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REVIEW

Purification, Characterization, and Comparison of Bacteriocins

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Biopreservatives are antimicrobial compounds of animal, plant, and microbial origin and have long been used in food without any known adverse effects on human health. Use of biopreservatives can enhance the safety and extend the shelf life of food (50). Tagg *et al.* (61) defined bacteriocins as bactericidal proteins with a narrow spectrum of activity targeted toward species related to the producer culture. Because bacteriocins are proteins and natural, there is tremendous interest in their use as a novel means to ensure the safety of food.

Recently many bacteriocins have been purified to homogeneity, and the amino acid sequences of many purified bacteriocins have been determined and compared. This has brought some order into the identification of unique bacteriocins. For example, the primary amino acid sequence of pediocin PA-1 reported by Henderson *et al.* (22) was identical to that of the bacteriocin produced by *Pediococcus acidilactici*, which was isolated from commercial cultures (36). Therefore, Lozano *et al.* (36) have also termed this bacteriocin pediocin PA-1 in order to avoid confusion. Many other bacteriocins also share homology with each other. Obtaining characterization data on purified bacteriocins will minimize the risk of overlapping of research and confusion in identification of these compounds. This review will summarize recent information on purification and characterization of bacteriocins.

Bacteriocins from Lactobacilli

Information on purification and characterization of bacteriocins produced by lactobacilli is summarized in Table 1.

Acidocin 8912, a bacteriocin produced by *Lactobacillus acidophilus* TK8912, was purified by ammonium sulfate precipitation and successive chromatographic steps on CM-Cellulose, Sephadex G-50, Sephadex G-25, and reversed-phase HPLC on Aquapore RP-300 (62). Reversed-phase HPLC, the final step, gave a single symmetrical peak of activity that was superimposable on a major protein peak. The overall procedure resulted in about 2,870-fold purification with a yield of 12%. The amino acid composition of acidocin 8912 was determined; the molecular weight was 5400. The sequence of 24 consecutive N-terminal amino acid residues of acidocin 8912 was identified as follows: NH_2-Lys-Thr-His-Tyr-Pro-Thr-Asn-Ala-Xaa-Lys-Ser-Leu-Arg-Lys-Gly-Phe-Xaa-Glu-Ser-Leu-Arg-Xaa-Thr-Asp (Xaa represents an unidentified residue). Acidocin A, a bacteriocin produced by *Lactobacillus acidophilus* TK9201, was purified by ammonium sulfate precipitation and sequential ion-exchange and reversed-phase chromatographies (31). The overall purification procedures resulted in a more than 3,000-fold increase in specific activity, with a recovery of 10%. The molecular mass (6,500 Da) was determined by high-performance liquid chromatography gel filtration. The sequence of the first 16 N-terminal amino acids was identified: NH_2-Lys-Thr-Tyr-Tyr-Gly-Thr-Asn-Gly-Val-His-Xaa-Xaa-Lys-Lys-Ser-Leu. The amino acid sequences at positions 4 to 11 of acidocin A showed homology with the consensus sequence (Tyr-Gly-Asn-Gly-Val-Xaa-Cys) in the N-terminal region of the anti-*Listeria* bacteriocins such as curvacin A (64), leucocin A-UAL187 (20), mesentericin Y 105 (21), pediocin PA-1 (18), and sakacin A (23).

Bavaricin A was produced during growth of *Lactobacillus bavaricus* MI401 (35). At 30°C, the highest activity (10,000 AU/ml) was detected in the late log phase. Bavaricin A was purified to homogeneity by ammonium sulfate precipitation, ion exchange, hydrophobic interaction and reverse-phase chromatography. Bavaricin A was eluted from the reverse-phase column at 31% (v/v) 2-propanol, with recovery of 80% of activity. SDS-PAGE of this bacteriocin showed a molecular weight of 3,500-4,000 Da. By amino acid sequencing 41 amino acids were determined. When the sequence was compared to the sequences of other proteins from lactic acid bacteria in the SWISS-PROT data bank, bavaricin A was found to share 66% homology with pediocin PA-1 produced by *Pediococcus acidilactici* (38) and 39% homology with leucocin A-UAL (20).

Two bacteriocin producers have been isolated by employing a catalase-containing bacteriocin-screening medi-

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Table 1. Purification, characterization, and comparison of bacteriocins from lactobacilli.

Bacteriocin	Producer	Medium[a]	Purification scheme[a]	Molecular mass	Amino acid analysis	Amino acid sequence	Reference
Acidocin 8912	*L. acidophilus* TK8912	MRS	ASP, IEC, GFC, reversed-phase HPLC	5,200 Da (by SDS-PAGE)	Yes (50 residues)	Yes (NS[c])	62
Acidocin A	*L. acidophilus* TK9201	MRS	ASP, CEC, RPC	6,500 Da (by gel filtration HPLC)	Yes	Yes	31
Bavaricin A	*L. bavaricus* MI401	MRS	ASP, CEC, HIC, RPC	3,500-4,000 Da (by SDS-PAGE)	ND[b]	Yes (41; SWISS-PROT)	35
Brevicin 27	*L. brevis* SB27	MRS	ASP, CEC	< 6.2 kDa (by SDS-PAGE)	ND	ND	5
Caseicin 80	*L. casei* B80	TJM	UF, IEC, Superose column	40-42 kDa (by GFC)	ND	ND	48
Curvacin A	*L. curvatus* LTH 1174	MRS	ASP, CEC, HIC, RPC	ND	Yes (38-41 residues)	Yes (30; SWISS-PROT)	64
Curvaticin 13	*L. curvatus* SB13	MRS	ASP, HIC	≥10 kDa (by UF)	ND	ND	59
Curvaticin FS47	*L. curvatus* FS47	MRS	ASP, SPE, reversed-phase HPLC	4.07 kDa (by MS)	ND	Yes (SWISS-PROT)	15
Helveticin J	*L. helveticus* 481	MRS	ASP, GFC	37 kDa (by SDS-PAGE)	ND	ND	29
Helveticin V1829	*L. helveticus* 1829	MRS	ASP, dialysis	ND	ND	ND	66
Lactacin B	*L. acidophilus* N2	MRS	IEC, UF, successive GFC	6,000-6,500 Da (by GFC)	ND	ND	4
Lactacin F	*L. acidophilus* 11088	MRS	ASP, GFC, reversed-phase HPLC	2.5 kDa (by SDS-PAGE)	Yes (56 residues)	Yes (25; NBRF)	42
Lactocin 27	*L. helveticus* strain LP27	APT	Chloroform precipitation, freeze-dry, GFC	12.4 kDa (by SDS-PAGE)	Yes	ND	65
Lactocin S	*L. sake* L45	MRS	ASP, IEC, HIC, RPC, GFC	ND	Yes (33 residues)	Yes (C-terminus; SWISS-PROT)	40
Plantaricin A	*L. plantaricin* C-11	MRS	ASP, CEC, HIC, RPC	2,687±30 Da (α) and 2,758±30 Da (β) (by MS)	ND	Yes (21 residues for α and 22 residues for β)	45
Plantaricin C	*L. plantarum* LL441	MRS (0.6% glucose)	ASP, HIC, CEC	3.5 kDa (by SDS-PAGE)	ND	Yes (SWISS-PROT)	17
Plantaricin LC74	*L. plantarum* LC74	MRS	ASP, CEC, HIC	≤5 kDa	ND	ND	49
Plantaricin S	*L. plantarum* LPCO10	MRS	ASP, UF	2.5 kDa (by SDS-PAGE)	ND	ND	28
Sakacin A	*L. sake* LB706	MRS	ASP, IEC, HIC, RPC	4.308 Da (by MS)	Yes (41 residues)	Yes (GCG program)	23
Sakacin M	*L. sake* 148	MRS	Concentration, lyophilization, GFC	4,640 Da (by GFC)	ND	ND	56
Sakacin P	*L. sake* LTH673	MRS	ASP, CEC, HIC, RPC	ND	Yes (36-38 residues)	Yes (41 residues; SWISS-PROT)	64

[a]Abbreviations: MRS, APT are commercially available media; TJM, tomato juice medium; ASP, ammonium sulfate precipitation; HIC, hydrophobic interaction chromatography; GFC, gel filtration chromatography; UF, ultrafiltration; CEC, cation exchange chromatography; IEC, ion exchange chromatography; RPC, reverse-phase chromatography; SPE, solid-phase extraction; HPLC, high-performance liquid chromatography; MS, mass spectrometry. [b]ND, Not determined. [c]NS, Not searched in computer databases.

um for lactobacilli. The bacteriocins (curvacin A and sakacin P) of both of these lactobacilli were produced in the late exponential growth phase. Both bacteriocins were purified to homogeneity by ammonium sulfate precipitation, cation exchange, hydrophobic interaction and reverse-phase chromatography. Finally, the specific activities of curvacin A and sakacin P increased by more than 15,000 and 5,000-fold, respectively, with yields of 64% and 21% at the end of purification. Amino acid composition and sequence analysis revealed that curvacin A and sakacin P are small peptides of 38-41 and 41 amino acid residues, respectively. In the N-terminal region, the two bacteriocins share the segment -Tyr-Gly-Asn-Gly-Val-. This conserved region is speculated to be responsible for the similar inhibitory spectra of curvacin A and sakacin P (64). The sequence of curvacin A and sakacin P had no similarity to the amino acid sequences of lactocin S or of other previously characterized bacteriocins (25, 30, 33, 42, 53) as revealed by a search of the SWISS-PROT data bank.

Curvaticin FS47, a bacteriocin produced by *Lactobacillus curvatus* FS47, was purified by 40% ammonium sulfate precipitation, solid-phase extraction on C_{18} Sep-Pak Cartridges (Millipore Corp., Milford, Mass.), and reversed-phase HPLC (15). The average mass of curvaticin FS47 was 4.07 kDa as determined by mass spectrometry. Actually, the size determined by mass spectrometry differed from that determined by SDS-PAGE (< 2 kDa). This difference has been attributed to the nonlinear migration of small peptides on SDS-PAGE (20, 22, 42, 57). Amino acid sequencing of this bacteriocin was performed by the Edman degradation reaction, and 31 residues were identified with confidence starting with NH_2-Tyr-Thr-Ala-Lys-Glu-. The partial sequence of curvaticin FS47 was compared with the sequences of other proteins by using four protein data bases (PDB, Swiss-Prot and Swiss-Prot Update, PIR, and GenPept and GenPept Update). No protein sequences with significant homology to curvaticin FS 47 were identified except for proteins with glycine-rich sequences that showed homology to the Gly residues in curvaticin FS47.

Lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088, was purified and characterized (42). Purification by ammonium sulfate precipitation, gel filtration, and reversed-phase HPLC resulted in a 474-fold increase in specific activity. The purified lactacin F was identified as a 2.5-kDa peptide by SDS-PAGE. Amino acid composition studies indicated that lactacin F may contain as many as 50 to 56 residues. In this study, amino acid sequence analysis of purified lactacin F identified 25 residues from the N-terminus. Sequence data showed that lactacin F contains an N-terminal arginine, atypical in nonprocessed gene-encoded proteins. The authors suggest that the purified lactacin F peptide may be the product of posttranslational processing. A computer search of the NBRF data base has not identified sequences that share significant homology with the partial sequence of lactacin F.

Isolation and characterization of lactocin 27 from a homofermentative *Lactobacillus helveticus* strain LP27 was performed (65). Lactocin 27 was purified by chloroform (25 ml/liter in H_2O) precipitation, freeze-drying, and successive gel filtration chromatography. Amino acid composition of purified lactocin 27 is quite similar to that of the bacteriocin produced by *L. fermenti* (10). Both have traces of methionine and quite high contents of glycine, alanine, and aspartate. Cysteine and cystine seem to be absent in both bacteriocins. The only apparent difference between the two bacteriocins was that an active protein was not dissociated from the lipocarbohydrate-protein complex of the *L. fermenti* bacteriocin by hydrolytic techniques (10), whereas lactocin 27 seems to be a small glycoprotein (65).

Lactocin S, a bacteriocin produced by *Lactobacillus sake* L45, was purified to homogeneity by ion exchange, hydrophobic interaction and reverse-phase chromatography, and gel filtration (40). The purification resulted in a 40,000-fold increase in specific acitvity. Amino acid composition analysis revealed that lactocin S contained approximately 33 amino acid residues, of which about 50% were nonpolar (alanine, valine, and leucine). Because the N-terminus was blocked, the amino acids at the C-terminus were determined, following cyanogen bromide cleavage at the internal methionine. The partial amino acid sequence of lactocin S is Met-Glu-Leu-Leu-Pro-Thr-Ala-Ala-Val-Leu-Tyr-Xaa-Asp-Val-Ala-Gly-Xaa-Phe-Lys-Tyr-Xaa-Ala-Lys-His-His, where Xaa represents unidentified amino acids associated with cysteine. This is indicated by the fact that two cysteic acids per molecule were found on performic acid oxidation of lactocin S. The partial amino acid sequence of lactocin S was determined to be unique when it was searched in the SWISS-PROT data bank, with three proteins showing partial homology with lactocin S: the pectate lyase B precursor isolated from *Erwinia caratovora*; the bacteriorhodopsin precursor isolated from *Halobacterium halobium*; and the 6-aminohexanoate-dimer hydrolase from *Flavobacterium* sp. strain K172. The sequences of the pectate lyase B precursor and the bacteriorhodopsin precursor are part of a signal sequence. The hydrophobic nature of lactocin S and its homology with these signal sequences suggest the cell membrane as a possible target for lactocin S (40).

Nissen-Meyer *et al.* (45) purified plantaricin A, a *Lactobacillus plantarum* bacteriocin, by ammonium sulfate precipitation, binding to a cation exchanger and octyl-sepharose (hydrophobic interaction), and reverse-phase chromatography. This resulted in a 1,300-fold increase in specific activity and a recovery of about 5% of the

activity. Interestingly, the bacteriocin activity was associated with two peptides (α and β), of which 21 and 22 amino acid residues have been sequenced. Nissen-Meyer *et al.* (45) suggested that the amino acid sequences of the α and β subunits indicate pore-forming toxins that create cell membrane channels through a 'barrel-stave' mechanism. No significant homology of plantaricin A to other known LAB bacteriocins was reported (45).

Gonzalez *et al.* (17) purified plantaricin C, a bacteriocin produced by a strain of *Lactobacillus plantarum*, by ammonium sulfate precipitation, hydrophobic interaction and cation exchange chromatography. Plantaricin C is a peptide of ca. 3,500 Da, according to SDS-PAGE. The sequence, obtained with amino terminal sequencing by automated Edman degradation, is NH_2-Lys-Lys-Thr-Lys-Lys-Asn-Xaa-Ser-Gly-Asp-, where Xaa represents an unidentified residue. After the 11th amino acid, the sequence was blocked. No homology to the N-terminal sequence of plantaricin C was found in the SWISS-PROT data bank.

Sakacin A, a bacteriocin produced by *Lactobacillus sake* LB706, was purified to homogeneity by ammonium sulfate precipitation, ion exchange, hydrophobic interaction and FPLC reversed-phase chromatography (23). An increase of more than 9,000-fold in specific activity, to a final value of 250 AU/μg protein, was obtained. According to complete amino acid sequence data for the purified bacteriocin, sakacin A consisted of 41 amino acid residues with a calculated MW of 4,308.7. Amino acid sequence comparisons with the GCG program package revealed no significant homology with other proteins. However, sakacin A has been shown to share some homology, especially in the N-terminal region, with the newly sequenced bacteriocins leucocin A-UAL187 (20), pediocin PA-1 (36) and sakacin P (64). Also, sakacin A appeared to be very similar in partial sequence to curvacin A (64). Lozano *et al.* (36) suggested that bacteriocins may be grouped into families by sequence similarity.

Bacteriocins from Lactococci

Information on purification and characterization of some of the bacteriocins from lactococci is summarized in Table 2.

Piard *et al.* (47) purified lacticin 481, a lanthionine-containing bacteriocin produced by *Lactococcus lactis* subsp. *lactis* CNRZ 481, by ammonium sulfate precipitation, gel filtration, and preparative and analytical reversed-phase HPLC. The overall purification scheme resulted in a 107,506-fold increase in specific activity. Lacticin 481 is a single peptide of 1.7 kDa, based on SDS-PAGE analysis. However, dimers of 3.4 kDa that also exhibit lacticin activity were detected. Amino acid

Table 2. Purification, characterization, and comparison of bacteriocins from lactococci.

Bacteriocin	Producer	Medium[a]	Purification scheme[a]	Molecular mass	Amino acid analysis	Amino acid sequence	Reference
Lacticin 481	*L. lactis* subsp. *lactis* CNRZ 481	Elliker	ASP, GFC, reversed-phase HPLC	1.7 kDa (by SDS-PAGE)	Yes (18 or 20 residues)	Yes (7 residues; NBRF and Gen Pro)	47
Lactococcin	*L. lactis* subsp. *lactis* ADRIA 85LO30	CG	Dialysis, CEC, GFC	2.3-2.4 kDa (by GFC)	ND[b]	ND	11
Lactococcin A (LCN-A)	*L. lactis* subsp. *cremoris* LMG 2130	M17	ASP, CEC, RPC	5,778 Da (by calculation)	ND	Yes (54 residues)	25
Lactococcin G	*L. lactis* LMG 2081	M17 (with 0.1% Tween 80)	ASP, CEC, HIC, RPC	4,376 Da for α_1 and 4,109 Da for β (by MS)	ND	Yes	44
Nisin[c]	*L. lactis* 354/07	LTB (2.5% glucose)	Extraction, IEC, acetone precipitation, CM-Cellulose chromatography	ND	ND	ND	3
Nisin	*L. lactis* subsp. *lactis* ATCC 11454	TGE buffered broth	Adsorption on producer cells by changing pH	ND	ND	ND	69

[a]Abbreviations: Elliker, APT, M17 are commercially available media; CG is a modified medium containing glucose, magnesium sulfate, K_2HPO_4, KH_2PO_4, and iron sulfate; LTB is the semidefined medium containing glucose, meat extract, yeast extract, NaCl and Na_2HPO_4; TGE buffered broth is TGE broth plus 0.5% sodium citrate, 0.1% sodium acetate, and 0.05% dipotassium phosphate (pH 6.5); IEC, ion exchange chromatography; CEC, cation exchange chromatography; GFC, gel filtration chromatography; ASP, ammonium sulfate precipitation; RPC, reverse phase chromatography; HPLC, high performance liquid chromatography; HIC, hydrophobic interaction chromatography; MS, mass spectrometry. [b]ND, Not determined. [c]Molecular mass (3,510 Da) and complete structure (34 residues) of nisin was revealed by Gross and Morell (19).

composition of purified lacticin 481 shows the presence of lanthionine residues, suggesting that lacticin 481 is a member of the lantibiotic family. No striking similarities were noted in amino acid composition between lacticin 481 and other lanthionine-containing peptides, such as nisin, subtilin, gallidermin (33), epidermin (2) or pep5 (30). Of a total of 18 or 20 amino acids, only seven were charged, while the remaining were uncharged or nonpolar. Only seven residues (NH_2-Lys-Gly-Gly-Ser-Gly-Val-Ile) of purified lacticin 481 were sequenced because the remaining peptide was not further degraded by the Edman reaction. No sequence homologous to this partial sequence of lactacin 481 was found in the National Biomedical Research Foundation (NBRF) or Gen Pro data bases.

Lactococcin A (LCN-A), a bacteriocin produced by *Lactococcus lactis* subsp. *cremoris* LMG 2130, was purified and characterized by Holo *et al.* (25). Complete purification was performed by ammonium sulfate precipitation, cation exchange chromatography and reversed-phase fast protein liquid chromatography (FPLC). The overall purification scheme resulted in about a 2,000-fold increase of specific activity, with a recovery of 16%. Based on the total amino acid sequence of the purified bacteriocin, lactococcin A contains 54 amino acid residues, has a calculated molecular weight of 5,778 and is rich in alanine and glycine residues (8 of each). LCN-A is definitely different from the two lactococcal bacteriocins nisin (19) and diplococcin. No significant sequence similarity was found to other proteins in the SWISS-PROT or NBRF data bases.

Lactococcin G was purified to homogeneity by ammonium sulfate precipitation, binding to a cation exchanger and octyl-sepharose CL-4B, and reverse-phase chromatography (44). A 7,000-fold increase in the specific activity was obtained with a yield of 20%. The bacteriocin activity of lactococcin G was associated with three peptides, termed α_1, α_2 and β. Nissen-Meyer *et al.* (44) found by amino acid sequence analysis that α_1 and α_2 were the same gene product. Molecular weights of 4, 376 and 4,109 for α_1 and β, respectively, were obtained by mass spectrometry. The complete amino acid sequences of the α_1 (39 amino acid residues) and β (35 amino acid residues) peptides and a major part of the sequence of the α_2 peptide were found. This study reported the first purification and characterization of a bacteriocin that requires for its activity the complementary action of two distinct peptides.

Bacteriocins from Leuconostocs

Information on the purification and characterization of some of the leuconostoc bacteriocins is summarized in Table 3.

Hastings *et al.* (20) reported that leucocin A-UAL 187, a bacteriocin produced by *Leuconostoc gelidum* UAL 187, was purified by 70% ammonium sulfate or acid (pH 2.5) precipitation, hydrophobic interaction chromatography, gel filtration, and reversed-phase HPLC, with a yield of 58% of the activity. In this study, ion-exchange chromatography, dialysis, and high pH conditions were avoided because these resulted in large losses in activity. Isocratic elution with 35% acetonitrile-0.15% trifluoroacetic acid (TFA) gave the best separation in reversed-phase HPLC. The molecular weight of leucocin A-UAL 187 was $3{,}930.3 \pm 0.4$ as determined by mass spectrometry. The N-terminal partial amino acid sequence identified 13 of the total 37 amino acid residues as follows: NH_2-Lys-Tyr-Tyr-Gly-Asn-Gly-Val-His-Cys-Thr-Lys-Ser-Gly-.

Table 3. Purification, characterization, and comparison of bacteriocins from leuconostocs.

Bacteriocin	Producer	Medium[a]	Purification scheme[a]	Molecular mass	Amino acid analysis	Amino acid sequence	Reference
Carnocin LA54A	*L. carnosum* LA 54A	MRS	HIC	4 kDa (by SDS-PAGE)	ND	ND	34
Leucocin A-UAL 187	*L. gelidum* UAL 187	CAA	ASP, HIC, GFC, reversed-phase HPLC	3,930.3±0.4 Da (by MS)	Yes	Yes (37 residues)	20
Leucocin B-Ta11a	*L. carnosum* Ta11a	MRS	NP[c]	ND	ND	Yes	13
Mesenterocin 52	*L. mesenteroides* FR52	MRS	ASP, GFC, CEC, HIC	6-7 kDa (by GFC)	ND	ND	60
Mesentericin Y105	*L. mesenteroides* ssp. *mesenteroides* Y105	MRS	ASP, UF, reversed-phase HPLC	3,666.6 Da (by sequence)	ND	Yes (36 residues; PIR)	21

[a]Abbreviations: MRS is commercially available medium; CAA is the defined medium containing casamino acids, yeast extract, glucose, dipotassium phosphate, Tween 80, diammonium citrate, magnesium sulfate, and manganous sulfate; ASP, ammonium sulfate precipitation; GFC, gel filtration chromatography; CEC, cationic exchange chromatography; HIC, hydrophobic interaction chromatography; UF, ultrafiltration; HPLC, high performance liquid chromatography; MS, mass spectrometry. [b]ND, Not determined. [c]NP, Not purified.

Felix *et al.* (13) characterized leucocin B-Ta11a, a bacteriocin from *Leuconostoc carnosum* Ta11a isolated from meat. Nucleotide sequence analysis of the 8.1 kb recombinant plasmid (pJF8.1), which contains the genetic determinant of the leucocin B-Ta11a, was accomplished. Recently, pediocin PA (38), sakacin A (23), sakacin P (64), leucocin A-UAL 187 (20) and curvacin A (64) have been shown to have a consensus sequence of -Tyr-Gly-Asn-Gly-Val-Xaa-Cys- in their N-termini. The amino acid sequence of leucocin B-Ta11a is significantly homologous to the sequence of leucocin A-UAL 187. The 37-amino acid structural proteins are identical, but the N-terminal extension of leucocin B-Ta11a differs from that of leucocin A-UAL 187 by seven residues.

Characterization and purification of mesentericin Y105, an anti-listeria bacteriocin from *Leuconostoc mesenteroides*, was accomplished (21). Mesentericin Y105 was purified to homogeneity by affinity chromatography, ultrafiltration, and reversed-phase HPLC on a C_4 column. Amino acid sequencing work showed that mesentericin Y105 is a 36-amino acid polypeptide with a primary struture close to that of leucocin A-UAL 187, according to the EMBL data bank. Mesentericin Y105, however, appears to be bactericidal to *Listeria monocytogenes* E 20, whereas leucocin A-UAL 187 seems to have a wider range of action and a bacteriostatic activity. The molecular mass of mesentericin Y105 is 3,666.6 Da, based on sequencing data.

Bacteriocins from Pediococci

Information on purification and characterization of some of the bacteriocins from pediococci is summarized in Table 4.

Motlagh *et al.* (41) studied the nucleotide and amino acid sequences of the pap-gene and its product, pediocin AcH, in *Pediococcus acidilactici* H. Protein transferred to the PVDF membrane that corresponded to pediocin AcH activity was used to perform limited N-terminal amino acid sequencing. A partial amino acid sequence (23 residues) was determined: NH_2-Lys-Tyr-Tyr-Gly-Asn-Gly-Val-Thr-Cys-Gly-Lys-His-Ser-Cys-Ser-Val-Asp-Trp-Gly-Lys-Ala-Thr-Thr. The authors suggested that pediocin AcH is most likely translated as prepediocin with an 18-amino acid leader sequence that is removed as a step in post-translational processing.

A bacteriocin produced by *Pediococcus acidilactici* was purified to homogeneity by ammonium sulfate precipitation, cation exchange, hydrophobic interaction, and reverse-phase chromatography (36). The purification resulted in an 80,000-fold increase in specific activity and an approximately 6-fold increase in total activity. Determination of the amino acid composition of pediocin PA-1 showed that it has 41 residues. On the other hand, 43 amino acid residues were sequenced from the N-terminus. The primary amino acid sequence of this bacteriocin is identical to that of pediocin PA-1 as reported by Henderson *et al.* (22).

Pediococcus acidilactici strain L50 produces pediocin L50, a heat stable bacteriocin when grown at 8 to 32°C (7). Pediocin L50 was purified to homogeneity by ammonium sulfate precipitation followed by cation-exchange chromatography, hydrophobic interaction and reversed-phase chromatography. Its mass was determined to be 5,250 by mass spectrometry. A partial C terminal sequence of 42 amino acids after CNBr cleavage was determined and compared. The sequence showed no similarity to those of other bacteriocins.

Table 4. Purification, characterization, and comparison of bacteriocins from pediococci.

Bacteriocin	Producer	Medium[a]	Purification scheme[a]	Molecular mass	Amino acid analysis	Amino acid sequence	Reference
Pediocin AcH	*P. acidilactici* strain H	DCGB	ASP, dialysis, GFC, AEC	2,700 Da (by SDS-PAGE)	ND[b]	ND	6
Pediocin AcH	*P. acidilactici* H	TGE	ASP, dialysis, freeze-dry	2.5-3.4 kDa (by SDS-PAGE)	ND	Yes (by using PVDF membrane)	41
Pediocin L50	*P. acidilactici* strain L50	MRS	ASP, CEC, HIC, RPC	5,250 Da (by MS)	Yes (42 residues)	Yes	7
Pediocin PA-1	*P. acidilactici* PAC1.0	MRS	ASP, dialysis, IEC, dialysis	16.5 kDa (by GFC)	ND	ND	18
Pediocin PA-1	*P. acidilactici*	MRS	ASP, successive CEC, RPC	4,600 Da (predicted)	Yes (43-44 residues)	Yes (NS[c])	36
Pediocin SJ-1	*P. acidilactici* SJ-1	TGE	CEC	4 kDa (by SDS-PAGE)	ND	ND	54

[a]Abbreviations: MRS is a commercially available medium; TGE, DCGB (dialysed casein broth) are semidefined media; ASP, ammonium sulfate precipitation; IEC, ion exchange chromatography; GFC, gel filtration chromatography; AEC, anion exchange chromatography; CEC, cation exchange chromatography; HIC, hydrophobic interaction chromatography; RPC, reverse phase chromatography. [b]ND, Not determined. [c]NS, Not searched in computer databases.

Bacteriocins from Carnobacteria

Information on purification and characterization of some of the *Carnobacterium* bacteriocins is summarized in Table 5. The genus *Carnobacterium* was described as the atypically nonaciduric lactobacilli by Collins *et al.* (8). Knowledge of bacteriocins produced by this new group of bacteria is limited.

Carnobacteriocin A, a bacteriocin produced by *Carnobacterium piscicola* LV17A was purified using hydrophobic interaction and gel filtration chromatography, and reversed-phase high-performance liquid chromatography (68). Three different active peaks (A1, A2 and A 3) were identified, but the purified samples showed the same N-terminal amino acid sequences for the first 15 amino acids. Carnobacteriocin A contained 53 amino acid residues and a molecular mass of 5,053 Da by calculation. Mass spectrometric analysis showed that the molecular mass of the major component (A3) was 2 Da lower, suggesting the presence of a disulfide linkage between Cys 22 and Cys 51.

Piscicolin 61, a bacteriocin from *Carnobacterium piscicola* LV61 was purified to homogeneity by ammonium sulfate precipitation and sequential hydrophobic interaction and reversed-phase chromatography (24). Overall, greater than a 64,000-fold increase in specific activity (AU/OD_{280}) was obtained by the end of the purification sequence. Forty N-terminal amino acid residues of the purified bacteriocin were determined by Edman degradation. Piscicolin 61 consisted of one polypeptide chain of 53 amino acid residues with a calculated MW from the amino acid sequence of 5,052.6. No sequence similarities of piscicolin 61 with other known proteins in the SWISS PROT or PIR sequence databases were detected.

Other Potential Bacteriocins

Information on purification and characterization of bacteriocins from other microorganisms is summarized in Table 6.

Purification and characterization of acnecin, a bacteriocin produced by *Propionibacterium. acnes* CN-8, was studied (14). Acnecin was purified to homogeneity by ultrasonic treatment, ammonium sulfate precipitation, ion exchange and gel filtration chromatography. Specific activity of acnecin increased 72-fold in comparison with the crude extract. Acnecin consisted of five subunits with a MW of about 12,000. From amino acid composition analysis, aspartic acid, glutamic acid, glycine, and alanine were found to predominate.

Purification and characterization of linecin A, a bacteriocin produced by *Brevibacterium linens* ATCC 9175, was studied by Kato *et al.* (32). When mitomycin C was added to the culture broth at a final concentration of 0.3 μg/ml to cause release of intracellular linecin A, the extracellular linecin A activity (128 units/ml) increased by almost 15-fold. Kato *et al.* (32) purified linecin A to homogeneity by DEAE-Cellulofine, Sephacryl S-500, and Sephacryl S-300 column chromatography. The molecular weight (95 kDa) of linecin A was determined by gel filtration. Amino acid composition of linecin A, but not the amino acid sequence, has been determined.

Salivaricin A was purified from agar cultures of *Streptococcus salivarius* 20P3 by XAD-2 ion-exchange chromatography and reversed-phase HPLC (51). Molecular weight of salivaricin A has been determined as 2,315± 1.1 Da by mass spectrometry. Purified salivaricin A has an N-terminal partial amino acid sequence as follows: NH_2-Lys-Arg-Gly-Ser-Gly-Trp-Ile-Ala-Xaa-Ile-Xaa-Asp-Asp-Xaa-Pro-Asn. A search of protein and DNA data bases by using FASTA, and a comparison of salivaricin A with other previously sequenced lantibiotics by RDF2 analysis, showed no significant homology. The recently

Table 5. Purification, characterization, and comparison of bacteriocins from carnobacteria.

Bacteriocin	Producer	Medium[a]	Purification scheme[a]	Molecular mass	Amino acid analysis	Amino acid sequence	Reference
Carnobacteriocin A	*C. piscicola* LV17A	CAA	HIC, GFC, reversed-phase HPLC	5,050.8±0.3 Da (by MS)	Yes	Yes	68
Carnocin UI49	*C. piscicola* UI49	MRS	ASP, desalt on GFC, CEC	ND[b]	ND	ND	57
Carnocin UI49	*C. piscicola* UI49	GM17	XAD chromatography, CEC (large scale purification)	ND	ND	ND	58
Piscicolin 61	*C. piscicola* LV61	cMRS	ASP, HIC, reversed phase FPLC	5,052.6 Da	ND	Yes	24
unnamed	*C. piscicola* LV17	APT	ASP, dialysis	ND	ND	ND	1

[a]Abbreviations: MRS, APT are commecially available media; CAA is a semidefined medium; GM17, cMRS are modified media; HIC, hydrophobic interaction chromatography; ASP, ammonium sulfate precipitation; GFC, gel filtration chromatography; CEC, cation exchange chromatography. [b]ND, Not determined.

Table 6. Purification, characterization, and comparison of bacteriocins from other microorganisms.

Bacteriocin	Producer	Medium[a]	Purification scheme[a]	Molecular mass	Amino acid manlysis	Amino acid sequence	Reference
Acnecin	*P. acnes* CN-8	unnamed[a]	UT, ASP, IEC, GFC	12,000 Da (by SDS-PAGE)	Yes	ND[b]	14
Bacteriocin 16-2	*Rhizobium rhizobial* strain 16-2	NB	Sucrose gradient sedimentation	ND[b]	ND	ND	16
Carotovoricin Er	*Erwinia carotovora* AMS 6082	NB or M9	ASP, IEC, Sucrose density gradient centrifugaiton	ND	ND	ND	26
Enterocin 226NWC	*Enterococcus faecalis* 226	M17	ASP, dialysis	5,800 Da (by SDS-PAGE)	ND	ND	67
Linecin A	*Brevibacterium linens* ATCC9175	Bouillon	ASP, successive IEC, GFC	95 kDa (by GFC)	Yes	ND	32
Linenscin OC2	*Brevibacterium linens* OC2	TSBYE	ASP, SE, RPC	1,197 Da (by MS)	Yes (12 residues)	ND	37
Propionicin PLG-1	*Propionibacterium thoenii* P127	NLB	ASP, IEC, reversed-phase HPLC	9,328 Da	Yes (99 residues)	Yes (10 residues)	46
SA-FF22	*Streptococcus pyogenes* strain FF22	TSB	XAD-2, evaporation, CEC, reversed-phase HPLC	2,794 Da (by MS)	Yes	Yes	27
Salivaricin A	*Streptococcus salivarius* 20P3	MGA (0.5% glucose)	XAD-2, successive IEC, reversed-phase FPLC	2,315±1.1 Da (by MS)	Yes (15 residues)	Yes (8 residues)	51
Staphylo-coccin 1580	*Staphylococcus epidermidis* 1580	TSB	XAD-2, CEC, reversed-phase HPLC	2,000 Da (by SDS-PAGE)	Yes (15 residues)	Yes (NS[c])	52
Syringacin W-1	*Pseudomonas syringae* pv. *syringae* PsW-1	NBY	UF, Sucrose gradient centrifugation, DEAE-Cellulose chromatography	ND	Yes	ND	55
Thuricin	*Bacillus thuringiensis* HD-2	MTS	PEG and UF, ultrogel AcA34 chromatography	950 kDa (by GFC)	ND	ND	12
Xenorhabdicin	variants of *Xenorhabdus nematophilus* F1	LB	Mitomycin induction, PEG precipitation, DEAE chromatography	43+20 kDa (two major components)	ND	ND	63
unnamed	*Bacteroides ovatus* H47	BHI-S	ASP, GFC, Preparative PAGE	78 kDa (by SDS-PAGE)	ND	ND	39
unnamed	*Pseudomonas solanacearum* B1	CPG	ASP, AEC, MUF, preparative electrophoresis	65 kDa (by SDS-PAGE)	ND	ND	9

[a]Abbreviations: unnamed medium is a medium containing 3.7% brain heart infusion (Difco) supplemented with 0.2% yeast extract (Difco); BHI, NB, M9, M17, TSB, LB are commercially available media; TSBYE, MGA, NBY, MTS, BHI-S, CPG are modified media; Bouillon broth and NLB are semidefined media; UT, ultrasonic treatment, UF, ultrafiltration; ASP, ammonium sulfate precipitation; AEC, anion exchange chromatography; MUF, membrane ultrafiltration; IEC, ion exchange chromatography; GFC, gel filtration chromatography; CEC, cation exchange chromatography; SE, solvent extraction; PEG, polyethylene glycol; PAGE, polyacrylamide gel electrophoresis; MS, mass spectrometry. [b]ND, Not determined. [c]NS, Not searched in computer databases.

found N-terminal sequence (NH_2-Lys-Gly-Gly-Ser-Gly-Val-Ile) of the lanthionine-containing lactacin 481 differs from the corresponding region of salivaricin A only at positions 2 and 6. However, the reported amino acid composition of lactacin 481 is totally different. The lack of sequence similarity between salivaricin A and other lantibiotics (nisin, subtilin, gallidermin, and epidermin) shows that salivaricin A does not share a common an-

cestry with these bacteriocins.

Sahl (52) reported that staphylococcin 1580 was purified to homogeneity by XAD-2 column separation, cation exchange chromatography, and reversed-phase HPLC. Analysis by SDS-PAGE showed that purified staphylococcin 1580 has an apparent MW of approximately 2,000. Amino acid composition analysis, determination of molecular mass (2,165 Da) and limited N-terminal sequencing (NH_2-Ala-Xaa-Lys-Phe-Ile-Xaa-Xaa-Pro-Gly-Xaa-Ala-Lys-block) demonstrated that staphylococcin 1580 is identical to epidermin, a lantibiotic.

The antibacterial peptide SA-FF22, produced by *Streptococcus pyogenes* strain FF22 was purified and characterized (27). Amino acid analysis showed the presence of the unusual amino acids, suggesting that this bacteriocin is a lantibiotic. A 22 N-terminal amino acid sequences were determined by automated gas-phase Edman degradation, but it was blocked beyond position 22.

Linenscin OC2, synthesized by *Brevibacterium linens* OC2 was purified by ammonium sulfate precipitation, 2-propanol (90%, v/v) extraction, and finally reversed-phase chromatography (37). The final specific activity of linenscin OC2 was increased 47,700 times when compared to culture supernatant, with a recovery of 2%. Molecular mass (1,196 Da) was determined by mass spectrometry. Amino acid analysis showed that linenscin OC2 may contain 12 residues.

Purification and characterization of xenorhabdicin, a phage tail-like bacteriocin, produced by variants of *Xenorhabdus nematophilus* F1 were reported (63). Electrophoresis of xenorhabdicin characterized two major subunits (43 and 20 kDa) and at least five minor subunits (67, 54, 35, 28 and 16 kDa).

Purification and characterization of propionicin PLG-1, a bacteriocin produced by *Propionibacterium thoenii* P 127 was reported (46). Propionicin PLG-1 was purified to homogeneity by ammonium sulfate precipitation followed by ion-exchange chromatography and reversed-phase HPLC. Amino acid composition analysis showed that propionicin PLG-1 contains 99 residues and has a calculated molecular weight of 9,328. A 10 N-terminal amino acid sequence was identified and propionicin PLG-1 was confirmed as a new bacteriocin in a search of the SWISS-PROT data bank.

Perspectives

During the past decade, bacteriocins have become a primary focus of research because of their potential use as nontoxic biopreservatives. To date, many bacteriocins have been optimized for production, purified to homogeneity, characterized and compared with other bacteriocins. Future efforts directed toward molecular characterization of the structure, function, and regulation of purified bacteriocin will accelerate efforts to engineer innovative antimicrobial peptides with enhanced capabilities and diverse applications (43). Manipulation of genes for bacteriocin production and immunity is expected to provide the opportunity for drastic improvement of bacteriocin production, and expansion of the inhibitory spectrum.

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