# Nisin production in acid whey with Lactococcus strains

Diana Paveljšek<sup>1</sup>, Jernej Oberčkal<sup>1</sup>, Nika Lekan<sup>1</sup>, Petra Mohar Lorbeg<sup>1</sup>, Marko Kete<sup>2</sup>, Maja Zupančič Justin<sup>2</sup>, Bojana Bogovič Matijašić<sup>1</sup>

<sup>1</sup>University of Ljubljana, Biotechnical faculty, Slovenia; <sup>2</sup>Arhel Ltd, Ljubljana, Slovenia

#### University of Ljubljana Biotechnical Faculty Microbiological Societies

## Introduction

Acid whey, a nutritious by-product of the fresh curd cheese production, represents a technological challenge further for processing in the dairy industry because of

#### **Objectives**

We further used acid whey for the cultivation of **nisin** producing *Lactococcus* strains and additional isolation of nisin. Nisin is a very vigorous **bacteriocin**,

### **Methods**

Our own isolates from sour milk, were inoculated into enriched (yeast extract, mineral salts), neutralized filterand sterilized or autoclaved acid whey. After

its high lactic acid content and bacterial **load**. Therefore, acid whey is often discarded as a **biological waste**.

belonging to a class of polycyclic peptide antibiotics – lantibiotics, and is because of its robustness highly demanded in the **food** industry.

Results

Lactococcus strains were cultivable in whey without additional supplements and were able to produce **nisin**, but the growth and amount of nisin was improved when **yeast extract** was included in the medium. We were able to concentrate nisin with ammonium sulfate **precipitation** and optimized detection with SDS-PAGE and RP-HPLC. We up-scaled production process in 2.5 L bioreactors.

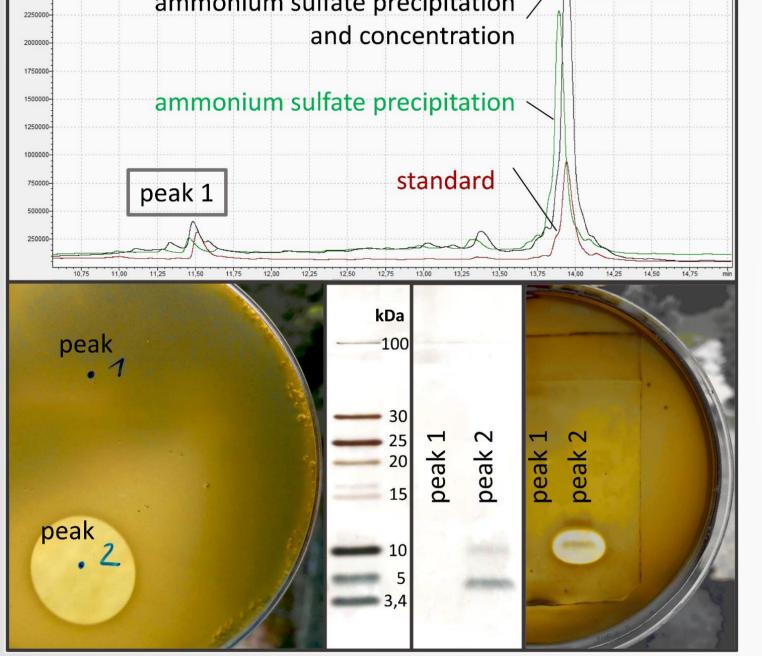
incubation, nisin was semi-purified and monitored for abundance and purity with SDS-PAGE or HPLC. Antimicrobial activity against the indicator strain Lactobacillus sakei ATCC 15521 was aslo determined.

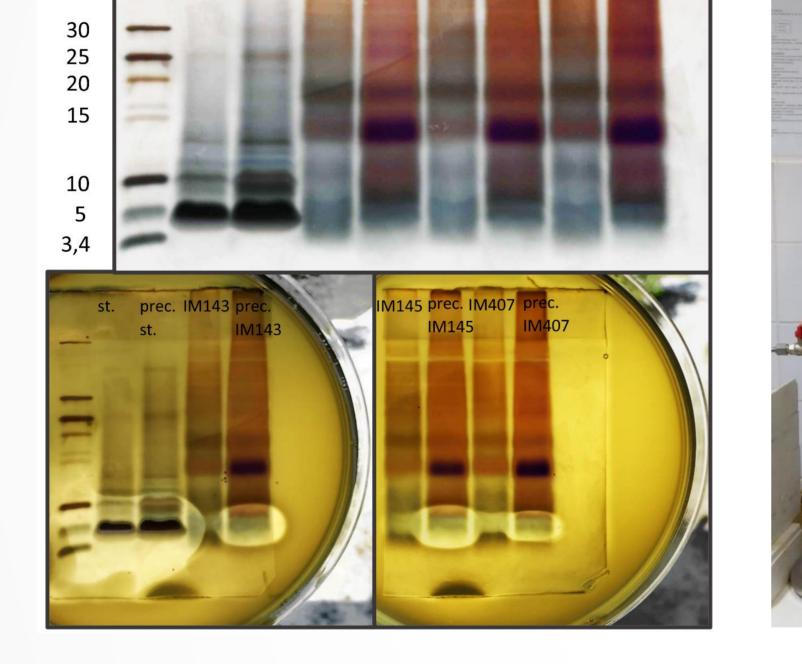
### Conclusions

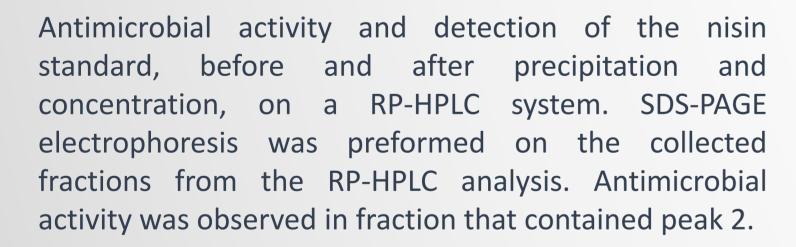
Subsequent use of acid whey for the production nisin represents an alternative approach in the dairy waste treatment and helps in the reduction of environmental burden.

-Data4:nizin std pred	centrat 30 procent.lcd PDA Ch1 214nm,8nm 1.lcd PDA Ch1 214nm,8nm c 30 procent.lcd PDA Ch1 214nm,8nm	 		Time 12,011 Inten.	13
50000		 	 	neak 2	
				peak z	

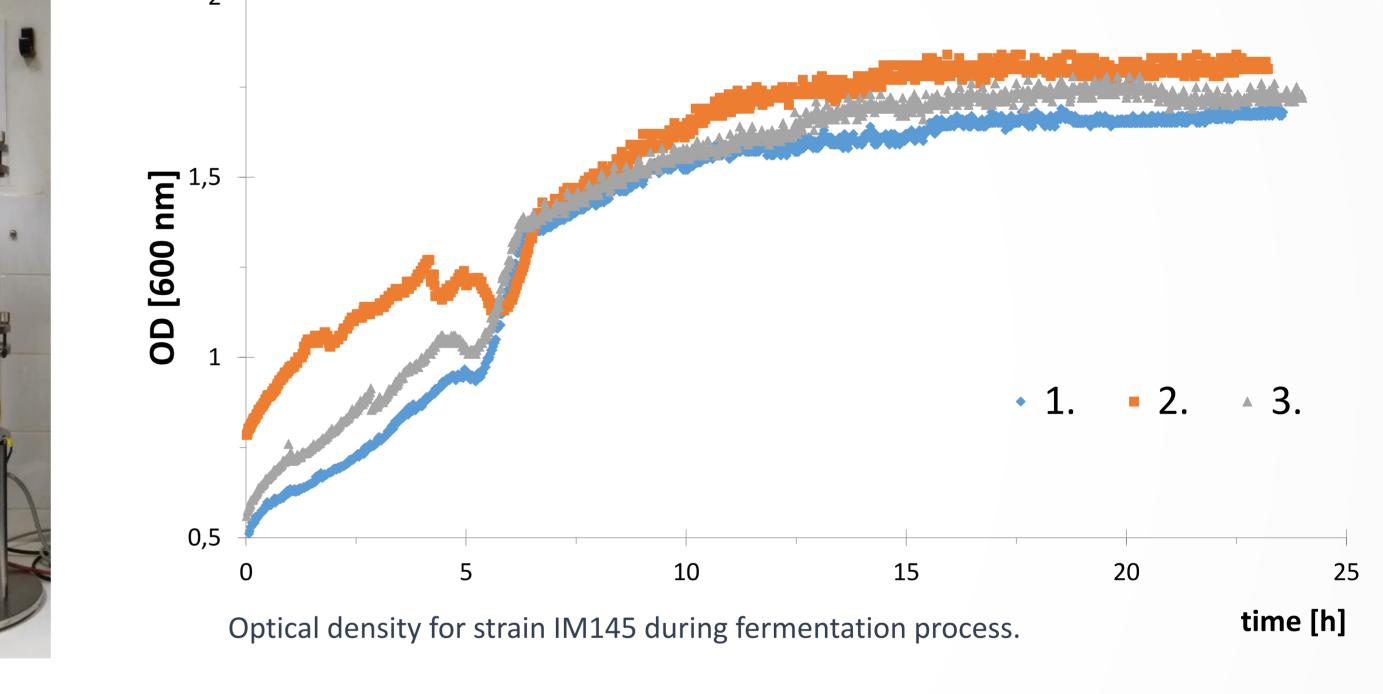
IM143 prec. IM145 prec.



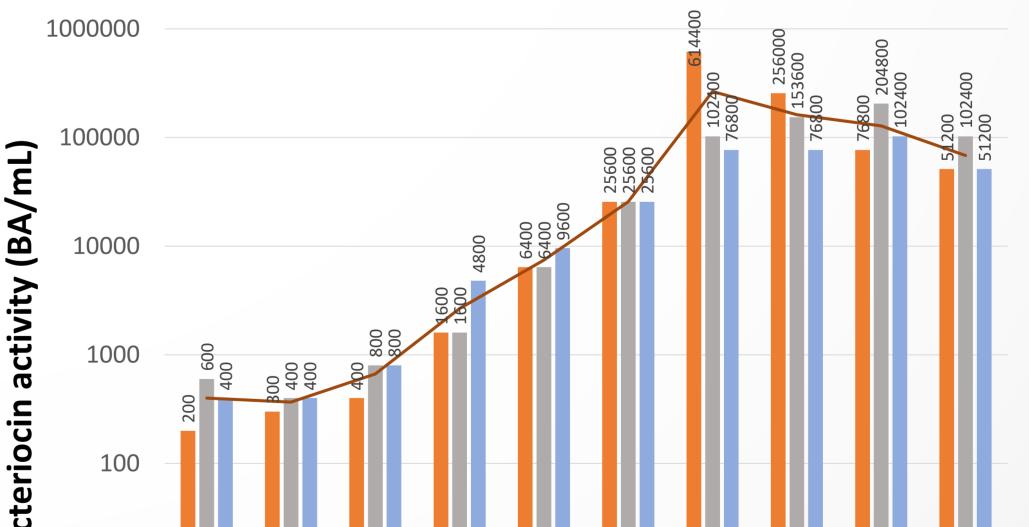


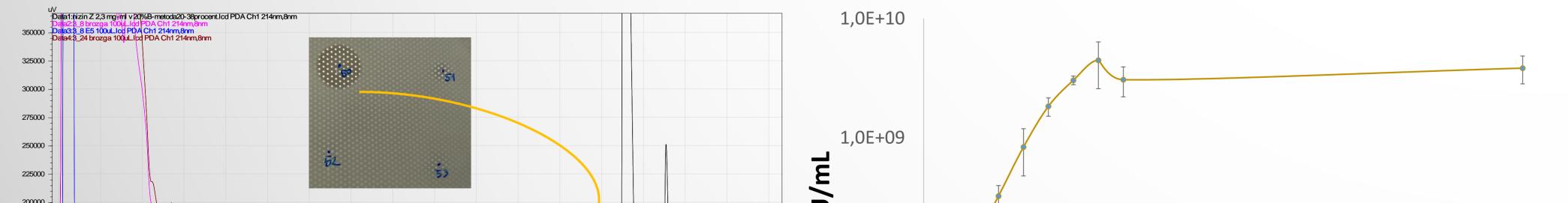


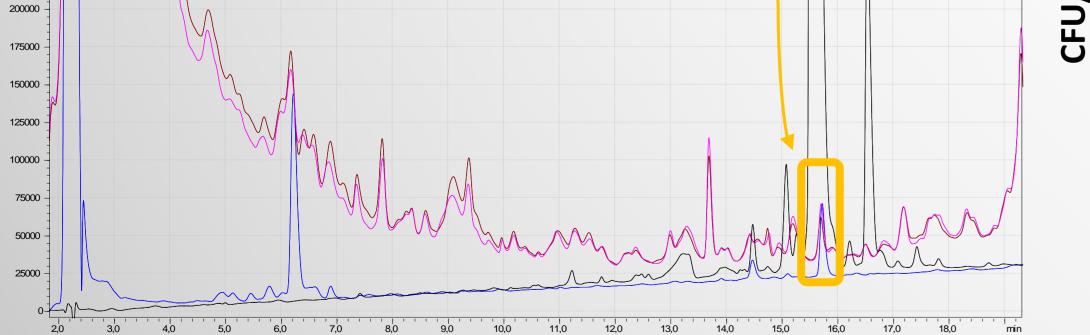
Antimicrobial activity SDS-PAGE and electrophoresis – detection of nisin before and after precipitation. Nisin was obtained by fermentation of whey with strains Lc. lactis IM143, Lc. lactis IM145, Lc. lactis IM407. St. - standard, prec. – precipitation.

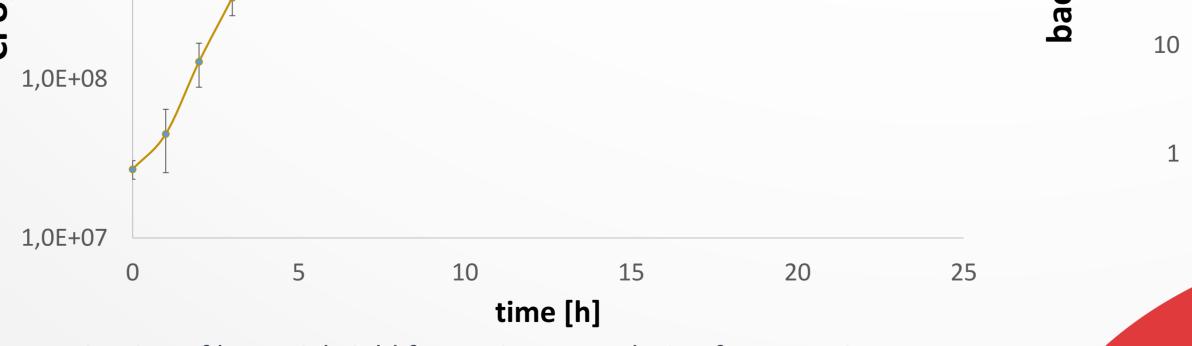


Determination of bacteriocin activity (BA) for nisin, obtained in bioreactor with strain IM145. BA was determined each h until stationary phase.









Determination of bacterial yield for strain IM145 during fermentation process RP-HPLC detection of nisin, produced with strain Lc. lactis IM145 in bioreactor. Standard deviation is shown for 3 biological replicates.



#### REFERENCES

after 8 h in 2.5 L bioreactor.

Bhunia, A. K. & Johnson, M. G. A modified method to directly detect in SDS-PAGE the bacteriocin of Pediococcus acidilactici. Lett. Appl. Microbiol. 15, 5-7 (1992).

Jin, Y., Wen, M., Yuan, Q., Zhang, J. & Tan, W. Beneficial effects of Coomassie staining on proteomic analysis employing PAGE separation followed with whole-gel slicing, in-gel digestion and quantitative LC-MS/MS. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1110–1111, 25–35 (2019). Dussault, D., Vu, K. D. & Lacroix, M. Enhancement of Nisin Production by Lactococcus lactis subsp. lactis. *Probiotics Antimicrob. Proteins* 8, 170–175 (2016).



#### ACKNOWLEDGEMENTS

This research was supported by LIFE16 ENV/SI/000335 LIFE for Acid Whey