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Effect of Growth Medium on Bacteriocin Production by *Lactobacillus plantarum* ST194BZ, a Strain Isolated from Boza**

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Summary

The cell-free supernatant containing bacteriocin ST194BZ, produced by *Lactobacillus plantarum* ST194BZ, inhibits the growth of *Lactobacillus casei*, *Lactobacillus sakei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. Strain ST194BZ produces two bacteriocins, *viz.* ST194BZ(a) of 3.3 kDa and ST194BZ(b) of 14.0 kDa, based on tricine-SDS-PAGE. Reduction in bacteriocin activity was observed after treatment with proteinase K, trypsin and pronase, but not with catalase and α-amylase. A maximum total bacteriocin activity of 12 800 AU/mL was recorded after 14 h in MRS broth. In MRS broth adjusted to pH=5.5, 6.0 or 6.5, an equal level of bacteriocin production of 12 800 AU/mL was recorded. Optimal production (12 800 AU/mL) was recorded in the presence of tryptone (20 g/L), a combination of tryptone and meat extract (1:0.6), or tryptone and yeast extract (1:0.6). Growth of strain ST194BZ in the presence of 10 or 20 g/L of D-mannose yielded bacteriocin levels of 12 800 AU/mL. In the presence of 30 or 40 g/L of mannose the activity levels doubled to 25 600 AU/mL. No difference in antibacterial activity was recorded when strain ST194BZ was grown in the presence of 2 g/L of K_2HPO_4 and 2 g/L of KH_2PO_4. Concentrations of 10, 20 and 50 g/L of KH_2PO_4 yielded double activity (25 600 AU/mL). Supplementing MRS with 1 g/L or more glycerol inhibited the production of bacteriocin. Growth in the presence of vitamins did not stimulate bacteriocin production. No plasmids were recorded for strain ST194BZ, suggesting that the genes encoding bacteriocin production are located on the genome.

Key words: bacteriocin ST194BZ, *Lactobacillus plantarum*, boza

Introduction

Lactic acid bacteria are widely used as starter cultures and play an important role in food preservation, microbiological stability and production of aroma compounds (*1–3*). Many of these lactic acid bacteria produce bacteriocins (*2,3*). By definition, bacteriocins are small proteins with bactericidal or bacteriostatic activity against genetically closely related species (*4*).

Countries of the Eastern Balkan region are well known for the production of food and beverages fermented with lactic acid bacteria. Boza is one such traditional drink, produced by the fermentation of different cereals with yeast and lactic acid bacteria. Only a few papers have been published on the microbial composition of boza (*5–8*). Most of the lactic acid bacteria that

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have been isolated from boza belong to the genera *Lactobacillus*, *Lactococcus* and *Leuconostoc*. As many as 33 strains isolated from boza showed antibacterial activity against various Gram-positive bacteria, including *Listeria innocua*, and Gram-negative bacteria such as *Escherichia coli* (*7*). A bacteriocin produced by *Lactococcus lactis* subsp. *lactis* 14 was partially characterized (*7*).

Lactobacillus plantarum has been isolated from various habitats and several bacteriocins (antimicrobial peptides) have been described in strains from fermented meat products (*9*), milk (*10*), cheese (*11*), fermented cucumber (*12*), olives (*13*), dough (*14*), pineapple (*15*), grapefruit juice (*16*) and sorghum beer (*17*).

Apart from the studies conducted on the effect of nitrogen and carbon sources on the production of plantaricin S (*13*), plantaricin 149 (*15*), plantaricin KW30 (*16*), plantaricin ST31 (*18*), plantaricin 423 (*19*), plantaricin UG1 (*20*), and plantaricin A (*21*), little is known about the growth conditions required for optimal production of bacteriocins by *L. plantarum*. Studies conducted on bacteriocins from other lactic acid bacteria, *e.g.* pediocin AcH (*22*), pediocin PD-1 (*23*), enterocin 1146 (*24*), enterocin AS-48 (*25*), enterocin P (*26*), sakP (*27*) and bacteriocins produced by *Leuconostoc mesenteroides* L124 (*28*) have shown that the production is often regulated by pH growth and temperature. In some cases, higher bacteriocin activity has been recorded at suboptimal growth conditions (*18*,*24*,*27*,*29–36*).

In this paper we report on bacteriocins ST194BZ(a) and ST194BZ(b) from *L. plantarum* ST194BZ, a strain isolated from boza, traditionally fermented cereal beverage from Belogratchik, northwest of Bulgaria. The effects that nutrients and medium pH have on bacteriocin activity were determined.

Materials and Methods

Bacterial strains and growth media

The bacteriocin-producing strain was identified according to its physiological and biochemical characteristics as described by Schillinger and Lücke (*37*), Stiles and Holzapfel (*38*) and Collins *et al.* (*39*). Sugar fermentation reactions were recorded by using the API 50 CHL test strips (bioMérieux, Marcy-l'Etiole, France). Further identification was done by using species-specific primers of *L. plantarum* as described by Torriani *et al.* (*40*). A 50-bp DNA fragment (Amersham Bioscience, UK Limited, Budunghamshire, UK) was used as a marker. Strain ST194BZ was grown in MRS medium (Biolab, Biolab Diagnostics, Midrand, South Africa) at 30 °C without rotation.

The growth media, incubation temperature and origin of the strains included in this study are listed in Table 1. Strains were stored at –80 °C in spent MRS broth in the presence of 15 % glycerol. MRS medium (Biolab) was used for all experiments, except for carbon and nitrogen optimization, in which case MRS described by De Man *et al.* (*41*) was used.

Bacteriocin bioassay

Bacteriocin screening was performed by using the agar-spot test and the well diffusion method, as described by Ivanova *et al.* (*42*). Adjustment of the cell-free supernatant to pH=6.0 with 1 M NaOH prevented the inhibitory effect of lactic acid. Antimicrobial activity was expressed as arbitrary units (AU) per mL. One AU was defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition (*17*). The indicator strains are listed in Table 1.

Table 1. Growth medium and incubation temperature of indicator strains, and inhibitory spectrum of the cell-free supernatant of strain ST194BZ

Indicator strain	Medium and temperature/ °C	Origin, collection	Bacteriocin activity
Acinetobacter bauinanii 19	BHI, 37	Human middle ear, SU	–
Enterobacter cloacae 15	BHI, 37	Human middle ear, SU	+
Enterococcus faecalis 20, 21	MRS, 37	Human middle ear, SU	+
Escherichia coli 40	BHI, 37	Human middle ear, SU	–
Escherichia coli 8	BHI, 37	Mastitic milk, SU	+
Klebsiella pneumoniae 31	BHI, 37	Human middle ear, SU	–
Lactobacillus casei LHS	BHI, 37	Wine, SU	+
Lactobacillus curvatus DF38	MRS, 30	Salami, SU	–
Lactobacillus delbrueckii subsp. *bulgaricus* 1	MRS, 30	Yoghurt, SU	+
Lactobacillus sakei DSM 20017	MRS, 30	Meat, DSM	+
Pseudomonas aeruginosa 7	BHI, 37	Mastitic milk, SU	+
Pseudomonas aeruginosa 22	BHI, 37	Human middle ear, SU	–
Pseudomonas sp. 25, 28	BHI, 37	Human middle ear, SU	–
Staphylococcus aureus 5	BHI, 37	Mastitic milk, SU	–
Streptococcus pneumoniae 29	MRS, 37	Human middle ear, SU	–

+, inhibition zone; –, no inhibition zone recorded
SU, Collection of Department of Microbiology, Stellenbosch University, Stellenbosch, South Africa; DSM, Deutsche Sammlung von Mikroorganismen

Molecular size of the bacteriocins

Strain ST194BZ was grown in MRS broth for 20 h at 30 °C. The cells were harvested by centrifugation (8000 x *g*, 10 min, 4 °C) and the bacteriocins precipitated from the cell-free supernatant with 40 % ammonium sulphate. The precipitate was resuspended in one-tenth volume of 25 mM ammonium acetate (pH=6.5) and then desalted by using a 1000 Da cut-off dialysis membrane (Spectrum Inc., CA, USA). Peptides were separated by tricine-SDS--PAGE, as described by Schägger and Von Jagow (*43*). A low molecular mass marker with sizes ranging from 2.35 to 46 kDa (Amersham International, UK) was used. The gels were fixed and one half stained with Coomassie Blue R250 (Saarchem, Krugersdorp, South Africa) and the position of the active bacteriocin determined on an unstained gel, as described by Van Reenen *et al.* (*17*). *Lactobacillus casei* LHS (10^6 CFU/mL), suspended in BHI broth (Biolab) and supplemented with 1 % agar, was used as sensitive strain.

Effect of enzymes on bacteriocin activity

Strain ST194BZ was grown in MRS broth at 30 °C for 24 h, the cells were harvested by centrifugation (8000 x *g*, 10 min, 4 °C), and the cell-free supernatant was adjusted to pH=6.0 with 6 M NaOH. Cell-free supernatant (1 mL) was incubated for 2 h in the presence of 1 and 0.1 mg/mL of proteinase K (Roche, Indianopolis, IN, USA), pronase and trypsin (Boehringer Mannheim GmbH, Germany), α-amylase (Sigma Diagnostics, St. Louis, MO, USA) and catalase (Boehringer Mannheim), respectively, according to Ivanova *et al.* (*42*). Antimicrobial activity was recorded by using the agar-spot test method as described before.

Bacteriocin production in different growth media and at different initial growth pH

An 18-hour-old culture of strain ST194BZ was inoculated (2 %) into MRS broth, BHI broth, M17 broth (Merck, Darmstadt, Germany), soy milk (10 %, soy flour) and molasses (2 to 10 %, with 2 % intervals), respectively. It was incubated at 30 and 37 °C, respectively, without agitation, for 28 h. Samples were taken every hour and examined for bacterial growth (at A_{600}), changes in culture pH, and antimicrobial activity (AU/mL) against *L. casei* LHS. The agar-spot test method was used as described before.

In a separate experiment, the effect of initial medium pH on bacteriocin production was tested. Volumes of 300 mL of MRS broth were adjusted to pH=4.5, 5.0, 5.5, 6.0 and 6.5, respectively, with 6 M HCl or 6 M NaOH and then autoclaved. Each flask was inoculated with 2 % of an 18-hour-old culture of strain ST194BZ and incubated at 30 °C for 20 h, without agitation. Changes in culture pH and bacteriocin production (AU/mL) were determined every hour, as described before.

Effect of medium composition on bacteriocin production

Strain ST194BZ was grown in 10 mL of MRS broth for 18 h at 30 °C, the cells were harvested by centrifugation (8000 x *g*, 10 min, 4 °C), and the pellet was resuspended in 10 mL of sterile peptone water. A volume of 4 mL of this cell suspension was used to inoculate 200 mL of the following media: (*i*) MRS broth (*41*), without organic nutrients, supplemented with tryptone 20 g/L, meat extract 20 g/L, yeast extract 20 g/L, tryptone 12.5 g/L plus meat extract 7.5 g/L, tryptone 12.5 g/L plus yeast extract 7.5 g/L, meat extract 10 g/L plus yeast extract 10 g/L, or a combination of tryptone 10 g/L, meat extract 5 g/L and yeast extract 5 g/L, respectively; (*ii*) MRS broth with glucose 20 g/L; (*iii*) MRS broth without glucose, supplemented with 20 g/L of fructose, sucrose, lactose, mannose, and maltose, respectively; (*iv*) MRS broth with 0.5–40 g/L of maltose as sole carbon source; (*v*) MRS broth with 2 g/L of K_2HPO_4 or 2–50 g/L of KH_2PO_4; pH was corrected to pH=6.5 with 0.1 M HCl; and (*vi*) MRS broth supplemented with 1–50 g/L of glycerol. In a separate experiment, the vitamins cyanocobalamin (Sigma, St. Louis, Mo.), L-ascorbic acid (BDH Chemicals Ltd, Poole, UK), thiamine (Sigma), DL-6,8-thioctic acid (Sigma) and vitamin K_1 (Fluka Chemie AG, CH-9471 Buchs, Switzerland) were filter-sterilised and added to MRS broth at 1 mg/L (final concentration). Incubation for all tests was at 30 °C for 20 h. Activity levels of bacteriocin ST194BZ were determined as described before.

Plasmid isolation

Plasmid DNA was isolated according to the method described by Burger and Dicks (*44*), followed by CsCl density gradient centrifugation (*45*). The DNA was separated on an agarose gel, according to Ausubel *et al.* (*45*).

Results and Discussion

Strain ST194BZ was identified as *L. plantarum*, based on API 50 CHL profiles (not shown) and PCR with species-specific primers (Fig. 1).

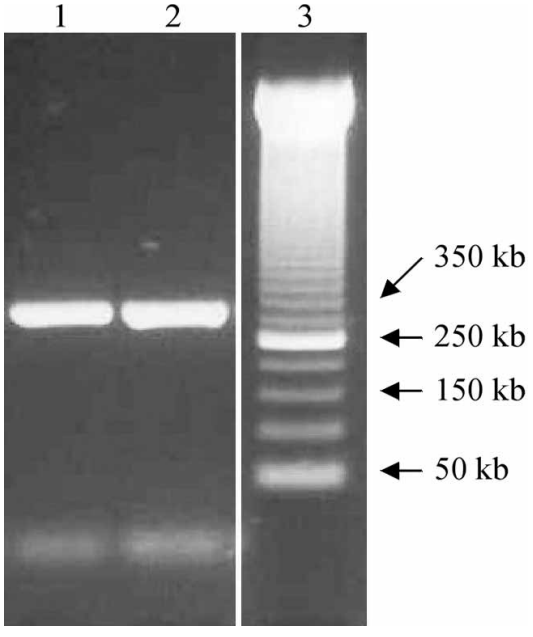

Fig. 1. DNA banding patterns obtained after PCR with species--specific primers. Lane 1: Strain ST194BZ, lane 2: *L. plantarum* ATCC 14917, lane 3: marker DNA (Amersham). ATCC, American Type Culture Collection

The cell-free supernatant of strain ST194BZ inhibited the growth of *L. casei* LHS, *Lactobacillus sakei* DSM 20017, *L. delbrueckii* subsp. *bulgaricus* 1, *Enterococcus faecalis* 20 and 21, *Escherichia coli* 8, *Enterobacter cloacae* 15 and *Pseudomonas aeruginosa* 7, but none of the other species (Table 1). Activity against Gram-negative bacteria is an unusual phenomenon and so far it has only been reported for termophilin 81, produced by *Streptococcus thermophilus*, bacteriocins produced by *Lactobacillus paracasei* subsp. *paracasei* L126 and L134, a bacteriocin produced by *L. lactis* KCA2386, and plantaricin 35d produced by *L. plantarum* (*42*,*46*–*48*).

According to tricine-SDS-PAGE, *L. plantarum* ST194BZ produces two bacteriocins, ST194BZ(a) and ST194BZ(b) with a molecular mass of approximately 3.3 and 14 kDa, respectively (Fig. 2). The latter is larger than most bacteriocins described for *L. plantarum* (*49*). Further purification and separate testing of the peptides will have to be done to determine if they have different spectra of activity. It may be possible that the two peptides act synergistically, as recorded for »two-peptide« bacteriocins of which lactacin F and lactococcin G (*49*) are examples.

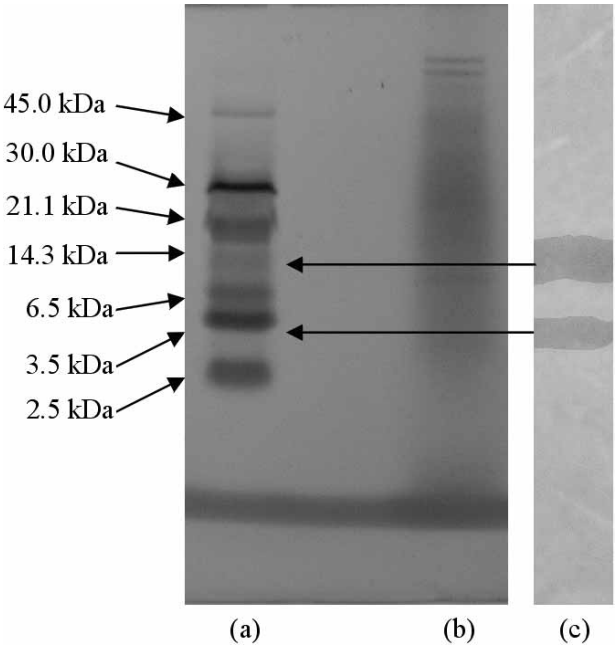

Fig. 2. Tricine-SDS PAGE of bacteriocins ST194BZ(a) and ST194-BZ(b). Lane A: molecular mass marker, lane B: peptide bands stained with Coomassie Blue R250, lane C: zone of growth inhibition, corresponding to the position of the peptide bands in lane B. The gel was covered with viable cells of *L. casei* LHS (approx. 10^6 CFU/mL), imbedded in MRS agar. Incubation was at 30 °C for 24 h

Complete inactivation or significant reduction in antimicrobial activity was observed after treatment of the cell-free supernatant with proteinase K, pronase and trypsin (Fig. 3). Treatment with catalase resulted in no activity change (Fig. 3), indicating that H_2O_2 was not responsible for the inhibition. Treatment with α-amylase did not alter the antimicrobial activity (Fig. 3), suggesting that bacteriocin ST194BZ is not glycosylated and is thus similar to most other bacteriocins (*49*). Leuconocin S, produced by *Leuconostoc paramesenteroides* (*50*) and

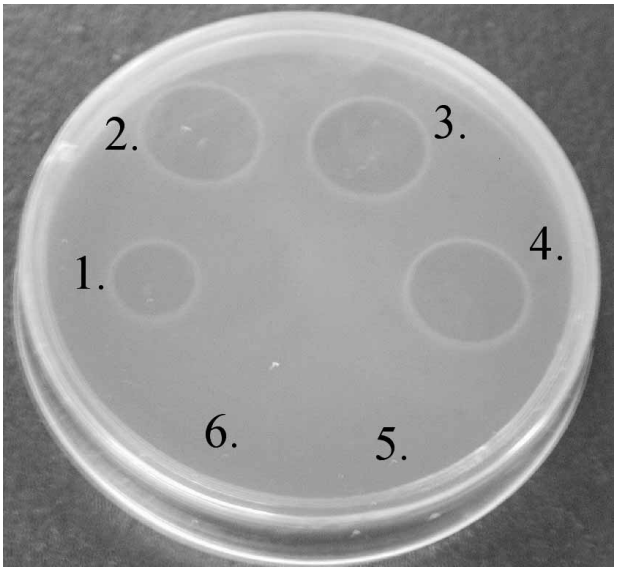

Fig. 3. Antimicrobial activity of bacteriocins ST194BZ(a) and ST194-BZ(b). Positions 1 and 2: cell-free supernatant; 3: cell-free supernatant treated with α-amylase (0.1 mg/mL); 4: cell-free supernatant treated with catalase (0.1 mg/mL); 5: cell-free supernatant treated with pronase (0.1 mg/mL); and 6: cell-free supernatant treated with proteinase K (0.1 mg/mL). *L. casei* LHS (approx. 10^6 CFU/mL) was used as sensitive strain

carnocin 54, produced by *Leuconostoc carnosum* (*51*) are typical examples of amylase-sensitive bacteriocins.

Strain ST194BZ produces low bacteriocin activity (400 AU/mL) when grown in BHI and M17 broth, despite good growth. Similar results have been recorded in the presence of 10 % molasses (results not shown). Good growth has been recorded in 10 % soy milk, but without the production of bacteriocins. The low activity levels recorded in M17 broth, BHI broth, soy milk and molasses, despite relatively good growth, suggest that specific nutrients are required for bacteriocin production. Furthermore, with higher levels of bacteriocin production recorded in MRS (Biolab) at 30 °C (12 800 AU/mL) and lower at 37 °C (6400 AU/mL), growth temperature seems to play an important role. Growth temperature and bacteriocin production are often correlated, as observed for lactocin A (*36*), enterocin 1146 (*24*), lactocin S (*35*), amylovorin 1471 (*31*) and nisin Z (*34*).

The highest yield (12 800 AU/mL) of total bacteriocin activity (ST194BZ(a) plus ST194BZ(b)) was recorded after 14 h in MRS broth (Biolab), and only when incubated at 30 °C. During 28 h of growth, the pH decreased from pH=6.0 to 3.8 and the cell density increased from 0.04 to 9.68 (Fig. 4). Low levels of bacteriocin activity (approximately 400 AU/mL) were detected after 6 h of growth in MRS broth, with maximal activity (12 800 AU/mL) after 13 h (at a pH of approximately 4). This suggests that the peptide is a primary metabolite. Similar results were reported for plantaricin Y (*52*) and bacteriocins produced by *P. acidilactici* (*53*). The stable bacteriocin activity levels observed at pH values below 4.5 may indicate that the production of the peptide is in some way blocked. Genetic studies on the expression of the genes encoding bacteriocin production will have to be done to find the answer to the latter hypothesis.

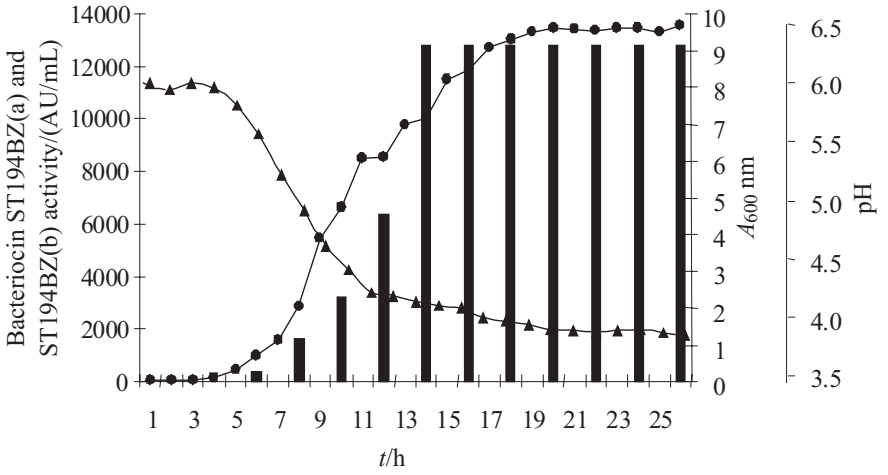

Fig. 4. Growth of strain ST194BZ in MRS broth (●), production of bacteriocins ST194BZ(a) and ST194BZ(b) (■) and changes in culture pH (▲)

ST194BZ does not hydrolyse casein on agar plates, suggesting that it does not produce extracellular proteases. The activity of bacteriocin ST194BZ did not decrease further during 48 h of incubation at room temperature (data not shown), suggesting that specific peptidases active against the bacteriocin were not produced. Furthermore, since no cell lysis occurred after 23 h of growth, based on asorbance readings (Fig. 4), it is safe to assume that there is no loss of activity due to degradation by intracellular proteases.

In MRS broth (Biolab) adjusted to pH=5.5, 6.0 or 6.5, bacteriocin production of 12 800 AU/mL was recorded (Table 2). However, at pH=4.5 low levels of bacteriocin ST194BZ (3200 AU/mL) were recorded (Table 2). Similar results were reported for other bacteriocins produced by *L. plantarum* (*13,15,16,18,21*). The end-pH values of the cultures ranged between 3.6 and 3.8 (Table 2), irrespective of the initial growth pH. From these results and the literature (*13,15,16,18,21*), it can be concluded that optimal production of *L. plantarum* bacteriocins occurs during early logarithmic growth, usually at a pH above 4.5.

Inclusion of Tween 80 in the growth medium increased bacteriocin ST194BZ production by more than 50 % (results not shown). Similar results were recorded for plantaricin 423 (*19*), pediocin AcH (*22*), lactacin B (*54*) and lactocin 705 (*55*). The reason for increased bacteriocin production is not clear. One possible explanation may be that Tween 80, which is a surfactant, facilitates the discharging of the bacteriocin from the cell surface of the producer strain.

Growth of strain ST194BZ in the presence of meat extract as the only nitrogen source resulted in a 75-percent reduction of activity (3200 AU/mL), compared to the 12 800 AU/mL recorded in MRS broth (Biolab) with meat extract, yeast extract and tryptone (Table 3). In the presence of yeast extract as the only nitrogen source, only 1600 AU/mL were recorded, corresponding to an 87.5-percent reduction in activity. However, in the presence of tryptone (20 g/L), a combination of tryptone and meat extract (1:0.6), or tryptone and yeast extract (1:0.6), production levels of 12 800 AU/mL were recorded. Growth in MRS with a combination of meat extract and yeast extract (1:1) resulted in only 800 AU/mL, corresponding to an activity level of 6.25 %. From these results it can be concluded that bacteriocin ST194BZ production is stimulated by tryptone and not yeast extract or meat extract. Similar results were recorded for plantaricin 423 production. In the latter case, higher activity levels were recorded when the producer strain was grown in the presence of tryptone compared to meat extract (*19*). Growth in the presence of bacteriological peptone or casamino acids yielded even higher activity levels (*19*).

Growth of strain ST194BZ in the presence of 10 or 20 g/L of D-mannose yielded bacteriocin levels of 12 800 AU/mL (Table 3). In the presence of 30 or 40 g/L of mannose the activity levels doubled to 25 600 AU/mL. At lower concentrations of mannose (1 and 5 g/L), bacteriocin levels of 800 AU/mL were recorded, clearly indicating that mannose stimulates bacteriocin production. As far as we can determine, no results have been published on the effect that mannose has on bacteriocin production. From our findings it can be concluded that only concentrations higher than 30 g/L stimulated bacteriocin production, which suggests that co-regulation of bacteriocin gene expression may be involved. Increased growth (higher biomass) of the producer strain may be

Table 2. Influence of initial growth pH on the antimicrobial activity of bacteriocins ST194BZ(a) and ST194BZ(b)

Initial pH	4.5	5.0	5.5	6.0	6.5
Final pH	3.6	3.6	3.7	3.8	3.8
Bacteriocin activity/(AU/mL)	3 200	6 400	12 800	12 800	12 800
Bacteriocin activity*/%	25	50	100	100	100

*Compared to the highest activity (12 800 AU/mL)

Table 3. Influence of organic nitrogen, carbohydrates, potassium and vitamins on bacteriocin ST194BZ(a) and ST194BZ(b) production

Component	Concentration/(g/L)	Activity/(AU/mL)	Bacteriocin activity*/%
Tryptone	20.0	12 800	100
Meat extract	20.0	3 200	25
Yeast extract	20.0	1 600	12.5
Tryptone + meat extract	12.5 + 7.5	12 800	100
Tryptone + yeast extract	12.5 + 7.5	12 800	100
Meat extract + yeast extract	10.0 + 10.0	800	6.25
Tryptone + meat extract + yeast extract	10.0 + 5.0 + 5.0	12 800	100
Glucose	20.0	6 400	50
Fructose	20.0	1 600	12.5
Saccharose	20.0	6 400	50
Lactose	20.0	6 400	50
Gluconate	20.0	800	6.25
Maltose	20.0	12 800	100
Mannose	1.0, 5.0	800	6.25
Mannose	10.0, 20.0	12 800	100
Mannose	30.0, 40.0	25 600	200
K_2HPO_4	2.0	12 800	100
KH_2PO_4	2.0, 5.0	12 800	100
KH_2PO_4	10.0, 20.0, 50.0	25 600	200
Glycerol	0	12 800	100
Glycerol	1.0	6 400	50
Glycerol	2.0, 5.0, 20.0	3 200	25
Glycerol	50.0	1 600	12.5
	Concentracion/ppm		
Cyanocobalamin (vit. B_{12})	1.0	12 800	100
Thiamine (vit. B_1)	1.0	12 800	100
DL-6,8-thioctic acid	1.0	6 400	50
L-ascorbic acid (vit. C)	1.0	6 400	50
Control	0	12 800	100

*Compared to the highest activity (12 800 AU/mL), as recorded with the control (MRS, Biolab)

another explanation. The latter hypothesis is less likely, since a mannose increase from 10 to 20 g/L did not result in increased levels of bacteriocin production.

Optimal bacteriocin production (12 800 AU/mL) was also recorded in the presence of 20 g/L of maltose (Table 3). However, in the presence of glucose 20 g/L, sucrose 20 g/L or lactose 20 g/L as sole carbon source, only 6400 AU/mL were recorded. From these results it can be concluded that glucose is only stimulating when present as a disaccharide (maltose) and not when in combination with fructose (as in sucrose) or gluconate (as in lactose). The effect of glucose on bacteriocin production has been reported for sakacin P (*27*), enterocin 1146 (*56*), plantaricin UG1 (*20*), plantaricin 149 (*15*), plantaricin KW30 (*16*) and plantaricin ST 31 (*18*).

Little is known about the influence of potassium ions on the production of bacteriocins. No difference in antibacterial activity was recorded when strain ST194BZ was grown in the presence of 2 g/L of K_2HPO_4 and 2 g/L of KH_2PO_4 (Table 3). Concentrations of 10, 20 and 50 g/L of KH_2PO_4 yielded double activity (25 600 AU/mL). The increase in activity cannot be due to pH changes caused by higher potassium levels, since all media have been adjusted to pH=6.5 before inoculation. In the case of plantaricin UG1, 7 g/L of K_2HPO_4 resulted in increased activity (*20*). The optimal level of K_2HPO_4 recorded for plantaricin ST31 was between 2 and 5 g/L (*18*).

In the presence of glycerol at 1 g/L and higher, bacteriocin ST194BZ production was inhibited (Table 3). Similar results were reported for the production of plantaricin ST31, when grown in the presence of 2 g/L and higher glycerol concentrations, leading to a decrease in activity (*18*). Glycerol is not used as carbon source. The decrease in bacteriocin production may be due to changes in osmotic stress and merits further research.

Maximal bacteriocin activity (12 800 AU/mL) was recorded in MRS without the added vitamins (Table 3). The same level of activity (12 800 AU/mL) was recorded when strain ST194BZ was grown in MRS broth (Biolab) enriched with either 1 ppm of cyanocobalamin or 1 ppm of thiamine. The addition of L-ascorbic acid and

DL-6,8-thioctic acid inhibited bacteriocin production by 50 %.

Strain ST194BZ does not contain plasmids, suggesting that the genes encoding bacteriocin production are located on the genome. Similar results were reported for plantaricin ST31 produced by *L. plantarum* ST31 (*14*). However, in case of plantaricin 423, the genes encoding bacteriocin production are plasmid bound (*17*).

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Utjecaj hranjive podloge na proizvodnju bakteriocina s pomoću *Lactobacillus plantarum* ST194BZ iz boze

Sažetak

Supernatant kulture *Lactobacillus plantarum* ST194BZ inhibira rast *Lactobacillus casei*, *Lactobacillus sakei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter cloacae* i *Pseudomonas aeruginosa.* Na osnovi rezultata dobivenih na tricin-SDS-PAGE ustanovljeno je da soj ST194BZ proizvodi dva bakteriocina, i to ST194BZ(a) od 3,3 kDa i ST194BZ(b) od 14,0 kDa. Smanjenje aktivnosti bakteriocina opaženo je nakon obrade s proteinazom K, tripsinom i pronazom, a ne s dodatkom katalaze i α-amilaze. Maksimalna ukupna aktivnost bakteriocina od 12 800 AU/mL dobivena je nakon 14 sati uzgoja u MRS-podlozi. Ista aktivnost dobivena je u MRS-podlozi pri pH=5,5, 6,0 i 6,5. Optimalna proizvodnja (12 800 AU/mL) opažena je u prisutnosti triptona (2 g/L), u kombinaciji triptona i mesnog ekstrakta (1:0,6) i triptona i ekstrakta kvasca (1:0,6). Tijekom

rasta soja ST194BZ u prisutnosti 10 ili 20 g/L manoze aktivnost bakteriocina iznosila je 12 800 AU/mL. U prisutnosti 30 ili 40 g/L manoze aktivnost je udvostručena. Uzgojem soja ST194BZ u prisutnosti 2 g/L K_2HPO_4 i 2 g/L KH_2PO_4 nije opažena promjena antibakterijske aktivnosti. Pri koncentracijama KH_2PO_4 od 10, 20 i 50 g/L postignuta je dvostruka aktivnost bakteriocina (25 600 AU /mL). Dodatak glicerola od 1 g/L ili više u MRS-podlogu inhibira proizvodnju bakteriocina, dok rast u prisutnosti vitamina ne stimulira proizvodnju. Soj ST194BZ ne sadrži plazmide, što znači da su geni koji kodiraju proizvodnju bakteriocina smješteni na genomu.