Natural Antimicrobial Peptides to Prevent Clostridium Cheese Spoilage

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INTRODUCTION

- ***Clostridium*** spores cause late-blowing defects during ripening
- **Antimicrobial peptides (AMP)** from lactic acid bacteria offer a sustainable strategy, though their efficacy depends on cheese composition and microbiota.

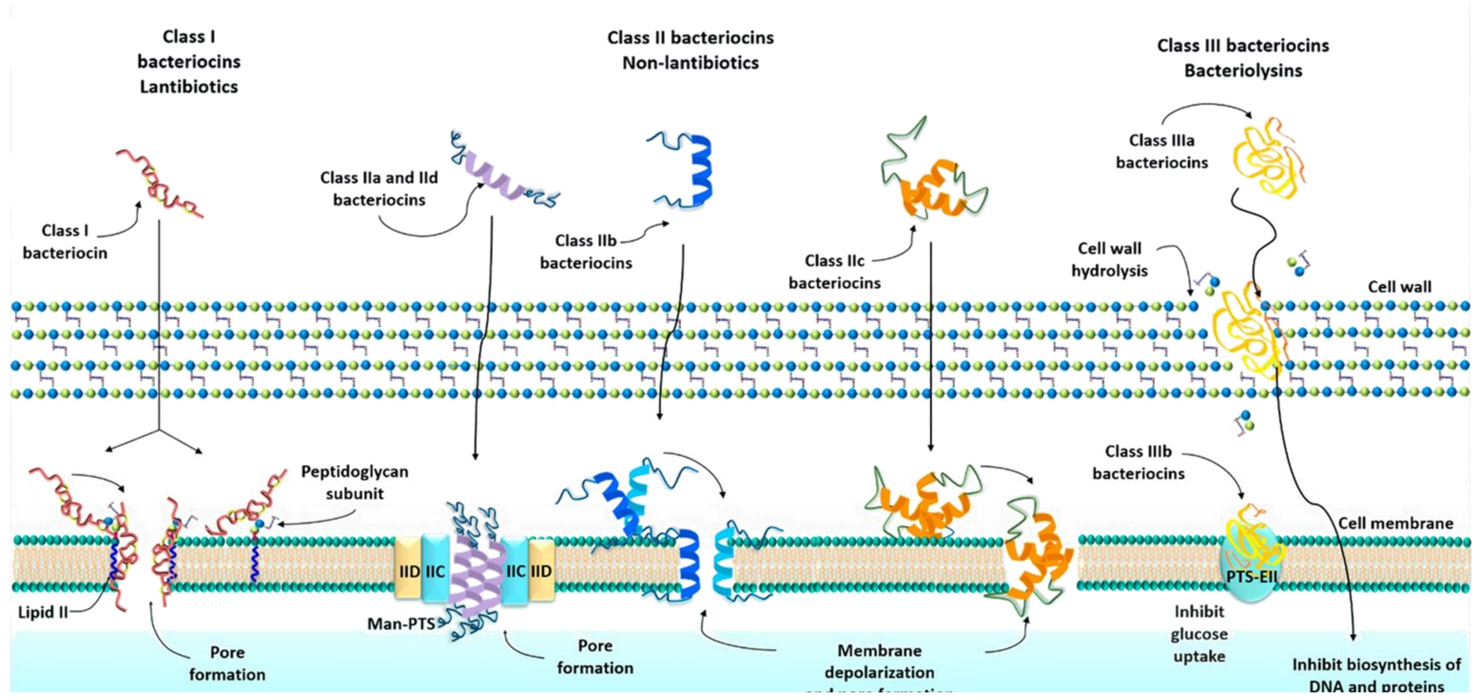

Figure 1: Different mechanisms of action of bacteriocins [1]

- Probable interaction of AMPs with cheese components.
- Increasing cheese shelf life and reducing waste.

OBJECTIVE

- Evaluate the inhibitory effects of natural AMP (nisin, enterocin, and brevibacilin) against *Clostridium spp.* both in vitro and in the cheese model.

METHODS

- Nisin, Brevibacillin, and Enterocin production [2]
- AMP's activity against *C. tyrobutyricum* ATCC 25755, *C. beijerinckii* ATCC 25752, and *C. sporogenes* ATCC 3584 [3]
- AMP's inhibitory effect against *Clostridium* consortium (10^7 CFU/mL) in a cheese model.

RESULTS

- Purity of the AMP were confirmed by HPLC

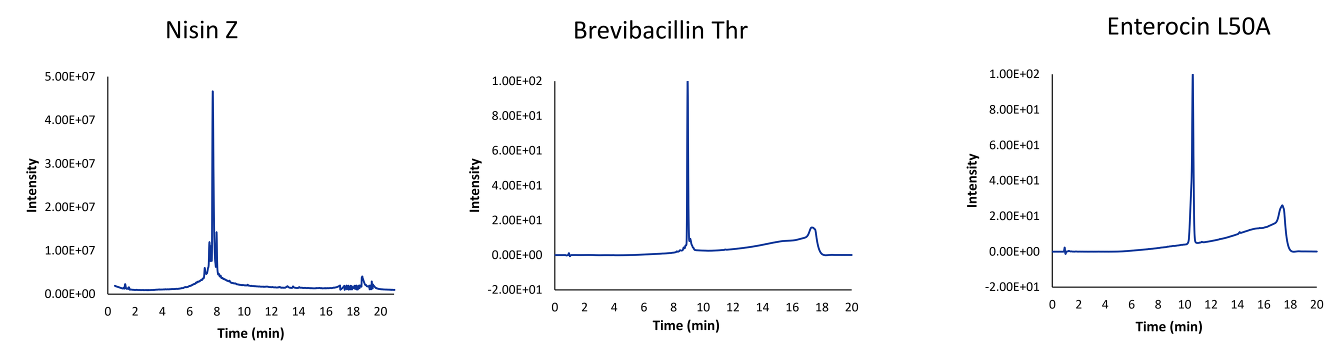

Figure 2: HPLC profile of Nisin Z, brevibacillin Thr, and enterocin L50A

- Minimum inhibitory concentration (MIC): The growth of each *Clostridium spp.* and their consortium was measured at 600 nm.

Table 1: MIC of nisin, enterocin, brevibacillin, and lysozyme against *Clostridium spp.*

	MIC (µg/mL)			
	Nisin	Enterocin	Brevibacillin	Lysozyme
C. Tyrobutyricum (ATCC 25755)	3.125	1.56	1.56	62.5
C. Sporogenes (ATCC 3584)	0.78	0.19	0.39	62.5
C. Beijerinckii (ATCC 25752)	0.78	0.39	0.39	62.5

Table 2: MIC of nisin, enterocin, brevibacillin, and lysozyme against *Clostridium consortium*

	MIC (µg/mL)			
	Nisin	Enterocin	Brevibacillin	Lysozyme
Clostridium consortium	0.97	15.62	3.9	62.5

- Agar diffusion assay: Nisin showed the highest inhibitory activity, followed by brevibacillin and enterocin.

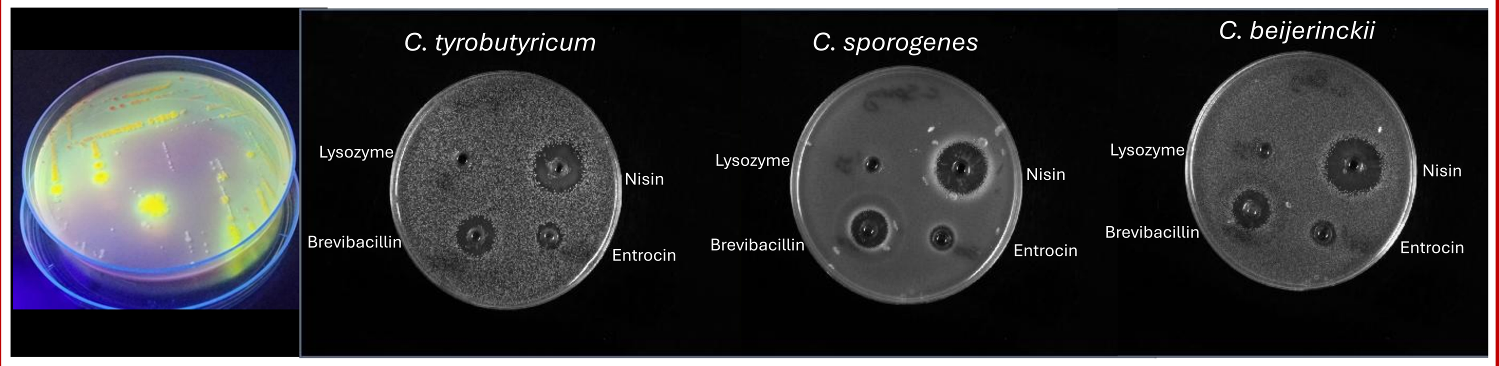

Figure 3: Agar diffusion assay of the effect of nisin, brevibacillin, entrocin, and lysozyme against *C. tyrobutyricum, C. sporogenes* & *C. beijerinckii*

- Nisin at 1,000 MIC significantly inhibited *Clostridium* consortium, with an average 2 log reduction compared to the control.
- At lower concentrations the effect was limited, likely due to interactions between bacteriocins and lipids in the cheese matrix.

Figure 4: Inhibitory effect of AMP against clostridial activity and gas production

Table 3: Inhibitory activity of different AMP against *Clostridium* consortium in a cheese model.

Treatment	CFU
Control negative	0
Control positive	9×10^7
Lysozyme (300ppm/g cheese)	3.3×10^6
Nisin 200 MIC	2.8×10^7
Nisin 500 MIC	3.9×10^6
Nisin 1000 MIC	6×10^5
Ent 200 MIC	2.8×10^7
Ent 500 MIC	3×10^7
Ent 1000 MIC	2.7×10^7
Brv 200 MIC	1.5×10^7
Brv 500 MIC	2×10^7
Brv 1000 MIC	1.8×10^7

CONCLUSION

Nisin had the strongest and most consistent anticlostridial activity across assays, while brevibacillin and enterocin showed limited effects and lysozyme was ineffective in preventing cheese spoilage.

REFERENCES

1) Hernández-González, J. C., Martínez-Tapia, A., Lazcano-Hernández, G., García-Pérez, B. E., & Castrejón-Jiménez, N. S. (2021). Bacteriocins from lactic acid bacteria. A powerful alternative as antimicrobials, probiotics, and immunomodulators in veterinary medicine. Animals, 11(4), 979., 2) Fliss O, Guay LD, Fliss I, Biron É. Synthesis and structure–activity study of the antimicrobial lipopeptide brevibacillin. RSC medicinal chemistry. 2024;15(12):4168-79., 3) Hassan, H., St-Gelais, D., Gomaa, A., & Fliss, I. (2021). Impact of nisin and nisin-producing Lactococcus lactis ssp. lactis on Clostridium tyrobutyricum and bacterial ecosystem of cheese matrices. Foods, 10(4), 898.

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