APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1991, p. 1829–1834
0099-2240/91/061829-06$02.00/0

Vol. 57, No. 6

Purification and Amino Acid Sequence of Lactocin S, a Bacteriocin Produced by *Lactobacillus sake* L45

C. I. MØRTVEDT,[1] J. NISSEN-MEYER,[2] K. SLETTEN,[3] AND I. F. NES[2]*

Norwegian Food Research Institute, N-1430 Ås,[1] Laboratory of Microbial Gene Technology, N-1432 Ås-NLH,[2] and Department of Biochemistry, University of Oslo,[3] Oslo, Norway

Received 31 December 1990/Accepted 10 April 1991

Lactocin S, a bacteriocin produced by *Lactobacillus sake* L45, has been purified to homogeneity by ion exchange, hydrophobic interaction and reverse-phase chromatography, and gel filtration. The purification resulted in approximately a 40,000-fold increase in the specific activity of lactocin S and enabled the determination of a major part of the amino acid sequence. Judging from the amino acid composition, lactocin S contained approximately 33 amino acid residues, of which about 50% were the nonpolar amino acids alanine, valine, and leucine. Amino acids were not detected upon direct N-terminal sequencing, indicating that the N-terminal amino acid was blocked. By cyanogen bromide cleavage at an internal methionine, the sequence of the 25 amino acids (including the methionine at the cleavage site) in the C-terminal part of the molecule was determined. The sequence was 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 residues. It is likely that the unidentified residues are modified forms of cysteine or amino acids associated with cysteine, since two cysteic acids per lactocin S molecule were found upon performic acid oxidation of lactocin S. The sequence was unique when compared to the SWISS-PROT data bank.

Lactic acid bacteria produce a number of antimicrobial substances such as lactic acid, H_2O_2, diacetyl, and bacteriocins (18). Bacteriocins are proteinaceous compounds that act bactericidally against closely related species. They may be produced by both gram-positive and gram-negative organisms (3, 32). Bacteriocin-producing lactic acid bacteria may prove to be useful in improving the safety of feed and food fermentation products. Today only one bacteriocin from lactic acid bacteria, nisin, has found practical application. In the early 1950s, Hirsch et al. (12) suggested the use of inhibitory streptococci for food preservation. Nisin-producing starter cultures were successfully used to prevent gas production of clostridia in cheese (23). Various applications of nisin in the production of food commodities have since been developed. Although a great number of reports on the existence of new bacteriocins have been published, very little information on their chemical composition, mode of action, and genetics has emerged. The molecular structure of nisin, however, has been determined by Gross and Morell (10). Nisin was shown to be a 3,500-Da peptide consisting of unusual amino acids, and the gene encoding this bacteriocin was recently cloned (4, 9, 16).

Within the genus *Lactobacillus*, a number of bacteriocinogenic substances have been reported in *L. acidophilus* (1, 26), *L. helveticus* (14, 33), *L. fermenti* (7), *L. plantarum* (6), and *L. sake* (24, 30). Only a few of these substances have been partially purified and characterized. From thermophilic lactobacilli, however, genes of two bacteriocins have been cloned and sequenced: helveticin J from *L. helveticus* (15) and lactacin F from *L. acidophilus* (28).

Mørtvedt and Nes (24, 25) have described an *L. sake*-produced bacteriocin, termed lactocin S, which inhibits growth of selected species of the genera *Lactobacillus*, *Pediococcus*, and *Leuconostoc*. The lactocin S production and its immunity factor were shown to be associated with an unstable 50-kb plasmid (25). This study describes for the first time a procedure for purifying to homogeneity a bacteriocin from a facultative heterofermentative *Lactobacillus* sp. and determination of its amino acid composition. Approximately 75% of the amino acid sequence of lactocin S was determined.

MATERIALS AND METHODS

Bacterial cultures and media. *L. sake* L45, isolated from natural fermented sausage as previously described (25), was propagated at 30°C in MRS broth without Tween 80 (prepared from the basic ingredients obtained from Difco Laboratories, Detroit, Mich.). *Pediococcus acidilactici* Pac 1.0, which was used as the indicator strain, was obtained from M. Daeschel (North Carolina State University, Raleigh), and propagated in MRS broth (Difco) at 30°C.

Bacteriocin assay. The bacteriocin, lactocin S, was quantified in a microtiter plate assay system as previously described (25). Twofold dilutions of bacteriocin extracts (100 μl) in MRS broth were prepared in microtiter plates (Costar, Cambridge, Mass.). Twenty microliters of fresh indicator culture (A_{600} = 0.1 to 0.4) and 80 μl of MRS broth were added, and the plates were incubated overnight at 30°C. Growth inhibition of the indicator organism was then measured spectrophotometrically at 600 nm by using a Titertek Multiskan (Flow Laboratories, Helsinki, Finland). One bacteriocin unit (BU) was defined as the amount of bacteriocin which inhibited growth of the indicator organism by 50% (50% of the turbidity of the control culture without bacteriocin) under standard assay conditions.

Ammonium sulfate precipitation. All the purification steps were performed at room temperature if not otherwise stated. All the chromatographic equipment was obtained from Pharmacia-LKB Biotechnology (Uppsala, Sweden). MRS broth without Tween 80 was inoculated with *L. sake* L45 (0.2% inoculum) and incubated overnight at 30°C. Overnight cultures contained a bacteriocin titer of 300 to 500 BU/ml. The

* Corresponding author.

TABLE 1. Purification of lactocin S

Purification stage	Vol (ml)	Total A_{280}[a]	Total activity (BU)	Sp act[b]	Increase in sp act[b]	Yield (%)
Culture supernatant	24,000	547,000	11,700,000	21	1	100
Fractions						
I (Ammonium sulfate precipitation)	900	19,700	11,100,000	560	27	95
II (Anion exchange)	1,400	5,000	8,200,000	1,600	76	70
III (Cation exchange)	450	1,650	9,200,000	5,600	270	79
IV (Phenyl Sepharose)	200	115	4,200,000	36,000	1,700	36
V (Gel filtration)[c]	42	7	1,700,000	240,000	11,000	15
VI (Phenyl Superose)	22	1.3	700,000	540,000	26,000	6
VII (C_2/C_{18} column)	2	0.35	310,000	900,000	40,000	3

[a] Total A_{280} equals the optical density at 280 nm multiplied by the volume in milliliters.
[b] Specific activity is bacteriocin units (BU) divided by the optical density at 280 nm.
[c] Prior to gel filtration, fraction IV was transferred to distilled water by gel filtration using PD-10 columns and concentrated to 7 ml by lyophilization.

cells from between 20 and 25 liters of overnight cultures were removed by centrifugation at 16,000 × *g* for 10 min at 4°C. The supernatant was adjusted to pH 6.5, and ammonium sulfate was added to a final concentration of 20% (wt/vol) at 4°C. Lactocin S was pelleted by centrifugation at 8,000 × *g* for 20 min and dissolved in 20 mM sodium phosphate buffer, pH 7.5 (375 ml per 10-liter culture) (fraction I).

Ion-exchange chromatography. Traces of ammonium sulfate in fraction I were removed by passing it through Sephadex G-25 PD-10 columns equilibrated with 20 mM sodium phosphate, pH 7.5. The lactocin S preparation was subsequently applied to an 80 ml Q-Sepharose anion-exchange column equilibrated with the phosphate buffer. The flowthrough fraction (fraction II), containing lactocin S activity, was adjusted to pH 5.2 and applied to an 80-ml S-Sepharose cation-exchange column equilibrated with 20 mM sodium phosphate, pH 5.2. The activity was eluted with the same buffer containing 1.0 M NaCl (fraction III).

Hydrophobic interaction and reverse-phase chromatography and gel filtration. Ammonium sulfate was added to fraction III to a final concentration of 6% (wt/vol) and was subsequently applied to a Phenyl Sepharose CL-4B column (18-ml column volume per 6-liter culture) equilibrated with 20 mM sodium phosphate, pH 5.2, and 6% (wt/vol) ammonium sulfate. The activity was eluted from the column by gradually decreasing the ammonium sulfate concentration (total gradient volume, 136 ml). The flow rate was 4.0 ml/min, and 8-ml fractions were collected. The lactocin S-containing fractions (fraction IV) were transferred to distilled water by gel filtration using the PD-10 columns as described above, concentrated by lyophilization, dissolved in 7 ml of 20 mM sodium phosphate (pH 6.9)–0.1 M NaCl, and finally applied to a Sephacryl S-100 HR column (2.6 by 90 cm) equilibrated with 0.1 M NaCl in 20 mM sodium phosphate, pH 6.9. Six-milliliter fractions were collected, and the bacteriocin-containing fractions were pooled (fraction V). The column was calibrated with bovine serum albumin (molecular weight, 67,000), ovalbumin (molecular weight, 43,000), chymotrypsinogen (molecular weight, 25,000), and RNase A (molecular weight, 13,700). Ammonium sulfate was added to fraction V to a final concentration of 6% (wt/vol), followed by application to a Phenyl Superose HR 5/5 column, equilibrated with 20 mM sodium phosphate, pH 6.9, and 6% (wt/vol) ammonium sulfate. The flow rate was 0.8 ml/min, and the lactocin S activity was eluted by gradually decreasing the ammonium sulfate concentration. Fractions containing lactocin S activity (fraction VI) were directly chromatographed on a C_2/C_{18} reverse-phase column, PepRPC HR 5/5, equilibrated with 5 mM sodium phosphate, pH 6.9. The flow rate was 1 ml/min, and lactocin S activity was eluted with a linear methanol gradient (total volume of 30 ml). Hydrophobic interaction, reverse-phase chromatography, and gel filtration were performed with the use of the FPLC system (Pharmacia-LKB Biotechnology, Uppsala, Sweden).

Amino acid composition and sequence analysis. After chromatography on the C_2/C_{18} column, purified lactocin S was concentrated in a SpeedVac Concentrator (Savant Instruments Inc., Farmingdale, N.Y.). For determination of the amino acid composition, the polypeptides were hydrolyzed in 6 N HCl (or in 6 N HCl containing 3% [vol/vol] thioglycollic acid for quantitation of tryptophan) under vacuum at 110°C for 24 h (22). The half-cystine content was determined as cysteic acid after performic acid oxidation (11). The amino acid analysis was performed on a Biotronik LC5000 analyzer, which was connected to a computing integrator system (SP 4100 Computing Integrator; Spectra Physics Inc., San Jose, Calif.). The polypeptides were cleaved with cyanogen bromide as described by Sletten and Husby (31). The amino acid sequence was determined by Edman degradation using an Applied Biosystems 477A automatic sequence analyzer with an on-line 120A phenylthiohydantoin amino acid analyzer (5).

The hydropathic index of lactocin S was determined by using the SOAP program of PC Gene (IntelliGenetics, Mountain View, Calif.) (19, 20). The sequence was compared with the content of the SWISS-PROT data base by using the DNASIS sequence program package (Pharmacia-LKB) on a VAX/780 computer with the University of Wisconsin Genetics Computer Group program package (8).

RESULTS

Purification of lactocin S. Lactocin S, which is found in the media in the late-exponential phase of growth (24), was concentrated approximately 25-fold from the culture media by ammonium sulfate precipitation (fraction I). This resulted in a 25- to 30-fold increase in the specific activity with a recovery of approximately 95% of the activity (Table 1, fraction I). After fraction I was passed through an anion-exchange column, followed by binding to a cation exchanger, the specific activity of lactocin S increased by 250- to 300-fold and the recovery was 70 to 80% (Table 1, fraction III). Upon subsequent hydrophobic interaction chromatography on Phenyl Sepharose, lactocin S eluted between 1.2

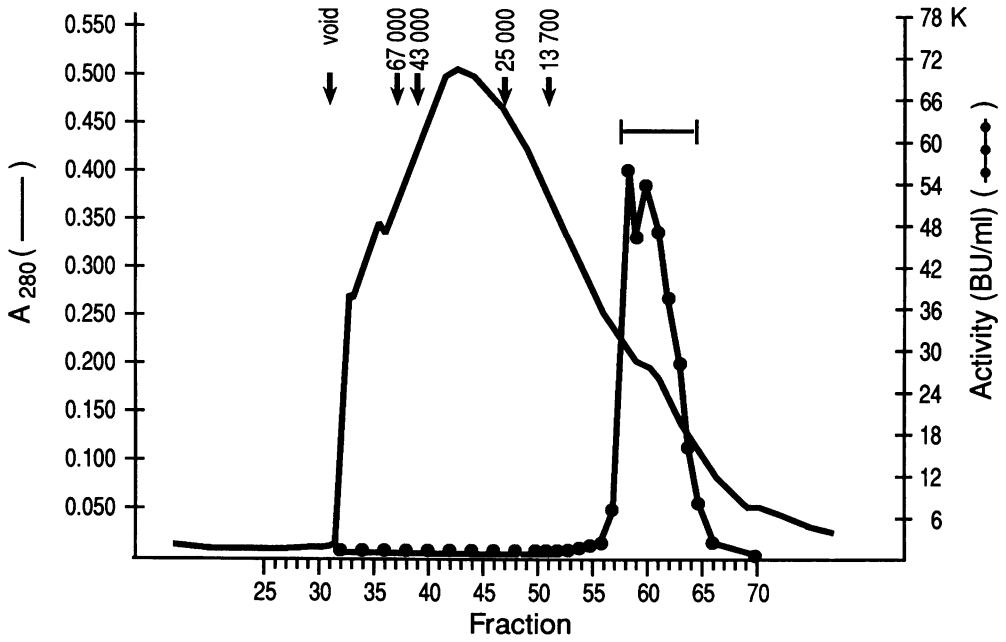

FIG. 1. Gel filtration on a Sephacryl S-100 column of fraction IV of lactocin S. The bar indicates which fractions were collected for further purification. The amount applied to the column represents that obtained from an approximately 24-liter culture. Six-milliliter fractions were collected.

and 0% (wt/vol) ammonium sulfate (data not shown). The specific activity was at this stage about 1,700-fold higher than that of the culture supernatant, and the recovery was 36% (Table 1, fraction IV).

Upon gel filtration of fraction IV, the activity eluted as a peak with a molecular weight less than that of the smallest protein standard, RNase A (molecular weight, 13,700) (Fig. 1). At this stage, a shoulder on the main, broad absorbance peak coincided with the peak of lactocin S activity (Fig. 1). After gel filtration, the specific activity had increased approximately 11,000-fold and the recovery was about 15% (Table 1, fraction V). Lactocin S was then subjected to hydrophobic interaction chromatography on a Phenyl Superose column, and it eluted between 3.5 and 3.0% ammonium sulfate (data not shown). A distinct absorbance peak coincided with the activity peak. The specific activity had increased about 26,000-fold, and the recovery was 6% (Table 1, fraction VI). The final purification step, resulting in pure lactocin S, was reverse-phase chromatography. The activity peak, which was recovered in one fraction at a methanol concentration of approximately 87% (vol/vol), coincided completely with the absorbance peak (Fig. 2). The final specific activity of pure lactocin S was 40,000-fold greater than in the culture supernatant, and the recovery was 3% (Table 1, fraction VII).

Amino acid composition and sequence analysis. The amino acid composition of lactocin S obtained after the final reverse-phase chromatography step (fraction VII) was determined (Table 2). Calculation of the number of probable amino acid residues in the bacteriocin molecule revealed that lactocin S most likely contains 33 amino acids. No tryptophan residues were found upon specific analysis for the presence of this amino acid. The amino acid analysis resulted in one extra unknown peak eluting between proline and glycine which disappeared after performic acid oxidation of lactocin S (data not presented). Performic acid-oxidized material revealed two residues of cysteic acid per molecule. This suggests that two modified cysteine residues are present in the bacteriocin molecule.

Amino acids were not detected upon direct N-terminal amino acid sequencing of lactocin S, indicating that the N-terminal amino acid was blocked. According to the amino acid composition, one methionine was present in lactocin S. Lactocin S was therefore subjected to cleavage with cyanogen bromide, which is specific at methionine residues. Amino acid sequencing was performed on the cleaved product. The sequencing revealed only one sequence, which means that the N-terminal blocking still was present. From the C-terminal part of the lactocin S molecule 25 amino acids were obtained, including the cleaved methionine and 3 amino acid residues which could not be identified. The sequence was 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 residues (Fig. 3A). The hydropathic index of lactocin S as determined from the known amino acid sequence was 1.37, indicating a hydrophobic nature (20).

DISCUSSION

This is the first report on the purification to homogeneity of a bacteriocin from a facultative heterofermentative *Lactobacillus* ("*Streptobacterium*") sp. The purification of the bacteriocin—lactocin S, produced by *L. sake*—resulted in a 40,000-fold increase in the specific activity and enabled the determination of a major part of its amino acid sequence.

Initial work on concentrating the bacteriocin from regular MRS culture medium was not reproducible, and the recovery was often not more than 20%. Various amounts of lactocin S activity were found in the foam and in a floating lipidlike material after the ammonium sulfate precipitate was pelleted; this activity might be due to the hydrophobic

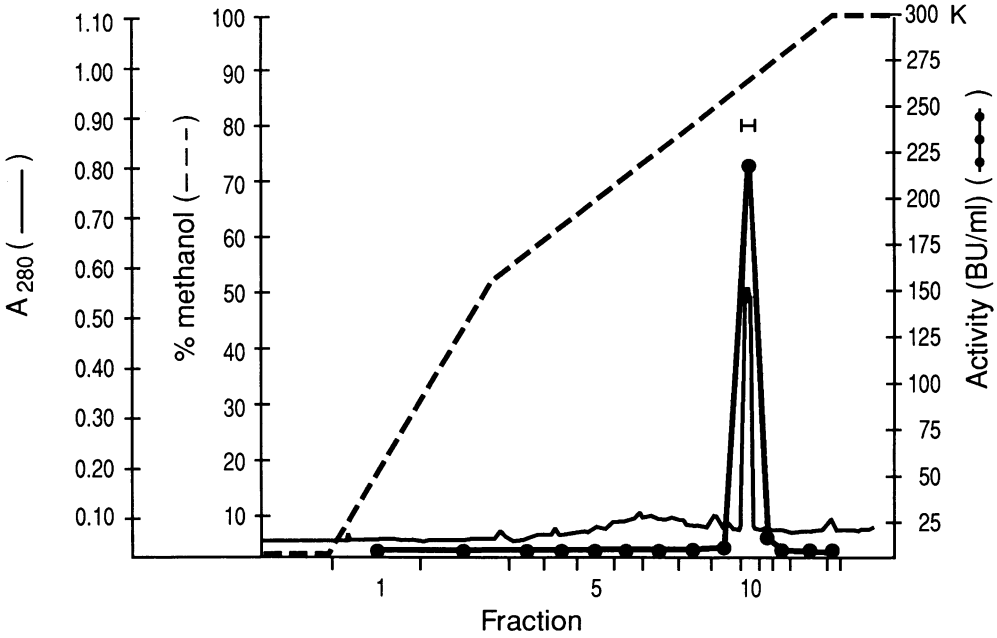

FIG. 2. Reverse-phase chromatography on a C_2/C_{18} column of fraction VI of lactocin S. The amount applied to the column represents that obtained from an approximately 16-liter culture. No activity was detected in the flowthrough. The gradient volume was 25 ml, and the bacteriocin was collected in one 1-ml fraction.

character of lactocin S. Lactacin F produced by *L. acidophilus* has also been reported to be lost in the floating fraction upon ammonium sulfate precipitation, and this bacteriocin was found to be associated with lipidlike material (26a). Exclusion of the nonionic detergent, Tween 80, from the MRS broth resulted in a high, reproducible recovery of lactocin S in the ammonium sulfate precipitate.

The apparent molecular weight of lactocin S as determined by gel filtration varied. After ammonium sulfate precipitation from the culture medium, lactocin S has previously been shown to elute with a molecular weight of about 30,000 (24), whereas in a more purified state it always eluted at a molecular weight of less than 13,700 (RNase A). It appeared that the more crude the bacteriocin was during gel filtration, the higher the apparent molecular weight, suggesting that it associates with other macromolecules. Its behavior upon gel filtration in a partially purified state (Fig. 1) indicates that it perhaps also associates with itself and forms dimers or trimers. Other bacteriocins produced by lactobacilli also aggregate or associate with other substances under nondissociating conditions. Upon gel filtration, lactocin 27 elutes with a molecular weight of more than 200,000, whereas under dissociating conditions a glycoprotein with an apparent molecular weight of 12,400 exhibits bacteriocin activity (33). Ultrafiltration experiments of crude extracts of lactacin B suggested a molecular weight of 100,000 (1). However, further analysis of a more pure bacteriocin preparation revealed a molecular weight of 6,500 (2). Also, helveticin J was present as an aggregate with a molecular weight above 300,000. Upon purification and sodium dodecyl sulfate-gel electrophoresis, however, the bacteriocin migrated as a 37,000-Da protein that retained the inhibitory activity (14).

TABLE 2. Amino acid composition

Amino acid	Residues per molecule[a]
Ala	7.60 (7–8)
Asp	0.92 (1)
Glu	1.06 (1)
Gly	1.10 (1)
His	2.03 (2)
Leu	3.80 (4)
Lys	2.09 (2)
Met	1.05 (1)
Phe	1.04 (1)
Pro	1.9 (2)
Thr	0.90 (1)
Tyr	1.84 (2)
Val	4.5 (4–5)
Cys[b]	1.8 (2)

[a] Mean values from three runs. The values in parentheses are the numbers of the individual amino acids in lactocin S.

[b] Determined as *cysteic acid after* performic acid oxidation of the polypeptide.

The amino acid composition indicated that lactocin S consists of 33 amino acids, of which about 50% are the nonpolar amino acids alanine, valine, and leucine (Table 2). The hydropathic index (20) was calculated from the sequenced part of the molecule to be 1.37, and the hydrophobic character of the molecule is consistent with its relatively high affinity to the phenyl and C_2/C_{18} columns. The 25-residue C-terminal part of lactocin S which was sequenced contained three amino acid residues that were not identified. It is likely that the unidentified residues are modified forms of cysteine and/or amino acids associated with cysteine in a manner similar to that seen in lanthionine residues present in nisin, since two cysteic acids per lactocin S molecule were found upon performic acid oxidation of lactocin S. In the N terminus, lactocin S consists of approximately 8 amino acids of unknown sequence. From the amino acid composition, this sequence is highly nonpolar, consisting mainly of 3 to 4 alanine and 2 to 3 valine residues.

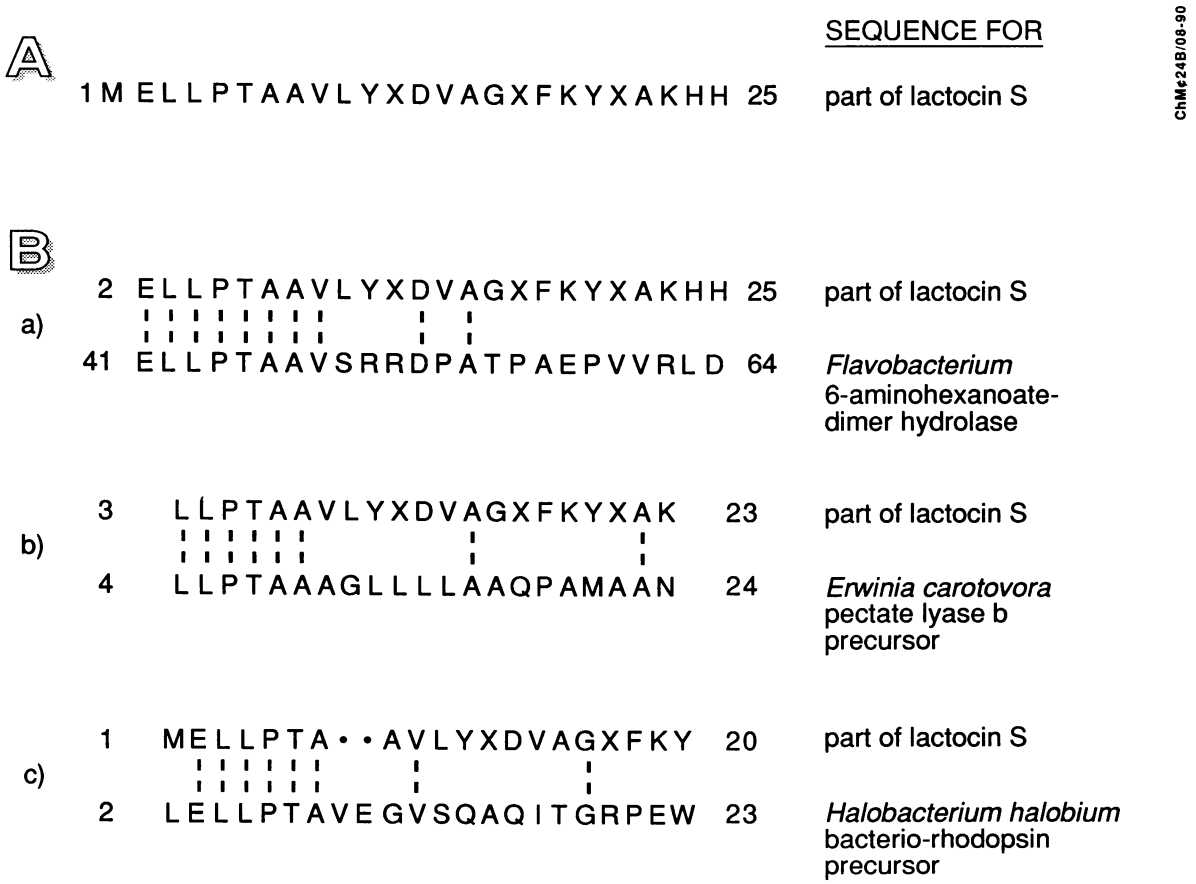

FIG. 3. (A) Amino acid sequence of the C-terminal part of lactocin S. (B) Comparison of lactocin S amino acid sequence with homologous sequences found in three bacterial proteins.

Nisin is the only bacteriocin from lactic acid bacteria for which the primary amino acid structure has been published. Lactocin S does not share any homology to nisin. A new lactococcal bacteriocin consisting of 54 amino acids has been isolated and sequenced (13). This lactococcal bacteriocin also contains two histidine residues at the very C-terminal end of the molecule, but no other homology to lactocin S was found (13). Van Belkum et al. published the sequences of two bacteriocins from *L. lactis* subsp. *cremoris*. These lactococcal bacteriocins differ from lactocin S, but one of these may also contain two histidine residues at the very C-terminal end of the molecule (34). Since this work was submitted, Muriana and Klaenhammer (28) published the sequence of 25 consecutive N-terminal amino acids of lactacin F (27) and the nucleotide sequence of this bacteriocin gene (28). Lactacin F is certainly different from lactocin S.

The known amino acid sequence of lactocin S was unique when compared to the SWISS-PROT data bank. Three proteins, however, revealed partial homology with lactocin S: the pectate lyase B precursor isolated from *Erwinia carotovora* (21), the bacteriorhodopsin precursor isolated from *Halobacterium halobium* (17), and the 6-aminohexanoate-dimer hydrolase from *Flavobacterium* sp. strain K172 (29) (Fig. 3B). Two of these sequences, 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 signal sequences suggest the cell membrane as a possible target for lactocin S. Because of the potential use of lactic bacteriocins, it is of great importance to gain insight into their genetics, chemical nature, and mode of action. This study presents biochemical characteristics and a major part of the amino acid sequence of a new bacteriocin found in *L. sake*, information which is of importance for elucidating the genetics and mode of action of this bacteriocin.

ACKNOWLEDGMENTS

This study was supported by both a fellowship to C.I.M. and a research grant from the Agricultural Research Council of Norway.

REFERENCES

1. **Barefoot, S. F., and T. R. Klaenhammer.** 1983. Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. **45**:1808–1815.
2. **Barefoot, S. F., and T. R. Klaenhammer.** 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. Antimicrob. Agents. Chemother. **26**:328–334.
3. **Bradley, D. E.** 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. **31**:230–314.
4. **Buchman, G. W., S. Banerjee, and J. N. Hansen.** 1988. Structure, expression, and evolution of a gene encoding the precursor nisin, a small protein antibiotic. J. Biol. Chem. **263**:16260–16266.
5. **Cornwell, G. G., K. Sletten, B. Johansson, and P. Westermark.** 1988. Evidence that the amyloid fibril protein in senile systemic amyloidosis is derived from normal prealbumin. Biochem. Biophys. Res. Commun. **154**:648–653.

6. **Daeschel, M. A., M. C. McKenny, and L. C. McDonald.** 1990. Bacteriocidal activity of *Lactobacillus plantarum* C-11. Food Microbiol. **7**:91–98.
7. **DeKlerk, H. C., and J. A. Smit.** 1967. Properties of a *Lactobacillus fermenti* bacteriocin. J. Gen. Microbiol. **48**:309–316.
8. **Devereux, J., P. Haeberli, and O. A. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
9. **Dodd, H. M., N. Horn, and M. J. Gasson.** 1990. Analysis of the genetic determinant for production of the peptide antibiotic nisin. J. Gen. Microbiol. **136**:555–566.
10. **Gross, E., and J. Morell.** 1971. The structure of nisin. J. Am. Chem. Soc. **93**:4634–4635.
11. **Hirs, C. H. W.** 1956. Oxidation of ribonuclease. J. Biol. Chem. **219**:611–621.
12. **Hirsch, A.** 1951. Growth and nisin production of a strain of *Streptococcus lactis*. J. Gen. Microbiol. **5**:208–221.
13. **Holo, H., Ø. Nilssen, and I. F. Nes.** 1991. Lactococcin A, a new bacteriocin from *Lactococcus lactis* subsp. *cremoris*: isolation and characterization of the protein and its gene. J. Bacteriol., in press.
14. **Joerger, M. C., and T. R. Klaenhammer.** 1985. Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. J. Bacteriol. **167**:439–446.
15. **Joerger, M. C., and T. R. Klaenhammer.** 1990. Cloning, expression, and nucleotide sequence of the *Lactobacillus helveticus* 481 gene encoding the bacteriocin helveticin J. J. Bacteriol. **172**:6339–6347.
16. **Kaletta, C., and K.-D. Entian.** 1989. Nisin, a peptide antibiotic: cloning and sequencing of the *nisA* gene and posttranslational processing of its peptide product. J. Bacteriol. **171**:1597–1601.
17. **Katre, N. V., P. K. Wolber, W. Stoeckenius, and R. M. Stroud.** 1981. Attachment site(s) of retinal in bacteriorhodopsin. Proc. Natl. Acad. Sci. USA **78**:4068–4072.
18. **Klaenhammer, T. R.** 1988. Bacteriocins of lactic acid bacteria. Biochimie **70**:337–349.
19. **Klein, P., M. Kanehisa, and C. DeLisi.** 1985. The detection and classification of membrane-spanning proteins. Biochim. Biophys. Acta **815**:468–476.
20. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
21. **Lei, S.-P., H.-C. Lin, S.-S. Wang, J. Callaway, and G. Wilcox.** 1987. Characterization of the *Erwinia carotovora pelB* gene and its product pectate lyase. J. Bacteriol. **169**:4379–4383.
22. **Matsubara, H., and R. M. Sasaki.** 1969. High recovery of tryptophan from acid hydrolysates of proteins. Biochem. Biophys. Res. Commun. **35**:175–181.
23. **McClintock, M., L. Serres, J. J. Hirsch, and G. Mocquot.** 1952. Action inhibitrice des streptocoques producteurs de nisine sur le développement des sporulés anaérobies dans le fromage de gruyère fondu. J. Dairy Res. **19**:187–193.
24. **Mørtvedt, C. I., and I. F. Nes.** 1989. Bacteriocin production by a lactobacillus strain isolated from fermented meat. Eur. Food Chem. Proc. **1**:336–341.
25. **Mørtvedt, C. I., and I. F. Nes.** 1990. Plasmid-associated bacteriocin production by a *Lactobacillus sake* strain. J. Gen. Microbiol. **136**:1601–1607.
26. **Muriana, P. M., and T. R. Klaenhammer.** 1987. Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. Appl. Environ. Microbiol. **53**:553–560.

26a. **Muriana, P. M., and T. R. Klaenhammer.** 1989. Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, abstr. P-43, p. 326.

27. **Muriana, P. M., and T. R. Klaenhammer.** 1991. Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. Appl. Environ. Microbiol. **57**:114–121.
28. **Muriana, P. M., and T. R. Klaenhammer.** 1991. Cloning, phenotypic expression, and DNA sequence of the gene for lactacin F, a bacteriocin produced by *Lactobacillus acidophilus*. J. Bacteriol. **173**:1779–1788.
29. **Okada, H., S. Negoro, H. Kimura, and S. Nakamura.** 1983. Evolutionary adaption of plasmid-encoded enzymes for degrading nylon oligomers. Nature (London) **306**:203–206.
30. **Schillinger, U., and F. K. Lucke.** 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. **55**:1901–1906.
31. **Sletten, K., and G. Husby.** 1974. The complete amino-acid sequence of non-immunoglobulin amyloid protein AS in rheumatoid arthritis. Eur. J. Biochem. **41**:117–125.
32. **Tagg, J. R., A. S. Dajani, and L. W. Wannamaker.** 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev. **40**:722–756.
33. **Upreti, G. C., and R. D. Hinsdill.** 1973. Isolation and characterization of a bacteriocin from a homofermentative *Lactobacillus*. Antimicrob. Agents Chemother. **4**:487–494.
34. **Van Belkum, M. J., B. J. Hayema, R. E. Jeeninga, J. Kok, and G. Venema.** 1991. Organization and nucleotide sequences of two lactococcal bacteriocin operons. Appl. Environ. Microbiol. **57**:492–498.