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# Factors Affecting the Adsorption of Bacteriocins to *Lactobacillus sakei* and *Enterococcus* sp.

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**Abstract** Bacteriocins bacJW3BZ and bacJW6BZ produced by *Lactobacillus plantarum*, and bacJW11BZ and bacJW15BZ produced by *Lactobacillus fermentum*, inhibit Gram-positive and Gram-negative bacteria. Treatment of *Enterococcus* sp. HKLHS and *Lactobacillus sakei* DSM 20017 with these bacteriocins deformed the cells and resulted in DNA and β-galactosidase leakage. The bacteriocins adsorbed to sensitive and resistant strains. Optimal adsorption of bacJW3BZ and bacJW6BZ to *Enterococcus* sp. HKLHS was recorded at pH 10.0, whereas adsorption of bacJW11BZ and bacJW15BZ was favored at pH 4.0–8.0 and 2.0–4.0, respectively. Adsorption to *L. sakei* DSM 20017 was less influenced by pH. Incubation temperature had a major influence on the adsorption of bacJW6BZ and bacJW11BZ to sensitive cells, with better results recorded below 30°C. Although variable results were recorded for bacJW3BZ and bacJW15BZ, optimal adsorption occurred between 37 and 60°C. Variable levels of adsorption were recorded in the presence of inorganic salts and solvents, and this seems to be species-specific. Maximal adsorption (100%) was recorded for bacJW3BZ and bacJW15BZ to *L. sakei* DSM 20017 in the presence of most inorganic salts and solvents tested. Maximal adsorption of bacJW6BZ to *Enterococcus* sp. HKLHS (50%) was recorded in the presence of Triton X-114 and little (17%) or no adsorption in the presence of other reagents.

**Keywords** Bacteriocins JW3BZ · JW6BZ · JW11BZ · JW15BZ · Adsorption

## Introduction

A number of bacteriocins have been described for lactic acid bacteria. Traditionally, bacteriocins have been defined as ribosomal synthesized polypeptides with bactericidal or bacteriostatic activity against genetically closely related strains [1, 2]. Klaenhammer [2] classified these peptides into four groups, based on their spectrum of activity, presence of lanthionine, heat resistance, and complexity.

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*Lactobacillus plantarum* JW3BZ and JW6BZ and *Lactobacillus fermentum* JW11BZ and JW15BZ isolated from boza, a cereal-based fermented beverage from Bulgaria, produce bacteriocins JW3BZ (2.3 kDa), JW6BZ (3.0 kDa), JW11BZ (2.3 kDa), and JW15BZ (3.0 kDa), respectively [3]. All four bacteriocins inhibit the growth of *Lactobacillus casei*, *Lactobacillus sakei*, *Lactococcus lactis* subsp. *lactis*, *Listeria innocua*, *Enterococcus faecalis*, *Streptococcus caprinus*, and *Klebsiella pneumoniae* [3]. Little is known about the adsorption of bacteriocins to target (sensitive) cells. Pediocin N5p, a bacteriocin produced by *Pediococcus pentosaceus*, adsorbed to Gram-positive and Gram-negative bacteria [4]. Adsorption of pediocin N5p to *P. pentosaceus* increased by 80 and 100% in the presence of $Mg^{2+}$ and $Mn^{2+}$, respectively. Treatment of target strains with 1% (m/v) sodium dodecyl sulfate (SDS) increased adsorption by 25% [4]. Adsorption of buchnericin LB, produced by *Lactobacillus buchneri*, was stimulated at pH 5.0 to 8.0 and reduced or prevented in the presence of anions and lipoteichoic acid [5]. Treatment of target cells with detergents or organic solvents had no effect on the adsorption of buchnericin LB [5].

In this study, the conditions required for bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ to adsorb to sensitive cells were determined. *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 were used as model target strains.

## Materials and Methods

### Growth Conditions and Preparation of Bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ

*Lactobacillus plantarum* JW3BZ and JW6BZ and *L. fermentum* JW11BZ and JW15BZ were grown in de Man–Rogosa–Sharpe (MRS) broth (Oxoid, Oxoid, Basingstoke, Hampshire, England). Other strains used in this study (Table 1) were grown in MRS broth (Oxoid) or brain–heart infusion (BHI) broth (Oxoid) and at temperatures indicated in the respective culture collection catalogues. All strains were stored at −80°C in the presence of sterile 15% (v/v) glycerol.

Strains JW3BZ, JW6BZ, JW11BZ, and JW15BZ were each inoculated (2%, v/v) into 100 ml MRS broth and incubated at 30°C for 24 h. Cells were harvested (10,000×*g*, 15 min, 4°C) and the pH of the cell-free supernatants adjusted to 6.0 with sterile 1 M NaOH, and the cell-free supernatants were heated for 10 min at 80°C and filter-sterilized (0.20 μm pore size nitrocellulose membrane, Minisart®, Sartorius, Göttingen, Germany).

### Bacteriocin Bioassay

Bacteriocin activity in cell-free supernatants were determined by using the agar-spot test and the well-diffusion methods [6]. Cell-free supernatants were adjusted to pH 6.0 with sterile 1 M NaOH before testing. Target strains (Table 1) were cultured in BHI broth (non-lactic acid bacteria) or MRS broth (lactic acid bacteria). Antimicrobial activity was expressed as arbitrary units (Au) per milliliter according to the method described by Todorov and Dicks [7].

### Effect of Bacteriocins on Cell Growth

Twenty milliliters of filter-sterilized cell-free supernatants containing bacteriocins JW3BZ (6,400 Au/ml), JW6BZ (25,600 Au/ml), JW11BZ (12,800 Au/ml), and JW15BZ

**Table 1** Activity spectrum of bacteriocins and their adsorption to target cells (expressed as a percentage value).

| Target organism | BacJW3BZ | | BacJW6BZ | | BacJW11BZ | | BacJW15BZ | |
|---|---|---|---|---|---|---|---|---|
| | Sensitivity | Adsorption (%) | Sensitivity | Adsorption (%) | Sensitivity | Adsorption (%) | Sensitivity | Adsorption (%) |
| *Enterococcus faecalis* BFE 1071 | − | 25 | − | 17 | − | 60 | − | 40 |
| *Enterococcus mundtii* ST4SA | + | 25 | + | 17 | + | 40 | + | 40 |
| *Enterococcus* sp. HKLHS | + | 50 | + | 33 | + | 60 | + | 60 |
| *Enterococcus faecalis* FAIR E92 | − | 25 | + | 0 | + | 60 | + | 40 |
| *Lactobacillus curvatus* DF38 | − | 25 | − | 0 | − | 20 | − | 40 |
| *Lactobacillus plantarum* LMG 13556 | + | 75 | + | 66 | + | 40 | + | 20 |
| *Lactobacillus sakei* DSM 20017 | + | 75 | + | 50 | + | 80 | + | 80 |
| *Lactobacillus salivarius* 241 | − | 25 | − | 0 | − | 20 | − | 20 |
| *Lactococcus lactis* subsp. *lactis* HV219 | − | 25 | + | 17 | + | 40 | + | 40 |
| *Listeria innocua* F | + | 0 | − | 17 | − | 60 | + | 80 |
| *Streptococcus caprinus* ATCC 700066 | − | 0 | + | 17 | + | 40 | + | 60 |

− represents growth in the presence of bacteriocin, + represents growth inhibited by bacteriocin. All strains besides those from BFE, LMG, DSM, and ATCC were from our own culture collection.

BFE = Bundesforschungsanstalt für Ernährung, Karslruhe, Germany; LMG = Labotarory of Microbiology, University of Ghent, Ghent, Belgium; DSM = Deutsche Sammlung von Mikroorganismen und Zellculturen; ATCC = American Type Culture Collection.

(12,800 Au/ml) were added, individually, to 100 ml of 3-h-old and 4-h-old cultures ($OD_{600}$= 0.12) of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017, respectively. Optical density of the cultures was determined hourly over 13 h. In a similar experiment, the effect of the four bacteriocins were tested on stationary-phase cells ($OD_{600}$=1.2–1.5) of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017, respectively.

## Effect of Bacteriocins on the Morphology of *L. sakei*

*Lactobacillus sakei* DSM 20017 was grown in 20 ml MRS broth at 37°C for 18 h. The cells were harvested (8,000×*g*, 10 min, 4°C) and washed five times with 20 ml sterile distilled water. Cells were then resuspended in 40 ml sterile distilled water and divided into four equal volumes. Bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ were each diluted with sterile distilled water to 3,200 Au/ml, adjusted to pH 6.0 with 1 M NaOH, and filter-sterilized as described before. Ten milliliters of each bacteriocin was added to the cell suspensions (final activity=1,600 Au/ml) and incubated for 1 h at 4°C. The cells were harvested (8,000×*g*, 10 min, 4°C), washed five times with 10 ml sterile distilled water, and resuspended in 1 ml sterile distilled water. Cell suspensions were applied onto a freshly cleaved mica surface and allowed to dry for 5 min before subjected to atomic force microscopy (AFM) in a Multimode AFM (Veeco, Santa Barbara, CA, USA). All images were obtained in air and with tapping mode. A silicon noncontact 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 on Cell Permeability

Twenty milliliters of *Enterococcus* sp. HKLHS ($OD_{600}$=0.8–1.0) was harvested (10,000×*g*, 15 min, 4°C), washed twice with 10 ml sterile 5 mM phosphate buffer (pH 6.5), and resuspended into 40 ml 5 mM phosphate buffer (pH 6.5). The cell suspension was divided into four equal volumes and each sample was treated with 1 ml bacteriocin (6,400 Au/ml bacJW3BZ, 25,600 Au/ml bacJW6BZ, 12,800 Au/ml bacJW11BZ, and 12,800 Au/ml bacJW15BZ) to yield final concentrations of 640, 2,560, 1,280, and 1,280 Au/ml, respectively. After 1 h of incubation at 37°C, the cells were harvested (10,000×*g*, 15 min, 4°C) and the supernatants filtered through a 0.20-μm nitrocellulose membrane (Minisart®). Absorbance readings of the filtrate were recorded at 260 nm. The experiment was repeated with *L. sakei* DSM 20017 as target strain. Controls were *L. sakei* DSM 20017 and *Enterococcus* sp. HKLHS suspended in 5 mM phosphate buffer, but without bacteriocins, and in the same buffer containing bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ, but without cells.

In a separate experiment, the level of β-galactosidase secreted from damaged cells was determined. Twenty milliliters of a log-phase culture of *Enterococcus* sp. HKLHS ($OD_{600}$= 0.8–1.0) was harvested, the cells were washed twice with 20 ml 0.03 M sodium phosphate buffer (pH 6.5), and the pellet was resuspended into 8 ml of the same buffer. The cell suspension was divided into four equal parts and each was treated with equal volumes of bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ to yield final concentrations of 3,200, 12,800, 6,400, and 6,400 Au/ml, respectively. 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 were 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×*g*, 15 min, 25°C) and absorbance readings of cell-free supernatants were recorded at 420 nm. The experiment was repeated with *L. sakei* DSM 20017. Controls were *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 prepared the same way, but not treated with bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ.

### Adsorption of Bacteriocins to Target Cells

Adsorption of bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ to target cells was performed according to the method described by Yildirim, Johnson, and Winters [8]. Target strains (Table 1) were grown overnight in 10 ml MRS broth (Oxoid) or BHI broth (Oxoid) and then harvested (10,000×*g*, 15 min, 4°C). Cells were washed twice with 10 ml sterile 5 mM phosphate buffer (pH 6.5) and resuspended to the original volume in the same buffer. Each cell suspension (0.7 ml) was treated with 0.7 ml JW3BZ (6,400 Au/ml), JW6BZ (25,600 Au/ml), JW11BZ (12,800 Au/ml), and JW15BZ (12,800 Au/ml), respectively, and incubated for 1 h at 37°C. Cells were then harvested (10,000×*g*, 15 min, 25°C) and the activity of unbound bacteriocins in the supernatant was determined as described before.

The percentage adsorption of bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ to target cells was calculated according to the following formula:

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

### Effect of pH and Temperature on the Adsorption of Bacteriocins

Bacteriocin JW3BZ (6,400 Au/ml) was added to *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 to yield a final concentration of 3,200 Au/ml. The cells were incubated for 1 h at 4, 10, 25, 30, 37, 45, and 60°C (pH 6.0) and, in a separate experiment, at pH 2.0, 4.0, 6.0, 8.0, and 10.0 (30°C). The cells were then harvested (10,000×*g*, 15 min, 25°C), the pH of the cell-free supernatant was adjusted to 6.0 with sterile 1 M NaOH, and bacteriocin activity was determined as described before. The experiment was repeated with bacteriocins JW6BZ (25,600 Au/ml), JW11BZ (12,800 Au/ml), and JW15BZ (12,800 Au/ml).

### Effect of SDS, Inorganic Salts, and Organic Compounds on the Adsorption of Bacteriocins

Eighteen-hour-old cells of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 were treated with 1% (m/v) NaCl, $Na_2HPO_4$, $NaH_2PO_4$, $MgCl_2$, KCl, KI, Tris, $(NH_4)_3C_6H_5O_7$, $CH_3COOK$, $Na_2CO_3$, EDTA ($C_{10}H_{16}O_8N_2$), SDS, 1% (v/v) Triton X-100, Triton X-114, β-mercaptoethanol, 80% ethanol, methanol, and chloroform. Bacteriocin JW3BZ (6,400 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 bacJW3BZ in the cell-free supernatant was determined as described before. The experiment was repeated with bacteriocins JW6BZ (25,600 Au/ml), JW11BZ (12,800 Au/ml), and JW15BZ (12,800 Au/ml).

## Results and Discussion

All data represent an average of three repeats. The optical density and pH values recorded in each experiment did not vary by more than 4% and standard deviation values were not

presented. In case of bacteriocin production (AU/ml), all three repeats produced the same results.

## Effect of Bacteriocins on Cell Growth

Immediate growth inhibition for at least 10 h was observed after the addition of bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ to early logarithmic-phase cells of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 (Fig. 1). The mode of action is bacteriostatic. Similar results have been reported for pediocin N5p against *P. pentosaceus* E5p [4], plantaricin 423 against *Oenococcus oeni* 19Cl [9], and buchnericin LB against *Listeria monocytogenes* and *Bacillus cereus* [5].

Treatment of stationary-phase cells of *Enterococcus* sp. HKLHS for 1 h with bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ decreased the number of viable cells by 3.4, 4.9, 2.4, and 2.3 log cycles, respectively (Table 2). A similar decrease in the number of viable cells (log 2.4, 4.8, 3.8, and 3.8) was observed when stationary-phase cells of *L. sakei* DSM 20017 were treated with bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ, respectively (Table 2). *Listeria monocytogenes* treated with 640, 1,280, and 2,560 Au/ml buhnericin LB decreased by 0.8, 1.9, and 3.1 log cycles [5].

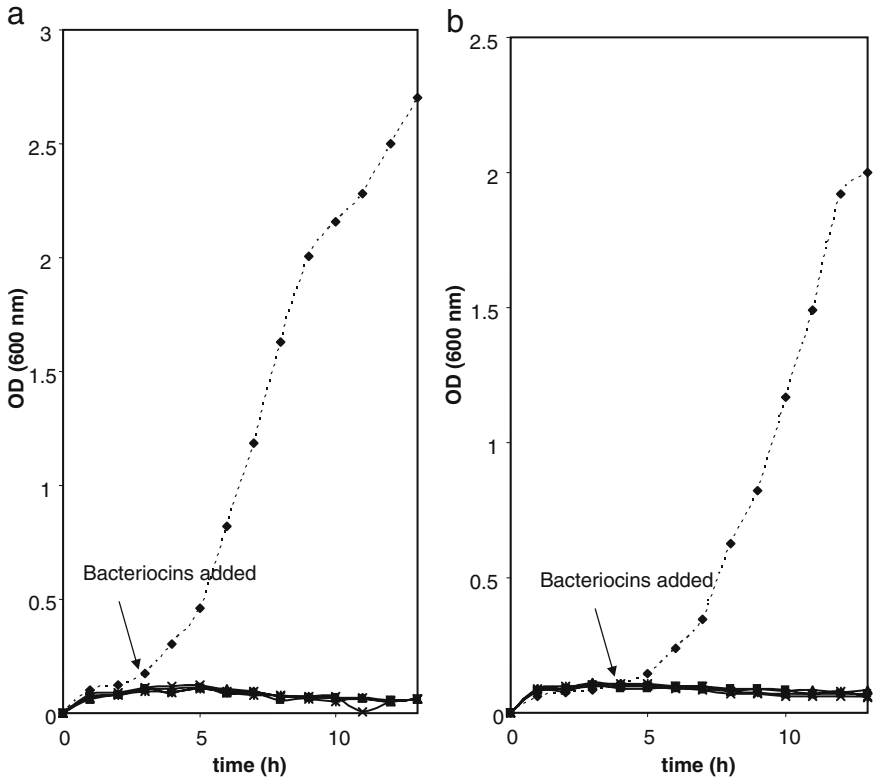


**Fig. 1** Effect of bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ on the growth of **a** *Enterococcus* sp. HKLHS and **b** *L. sakei* DSM 20017. *Diamonds*, in the absence of bacteriocins; *squares*, in the presence of bacJW3BZ; *triangles*, in the presence of bacJW6BZ; ×, in the presence of bacJW11BZ; *circles*, in the presence of bacJW15BZ

**Table 2** Changes in the number of viable cells recorded after 1 h in the presence of bacteriocins.

| Test microorganism | Before treatment | After 1 h in the presence of | | | |
|---|---|---|---|---|---|
| | | Bac JW3BZ | Bac JW6BZ | Bac JW11BZ | Bac JW15BZ |
| *Enterococcus* sp. HKLHS | $8.7\times10^{9}$ | $3.8\times10^{6}$ (0.043%) | $1.1\times10^{5}$ (0.001%) | $3.7\times10^{7}$ (0.430%) | $4.2\times10^{7}$ (0.500%) |
| *L. sakei* DSM 20017 | $1.0\times10^{9}$ | $4.3\times10^{6}$ (0.400%) | $1.8\times10^{4}$ (0.002%) | $1.6\times10^{5}$ (0.016%) | $1.8\times10^{5}$ (0.018%) |

Survival ratio indicated in parenthesis.

## Effect of Bacteriocins on Cell Morphology

Deformation of *L. sakei* DSM 20017 cells was observed after treatment with bacteriocins JW3BZ, JW6BZ, JW11BZ, or JW15BZ, (Fig. 2). Cytoplasm leakage of exponential-growing cells of *L. sakei* DSM 20017 was observed after treatment with bacteriocins JW3BZ and JW6BZ.

## Effect of Bacteriocins on Cell Membrane Permeability

Leakages of DNA and β-galactosidase were observed when *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 were treated with bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ (Table 3). Bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ most probably destabilize the permeability of the cell membrane. Similar results have been reported for buchnericin LB [5, 8] and pediocin AcH [10].

## Adsorption of Bacteriocins to Target Cells

Bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ adsorbed to sensitive and resistant cells of Gram-positive bacteria (Table 1). Higher levels of adsorption were observed for strains sensitive to bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ, compared to cells resistant to the peptides. Similar results have been reported by Yildirim, Avşar, and Yildirim [5]. In the case of buhnericin LB, 100% adsorption to *L. plantarum*, *Pediococcus dextranicus*, *O. oeni*, and *E. faecalis* have been reported [5]. The authors also reported 100% adsorption of the peptide to a strain of *Pediococcus cerevisiae* insensitive to buchnericin LB. Adsorption of 100% was recorded for *O. oeni* X2L, 80% for *Lactobacillus hilgardii* and *O. oeni* L10, and 70% for *L. hilgardii* 6D [4]. Pediocin N5p adsorbed to resistant bacteria at levels less than 20% [4].

## Effect of pH, Temperature, SDS, and Salts on the Adsorption of Bacteriocins to Target Cells

Optimal adsorption of bacJW3BZ (100%) and bacJW6BZ (83%) to *Enterococcus* sp. HKLHS was recorded at pH 10.0, whereas optimal adsorption of bacJW11BZ (60%) and bacJW15BZ (80%) to *Enterococcus* sp. HKLHS was observed at pH values ranging from pH 4.0 to 8.0 and pH 2.0 to 4.0, respectively (Table 4). Lower adsorption levels were recorded for bacJW3BZ (50%), bacJW6BZ (33%), bacJW11BZ (20%), and bacJW15BZ (60%) to *Enterococcus* sp. HKLHS at pH 2.0–8.0, 6.0, 2.0, and 6.0–10.0, respectively (Table 4). The highest adsorption (100%) to *Enterococcus* sp. HKLHS was recorded for bacteriocin JW3BZ at pH 10.0.

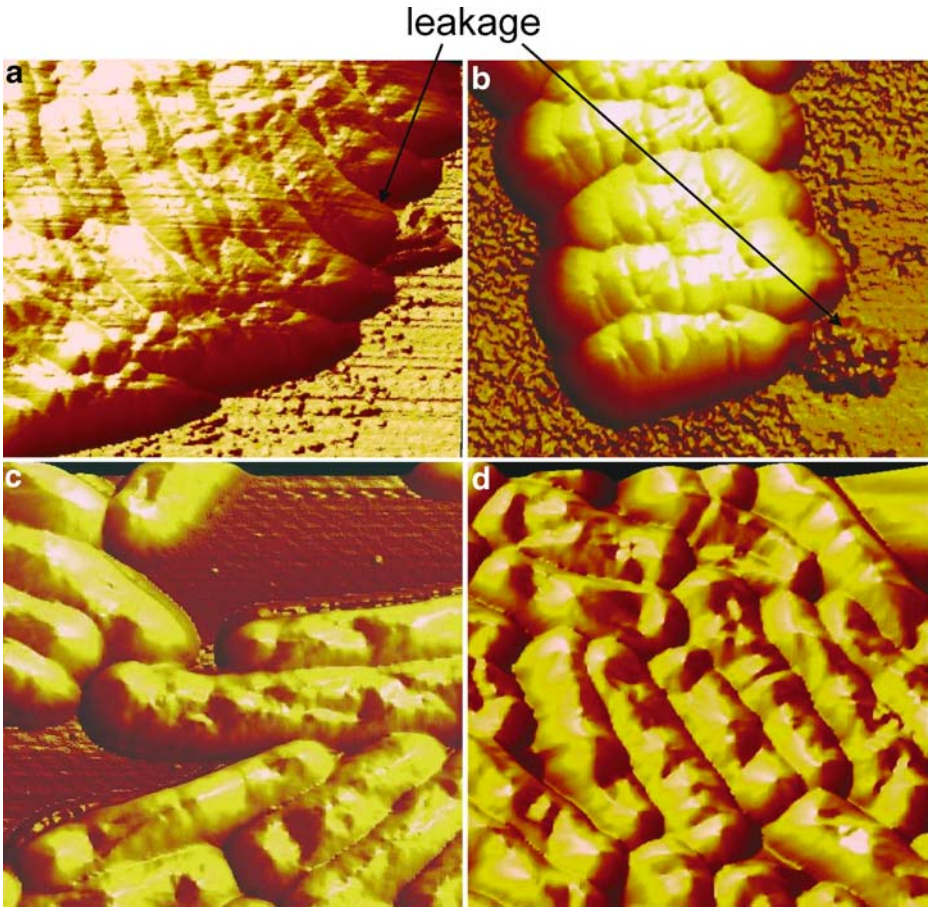


**Fig. 2** Morphology of *L. sakei* DSM 20017 after treatment with **a** bacJW3BZ, **b** bacJW6BZ, **c** bacJW11BZ, and **d** bacJW15BZ. The images were taken with an AFM

In the case of *L. sakei* DSM 20017, optimal adsorption of bacJW3BZ (75%), bacJW6BZ (83%), bacJW11BZ (80%), and bacJW15BZ (80%) were recorded at pH 6.0, 10.0, 2.0–10.0, and 4.0–10.0, respectively (Table 4). No adsorption was recorded for bacJW3BZ to cells of *L. sakei* DSM 20017 at pH 2.0. Lowest adsorption (33%) was recorded for bacJW6BZ at pH 2.0–4.0. In the case of bacJW15BZ, 60% absorption was detected at pH 2.0. Buchnericin LB adsorbed the best to *L. plantarum* at pH 5.0–8.0 [5].

Incubation temperature has a significant effect on the adsorption of bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ to *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 (Table 4). Optimal adsorptions to *Enterococcus* sp. HKLHS for bacJW6BZ (50%), bacJW11BZ (80%), and bacJW15BZ (80%) were recorded at 4–10, 37–60, and 25–30°C, respectively. Adsorption of bacJW3BZ (50%) to *Enterococcus* sp. HKLHS was not affected by incubation temperatures. In the case of *L. sakei* DSM 20017, optimal adsorption of bacJW3BZ (75%), bacJW6BZ (66%), bacJW11BZ (80%), and bacJW15BZ (80%) was recorded at 37–45, 4–25, 25–37, and 4–37°C, respectively. Reasons for the differences in adsorption rates are not known, but may be due to specific interactions between the bacteriocins and the target cells. In the case of buchnericin LB, identical adsorption

**Table 3** Extracellular levels of DNA and β-galactosidase recorded after treatment of *Enterococcus* sp. HKLHS and *L. sakei* DSM 20017 with bacteriocins.

| | Absorbance at 260 nm (DNA detection) | | Absorbance at 420 nm (β-galactosidase detection) | |
|---|---|---|---|---|
| | *Enterococcus* sp. HKLHS | *L. sakei* DSM 20017 | *Enterococcus* sp. HKLHS | *L. sakei* DSM 20017 |
| BacJW3BZ | | | | |
| Treated cells | 3.14 | 3.29 | 1.19 | 1.16 |
| Untreated cells | 0.46 | 0.62 | 0.12 | 0.23 |
| Bacteriocin JW3BZ, no cells | 1.13 | 1.13 | 0.65 | 0.65 |
| BacJW6BZ | | | | |
| Treated cells | 3.09 | 3.23 | 1.12 | 1.29 |
| Untreated cells | 0.46 | 0.62 | 0.12 | 0.23 |
| Bacteriocin JW6BZ, no cells | 0.73 | 0.73 | 0.64 | 0.64 |
| BacJW11BZ | | | | |
| Treated cells | 3.01 | 3.06 | 1.68 | 1.74 |
| Untreated cells | 0.46 | 0.63 | 0.12 | 0.23 |
| Bacteriocin JW11BZ, no cells | 0.95 | 0.95 | 0.84 | 0.84 |
| BacJW15BZ | | | | |
| Treated cells | 3.03 | 3.05 | 1.00 | 1.41 |
| Untreated cells | 0.46 | 0.62 | 0.12 | 0.23 |
| Bacteriocin JW15BZ, no cells | 1.01 | 1.01 | 0.69 | 0.69 |

to *L. plantarum* was recorded after treatment at 0, 10, 25, 50, and 80°C [5]. Relative good adsorption at low pH and 37°C was recorded for bacteriocins JW3BZ, JW6BZ, JW11BZ, and JW15BZ, suggesting that the strains could be used as probiotics.

A reduction in adsorption of bacJW3BZ to *Enterococcus* sp. HKLHS was observed when cells were treated with $(NH_4)_3C_6H_5O_7$, Tris, KCl, and β-mercaptoethanol (Table 4). Treatment of HKLHS cells with KI, $MgCl_2$, $Na_2HPO_4$, NaCl, methanol, 80% ethanol, SDS, $NaCO_3$, and $CH_3COOK$ did not change the adsorption levels of bacJW3BZ (Table 4). Increased adsorption of bacJW3BZ to *Enterococcus* sp. HKLHS was observed in the presence of $NaH_2PO_4$, chloroform, Triton X-100, Triton X-114, and EDTA (Table 4).

Salts and SDS led to a reduction of bacJW6BZ adsorption to *Enterococcus* sp. HKLHS. Triton X-114, on the other hand, led to an increase in bacJW6BZ adsorption. Reduction in bacJW11BZ adsorption to *Enterococcus* sp. HKLHS was observed when cells were treated with Tris, KCl, $MgCl_2$, $Na_2HPO_4$, NaCl, and SDS (Table 4). Treatment of the cells with $(NH_4)_3C_6H_5O_7$, KI, $NaH_2PO_4$, methanol, Triton X-100, EDTA, $Na_2CO_3$, and $CH_3COOK$ yielded the same levels of adsorption compared to untreated cells of *Enterococcus* sp. HKLHS (Table 4). An increase in adsorption of bacJW11BZ to *Enterococcus* sp. HKLHS was observed in the presence of chloroform, 80% ethanol, β-mercaptoethanol, and Triton X-114 (Table 4).

No reduction in the adsorption of bacteriocin JW15BZ to *Enterococcus* sp. HKLHS was recorded in the presence of salts and SDS (Table 4). Treatment of the cells with $(NH_4)_3C_6H_5O_7$, Tris, KCl, SDS, and $Na_2CO_3$ yielded adsorption levels similar to that observed with untreated cells of *Enterococcus* sp. HKLHS. An increase in adsorption was observed in the presence of KI, KCl, $NaH_2PO_4$, $Na_2HPO_4$, NaCl, chloroform, methanol, 80% ethanol, β-mercaptoethanol, Triton X-100, Triton X-114, EDTA, and $CH_3COOK$ (Table 4).

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

| | BacJW3BZ | | BacJW6BZ | | BacJW11BZ | | BacJW15BZ | |
|---|---|---|---|---|---|---|---|---|
| | *Enterococcus* sp. HKLHS | *L. sakei* DSM 20017 | *Enterococcus* sp. HKLHS | *L. sakei* DSM 20017 | *Enterococcus* sp. HKLHS | *L. sakei* DSM 20017 | *Enterococcus* sp. HKLHS | *L. sakei* DSM 20017 |
| pH | | | | | | | | |
| 2 | 50 | 0 | 50 | 20 | 20 | 100 | 80 | 60 |
| 4 | 50 | 50 | 50 | 20 | 60 | 100 | 80 | 80 |
| 6 | 50 | 75 | 33 | 40 | 60 | 100 | 60 | 80 |
| 8 | 50 | 50 | 50 | 40 | 60 | 100 | 60 | 80 |
| 10 | 100 | 50 | 83 | 80 | 40 | 100 | 60 | 80 |
| Temperature (°C) | | | | | | | | |
| 4 | 50 | 50 | 50 | 66 | 60 | 80 | 60 | 80 |
| 10 | 50 | 50 | 50 | 66 | 40 | 60 | 60 | 80 |
| 25 | 50 | 50 | 33 | 66 | 40 | 80 | 80 | 80 |
| 30 | 50 | 50 | 33 | 50 | 40 | 80 | 80 | 80 |
| 37 | 50 | 75 | 33 | 50 | 60 | 80 | 60 | 80 |
| 45 | 50 | 75 | 33 | 50 | 60 | 60 | 60 | 60 |
| 60 | 50 | 50 | 17 | 50 | 60 | 60 | 60 | 60 |
| $CH_3COOK$ | 50 | 100 | 0 | 17 | 60 | 80 | 80 | 100 |
| $Na_2CO_3$ | 50 | 50 | 0 | 33 | 60 | 80 | 60 | 100 |
| EDTA (-Na) | 75 | 100 | 0 | 50 | 60 | 100 | 80 | 100 |
| SDS | 50 | 75 | 0 | 17 | 40 | 40 | 60 | 60 |
| Triton X-100 | 100 | 100 | 0 | 50 | 60 | 80 | 80 | 100 |
| Triton X-114 | 100 | 100 | 50 | 100 | 100 | 100 | 100 | 100 |
| 2-mercapto-ethanol | 25 | 100 | 17 | 83 | 80 | 100 | 80 | 80 |
| 80% Methanol | 50 | 100 | 0 | 66 | 80 | 100 | 80 | 80 |
| Methanol | 50 | 100 | 0 | 66 | 60 | 100 | 80 | 100 |
| Chloroform | 100 | 100 | 0 | 83 | 80 | 100 | 100 | 100 |
| NaCl | 50 | 100 | 0 | 17 | 40 | 80 | 100 | 100 |
| $Na_2HPO_4$ | 50 | 100 | 0 | 17 | 40 | 80 | 80 | 100 |
| $NaH_2PO_4$ | 75 | 100 | 17 | 33 | 60 | 80 | 100 | 100 |
| $MgCl_2$ | 50 | 75 | 0 | 33 | 40 | 60 | 60 | 80 |
| KCl | 25 | 100 | 0 | 17 | 10 | 80 | 80 | 100 |
| KI | 50 | 100 | 0 | 17 | 60 | 80 | 80 | 100 |
| Tris | 25 | 50 | 0 | 33 | 40 | 80 | 60 | 100 |
| $NH_4$-citrate | 25 | 100 | 0 | 17 | 60 | 80 | 60 | 100 |
| Control (not treated) | 50 | 75 | 0 | 50 | 60 | 80 | 60 | 80 |

Reduction in adsorption of bacteriocin JW3BZ to *L. sakei* DSM 20017 was observed when cells were treated with Tris and $Na_2CO_3$ (Table 4). The same levels of adsorption compared to untreated cells of *L. sakei* DSM 20017 was observed in the presence of $MgCl_2$ and SDS, whereas an increase in adsorption of bacteriocin JW3BZ to *L. sakei* DSM 20017 was observed when cells were treated with $(NH_4)_3C_6H_5O_7$, KI, KCl, $NaH_2PO_4$, $Na_2HPO_4$, NaCl, chloroform, methanol, 80% ethanol, β-mercaptoethanol, Triton X-100, Triton X-114, EDTA, and $CH_3COOK$ (Table 4).

Treatment of *L. sakei* DSM 20017 with $(NH_4)_3C_6H_5O_7$, Tris, KI, KCl, $MgCl_2$, $NaH_2PO_4$, $Na_2HPO_4$, NaCl, SDS, $Na_2CO_3$, and $CH_3COOK$ led to a decrease in the adsorption of bacteriocin JW6BZ (Table 4), whereas treatment with Triton X-100 and EDTA yielded the same level of adsorption compared to untreated cells of *L. sakei* DSM 20017 (Table 4). However, an increase in adsorption of bacteriocin JW6BZ to *L. sakei* DSM 20017 was recorded in the presence of chloroform, methanol, 80% ethanol, β-mercaptoethanol, and Triton X-114 (Table 4).

Reduction in adsorption of bacteriocin JW11BZ to *L. sakei* DSM 20017 was observed when cells were treated with $MgCl_2$ and SDS (Table 4). Treatment with $(NH_4)_3C_6H_5O_7$, Tris, KI, KCl, $NaH_2PO_4$, $Na_2HPO_4$, NaCl, Triton X-100, $Na_2CO_3$, and $CH_3COOK$ yielded the same levels of adsorption compared to untreated cells of *L. sakei* DSM 20017, whereas an increase in adsorption of bacteriocin JW11BZ was observed in the presence of chloroform, methanol, 80% ethanol, β-mercaptoethanol, Triton X-114, and EDTA (Table 4).

In the case of JW15BZ, reduction in adsorption to *L. sakei* DSM 20017 was observed in the presence of SDS. The same levels of adsorption compared to untreated cells of *L. sakei* DSM 20017 was observed in the presence of $MgCl_2$, 80% ethanol, and β-mercaptoethanol, whereas an increase in adsorption of bacteriocin JW15BZ was recorded in the presence of $(NH_4)_3C_6H_5O_7$, Tris, KI, KCl, $NaH_2PO_4$, $Na_2HPO_4$, NaCl, chloroform, methanol, Triton X-100, Triton X-114, EDTA, $Na_2CO_3$, and $CH_3COOK$ (Table 4).

Adsorption of buchnericin LB to *L. plantarum* was reduced by NaCl, $NH_4Cl$, $MgCl_2$, KCl, KI, and Tris. Treatment of cells with $NH_4$-citrate, Na-acetate, $NaCO_3$, EDTA SDS, Triton-X, β-mercaptoethanol, 80% ethanol, and 80% methanol had no effect on adsorption of buchnericin LB to *L. plantarum* [5].

The adsorption of pediocin N5p to *P. pentosaceus* E5p increased in the presence of $MgCl_2$, $MgSO_4$, $MnCl_2$, $MnSO_4$, whereas NaCl, KCl, KI, $NH_4Cl$, $CaCl_2$, $Na_3PO_4$, $Na_2SO_4$, EDTA, and ethanol had no affect on its adsorption [4]. Organic salts and Na-acetate reduced pediocin N5p adsorption to the target cells. Adsorption of pediocin N5p increased with 25% in the presence of SDS [4].

Different results recorded for adsorption of the four bacteriocins suggests that the conditions required for adsorption to target strains are species-specific. Relative good adsorption of the bacteriocins to target strains at low pH and at 37°C suggests that strains JW3BZ, JW6BZ, JW11BZ, and JW15BZ may be used as probiotic starter cultures. All four bacteriocins are active over a broad pH spectrum and can be incorporated in a variety of food products. Incorporation of EDTA, KCl, emulsifiers, sodium phosphates, and citrate in food products may enhance the adsorption of bacteriocins to spoilage and pathogenic bacteria, thereby increasing the shelf life.

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