

# Influence of temperature and pH on anti-listerial activity of bacteriocin isolated from *Lactobacillus sakei* I 154

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## Abstract

Bacteriocin-producing strain *Lactobacillus sakei* I 154 with high anti-listerial effect has been isolated from authentic Serbian traditionally fermented sausage („Sremska“). Semi-purified bacteriocin (sakacin) with the strength value of 640 AU/mL was obtained by precipitation with ammonium sulphate. Considering that *L. monocytogenes* presents main threat in meat industry today, due to its wide spread, high resistance on environmental conditions and high fatality rate in humans, we set that task to investigate its sensitivity towards isolated bacteriocin. Experiment was carried out *in vitro* on media that approximated the composition of „Sremska“ sausage. *L. monocytogenes* (18 hours old culture) was inoculated to BHI broth „enriched“ with salts (5.2% of NaCl and 150 ppm of NaNO<sub>2</sub>) in concentration of 10<sup>3</sup> cells/mL of medium. One mL of bacteriocin was added to each broth. Anti-listerial activity of sakacin was determined at 12°C (experiment lasted 10 days), 18°C and 25°C (5 days) at different pH values (5, 5.5 and 6). *L. monocytogenes* count was determined daily in accordance with ISO standard 11290-1.2. The results of investigation showed that with higher temperature, anti-listerial effect of sakacin increases. Also, intensity of anti-listerial activity increased with increase of pH value.

## Introduction

Lactic acid bacteria (LAB) play an essential role in production of raw, fermented sausages either as a part of accidentals present microflora or especially selected and added microorganisms – starter cultures. Inhibitory, bioprotective effect towards some pathogenic bacteria as well as towards spoilage bacteria, LAB achieve through the activity of its specific and unspecific metabolites (Lindgren & Dobrogosr, 1990). Non-specific activity (acidification due to production of lactic, acetic and other organic acids, production of H<sub>2</sub>O<sub>2</sub>, diacetyl and other compounds) and specific activity (production of specific compounds – bacteriocins) results in the fact that optimally produced fermented sausages are considered as safe products (De Vuyst & Vandamme, 1994; Stiles, 1994).

From domestic Serbian sausage ("Sremska") fermented on traditional way, bacteriocin producing strain of *Lactobacillus sakei* I 154 with high antilisterial effect has been isolated (S.Veskovic, 2005).

It is known that *L. monocytogenes* causes serious human disease (listeriosis) and contaminated food is often responsible for its occurrence. Fatality of the disease as well as properties of this foodborne pathogen (widespreadness, survival in non-favourable conditions of the environment: low pH, wide temperature limits of growth and increased salt content etc) lead to the situation where it became necessary to consider application of LAB bacteriocin in order to get safe food and decrease the risk of this pathogen (Cintas *et al.*, 2001; Cleveland *et al.*, 2001; Diep & Nes, 2002).

Antilisterial effect of the isolated bacteriocin from *Lb. sakei* I 154 was investigated in laboratory conditions "*in vitro*" on various media and in various conditions that simulated production process of the traditional "Sremska" sausages. Obtained results represented the guidelines for continuing the experiment in industrial conditions.

## Material and methods

Isolation of semipurified bacteriocin from *Lb. sakei* I 154 was carried out by the method of precipitation with ammonium sulphate and creating the 70% saturated solution – 472,7 g/L of ammonium sulphate was used. Procedure of obtaining bacteriocin from LAB was based on the method by Schilinger and Lücke (1989) adapted according to given laboratory conditions (S. Veskovic, 2005, 2007).

Strength of isolated bacteriocin to *L. monocytogenes* NCTC 10527 was determined by Agar Well Diffusion Assay. Reaction was based on founding of maximal dilution of bacteriocin portion (50 µL) that gave antilisterial effect in the medium. Activity of isolated bacteriocin was expressed through arbitrary units (AU/mL) using the formula  $AU/mL = 2^n \times (1000 \mu L / 50 \mu L)$  where "n" is maximal dilution of bacteriocin that gave inhibition zone of the growth of test microorganism larger than 2 mm.

Antilisterial effect of isolated bacteriocin was investigated in laboratory conditions that simulated production process of "Sremska" sausage. To the BHI broth was added 5.2 % NaCl and after sterilization to the cooled medium 150 ppm of NaNO<sub>2</sub> solution previously sterilized by membrane filtration was added. In 20mL salt purched BHI broth, 18<sup>h</sup> culture of *L. monocytogenes* NCTC 10527 was inoculated in concentration of 10<sup>3</sup> cells/mL of medium. In every sample 1 mL of bacteriocin was added. At the same time, control samples (without bacteriocin) were investigated. Experiment was designed in such a way to monitor antilisterial effect of bacteriocin at different temperatures (12, 18 and 25°C) and pH values (5.0; 5.5; 6.0). Experiment that monitored antilisterial activity at 12°C lasted for 10 days and investigation of bacteriocin activity at 18°C and 25°C lasted 5 days. In that period (10 and 5 days) count of *L. monocytogenes* was determined according to ISO standard 11290-1,2 (1998). Experiment was repeated 3 times.

## Results

Agar Well Diffusion Assay showed that maximal dilution of bacteriocin isolated from *Lb. sakei* I 154 that shows antilisterial effect was 1:32 (2<sup>5</sup>). Strength of bacteriocin expressed through arbitrary units was cca 640 AU/mL.

Tables 1, 2 and 3 show the results of antilisterial activity of bacteriocin isolated from *Lb. sakei* I-154 at the temperatures of 12, 18 and 25°C and pH values of 5.0; 5.5 and 6.0. At the same time, growth intensity of *L. monocytogenes* is shown in control mediums at the same temperature and pH values.

**Table 1.** Antilisterial activity of bacteriocin isolated from *Lactobacillus sakei* I154 at the temperature of 12°C and pH values of 5.0, 5.5 i 6.0

pH	Model	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
		log±SD	log±SD	log±SD	log±SD	log±SD	log±SD	log±SD	log±SD	log±SD	log±SD
5.0	I 154	3.477±0.014	3.698±0.000	3.845±0.012	4.000±0.000	4.079±0.073	4.477±0.000	3.698±0.017	3.698±0.107	3.602±0.032	3.301±0.000
	C	3.477±0.014	3.954±0.009	3.954±0.004	4.602±0.000	4.698±0.008	4.740±0.015	4.602±0.010	3.954±0.000	3.913±0.005	3.903±0.000
5.5	I 154	3.602±0.000	3.903±0.021	3.929±0.012	3.977±0.018	3.778±0.000	3.602±0.000	3.544±0.088	3.556±0.024	3.602±0.000	3.602±0.000
	C	3.778±0.029	3.977±0.004	4.255±0.048	4.301±0.000	4.301±0.000	4.301±0.111	4.477±0.073	4.602±0.000	4.623±0.032	4.301±0.021
6.0	I 154	3.755±0.015	3.954±0.000	3.963±0.009	3.986±0.008	4.000±0.000	4.477±0.000	4.778±0.000	4.903±0.054	4.778±0.000	4.903±0.010
	C	3.778±0.036	4.255±0.004	4.778±0.000	4.778±0.000	4.792±0.014	4.845±0.012	4.903±0.000	4.977±0.009	4.924±0.020	4.929±0.005

I 154 – Bacteriocin isolated from *Lb. sakei* I 154

C – Control (broth with inoculated *L. monocytogenes* NCTC 10527)

SD – Standard error

**Table 2.** Antilisterial activity of bacteriocin isolated from *Lactobacillus sakei* I154 at the temperature of 18°C and pH values of 5.0, 5.5 i 6.0

pH	Model	1st day	2nd day	3rd day	4th day	5th day
		log±SD	log±SD	log±SD	log±SD	log±SD
5.0	I 154	3.602±0.010	3.778±0.000	3.903±0.016	3.845±0.012	3.845±0.000
	C	3.845±0.000	3.903±0.010	3.903±0.000	3.903±0.000	3.903±0.000
5.5	I 154	3.602±0.010	3.903±0.027	3.929±0.020	3.903±0.000	3.954±0.019
	C	3.845±0.000	3.929±0.020	4.000±0.043	3.954±0.000	4.954±0.009
6.0	I 154	3.698±0.043	3.908±0.020	3.954±0.014	3.968±0.018	4.000±0.092
	C	3.845±0.000	3.954±0.009	4.000±0.025	4.477±0.052	6.954±0.055

**Table 3.** Antilisterial activity of bacteriocin isolated from *Lactobacillus sakei* I154 at the temperature of 25°C and pH values of 5.0, 5.5 i 6.0

pH	Model	1st day	2nd day	3rd day	4th day	5th day
		log±SD	log±SD	log±SD	log±SD	log±SD
5.0	I 154	3.602±0.025	3.740±0.039	3.954±0.048	4.176±0.058	3.875±0.029
	C	3.845±0.029	4.000±0.025	4.505±0.027	4.544±0.062	4.079±0.047
5.5	I 154	3.698±0.000	3.732±0.016	3.698±0.000	3.612±0.010	3.602±0.020
	C	3.977±0.013	4.176±0.088	7.322±0.020	7.954±0.014	8.929±0.015
6.0	I 154	3.857±0.012	3.892±0.016	3.954±0.024	4.041±0.039	4.301±0.043
	C	3.954±0.000	4.322±0.020	7.322±0.020	8.397±0.505	8.903±0.054

At 12°C, *L. monocytogenes* show the most intensive growth at pH value of 6.0 in the medium. Bacteriocin inhibits growth of test microorganism at pH 6.0 in the first five days. However, at the end of the experiment, growth intensity of *L. monocytogenes* is approximately the same in both cases. At pH 6, after six days, bacteriocin intensively inhibits the growth of *L. monocytogenes*. It also inhibits the growth of this pathogen at 18°C and 25°C in media with pH value of 5.0 and 5.5.

### Conclusions

Bacteriocin isolated from *Lb. sakei* I-154 have significant antimicrobial effect towards *L. monocytogenes* NCTC 10527

Activity of isolated bacteriocin has been shown at the temperatures of 12, 18 and 25°C and pH values of 5.0; 5.5 and 6.0. Temperature increase also increases its antilisterial effect. Intensity of antilisterial activity of examined bacteriocin is proportional with pH values.

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