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Factors affecting the adsorption of bacteriocins ST194BZ and ST23LD to *Lactobacillus sakei* and *Enterococcus* sp.

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Bacteriocins ST194BZ and ST23LD, produced by *Lactobacillus plantarum*, inhibit Gram-positive and Gram-negative bacteria. Images obtained by atomic force microscopy showed clear signs of membrane damage of *Lactobacillus sakei*, accompanied by the leakage of DNA and β -galactosidase. Adsorption of the bacteriocins to cells was increased when cells were treated with buffers at pH values above neutral. An increase in bacteriocin ST194BZ adsorption to cells of *Enterococcus* sp. and *L. sakei* was observed with an increase in incubation temperatures, but at different rates for the two species. Treatment of the two species with various inorganic salts and solvents gave different results regarding the adsorption of the two bacteriocins. In general, pre-treatment of the two sensitive cells with Triton X-100, Triton X-114 and chloroform increased the adsorption of the two bacteriocins. Increased adsorption of bacteriocin ST23LD to *L. sakei* was recorded when the cells were pre-treated with Tris and NH_4 -citrate. Treatment of *Enterococcus* sp. and *L. sakei* with Na-EDTA and SDS decreased the adsorption of the two bacteriocins. Variable results were recorded with inorganic salts.

Key Words—adsorption; bacteriocins ST194BZ and ST23LD; *Lactobacillus plantarum*

Introduction

The ability of lactic acid bacteria (LAB) to inhibit the growth of undesirable bacteria is well known. Inhibition may be due to production of organic acids, diacetyl, H_2O_2 , or bacteriocins. Bacteriocins are ribosomal synthesized polypeptides with activity against bacteria closely related to the producer strain (Klaenhammer, 1988). These peptides are divided into three categories, i.e. (i) peptides with a narrow inhibitory spectrum, affecting only closely-related species, (ii) peptides inhibitory to Gram-positive bacteria, including food-borne pathogens (Klaenhammer, 1988), and (iii)

peptides with an unusual spectrum of activity, including that against Gram-negative bacteria (Piard and Desmazeud, 1992; Todorov and Dicks, 2005c), viruses (Serkedjieva et al., 2000; Todorov et al., 2005; Wachsmann et al., 1999) and yeast (Okkers et al., 1999).

Lactobacillus plantarum ST194BZ, isolated from boza, a cereal-based fermented beverage from Bulgaria, produces two bacteriocins, viz. ST194BZ(a) of 3.3 kDa and ST194BZ(b) of 14.0 kDa (Todorov and Dicks, 2006). A combination of the two bacteriocins inhibits the growth of *Lactobacillus casei*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. A maximum total bacteriocin activity of 12,800 AU/ml was recorded in MRS broth (Todorov and Dicks, 2005b).

Bacteriocins ST194BZ(a) and ST194BZ(b) withstand 20 min at 121°C and incubation at pH 2–10, but

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Table 1. Changes in the number of viable cells recorded after 1 h in the presence of bacteriocins ST194BZ and ST23LD.

Test microorganism	Before treatment	After 1 h in the presence of 2,133 AU/ml	
		Bacteriocin ST194BZ	Bacteriocin ST23LD
<i>Enterococcus</i> sp. HKLHS	8.7×10^9	1.4×10^7	3.4×10^7
<i>L. sakei</i> DSM 20017	1.0×10^9	1.2×10^8	2.5×10^8

are inactivated when treated with proteolytic enzymes such as pepsin, papain, α -chymotrypsin, trypsin and Proteinase K (Todorov and Dicks, 2005b). The mechanism of activity is bactericidal, as determined against *L. casei* LHS (Todorov and Dicks, 2005b, 2006).

L. plantarum ST23LD was isolated from the brine of spoiled black olives and produces two bacteriocins (ST23LD(a) and ST23LD(b)) of approximately 3.0 and 14.0 kDa, respectively (Todorov and Dicks, 2005a). The peptides inhibit the growth of Gram-positive bacteria (*E. faecalis*, *L. casei*, and *Streptococcus pneumoniae*) and *E. coli* and *P. aeruginosa*. The combined maximum activity recorded for the two peptides was 25,600 AU/ml (Todorov and Dicks, 2005a).

Bacteriocins ST23LD(a) and ST23LD(b) withstand 20 min at 121°C and incubation at pH 2–10, but are inactivated when treated with papain, α -chymotrypsin, trypsin and Proteinase K. The mechanism of activity is bactericidal, as determined against *L. casei* LHS (Todorov and Dicks, 2005a).

A limited number of papers have been published on the adsorption of bacteriocins to target (sensitive) cells. Pediocin N5p, produced by *Pediococcus pentosaceus*, adsorbed selectively to Gram-positive and Gram-negative bacteria (Manca de Nadra et al., 1998). The presence of Mg^{2+} and Mn^{2+} increased pediocin N5p binding to *P. pentosaceus* by 80% and 100%, respectively. Treatment of the target strain with 1% SDS increased the adsorption of pediocin N5p by 25% (Manca de Nadra et al., 1998). Adsorption of buchnerin LB, produced by *Lactobacillus buchneri*, is influenced by pH and not temperature or contact time (Yildirim et al., 2002). Anions and lipoteichoic acid reduced or inhibited the adsorption of buchnerin LB to the target cells. Treatment of cells with detergents or organic solvents had no effect on the adsorption of buchnerin LB.

In this study, the factors affecting the adsorption of

bacteriocins ST194BZ(a), ST194BZ(b), ST23LD(a), and ST23LD(b) to target bacteria were determined. The bacteriocins were not separated and will be referred to as ST194BZ and ST23LD.

Materials and Methods

Growth conditions and preparation of bacteriocins ST194BZ and ST23LD. *Lactobacillus plantarum* ST194BZ and ST23LD were grown in MRS broth (Oxoid, Ltd., Basingstoke, Hampshire, England). The other strains used in this study (Table 1) were grown in MRS broth (Oxoid) or BHI broth (Oxoid), at temperatures indicated in the respective culture collection catalogues. All strains were stored at –80°C in MRS or BHI broth supplemented with 15% (v/v) glycerol.

L. plantarum ST194BZ and ST23LD were inoculated (2%, v/v, respectively) into 100 ml MRS broth and incubated at 30°C for 24 h. The cells were harvested ($1,000 \times g$, 15 min, 4°C), the pH of the cell-free supernatants adjusted to 6.0 with sterile 1 M NaOH, heated for 10 min at 80°C, and then filter-sterilized (nitrocellulose membrane, 0.20 μ m pore size, Minisart®, Sartorius).

Bacteriocin bioassay. Bacteriocin activity tests were performed on cell-free supernatants by using the agar-spot test and the well-diffusion method, respectively (Todorov and Dicks, 2005c). Cell-free supernatants were adjusted to pH 6.0 with sterile 1 M NaOH before testing. Target microorganisms (Table 1) were cultured in BHI (non-lactic acid bacteria) or MRS (lactic acid bacteria). Antimicrobial activity was expressed as arbitrary units (AU)/ml, calculated as follows: $a^b \times 100$, where “a” represents the dilution factor and “b” the last dilution that produces an inhibition zone of at least 2 mm in diameter. Activity was expressed per ml by multiplication with 100 (Todorov and Dicks, 2005c).

Effect of bacteriocins ST194BZ and ST23LD on cell

growth. Twenty milliliters filter-sterilized cell-free supernatant containing bacteriocin ST194BZ (12,800 AU/ml, pH 6.0), was added to 100 ml 3 h-old cultures ($OD_{600}=0.1-0.2$) of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017, respectively. Optical density readings were taken at 600 nm, hourly for 13 h. The experiment was repeated with bacteriocin ST23LD (12,800 AU/ml, pH 6.0). In a similar experiment, the effect of the two bacteriocins was tested on stationary-phase cells ($OD_{600}=1.2-1.5$) of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017.

Effect of bacteriocins ST194BZ and ST23LD on the cell morphology of L. sakei DSM 20017. *L. sakei* DSM 20017 was grown in 10 ml MRS broth at 37°C for 18 h. The cells were harvested ($8,000\times g$, 10 min, 4°C) and washed 5 times with 10 ml sterile distilled water. The cells were then re-suspended in 20 ml sterile distilled water and divided into two equal volumes. Bacteriocins ST194BZ and ST23LD were each diluted with sterile distilled water to 12,800 AU/ml, filter-sterilized and adjusted to pH 6.0 with 1 M NaOH. To each of the cell suspensions 10 ml of a bacteriocin was added (final activity=6,400 AU/ml) and incubated for 1 h at 4°C. The cells were then harvested ($8,000\times g$, 10 min, 4°C) and washed five times with 10 ml sterile distilled water. The cells were suspended in 1 ml sterile distilled water and visualized by atomic force microscopy (AFM) (Multimode AFM from Veeco, Santa Barbara, USA). The experiment was repeated with sterile distilled water (no bacteriocins) as a control. Cell suspensions were applied to a freshly cleaved mica surface and allowed to dry for 5 min before being subjected to AFM. All images were obtained in air and with tapping mode. A silicon non-contact cantilever from Nanosensors (Neuchatel, Switzerland) with a resonance frequency of 160 kHz and a spring constant of approximately 50 N/m was used. Height and size information was acquired by using the imaging software from Veeco.

Effect of bacteriocins ST194BZ and ST23LD on cell permeability. Log-phase cells ($OD_{600}=0.8-1.0$) of *Enterococcus* sp. HKLHS (10 ml) were harvested ($10,000\times g$, 15 min, 4°C), washed twice with 10 ml sterile 5 mM phosphate buffer (pH 6.5), and re-suspended in 20 ml 5 mM phosphate buffer (pH 6.5). The cell suspension was then divided into two equal volumes. One of the cell suspensions was treated with 1.0 ml bacteriocin ST194BZ (12,800 AU/ml) and the other with 1.0 ml bacteriocin ST23LD (12,800 AU/ml). The final

concentration of the two bacteriocins were 1,280 AU/ml. After 1 h of incubation at 37°C, the cells were harvested ($10,000\times g$, 15 min, 4°C) and the supernatant filtered through a 0.20 μ m nitrocellulose membrane (Minisart®, Sartorius). Absorbancy readings of the cell-free supernatant were recorded at 260 nm. The experiment was repeated with *L. sakei* DSM 20017 as a sensitive strain. Controls were *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 suspended in 5 mM phosphate buffer, but without bacteriocins, and in the same buffer containing bacteriocins ST194BZ and ST23LD, respectively, but without cells.

In a separate experiment, the level of β -galactosidase secreted from damaged cells was determined. Ten milliliters of a log-phase culture of *Enterococcus* sp. HKLHS ($OD_{600}=0.8-1.0$) was harvested, the cells washed twice with 10 ml 0.03 M sodium phosphate buffer (pH 6.5), the pellet re-suspended into 4 ml of the same buffer and divided into two equal volumes. One of the cell suspensions was treated with bacteriocin ST194BZ and the other with bacteriocin ST23LD (final concentrations=6,400 AU/ml). After 5 min of incubation at 25°C, 0.2 ml 0.1 M ONPG (*O*-nitrophenyl- β -D-galactopyranoside), dissolved in 0.03 M sodium phosphate buffer (pH 6.8), was added to each of the cell suspensions and the cells incubated for 10 min at 37°C. The β -galactosidase reaction was stopped by adding 2.0 ml 0.1 M sodium carbonate. The cells were harvested ($10,000\times g$, 15 min, 25°C) and absorbance readings of the cell-free supernatants recorded at 420 nm. The experiment was repeated with *L. sakei* DSM 20017. Controls were cells of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 prepared the same way, but not treated with bacteriocins ST194BZ or ST23LD (Hsu et al., 2005; Nagy et al., 2001).

Adsorption of bacteriocins ST194BZ and ST23LD to target cells. Adsorption of bacteriocins ST194BZ and ST23LD to target cells was performed according to the method described by Yildirim et al. (2002). The target strains (Table 1) were grown overnight in 10 ml MRS or BHI broth at 30°C or 37°C and then harvested ($10,000\times g$, 15 min, 4°C). The cells were washed twice with 10 ml sterile 5 mM phosphate buffer (pH 6.5) and re-suspended in the original volume in the same buffer. Each cell suspension (0.7 ml) was treated with 0.7 ml bacteriocin ST194BZ (12,800 AU/ml) and 0.7 ml bacteriocin ST23LD (12,800 AU/ml), respectively, and incubated for 1 h at 37°C. Cells were then harvested ($10,000\times g$, 15 min, 25°C) and the activity of unbound

bacteriocins in the supernatant determined as described before.

The percentage adsorption of bacteriocins ST194BZ and ST23LD to the target cells was calculated according to the following formula:

$$\% \text{adsorption} = 100 - \left(\frac{\text{bacteriocin activity after treatment}}{\text{original bacteriocin activity}} \times 100 \right)$$

Effect of pH and temperature on the adsorption of bacteriocins ST194BZ and ST23LD. Bacteriocin ST194BZ (12,800 AU/ml) was added to *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017, respectively, to a final concentration of 6,400 AU/ml. The cells were incubated for 1 h at 4, 10, 25, 30, 37, 45, and 60°C and, in a separate experiment, at pH 2.0, 4.0, 6.0, 8.0, and 10.0. The cells were then harvested (10,000×g, 15 min, 25°C), the pH of the cell-free supernatant adjusted to 6.0 with sterile 1 M NaOH and bacteriocin activity determined as described before. The experiment was repeated with bacteriocin ST23LD (12,800 AU/ml).

Effect of SDS, inorganic salts and organic compounds on the adsorption of bacteriocins ST194BZ and ST23LD to target cells. Eighteen-hour-old cells

of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 were treated with 1% (w/v) NaCl, K₂HPO₄, KH₂PO₄, MgCl₂, KCl, KI, Tris, (NH₄)₃C₆H₅O₇, CH₃COONa, Na₂CO₃, EDTA (C₁₀H₁₆O₈N₂), and SDS, 1% (v/v) Triton X-100, Triton X-114, β-mercaptoethanol, and 80% ethanol, methanol, and chloroform, respectively. Bacteriocin ST194BZ (12,800 AU/ml) was added to the treated cells, as described before, and incubated for 1 h at 37°C. The cells were then harvested (10,000×g, 15 min, 25°C) and the activity of the bacteriocins in the cell-free supernatant determined as described before. The experiment was repeated with bacteriocin ST23LD (12,800 AU/ml).

Results and Discussion

Effect of bacteriocins ST194BZ and ST23LD on cell growth

Addition of the bacteriocins ST194BZ and ST23LD to early logarithmic-phase cells of *Enterococcus* sp. HKLHS or *L. sakei* DSM 20017 resulted in immediate growth inhibition for at least 10 h (Fig. 1). Concluded from these results, the mode of action of the two bacteriocins is bactericidal. Similar results were recorded for the two bacteriocins against *L. casei* LHS (Todorov and Dicks, 2005a, 2006), plantaricin 423 against

Fig. 1. Effect of bacteriocins ST194BZ and ST23LD on the growth of (A) *Enterococcus* sp. HKLHS and (B) *L. sakei* DSM 20017.

Symbols: ♦, in the absence of bacteriocins; ■, in the presence of bacteriocin ST194BZ; ▲, in the presence of bacteriocin ST23LD.

Fig. 2. Morphology of *L. sakei* DSM 20017 after treatment with (A) bacteriocin ST194BZ and (B) bacteriocin ST23LD.

The images were taken with an atomic force microscope. The image size is $5 \times 5 \mu\text{m}$.

Oenococcus oeni 19Cl (Van Reenen et al., 1998), pediocin N5p against *Pediococcus pentosaceus* E5p (Manca de Nadra et al., 1998), and buchnericin LB against *Listeria monocytogenes* and *Bacillus cereus* (Yildirim et al., 2002).

Treatment of stationary-phase cells of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 for 1 h with bacteriocin ST194BZ decreased the number of viable cells by 2.8 and 0.9 log cycles, respectively. Similar results were recorded for cells treated with bacteriocin ST23LD (Table 1). *L. monocytogenes* treated with 640, 1,280, and 2,560 AU/ml buchnericin LB decreased by 0.8, 1.92, and 3.12 log cycles (Yildirim et al., 2002).

Effect of bacteriocins ST194BZ and ST23LD on cell morphology

Cells of *L. sakei* DSM 20017 treated with bacteriocins ST194BZ and ST23LD were clearly deformed, as visualized by AFM (Fig. 2). Treatment with bacteriocin ST194BZ caused the cells to collapse, accompanied by leakage of the cytoplasm from the cells (Fig. 2A). The surface of these cells appeared smooth. Treatment with bacteriocin ST23LD, on the other hand, caused vesiculation of the cell membrane with a vesicle diameter of 100–200 nm (Fig. 2B). The surface of these cells appeared granular, but the cells retained their shape. The morphology of cells treated with sterile distilled water (no bacteriocins) remained un-

changed.

Effect of bacteriocins ST194BZ and ST23LD on cell membrane permeability

Enterococcus sp. HKLHS and *Lactobacillus sakei* DSM 20017 treated with bacteriocins ST194BZ and ST23LD resulted in the leakage of DNA and β -galactosidase (Table 2). Concluded from these results, the mode of action of bacteriocins ST194BZ and ST23LD is most probably destabilizing of the permeability of the cell membrane. Similar results have been reported for buchnericin LB (Yildirim et al., 1999, 2002) and pediocin AcH (Bhunia et al., 1991).

Adsorption of bacteriocins ST194BZ and ST23LD to target cells

Bacteriocins ST194BZ and ST23LD adsorbed to sensitive and resistant cells of Gram-positive bacteria (Table 3). Adsorption of bacteriocin ST194BZ ranged from 0% for *Lactococcus lactis* subsp. *lactis* HV219 to 50% for *L. sakei* DSM 20017 and *Enterococcus* sp. HKLHS. Bacteriocin ST23LD adsorption ranged from 28% for *L. plantarum* LMG 13556 to 71% for *L. sakei* DSM 20017. Strains sensitive to bacteriocins ST194BZ and ST23LD revealed stronger adsorption of the peptides, compared to cells resistant to the peptide. Similar results have been reported by Yildirim et al. (2002). In the case of buchnericin LB, 100% adsorp-

Table 2. Extracellular levels of DNA and β -galactosidase recorded after treatment of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 with bacteriocins ST194BZ and ST23LD.

	Absorbance at 260 nm (DNA detection)		Absorbance at 420 nm (β -galactosidase detection)	
	<i>Enterococcus</i> sp. HKLHS	<i>L. sakei</i> DSM 20017	<i>Enterococcus</i> sp. HKLHS	<i>L. sakei</i> DSM 20017
Treated cells	3.00	3.11	1.20	1.17
Untreated cells	0.46	0.62	0.12	0.23
Bacteriocin ST194BZ, no cells	0.64	0.64	0.63	0.63

	Absorbance at 260 nm (DNA detection)		Absorbance at 420 nm (β -galactosidase detection)	
	<i>Enterococcus</i> sp. HKLHS	<i>L. sakei</i> DSM 20017	<i>Enterococcus</i> sp. HKLHS	<i>L. sakei</i> DSM 20017
Treated cells	2.79	3.13	1.13	1.18
Untreated cells	0.46	0.62	0.12	0.23
Bacteriocin ST23LD, no cells	0.73	0.73	0.64	0.64

Table 3. Activity spectrum of bacteriocins ST194BZ and ST23LD and their adsorption to target cells (expressed as a percentage value).

Target organism	Bacteriocin ST194BZ		Bacteriocin ST23LD	
	Sensitivity to bacteriocin ST194BZ ^a	Adsorption (%)	Sensitivity to bacteriocin ST23LD ^a	Adsorption (%)
<i>Enterococcus faecalis</i> BFE 1071	—	33	+	57
<i>Enterococcus mundtii</i> ST4SA	—	16	—	43
<i>Enterococcus</i> sp. HKLHS	+	50	+	57
<i>Enterococcus faecalis</i> FAIR E92	—	16	—	43
<i>Lactobacillus curvatus</i> DF38	—	16	+	57
<i>Lactobacillus plantarum</i> LMG 13556	—	16	—	28
<i>Lactobacillus sakei</i> DSM20017	+	50	+	71
<i>Lactobacillus salivarius</i> 241	—	16	+	57
<i>Lactococcus lactis</i> subsp. <i>lactis</i> HV219	—	0	—	43
<i>Listeria innocua</i> LMG 13568	+	33	+	57
<i>Streptococcus caprinus</i> ATCC 700066	—	16	—	43

^a— = not inhibited, and + = inhibited by bacteriocin ST194BZ or bacteriocin ST23LD, respectively.

tion to *L. plantarum*, *Pediococcus dextranicus*, *O. oeni*, and *E. faecalis* have been reported (Yildirim et al., 2002). The authors also reported 100% adsorption of the peptide to a strain of *Pediococcus cerevisiae* insensitive to buchnericin LB. In our experiments, bacte-

riocins ST194BZ and ST23LD adsorbed to the cells at levels less than 33%. Similar results have been recorded by Manca de Nadra et al. (1998). Higher adsorption of pediocin N5p was observed to sensitive strains. Adsorption of 100% was recorded for *O. oeni*

(previously *Leuconostoc oenos*) X2L, 80% for *Lactobacillus hilgardii* and *O. oeni* L10, and 70% for *L. hilgardii* 6D (Manca de Nadra et al., 1998). Pediocin N5p adsorbed to resistant bacteria at levels below 20% (Manca de Nadra et al., 1998).

Effect of pH, temperature, SDS and salts on the adsorption of bacteriocins ST194BZ and ST23LD to target cells

Optimal adsorption of bacteriocin ST194BZ to *Enterococcus* sp. HKLHS was recorded at pH 10.0 (66%).

Table 4. Effect of pH, temperature, SDS, inorganic salts, and solvents on the adsorption of bacteriocins ST194BZ and ST23LD to target cells (expressed as a percentage value).

	Bacteriocin ST194BZ		Bacteriocin ST23LD	
	<i>Enterococcus</i> sp. HKLHS	<i>L. sakei</i> DSM 20017	<i>Enterococcus</i> sp. HKLHS	<i>L. sakei</i> DSM 20017
Effect of pH:				
2	33	50	71	57
4	33	50	57	57
6	50	50	57	71
8	50	66	57	71
10	66	66	57	71
Effect of temperature (°C):				
4	33	50	57	71
10	33	50	71	71
25	33	50	71	57
30	50	50	57	57
37	50	50	57	71
45	33	66	57	86
60	33	83	57	86
Effect of SDS, inorganic salts and solvents:				
Na-acetate	50	66	42	71
Na ₂ CO ₃	50	66	42	71
EDTA(Na)	33	33	42	57
SDS	33	33	42	57
Triton X-100	83	83	71	86
Triton X-114	83	100	86	100
β-Mercaptoethanol	66	50	71	71
80% Ethanol	66	66	57	71
Methanol	50	50	42	71
Chloroform	66	100	71	86
NaCl	33	50	42	71
K ₂ HPO ₄	50	16	42	71
KH ₂ PO ₄	33	33	57	71
MgCl ₂	33	50	42	71
KCl	50	33	42	71
KI	50	33	28	71
Tris	50	50	57	86
NH ₄ -citrate	50	50	42	86
Control (not treated)	50	50	57	71

At pH 6.0 and 8.0 only 50% adsorption was detected (Table 4). In the case of *L. sakei* DSM 20017, optimal adsorption of bacteriocin ST194BZ was recorded between pH 8.0 and 10.0. Similar results were recorded for bacteriocin ST23LD. Optimal adsorption of bacteriocin ST23LD to *Enterococcus* sp. HKLHS (57%) was recorded at pH 4.0 to 10.0. The best adsorption (71%) to *L. sakei* DSM 20017 was recorded at pH 6.0 to 10.0. In the case of buchnericin LB, optimal levels of adsorption to *L. plantarum* were recorded at pH 5.0–8.0 (Yildirim et al., 2002).

Incubation temperature has a significant effect on the adsorption of bacteriocins ST194BZ and ST23LD to *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 (Table 4). Higher adsorption of bacteriocin ST194BZ to cells of *Enterococcus* sp. HKLHS (50%) and to *L. sakei* DSM 20017 (83%) was recorded at 30°C or 37°C and 60°C, respectively. In the case of bacteriocin ST23LD, higher adsorption was observed at 10°C or 25°C to cells of *Enterococcus* sp. HKLHS (71%) and at 45°C or 60°C to cells of *L. sakei* DSM 20017 (86%). Reasons for the latter differences in adsorption rates are not known, but may be due to specific interaction between bacteriocins ST194BZ and ST23LD and the target cells. In the case of buchnericin LB, identical adsorption to cells of *L. plantarum* was recorded after treatment at 0, 10, 25, 50, and 80°C (Yildirim et al., 2002).

Reduction in adsorption of bacteriocin ST194BZ to *Enterococcus* sp. HKLHS was observed when cells were treated with NaCl, KH₂PO₄, MgCl₂, EDTA, and SDS (Table 4). Treatment of the cells with Na-acetate, Na₂CO₃, methanol, K₂HPO₄, KCl, KI, Tris, and NH₄-citrate yielded the same levels of adsorption compared to untreated cells of *Enterococcus* sp. HKLHS (Table 4). An increase in the adsorption of bacteriocin ST194BZ to *Enterococcus* sp. HKLHS was observed in the presence of Triton X-100, Triton X-114, β -mercaptoethanol, 80% ethanol, and chloroform (Table 4).

Cells of *L. sakei* DSM 20017 treated with EDTA, SDS, K₂HPO₄, KH₂PO₄, KCl, and KI led to a reduction in bacteriocin ST194BZ adsorption (Table 4). No change in adsorption was observed in the presence of β -mercaptoethanol, methanol, NaCl, MgCl₂, Tris, or NH₄-citrate, whereas an increase in adsorption was observed in the presence of Na-acetate, Na₂CO₃, Triton X-100, Triton X-114, 80% ethanol, and chloroform (Table 4).

Reduction in adsorption of bacteriocin ST23LD to

Enterococcus sp. HKLHS was observed when cells were treated with Na-acetate, Na₂CO₃, EDTA, SDS, methanol, NaCl, K₂HPO₄, MgCl₂, KCl, KI, and NH₄-citrate (Table 4). Treatment of the cells with 80% ethanol, KH₂PO₄, and Tris yielded the same levels of adsorption compared to untreated cells of *Enterococcus* sp. HKLHS (Table 4). An increase in the adsorption of bacteriocin ST194BZ to *Enterococcus* sp. HKLHS was observed in the presence of Triton X-100, Triton X-114, β -mercaptoethanol, and chloroform (Table 4).

Cells of *L. sakei* DSM 20017 treated with EDTA and SDS led to a reduction in bacteriocin ST23LD adsorption (Table 4). No change in adsorption was observed in the presence of Na-acetate, Na₂CO₃, β -mercaptoethanol, 80% ethanol, methanol, NaCl, K₂HPO₄, KH₂PO₄, MgCl₂, KCl, or KI, whereas an increase in adsorption was observed in the presence of Triton X-100, Triton X-114, chloroform, Tris, and NH₄-citrate (Table 4).

Adsorption of buchnericin LB to *L. plantarum* was reduced by NaCl, NH₄Cl, MgCl₂, KCl, KI, and Tris. Treatment of cells with NH₄-citrate, Na-acetate, NaCO₃, EDTA, SDS, Triton-X, 2-mercaptoethanol, 80% ethanol, and 80% methanol had no effect on adsorption of buchnericin LB to *L. plantarum* (Yildirim et al., 2002).

The adsorption of pediocin N5p to *P. pentosaceus* E5p increased in the presence of MgCl₂, MgSO₄, MnCl₂, and MnSO₄, whereas NaCl, KCl, KI, NH₄Cl, CaCl₂, Na₃PO₄, Na₂SO₄, EDTA, and ethanol had no effect on its adsorption (Manca de Nadra et al., 1998). Organic salts and Na-acetate reduced pediocin N5p adsorption to the target cells. Adsorption of pediocin N5p increased by 25% in the presence of SDS (Manca de Nadra et al., 1998).

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