INFLUENCE OF GROWTH TEMPERATURE ON THE PRODUCTION OF BACTERIOCINS BY *Lactococcus* spp.

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Lactococcus spp. can produce bacteriocins, mainly nisins, belonging to the lantibiotics group. The use of nisin A as a food preservative is ruled down by EU. Nisin A exerts antimicrobial activity against Gram-positive bacteria, pathogens such as ***Listeria monocytogenes*** and ***Staphylococcus aureus***, and spore forming bacteria (i.e., ***Bacillus*** and ***Clostridium***). Therefore, the utilization of bacteriocin producer strains in dairy may positively impact cheesemaking and quality and safety of cheeses.
In this study, four strains of *Lc. lactis* and one strain of *Lc. cremoris* were considered. The strains harbored Nisin A (*Lc. cremoris*) or Z (*Lc. lactis*) genes. One *Lc. lactis* strain (VC106) also harbored Lactococcins B and Lacticin 481 structural genes. All the strains were screened for their ability to produce bacteriocins at the optimal growth temperature (30 °C) and at temperature **resembling those used in cheese-making** (18-45 °C) and **ripening** (8 °C) of semi-cooked cheese.

METHODS

Strains considered: *Lc. cremoris* FT27
Lc. lactis N16
Lc. lactis N26
Lc. lactis SV77
Lc. lactis VC106
Clostridium tyrobutyricum (10 ceppi)

Bacteriocins production: *Lactococcus* spp. inocula: 1 % w/w
Growth substrate: MRS broth + Tween 80 (0.2% w
Fermentation: MiniBio 500 (Applikon Biotechnolo
Optimal growth temperature: 30 °C
Cheese-making temperatures: 30 °C x 85'
45 °C x 15' (*cooking*)
18 °C x 24 h (*salting*)
8 °C x 170 days (*ripening*)

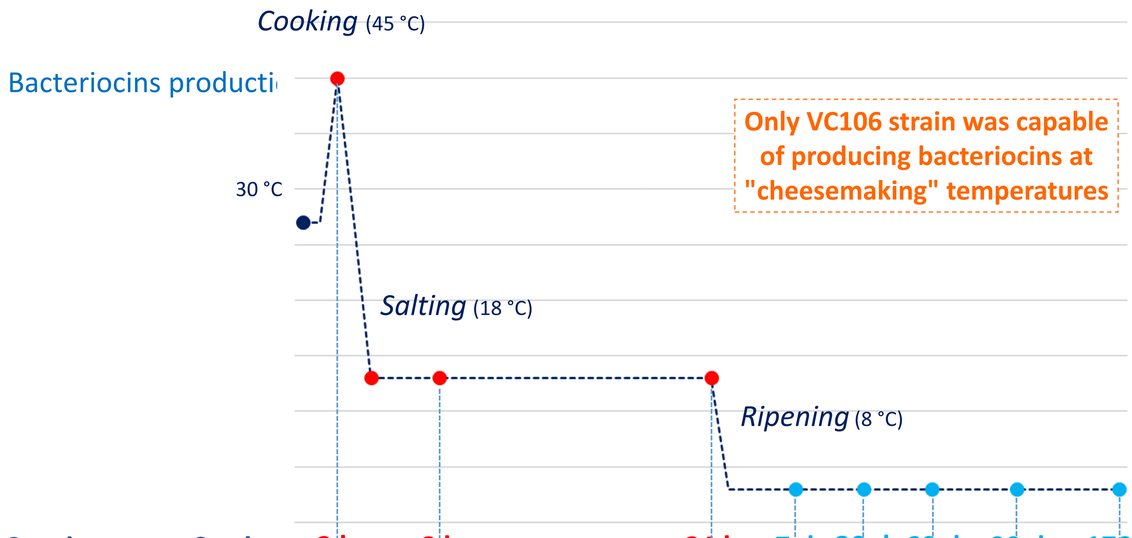
Nisin determination: Pongtharangkul & Demirci (2004); Murat & Hakkı (2021)
Screening of bacteriocin genes: Ghrairi et al. (2004); Dal Bello et al. (2010)
Nisin MIC assays against *Cl. tyrobutyricum* strains: Morandi et al. (2019)

SCREENING OF BACTERIOCIN GENES AND THEIR PRODUCTION AT *LACTOCOCCUS* SPP. AT OPTIMAL GROWTH TEMPERATURE (30 °C)

Species	Strain	Genes	Batteriocins (IU/mL)
Lc. cremoris	FT27	*nisA*	610
Lc. lactis	N16	*nisZ*	1.628
	N26	*nisZ*	227
	SV77	*nisZ*	1.250
	VC106	*nisZ*, *lct481*, *lcnB*	858

Nisin production was strain dependent and at the optimal growth temperature a great variability was observed among the biotypes (from 227 to 1.628 IU/mL). All strains harboured the gene coding for nisin production (nisin A in *Lc. cremoris* and nisin Z in all *Lc. lactis* strains); VC106 strains shows genes coding for lacticin 481 (*lct481*) and lactococcin B (*lcnB*) production.

BACTERIOCINS PRODUCTION BY *Lactococcus spp.* STRAINS AT TEMPERATURES RESEMBLING THOSE USED IN THE CHEESE-MAKING AND RIPENING (DATA EXPRESSED AS IU/ML)



Species	Strain	2 h	8 h	24 h	7 d	30 d	60 d	90 d	170 g
Lc. cremoris	FT2	0,4	2,1	2,3	2,4	2,6	3,4	1,9	-
Lc. lactis	N16	0,0	0,5	3,2	3,2	2,5	2,7	3,6	-
	N26	0,8	1,0	3,4	2,1	1,4	2,9	4,8	-
	SV77	0,4	0,5	0,6	1,5	0,7	0,9	1,0	-
	VC106	1,0	1,8	**729**	**452**	**311**	**165**	**127**	**72,7**

MINIMUM INHIBITORY CONCENTRATION (MIC) OF DI NISIN AGAINST THE *Cl. tyrobutyricum* SPORES PRODUCED BY STRAINS ISOLATED FROM DAIRY PRODUCTS

Species	Strain	MIC (IU/mL)	VC106 protection
Cl. tyrobytyricum	CL15b	28	170 days
	CL10	112	90 days
	CL12	112	90 days
	CL1	112	90 days
	CL2	112	90 days
	CL11	112	90 days
	CL29	112	90 days
	CL9	225	30 days
	CL167	225	30 days
	CL6a	225	30 days

Nisin MIC, evaluated on *Cl. tyrobutyricum* spores, ranged from 28 and 225 IU/mL. These values suggest that the amount of bacteriocin produced by VC106, was able to control the spore germination for up to 170 days at low temperatures.

CONCLUSIONS

These results highlight the need to better understand the bacteriocins production under real cheese-making and ripening conditions for using *Lc. lactis* and *Lc. cremoris* as bioprotective starter cultures.

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