

BACTERIOCINS OF *Lactobacillus* LF221 STRAIN

Bojana BOGOVIČ MATIJAŠIĆ^{a)} and Irena ROGELJ^{b)}

^{a)} Univ. of Ljubljana, Biotechnical Fac., Zootechnical Dept., Groblje 3, SI-1230 Domžale, Slovenia, Ph.D., M.Sc., B.Sc.Food Tech.

^{b)} Same address, Assos.Prof., Ph.D., B.Sc.Food Tech.

Received October 30, 1998, accepted November 12, 1998.

Delo je prispelo 1998-10-30, sprejeto 1998-11-12.

ABSTRACT

LF221 strain is a *Lactobacillus* isolate from a baby's faeces that was previously found to produce bacteriocin(s) with inhibitory activity against a wide range of bacterial species including some pathogenic and food-spoilage (*Bacillus cereus*, *Clostridium sp.*, *Listeria innocua*, *Staphylococcus aureus*). Isoelectric point and molecular mass of bacteriocin complex and subunits were estimated. Precipitation with ammonium sulphate, cation exchange chromatography, C₈ hydrophobic interaction chromatography and reverse-phase FPLC chromatography were used to isolate bacteriocins from the supernatant of MRS culture. Amino acid composition and partial N-terminal amino acid sequences of two isolated bacteriocins were determined. Bacteriocins were present in MRS supernatant as aggregates with Mw>150.000 and pI 5.1-5.3, while Mw of subunits was 3500-5000. A high content of glycine and alanine was observed for both bacteriocins. The N-terminal amino acid sequences showed little similarity with those of other bacteriocins. Acidocins LF221 A and B appear to belong to the II class of LAB bacteriocins.

Key words: microbiology / bacteria / *Lactobacillus* / bacteriocins / classification

BACTERIOCINI SEVA *Lactobacillus* LF221

IZVLEČEK

Za sev LF221, izolat *Lactobacillus* iz blata dojenčka, je bilo že ugotovljeno, da proizvaja bakteriocin(e) z zaviralno aktivnostjo proti vrsti bakterij, tudi nekaterih patogenih in kvarljivcev (*Bacillus cereus*, *Clostridium sp.*, *Listeria innocua*, *Staphylococcus aureus*). Ocenili smo izoelektrično točko in molekularno maso bakteriocinskega kompleksa ter njegovih podenot. Bakteriocine smo izolirali iz supernatanta kulture v bujonu MRS s precipitacijo z amonijevim sulfatom, kationsko izmenjevalno kromatografijo, kromatografijo s hidrofobnimi interakcijami in tekočinsko kromatografijo z obrnjenimi fazami FPLC. Ugotovili smo aminokislinsko sestavo dveh izoliranih bakteriocinov in delno zaporedje aminokislin na N-terminalnem delu molekul. Bakteriocini so bili v bujonu MRS v skupkih z Mw, večjo od 150.000, in pI 5,1-5,3, Mw podenot pa je bila 3500-5000. Pri obeh bakteriocinih smo ugotovili visoko zastopanost glicina in alanina. N-terminalni zaporedji aminokislin sta bili zelo malo podobni zaporedjem pri ostalih bakteriocinih. Kaže, da acidocina LF221 A in B sodita v II. skupino bakteriocinov MKB.

Ključne besede: mikrobiologija / bakterije / *Lactobacillus* / bakteriocini / razvrščanje

INTRODUCTION

Lactic acid bacteria (LAB) produce a variety of antimicrobial substances, such as organic acids, diacetyl, hydrogen peroxide and bacteriocins. Bacteriocins of LAB are proteinaceous compounds with a bactericidal activity against a narrow or, less common, a wide range of bacteria (Klaenhammer, 1993, Jack, 1976). Within the genus *Lactobacillus*, *L.acidophilus* and related species (*L.gasseri*, *L.johnsonii*, *L.amylovorus*) has been especially known to display antimicrobial activity against other LAB. The majority of bacteriocins produced by *L.acidophilus* are heat-stable, low-molecular mass, non-lantibiotic peptides which belong to class II on the basis of the recent classification for LAB bacteriocins (Klaenhammer, 1993). Beside bacteriocins of *L.acidophilus* with narrow range of activity, like lactacin F (Muriana and Klaenhammer, 1987, Muriana and Klaenhammer, 1991, 1991a), lactobin A (Contreras *et al.*, 1997), acidocin J1132 (Tahara *et al.*, 1996) and acidocin J1229 (Tahara and Kanatani, 1996), a few with wider spectra have been described recently. Acidocin A inhibits growth of selected species of LAB, food spoilage bacteria and food-borne pathogens, including *Listeria monocytogenes* (Kanatani *et al.*, 1995). *Clostridium sporogenes*, *L.monocytogenes* and *Brochotrix thermosphacta* were found to be sensitive to acidocin B (ten Brink *et al.*, 1994, van der Vossen *et al.*, 1994, Leer *et al.*, 1995).

The strain LF221 was isolated from an infant faeces and was identified as a member of *Lactobacillus acidophilus* group. In our previous studies the strain LF221 has been found to inhibit a wide spectrum of bacteria, including particular strains of the following species: *Bacillus cereus*, *Clostridium sp.*, *Listeria innocua*, *Staphylococcus aureus*, *Streptococcus D*. The inhibition of clostridia strains belonging to *C.sporogenes*, *C.perfringens*, *C.tyrobutyricum*, *C.feseri* and *C.difficile* species was of particular interest. Since the antimicrobial activity of LF221 bacteriocins was reduced by proteolytic enzymes (trypsin, pronase, proteinase K and pepsin) but was not affected by 5-min heating at 100°C, it was presumed that strain LF221 produced bacteriocin(s). Bacteriocin complex of the strain LF221 was produced during the logarithmic phase of growth. The optimum pH for the production of bacteriocin complex was 6.5 and optimum incubation time 12 h. The bacteriocin(s) remained active at pH from 2 to 9 and partially active at pH 10. In the present study, two new bacteriocins produced by *L.acidophilus* strain LF221 were purified, characterised and partially sequenced.

MATERIAL AND METHODS

Bacterial strains and media

Lactobacillus acidophilus LF221 (ZIM BI50, Collection of Industrial Microorganisms, Ljubljana, Slovenia) is an isolate from an infant faeces and was isolated at Istituto di microbiologia, Facoltà di Agraria, Università Cattolica dal Sacro Cuore, Piacenza, Italia. *Lactobacillus sake* NCDO 2714 and *Lactobacillus helveticus* ATCC 15009 were used as indicator strains. Lactobacilli were propagated in MRS broth (Difco) at 37°C (*L.acidophilus* LF221 and *Lactobacillus sake* NCDO 2714) or at 42°C (*L.helveticus* ATCC 15009). Stock cultures were stored in MRS broth with 20% of glycerol at -20°C and in liquid nitrogen.

Bacteriocin activity assay

Lactobacillus sake NCDO 2714 (National Collection of Dairy Organisms, Reading, England) was used as an indicator strain. A critical dilution method was done as described by Mortvedt *et al.* (1990) and Holo *et al.* (1991) and the following adaptation was used for our strain. Each cell

on the microtiter plate contained 50 µl of MRS broth, 1 µl of 2-fold serial dilutions of the sample with bacteriocin and 150 µl of the overnight *L.sake* NCDO 2714 culture in MRS broth, diluted 10 000-fold. Bacteriocin dilutions were made directly in MRS broth by microtiter plate assay. Microtiter plates were incubated for 18 h at 30°C and optical density was measured at 630 nm. One unit of bacteriocin activity (BA) was defined as the amount of bacteriocins causing 50% growth inhibition, compared with a control without bacteriocins.

Isoelectric focusing

Isoelectric focusing was done by Multiphor II system (Pharmacia). 1.5 mm thick polyacrylamide gel (5% acrylamide, 3% bis-acrylamide) with ampholine preblended mixture pH 3.5 - 9.5 was prepared on Gel Bond PAG Film (Pharmacia). One mol l⁻¹ NaOH and 1 mol l⁻¹ H₃PO₄ were used as cathode and anode buffers and IEF-MIX 3.5-9.3 (Sigma) marker proteins for calibration curve. The gel was prefocused 30 min at 11W. Focusing ran out for 1.5 hours at 20 W and 4°C. The maximum voltage was 1500. After the focusing a part of the gel was stained with Coomassie Blue R 250. The other part was rinsed with Milli Q water for 15 minutes and overlaid with soft agar inoculated with sensitive strain *L. helveticus* ATCC 15009. After incubation the position of the inhibition zone was observed and compared with the position of stained bands.

Determination of molecular mass: gel filtration, ultrafiltration, SDS-PAGE electrophoresis

Gel filtration chromatography of concentrated MRS culture supernatant was performed on Sephadex G100 column (90 cm x 1.4 cm, Pharmacia), equilibrated with 0.05 M K phosphate, pH 7.0. The flow rate was 20 ml/h and the column was calibrated with the following standard proteins: cytochrom c (12.400), α-amylase (200.000), alcoholic dehydrogenase (150.000), bovine albumin (66.000) and carbonic anhydrase (29.000).

Ultrafiltration of concentrated MRS culture supernatant of the strain LF221 was done with Minitan S system, using ultrafiltration membranes Millipore with 10K and 100 K MWCO.

Method of Schägger and von Jagow (1987) was used for SDS-PAGE electrophoresis. The gel was composed of 4% "stacking" gel and 16.5% "separating" gel. Electrophoresis was run at constant current 35 mA in "stacking" gel and at 50 mA in "separating" gel. 15 µl of the 20 fold concentrated bacteriocin sample was mixed with 15 µl of 2-fold concentrated sample buffer and boiled for 5 minutes. Two molecular mass standards were applied: MW-17 (Sigma), 2510-16950 Da and Low Molecular Mass Range (Sigma), 6500-66000 Da. After electrophoresis, the gel was divided into two parts. The first half with standards and bacteriocin sample was stained with Coomassie R250 stain. The other part contained only bacteriocin sample and was used for activity detection. It was placed into sterile mQ water for 24 hours, with frequent changing of water, and then overlaid with indicator strain *L.helveticus* ATCC 15009. After overnight incubation, the position of the inhibition zone was detected and compared with the stained part.

Purification of bacteriocins

The strain LF221 was grown in 2100 ml of MRS broth in fermentor with the pH controller under the optimal conditions for bacteriocin production that were determined previously (Bogovič-Matijašić and Rogelj, 1997): for 18 hours at 37°C, at constant pH 6.5 and under N₂ atmosphere. During fermentation, pH was controlled by the addition of 5 mol l⁻¹ NaOH solution. The cells were removed by centrifugation at 4000 g for 15 min at 4°C. Proteins were precipitated by addition of 300 g ammonium sulphate per litre of culture supernatant, followed by centrifugation at 7000 g for 20 min. The pellet was resuspended in 400 ml of 5 mmol l⁻¹ Na-phosphate buffer pH 5 (fraction I), and applied to a 10 ml S-Sepharose Fast Flow cation exchange column, equilibrated with 5 mmol l⁻¹ Na-phosphate buffer, pH 5. The activity was

eluted with 50 ml 1 mol l⁻¹ NaCl in 5 mmol l⁻¹ Na-phosphate buffer pH 5 (fraction II). Fraction II was applied to Octyl sepharose column, equilibrated with 1 mol l⁻¹ NaCl in 5 mmol l⁻¹ Na-phosphate buffer pH 5 and bacteriocin activity was eluted with 10 ml of 70% (v/v) ethanol (fraction III). Fraction III was further purified with the reverse-phase chromatography on a C₂/C₁₈ column, PepRPC HR 5/5, equilibrated with 0.1% (v/v) trifluoroacetic acid (TFA) in distilled water. Bacteriocins were eluted with a linear gradient ranging from 20 to 40% 2-propanol containing 0.1% TFA. Active fractions were diluted 4-5 fold in 0.1% (v/v) TFA and rechromatographed (a linear gradient was 30-50% (v/v) 2-propanol and 0.1% (v/v) TFA). Two active fractions were rechromatographed separately in more narrow linear gradient (30-40% (v/v) 2-propanol and 0.1% (v/v) TFA). The FPLC system (Pharmacia-LKB Biotechnology, Uppsala, Sweden) was used for the reverse-phase chromatography.

Amino acid composition and sequence analysis

The purified bacteriocin was hydrolysed and analysed as described previously (Fykse *et al.*, 1988). The NH₂-terminal amino acid sequences were determined by Edman degradation using an Applied Biosystems (Foster City, California) 477A automatic sequence analyser with an on-line 120A phenylthiohydantoin amino acid analyser as described previously (Cornwell *et al.*, 1988, Kok *et al.*, 1993). The amino acid sequences were compared with the others of the NCBI database using Blast program (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-blast>).

RESULTS

During gel filtration chromatography performed on Sephadex G100 column, almost all the activity was eluted at a void volume (blue dextran elution volume). The result indicates that most of bacteriocins were in the form of aggregates with molecular mass above 150.000. When the neutralised, catalase treated and cell free supernatant was concentrated 10-fold by ultrafiltration through the membranes with molecular mass exclusion limits 10 kDa and 100 kDa, almost all bacteriocins were retained in the retentate as its bacteriocin activity was increased about 8-fold.

The molecular mass of bacteriocin complex subunits was estimated to be 3500 - 5000 Da on the basis of the results of SDS-PAGE electrophoresis. Extensive washing of the gel with sterile water was necessary to wash out all SDS before activity detection, because SDS was very inhibitory for *L.helveticus* ATCC 15009 indicator strain. Although it was possible to detect the position of bacteriocin(s) on the gel by the activity testing, no visible bands were observed at the position of the inhibition zone after Coomassie blue staining. One clear band of inhibition of indicator strain *L.helveticus* ATCC15009 was detected in the gel after isoelectric focusing in the pH range 3.5 – 9.3. The active substance was found on the position corresponding pH values between 5.1 and 5.3.

During the purification, fraction volumes, bacteriocin activity and O.D.₂₈₀ were measured. Specific bacteriocin activity was expressed as activity per O.D.₂₈₀ (Table 1). The procedure described for the purification of LF221 bacteriocins resulted in two absorbance peaks after the first run of FPLC. The corresponding fractions with bacteriocin activity were repurified in additional two separate runs of FPLC to separate two bacteriocins. The amino acid composition of pure bacteriocins from fractions IV.A and IV.B was determined (Table 2). 17 normal amino acids were detected. Lanthionine or methyl-lanthionine was not detected. Partial sequences of 46 amino acid residues of acidocin LF221A and 35 residues of acidocin LF221B were obtained by Edman degradation (Fig. 1).

Table 1. Purification of acidocins LF221 A and B of strain *Lactobacillus acidophilus* LF221Preglednica 1. Čiščenje acidocinov LF221 A in B seva *Lactobacillus acidophilus* LF221

Purification stage	Vol (ml)	Activity (BA ml ⁻¹)	Yield (%)	O.D. ₂₈₀ ^a	Spec. act. ^b
Culture supernatant	2000	125,000	100	23.20	5,388
Fraction					
I. (NH ₄) ₂ SO ₄ precipitation	400	500,000	80	19.21	26,028
II. S-sepharose col. eluat	50	500,000	10	4.20	119,047
III. C ₈ col. eluat	10	2,000,000	8	2.38	840,336
IV A. FPLC fr. with acidocin LF221 A	1	50,000	0.020	0.13	384,615
IV B. FPLC fr. with acidocin LF221 B	1	150,000	0.060	0.28	535,714

^a O.D.₂₈₀, optical density at 280 nm^b Specific activity = activity (BA ml⁻¹) / O.D.₂₈₀

Table 2. Amino acid composition of acidocins LF221 A and B

Preglednica 2. Aminokislinska sestava acidocinov LF221 A in B

Amino acid	Content (%)	
	acidocin LF221 A	acidocin LF221 B
Asp	13.0	8.3
Ser	7.6	4.5
Glu	6.8	1.2
Lys	6.6	3.3
Gly	14.0	29.9
His	0.6	0.5
Arg	2.0	4.0
Thr	4.0	5.8
Ala	11.1	17.4
Pro	2.3	1.7
Tyr	5.3	3.3
Val	7.4	6.2
Met	1.9	1.5
Cys	5.2	1.7
Ile	5.2	4.1
Leu	5.5	4.3
Phe	1.9	2.4

(A) NNVNWGSVAG SXGKGAVMEI YFGNPILGXA NGAATSLVLX QTAXXI
(B)(N)KWG(N)AVIGA ATGATRGVSW X(A)(G)FGPWGMT AXG(L)(A)

Figure 1. (A) NH₂-terminal amino acid sequence of acidocin LF221 A
(B) NH₂-terminal amino acid sequence of acidocin LF221 B
Amino acids that were not identified with certainty but are probable are shown in parentheses; X - unidentified amino acid residue.

Slika 1. (A) NH₂-terminalna aminokislinska sekvenca bakteriocina A.
(B) NH₂-terminalna aminokislinska sekvenca bakteriocina B.
Aminokisliline, ki niso bile identificirane z gotovostjo, ampak so verjetne, so prikazane v oklepaju; X - neidentificirani aminokislinski ostanki.

DISCUSSION

Bacteriocins of LAB are a heterogeneous group of antibacterial compounds varying in their biochemical properties and, therefore, there are no uniform protocols for their purification. One of the problems that is widely reported is the visualisation of the bacteriocins after SDS-PAGE. Some of bacteriocins can not be seen either after Coomassie Blue or silver staining (Carolissen-Mackay, 1997). LF221 bacteriocins were detected in the gel by overlaying it with the indicator strain, but could not be stained with Coomassie Blue dye. Lactacin F, for example, could be detected by silver staining only (Muriana and Klaenhammer, 1991). Alcian blue staining was successful for leuconocin S detection (Lewus *et al.*, 1992). Some authors reported the difficulties in detecting bacteriocins in the gel after SDS-PAGE. Piva and Headon (1994) did not succeed to detect pediocin A in the SDS-PAGE gel by the most often used method described by Bhunia *et al.* (1987). SDS was probably responsible because it could inhibit indicator strain or bacteriocin. As indicator strain for LF221 bacteriocins *Lactobacillus helveticus* ATCC 15009 was also very sensitive to SDS, we solved this problem with extensive washing of the SDS-PAGE gel with sterilised water. In spite of the fact that bacteriocins were not fixed after electrophoresis, they did not diffuse from the gel and could be detected successfully.

SDS-PAGE electrophoresis and isoelectric focusing did not indicate that two bacteriocins were present, probably because of their similar biochemical characteristics. Only the final purification steps revealed that at least two bacteriocins were present in the supernatant.

The biochemical properties of two bacteriocins LF221 were similar to those of the other bacteriocins of *L.acidophilus* and related species that belong to the group II LAB bacteriocins: crude bacteriocins were found in a form of aggregates with Mw > 160.000, Mw of subunits was 3500-5000, they were thermostable, sensitive to trypsin, pronase, proteinase K and pepsin, and active at pH values from 2 to 9 (partially active at pH 10). Isoelectric point (5.1-5.3) only differed from the pI for bacteriocins of the II group, which is generally between the values 8 and 10 (Klaenhammer, 1993).

The recovery of acidocin LF221 A and B was low, but not unusual. Many authors reported high losses of bacteriocin activity during purification. Combination of ammonium sulphate precipitation, ion-exchange chromatography, hydrophobic interaction chromatography and RP-FPLC has been quite successful in the purification of plantaricin A, curvacin A, sakacin P, lactocin S and bavaricin A (Mortvedt *et al.*, 1991, Tichaczek *et al.*, 1992, Larsen *et al.*, 1993, Nissen-Meyer *et al.*, 1993). During the bavaricin A purification, the yield after ammonium sulphate precipitation was 25% and after cation exchange chromatography 9%. Curvaticin FS47

was purified by ammonium sulphate precipitation and RP-HPLC, and the final yield was only 0.0034% (Garver and Muriana, 1994). The result of acidocin A purification by ammonium sulphate precipitation, after cation exchange chromatography and RP-chromatography was pure bacteriocin with 10% of initial activity. For the plantaricin A similar purification steps were used as for LF221 bacteriocins and final yield was 0.1-1% (Nissen-Meyer *et al.*, 1993).

Ammonium sulphate precipitation was quite successful with 80% recovery of bacteriocin activity. Higher losses, although not unusual, occurred during cation exchange chromatography, when another 70% of initial bacteriocin activity was lost. Hydrophobic interaction chromatography was satisfactory. A specific activity increased during those first three steps of purification, but during 3 successive runs of reversed phase FPLC it decreased evidently. Similar observation was recorded by Fimland *et al.* (1996) during purification of curvacin A and pediocin PA-1 and was explained by the decreased activity of bacteriocin molecule as a result of methionine oxidation.

The analysis of amino acid composition for both LF221 bacteriocins showed a high content of glycine and alanine: acidocin LF221 A contains 14% of glycine and 11.1% of alanine and acidocin LF221 B 29.9% Gly and 17.4% Ala. This is a typical characteristic of LAB bacteriocins (Klaenhammer, 1993). Moreover, bacteriocin A contains 47 % of non-polar amino acid residues (G, A, V, L, I, M, F) and 18 % of hydrophobic (V, L, I). The content of non-polar amino acids in bacteriocin B is still higher, 65.8 %, while it contains 14.6 % of hydrophobic amino acids. Comparable results were reported for acidocin B, containing 30 % of alanine and 22 % of hydrophobic amino acids (Leer *et al.*, 1995). It was suggested that the extreme hydrophobic nature of LAB bacteriocins can explain their aberrant migration behaviour in SDS-polyacrylamide gels and their behaviour during purification, as the aggregation of bacteriocins and bacteriocins with the other proteins (van der Vossen *et al.*, 1994, Leer *et al.*, 1995).

Amino acid sequence of 35 N-terminal amino acid residues of acidocin LF221 B showed 26 % homology to brevicin (NCBI accession N^o 1361411). However, no significant homologies were found between 46 N-terminal amino acid residues of acidocin LF221 A and other bacteriocins. According to Klaenhammer (1993), the two bacteriocins of the strain LF221 probably belong to the class II bacteriocins, which are small, heat-stable, hydrophobic and are synthesised as precursors.

As *L.acidophilus* LF221 is a human intestinal isolate, resistant to the bile salts and low pH and produces at least two bacteriocins with a wide range of bactericidal activity, it is interesting as a probiotic strain. For possible future applications of the strain LF221, the contribution of individual bacteriocins to the total activity of the bacteriocin complex, mechanisms of action and the genetic determinants for the production of both bacteriocins need to be studied in detail.

POVZETEK

Bakteriocini mlečnokislinskih bakterij so beljakovine z baktericidnim delovanjem proti ozkemu ali, redkeje, širšemu izboru mikroorganizmov. V prejšnjih raziskavah smo ugotovili, da sev *Lactobacillus acidophilus* LF221, izolat iz blata dojenčka, inhibira celo vrsto bakterij, med katerimi so posamezni sevi *Bacillus cereus*, *Clostridium sp.*, *Listeria innocua*, *Staphylococcus aureus*, *Streptococcus D* in klostridijev (*C.sporogenes*, *C.perfringens*, *C.tyrobutyricum*, *C.feseri* in *C.difficile*). Ker so protimikrobno delovanje zmanjšali proteolitični encimi (tripsin, pronaza, pepsin, proteinaza K), ne pa toplotna obdelava za 5 minut pri 100°C, smo sklepali, da sev LF221 proizvaja bakteriocin(e). V predstavljeni raziskavi smo očistili dva bakteriocina seva *L. acidophilus* LF221 ter ugotovili njune osnovne lastnosti, sestavo in delno zaporedje aminokislin. Najprej smo ocenili velikost z gelsko filtracijo, ultrafiltracijo in SDS-PAGE, ter pI z izoelektričnim fokusiranjem. Bakteriocini so bili v skupkih z Mw > 160.000, Mw podenot pa je

bila 3500-5000. Izoelektrično točko smo ugotovili pri pH 5,1-5,3. Pri čiščenju smo uporabili precipitacijo z amonijevim sulfatom, kationsko izmenjevalno kromatografijo, kromatografijo s hidrofobnimi interakcijami in tekočinsko kromatografijo z obrnjenimi fazami FPLC. Šele zadnji koraki čiščenja so pokazali, da sta v supernatantu najmanj dva bakteriocina. Aminokislinsko sestavo obeh bakteriocinov smo ugotovili s hidrolitično razgradnjo, N-terminalno zaporedje aminokislin pa z Edmanovim razkrojem. Pri obeh bakteriocinih smo ugotovili močno zastopanost nepolarnih aminokislin, predvsem glicina in alanina. Ker bakteriocina še nista bila opisana, smo ju poimenovali acidocin LF221 A in acidocin LF221 B. Pri acidocinu LF221 B smo ugotovili 26% homolognost zaporedja 35 aminokislin na N-terminalnem koncu z brevicinom, podobnosti zaporedja 46 N-terminalnih aminokislin acidocina LF221 A z drugimi bakteriocini pa nismo ugotovili. Opisana bakteriocina sodita v II. skupino bakteriocinov po razdelitvi po Klaenhammerju (1993), ki so majhni, odporni proti toploti, hidrofobni in se sintetizirajo kot prekursorji.

REFERENCES

- Bhunia, A. K./ Johnson, M. C./ Ray, B. Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Ind. Microbiol.*, 2(1987), 319-322.
- Bogovič-Matijašič B./ Rogelj I. Bacteriocins of *Lactobacillus acidophilus* LF221 - Production studies in MRS media at different pH values and effect against *Lactobacillus helveticus* ATCC 15009. *Process Biochem.*, 33(1998), 345-352.
- Brink ten, B./ Minekus, M./ Vossen van der, J. M. B. M./ Leer, R. J. / Huis in't Veld, J. H. J. Antimicrobial activity of lactobacilli: preliminary characterization and optimization of production of acidocin B, a novel bacteriocin produced by *Lactobacillus acidophilus* M46. *J. Appl. Bacteriol.*, 77(1994), 140-148.
- Carolissen-Mackay, V./ Arendse, G./ Hastings, J.W. Purification of bacteriocins of lactic acid bacteria: problems and pointers. *Int. J. Food Microbiol.*, 34(1997), 1-16.
- Contreras, B. G. L./ Vuyst de, L./ Devreese, B./ Busanyova, K./ Raymaeckers, J./ Bosman, F./ Sablon, E./ Vandamme, E. J. Isolation, purification, and amino acid sequence of lactobin A, one of the two bacteriocins produced by *Lactobacillus amylovorus* LMG P-13139. *Appl. Environ. Microbiol.*, (1997)63, 13-20.
- Cornwell, G. G./ Sletten, K./ Johansson, K./ Westermarck, P. Evidence that the amyloid fibril protein in senile systemic amyloidosis is derived from normal prealbumin. *Biochem. Bioph. Res. Com.*, 154(1988), 648-653.
- Fykse, E. M./ Sletten, K./ Husby, D./ Cornwell, G. G. The primary structure of the variable region of an immunoglobulin IV light-chain amyloid-fibril protein (AL GIL). *Biochem. J.*, 256(1988), 973-980.
- Garver, K. I./ Muriana, P. M. Purification and partial amino acid sequence of curvaticin FS47, a heat-stable bacteriocin produced by *Lactobacillus curvatus* FS47. *Appl. Environ. Microbiol.*, 60(1994), 2191-2195.
- Holo, H./ Nilssen, O./ Nes, I. F. Lactococcin A, a new bacteriocin from *Lactococcus lactis subsp. cremoris*: isolation and characterization of the protein and its gene. *J. Bacteriol.*, 173(1991), 3879-3887.
- Jack, R. W./ Tagg, J. R./ Ray, B. Bacteriocins of Gram-Positive Bacteria. *Microbiol. Rev.*, 59(1995), 171-200.
- Kanatani, K./ Oshimura, M./ Sano, K. Isolation and characterization of acidocin A and cloning of the bacteriocin gene from *Lactobacillus acidophilus*. *Appl. Environ. Microbiol.*, 61 (1995), 1061-1067.
- Klaenhammer, T. R. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.*, 12(1993), 39-86.
- Kok, J./ Holo, H./ Belkum van, A. J./ Haandrikman, A. J./ Nes, I. F. Nonnisin bacteriocins in lactococci: biochemistry, genetics and mode of action. In: *Bacteriocins of lactic acid bacteria* (eds.: Hoover, D. G./ Steenson, L. R.). New York, Academic Press, 1993, 121-150.
- Larsen, A. G./ Vogensen, F. K./ Josephsen, J. Antimicrobial activity of lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* M1410. *J. Appl. Bacteriol.*, 75(1993), 113-122.
- Leer, R. J./ Vossen van der, J. M. B. M./ Giezen van, M./ Noort van, J. M./ Pouwels, P. H. Genetic analysis of acidocin B, a novel bacteriocin produced by *Lactobacillus acidophilus*. *Microbiology*, 141(1995), 1629-1635.
- Lewus, C. B./ Kaiser, A./ Montville, T. J. Inhibition of food borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. *Appl. Environ. Microbiol.*, 57(1991), 1683-1688.
- Mortvedt, C. I./ Nes, I. F. Plasmid-Associated bacteriocin produced by a *Lactobacillus sake* strain. *J. Gen. Microbiol.*, 136(1990), 1601-1607.
- Muriana, P. M./ Klaenhammer, T. R. Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. *Appl. Environ. Microbiol.*, 53(1987), 553-560.

- Muriana, P. M./ Klaenhammer, T. R. Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. *Appl. Environ. Microbiol.*, 57(1991), 114-121.
- Muriana, P. M./ Klaenhammer, T. R.. Cloning, phenotypic expression and DNA sequence of the gene for lactacin F, an antimicrobial peptide produced by *Lactobacillus* spp. *J. Bacteriol.*, 173(1991a), 1779-1788.
- Nissen-Meyer, J./ Granly Larsen, A./ Sletten, K./ Daeschel, M./ Nes, I. F. Purification and characterization of plantaricin A, a *Lactobacillus plantarum* bacteriocin whose activity depends on the action of two peptides. *J. Gen. Microbiol.*, 139(1993), 1973-1978.
- Piva, A./ Headon, D. R. Pediocin A, a bacteriocin produced by *Pediococcus pentosaceus* FBB61. *Microbiol.*, 140(1994), 697-702.
- Schägger, H./ Jagow von, G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.*, 166(1987), 368-372.
- Tahara, T./ Oshimura, M./ Umezawa, C./ Kanatani, K. Isolation, partial characterization and mode of action of acidocin j1132, a two-component bacteriocin produced by *Lactobacillus* JCM 1132. *Appl. Environ. Microbiol.*, 62(1996), 892-897.
- Tahara, T./ Kanatani, K. Isolation, partial characterization and mode of action of acidocin J1229, a bacteriocin produced by *Lactobacillus acidophilus* JCM 1229. *J. Appl. Bacteriol.*, 81(1996), 669-677.
- Tichaczek, P. S./ Nissen-Meyer, J./ Nes, I. F./ Vogel, R. F./ Hammes, W. P. Characterization of the bacteriocins curvacin A from *Lactobacillus curvatus* LTH 1174 and sakacin P from *Lactobacillus sake* LTH 673. *Syst. Appl. Microbiol.*, 15(1992), 460-468.
- Vossen van der, J.M.B.M./ Herwijnen van, M. H. M./ Leer, R. J./ Brink ten, B./ Pouwels, P. H./ Huis in 't Veld, J. H. J. Production of acidocin B, a bacteriocin of *Lactobacillus acidophilus* M46 is a plasmid encoded trait: plasmid curing, genetic marking by in vivo plasmid integration, and gene transfer. *FEMS Microbiol. Lett.*, 116(1994), 333-340.