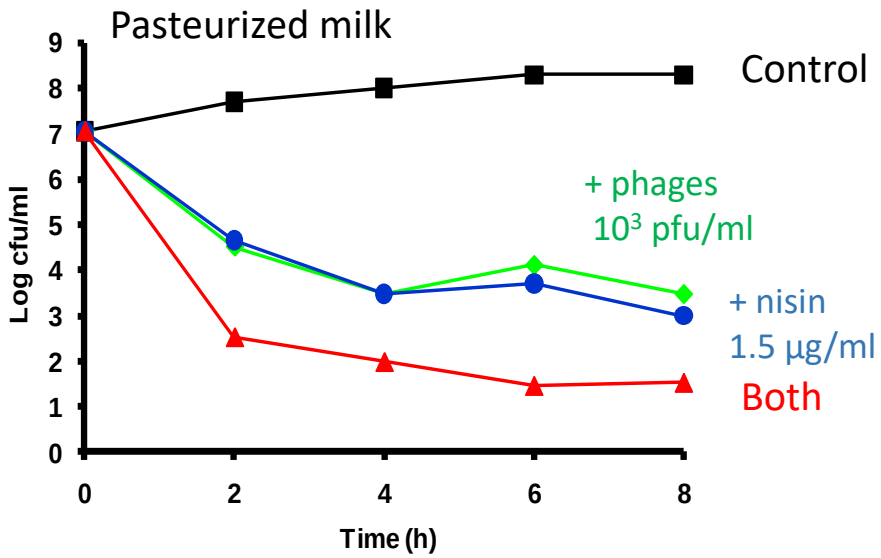


Gutiérrez et al., 2021. *Front. Microbiol.* 12:723834; Rodríguez-Rubio et al. 2012. AEM 78(7):2241

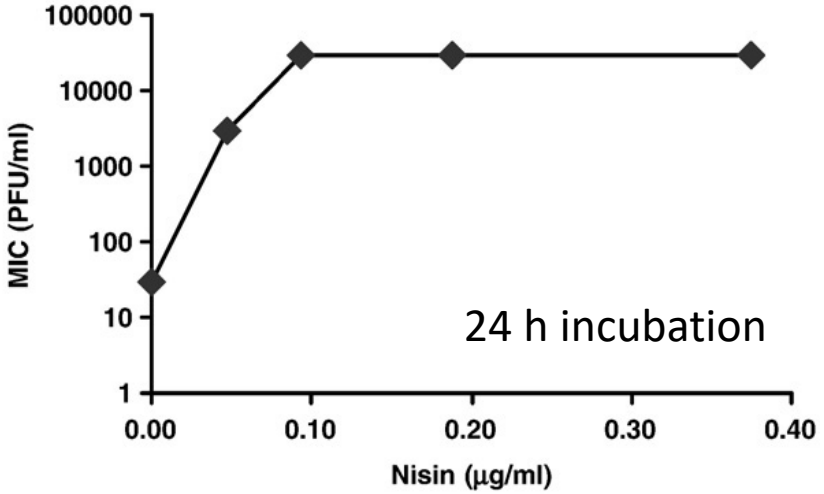
WHAT ABOUT ANTIMICROBIAL ACTIVITY WHEN COMBINED?

JOINING FORCES?

- Hurdle technology
  - Nisin + phages
  - *Staphylococcus aureus*



👍**Synergy**



S. aureus	Nisin	EOP [b]		Adsorption (%)	
	MIC [a] (µg/ml)	Φ35	Φ88	Φ35	Φ88
Sa9WT	0.75	1	1	99.9±0.1	99.7±0.5
Sa9R	>100	0.002±0.0001	0.5±0.03	56±0.5	96±0.3
Sa9SR	0.75	1.1±0.3	1±0.2	85.3±0.2	99.9±0.3
Sa9 BIM B2	0.75	0	0	97.6±0.3	13.9±3.0
Sa9 BIM B6	0.75	0	0	ND [c]	<3
Sa9 BIM B13	0.75	0	0	57.8±7.1	7.5±2.9

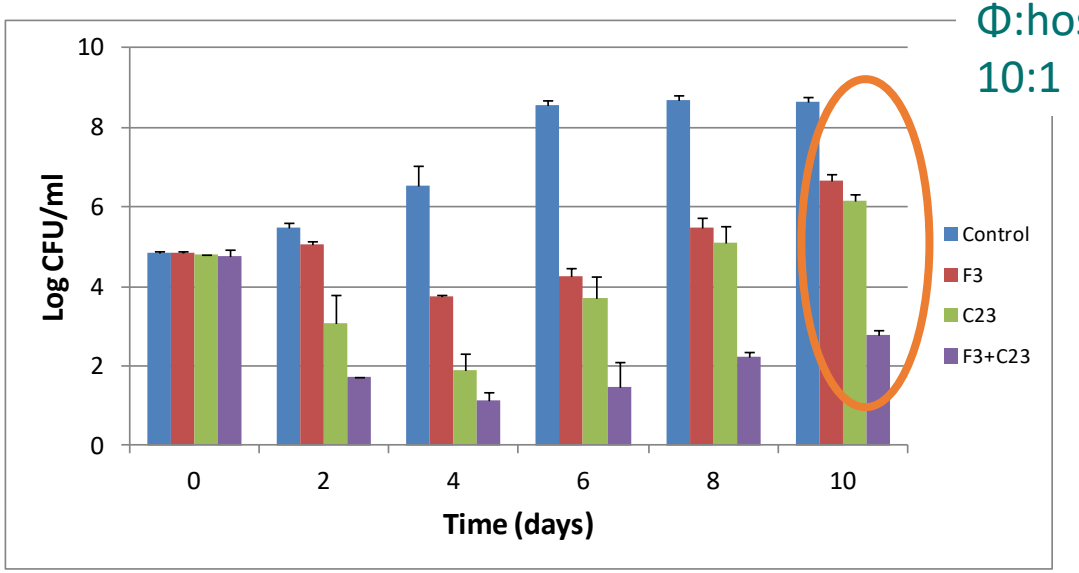
👎**Cross-tolerance**

👎**Change of surface properties**

Martínez et al., 2008. Int J Food Microbiol 122:253–258

JOINING FORCES

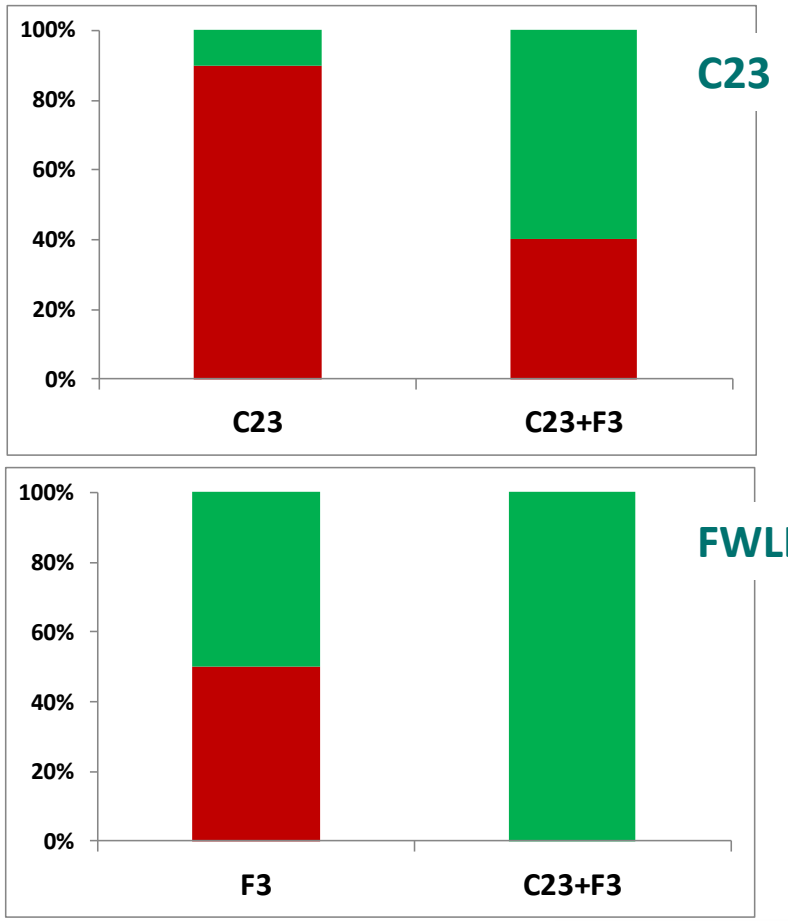
- Hurdle technology
  - Coagulin A (Pediocin-like) + phages
  - *Listeria monocytogenes*
  - Milk, 4 °C, 10 d



Φ:host 10:1

Randomly selected colonies

Resistant / Sensitive



Treatment

Synergy

Reduced frequency of resistance

Rodríguez-Rubio et al., 2015. Int J Food Microbiol 205:68–72

JOINING FORCES

- Hurdle technology
  - Lysin + Nisin
  - *S. aureus*
  - Milk, 37 °C

LysH5

Low ← Cell density → High

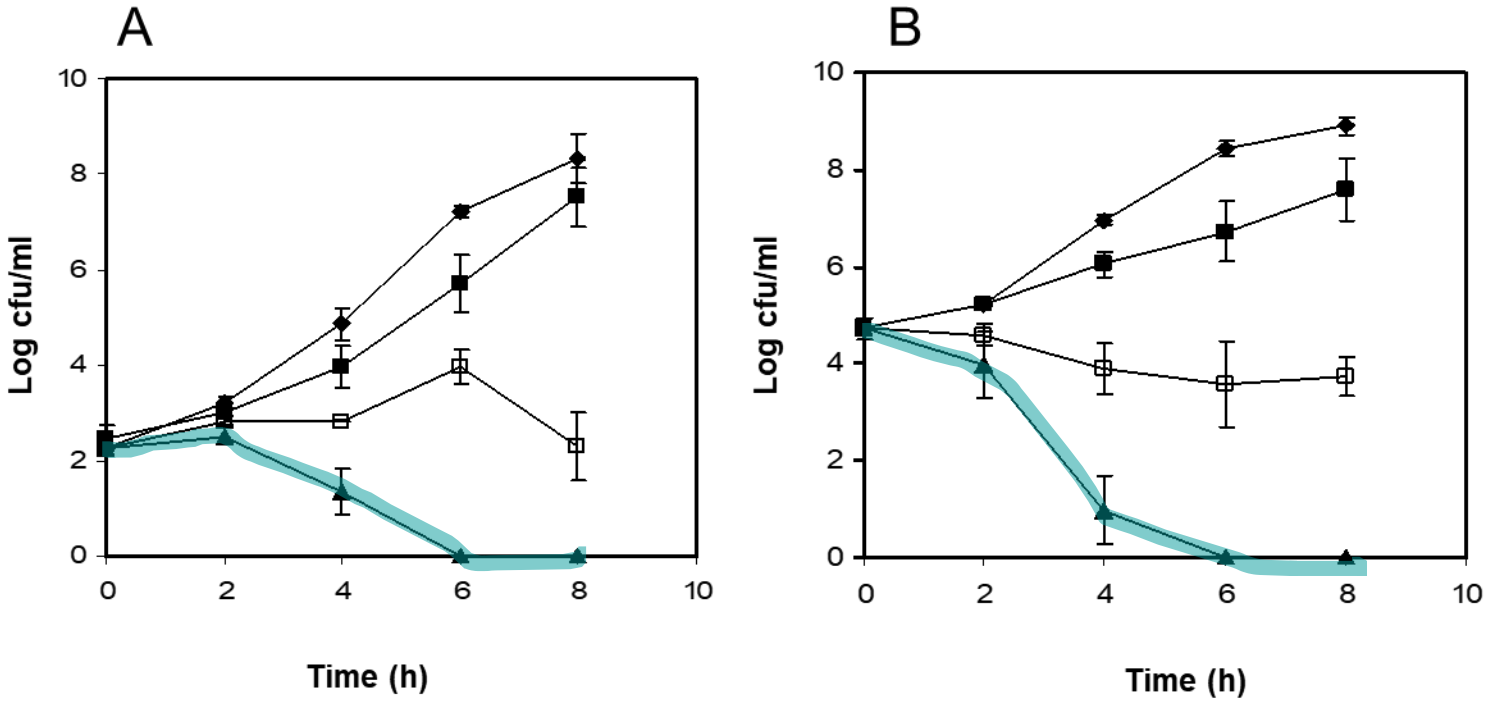
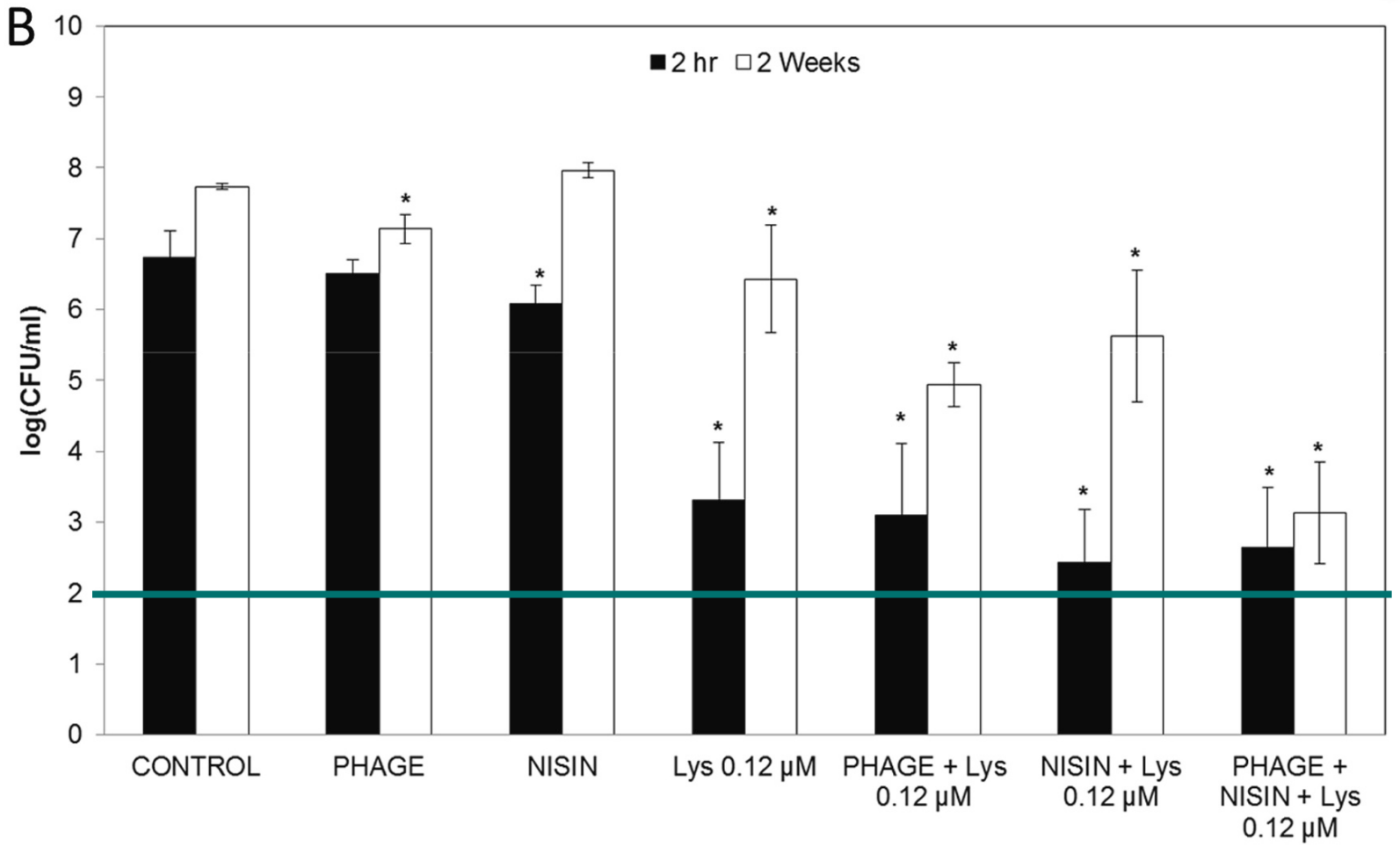


Fig. 3. Killing of *S. aureus* Sa9 with purified LysH5 and nisin in pasteurized whole milk. A) ♦, cell numbers of *S. aureus* Sa9; ■, LysH5 (7.5 U/ml); □, nisin (0.37 μg/ml); ▲, LysH5 (7.5 U/ml) and nisin (0.37 μg/ml). B) ♦, cell numbers of *S. aureus* Sa9; ■, LysH5 (15 U/ml); □, nisin (0.75 μg/ml); ▲, LysH5 (15 U/ml) and nisin (0.75 μg/ml). Values are the means of two independent experiments with standard deviation indicated by vertical bars.

García et al., 2010. Int J Food Microbiol 141: 150-155

JOINING FORCES

- Hurdle technology
  - Phage + Lysin + Nisin
  - *S. aureus*
  - Lab-scale cheese, 12 °C



Youssef et al., 2023. Int J Food Microbiol 399: 110227

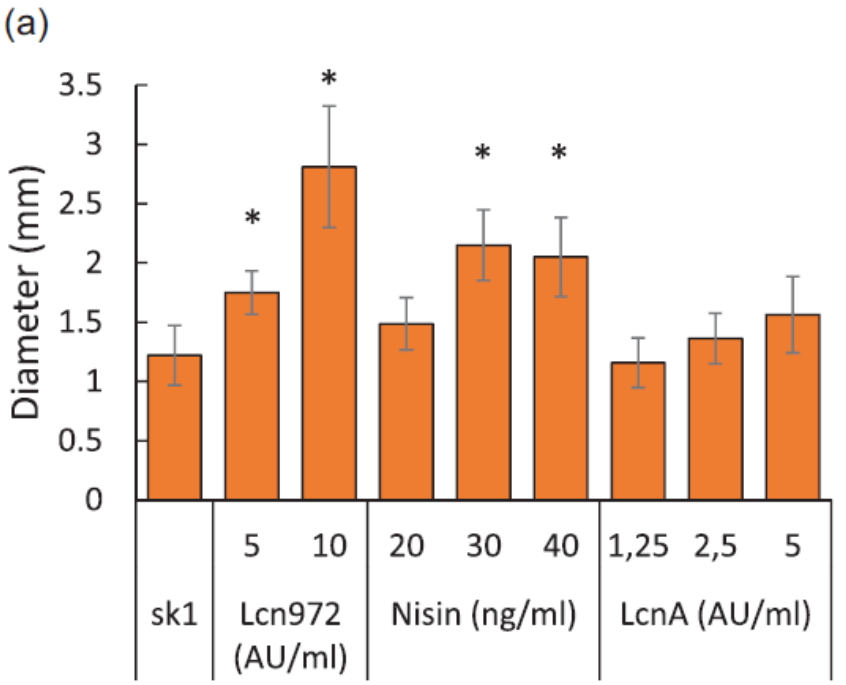
WHAT ABOUT DAIRY FERMENTATIONS?

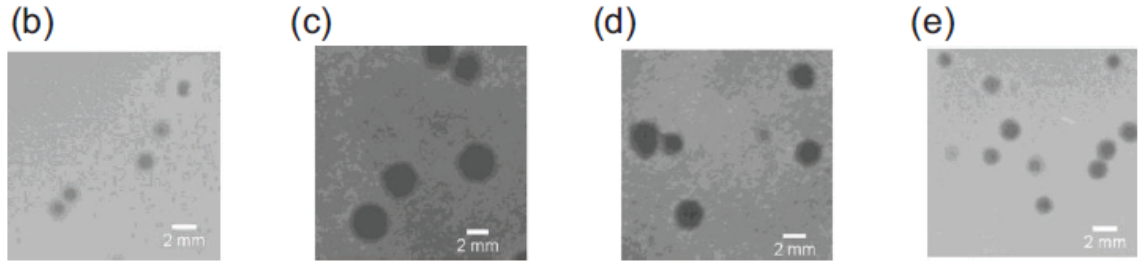
IN LACTOCOCCI

- *L. cremoris* MG1363
  - Phage sk1
  - Bacteriocins:
    - Nisin: pore + CW
    - Lcn972: CW
    - LcnA: Man-PTS
  - *In vitro*

TABLE 1 The burst size and latent period based on one-step curves of sk1 infecting *L. lactis* MG1363 in the absence or presence of Lcn972.

Condition	Burst size (pfu ml^{-1})	Latent period (min)
Control	43 ± 4	27 ± 3
Lcn972 (1.25 AU ml^{-1})	60* ± 10	28 ± 2
Lcn972 (5 AU ml^{-1})	61** ± 1	26 ± 2





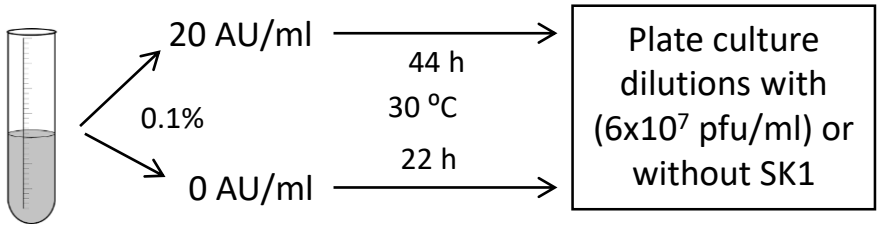
➔ Dependent of the main autolysin AcmA
➔ Not exclusive for the sk1-Lcn972

Rendueles et al., 2022. MicrobiologyOpen: 11:e1308

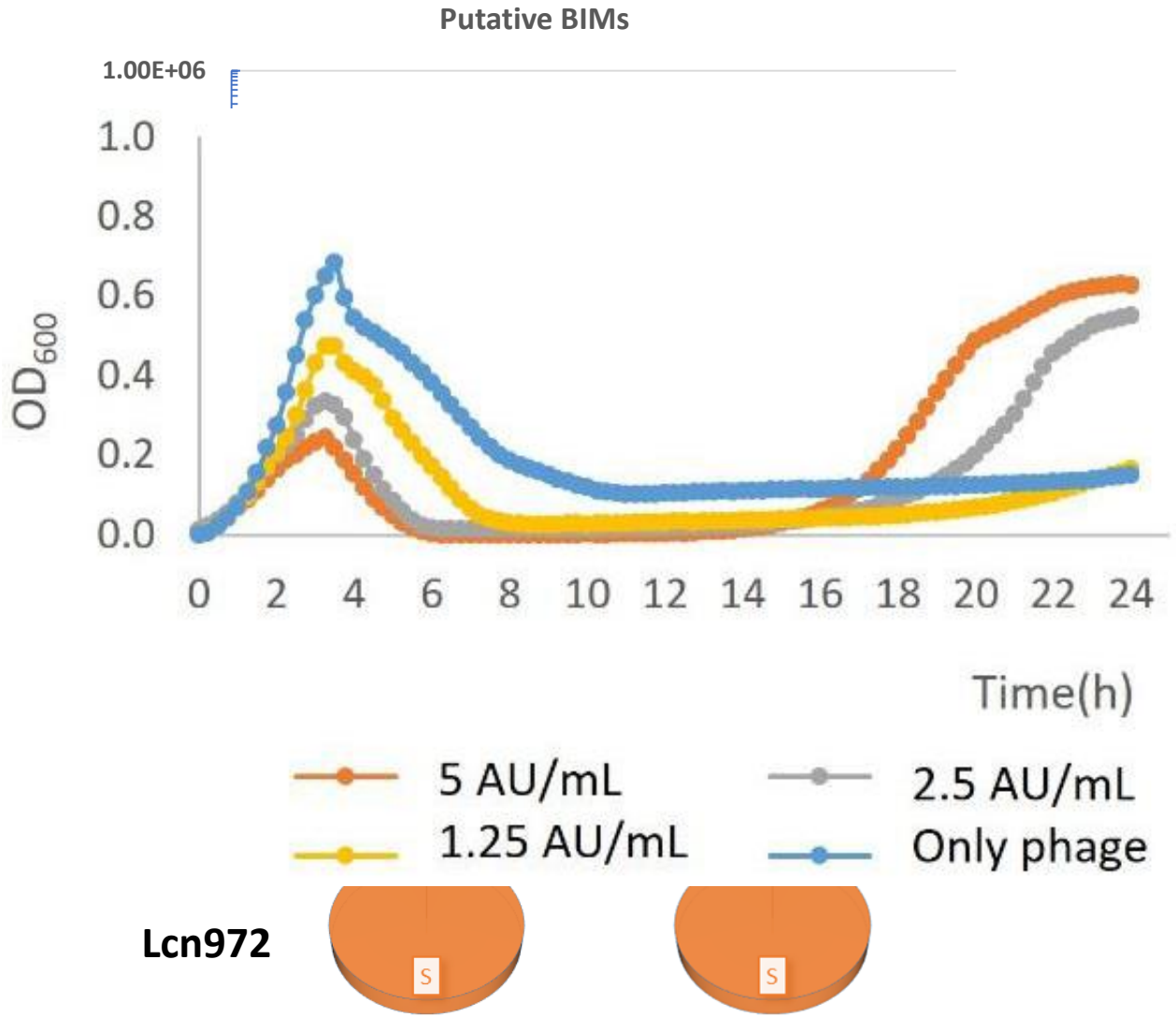
IN LACTOCOCCI

- *L. cremoris* MG1363
  - Phage sk1
  - Bacteriocins:
    - Lcn972: CW
  - *In vitro*

ADAPTATION to Lcn972



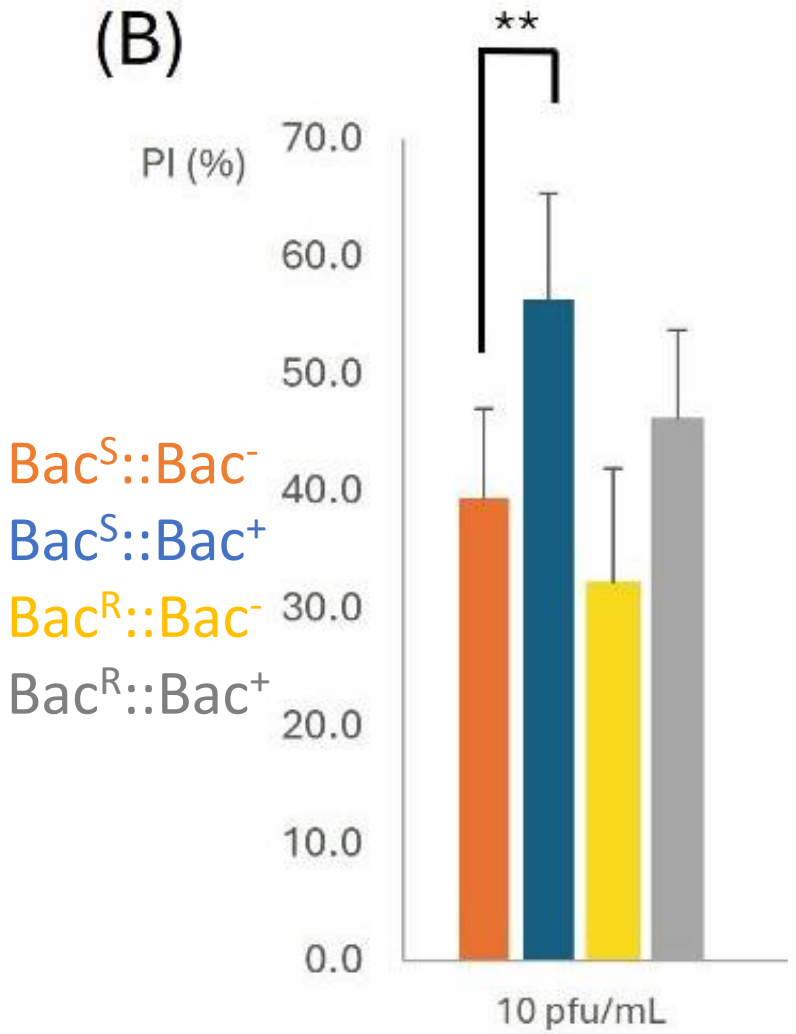
MG1363
overnight
GM17
30 °C



IN LACTOCOCCI

- *L. cremoris* MG1363
  - Phage sk1
  - Bacteriocins:
    - Lcn972: CW
  - Co-cultures Bac+/Bac- in milk

→ Slower acidification
→ Higher % inhibition



👎Increased risk of fermentation failure

Rendueles et al., 2025. Unpublished

IN SUMMARY: The Bac-Bac network

- Positive Bac-Bac interactions (BaPi) may be exploited in food biopreservation **BUT…**
  - Antagonism may occur
  - Case-by-case
- BaPi may be detrimental in starter-driven fermentations **BUT…**
  - Adaptation to Lcn972 may increase survival upon phage infection