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Detection and Activity of a Bacteriocin Produced by *Leuconostoc mesenteroides*

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***Leuconostoc mesenteroides* UL5 was found to produce a bacteriocin, referred as mesenterocin 5, active against *Listeria monocytogenes* strains but with no effect on several useful lactic acid bacteria. The antimicrobial substance is a protein, since its activity was completely destroyed following protease (pronase) treatment. However, it was relatively heat stable (100°C for 30 min) and partially denaturated by chloroform. The inhibitory effect of the bacteriocin on sensitive bacterial strains was determined by a critical-dilution micromethod. Mutants of *L. mesenteroides* UL5 which had lost the capacity to produce the bacteriocin were obtained. The mutant strain was stable and phenotypically identical to parental cells and remained resistant to the bacteriocin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to detect bacteriocin activity corresponding to an apparent molecular mass of about 4.5 kDa.**

The lactic acid bacteria represented by lactococci, lactobacilli, pediococci, and leuconostocs contribute to flavor development, as well as preservation of foods. Preservation is generally induced by organic acid formation, and the resulting pH decrease exhibits an antagonistic effect on microbial contaminants. Since organic acids do not invariably destroy spoilage organisms and pathogens (4) and a high level of acidity is not desirable in some products (25), the efficiency of organic acids as antimicrobial agents is relatively less significant in some cases.

Listeria monocytogenes has been recognized as a major food-borne pathogen with the ability to survive in various environmental conditions, such as refrigeration (15), at pHs as low as 3.6 in foods (24), in salt concentrations of up to 10% (22), in the presence of surfactant sanitizers (13), and at high temperatures (11, 21). In the latter case, it was recently shown that heat-stressed *L. monocytogenes* strains could be recovered from experimentally contaminated shrimps boiled for up to 5 min (21). *L. monocytogenes* has also been encountered in fermented products (cheese, yogurt, fermented milk, sausage) made from raw materials contaminated with the organism (4). All of these indicate that traditional methods of preservation are not sufficient to prevent growth of *L. monocytogenes* in foods.

Therefore, investigation of bacteriocins which inhibit pathogens, such as *L. monocytogenes*, becomes particularly attractive for the food industry. In fact, it has been shown that lactic acid bacteria could inhibit undesirable microorganisms through bacteriocins. Interest in the identification and characterization of bacteriocins of lactic acid bacteria has grown fairly rapidly in the last few years. The various bacteriocins have shown great diversity in their effects on bacterial species (8, 19, 26, 29, 31). Nisin, a bacteriocin that is generally regarded as safe and is produced by certain strains of *Lactococcus lactis* subsp. *lactis*, is the only bacteriocin that has been approved for food use in some countries (9, 12). It is expected that further investigations of other bacteriocins may aid in their successful exploitation as food preservatives.

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The general objectives in searching for a desirable bacteriocin are that it be produced extracellularly in sufficient quantities from a food-safe organism and that it effectively antagonize undesirable bacteria which contaminate the foods.

In our previous study on bacteriocin production by lactic acid bacteria (unpublished data), several gram-positive bacteria were screened for the ability to produce bacteriocin against *L. monocytogenes*. The main objective of this study was to characterize the mesenterocin 5 secreted by *Leuconostoc mesenteroides* UL5 and investigate its production in batch culture. A critical-dilution micromethod was used to define bacteriocin activity.

MATERIALS AND METHODS

Bacterial strains and media. *L. mesenteroides* UL5 was isolated from local Cheddar cheese and maintained as a part of our culture collection. It was characterized by its carbohydrate fermentation profile using API 50 (API Division of Sherwood Medical, Plainview, N.Y.) at both our laboratory and the Veterinary Pathology Laboratory of Agriculture Canada (St.-Hyacinthe, Quebec). For production of bacteriocin, single colonies were inoculated into MRS broth (Difco Laboratories, Detroit, Mich.) and incubated overnight at 30°C. A 1% inoculum of this overnight culture was added to an appropriate volume of fresh MRS broth, and the bacteriocin produced by this culture was tested in various assays as described below. *Listeria* strains used as indicator microorganisms were obtained from the Health Protection Branch (Health and Welfare Canada, Ottawa, Ontario) or from the Food Research and Development Center (Agriculture Canada). *Listeria* species were grown in Trypticase soy agar or broth (TSY) supplemented with 0.6% yeast extract (Difco). Several other lactic acid, food-spoiling, and pathogenic bacteria from different origins were tested in this study.

Detection of bacteriocin activity. A culture of *L. mesenteroides* UL5 was grown overnight in MRS broth at 30°C in a rotary shaker. Stationary cells were centrifuged at 10,000 × *g* for 20 min at 4°C. The culture supernatant was concentrated by ammonium sulfate precipitation or ultrafiltration.

In the first method, 50-ml aliquots of culture supernatant were made up to 60% saturation by addition of ammonium sulfate (Fisher Scientific, Fair Lawn, N.J.) and kept overnight at 4°C with gentle stirring. After centrifugation (10,000 × *g*, 20 min, 4°C), the sedimented pellet was recovered and suspended in 3 ml of 0.1 M potassium phosphate buffer at pH 6. The pellet suspension was dialyzed at 4°C with a dialysis membrane with a 3.5-kDa cutoff against 1.0 liter of 0.1 M potassium phosphate buffer at pH 6 for at least 18 h with two changes of buffer. After dialysis, the solution in the dialysis bag was sterilized by filtration through a 0.2-μm-pore-size filter (Sigma Chemical Co., St. Louis, Mo.). This cell-free extract was tested for bacteriocin activity against indicator bacteria by using a well diffusion assay (3, 27). A fresh overnight culture of indicator bacteria (200 μl) grown in an appropriate broth was mixed with 3 ml of fresh medium containing 0.7% agar (soft agar), cooled to 45°C, and poured into a petri dish which contained 20 ml of 1.5% agar medium. The soft agar was allowed to solidify, thus generating a potential mat of the indicator bacteria. With the broad end of a sterile Pasteur pipette, several wells were punched out of the agar. The resulting agar "buttons" were removed, and a drop of soft agar was placed into each well to plug the bottom of the well. The wells were then filled with the bacteriocin solution (100 μl). The plates were incubated for 18 h at optimal temperatures for the indicator bacteria. At the end of the incubation period, the diameters of the inhibition zones were measured.

For ultrafiltration, 10 ml of a cell-free culture of *L. mesenteroides* UL5 was passed through a type YM membrane with a 3-kDa cutoff by using a standard cell model (Amicon Corp., Danvers, Mass.) and concentrated to a final volume of 2 ml. To exclude inhibitory effects of hydrogen peroxide or organic acids, the concentrated supernatant was washed twice with 10 ml of 0.1 M potassium phosphate buffer at pH 6. The bacteriocin activity in the retentate was determined by the well diffusion assay described above. The pH of the ultrafiltration retentate was measured before and after washing.

Bacterial cell lysis. The method used for bacterial cell lysis was adapted from the method of Arora et al. (2). An overnight culture of *L. mesenteroides* UL5 (100 ml) was centrifuged at 10,000 × *g* for 20 min at 4°C, and the cell pellet was suspended in 100 ml of 0.1 M phosphate-buffered saline (pH 7). The centrifugation and washing were repeated, and the cells were pelleted once more. Then, 10 ml of phosphate-buffered saline and 5 g of glass beads (425 to 600 μm in diameter; Sigma) were added to the cell pellet and agitated on a G10 Gyratory shaker (New Brunswick Scientific Inc., Edison, N.J.) at 200 rpm and 4°C for 1 h. The suspension was centrifuged, and the supernatant was recovered for determination of antagonistic activity.

Bacteriocin activity assay. Bacteriocin activity was determined by a critical-dilution micromethod using a microtiter plate (10). Samples were dialyzed and filter sterilized. Serial twofold dilutions of the filtrate were made in 125-μl volumes of TSY in a 96-well Falcon microtiter plate (Becton Dickinson Labware, Lincoln Park, N.J.). Each well was then inoculated with 50 μl of a 100-fold-diluted overnight culture of the test organism *Listeria ivanovii* 28 with an initial optical density at 600 nm of 1.2. This strain was selectively used as indicator cells because of its high sensitivity to the bacteriocin (see Table 1). Assay microplates were incubated at 30°C for 18 h.

One arbitrary unit (AU) was defined as a 125-μl portion from the highest dilution of the bacteriocin preparation which prevented visible turbidity in a well at 18 h. The activity of the bacteriocin preparation expressed in AU per milliliter was calculated with the formula (1,000/125) × (1/*D*), where *D* is the highest dilution that allowed no growth of the test organism at 18 h of incubation.

Determination of MICs and MBCs. The micromethod described above was designed to evaluate the MIC and MBC of the active substance (10). In our experiments, we observed that mesenterocin 5 activity was proportional to the bacteriocin concentration. Therefore, MIC and MBC were directly determined from bacteriocin activity. The results shown (see Table 1) were calculated by using a dilution factor to account for the volume of the inoculum added. The dilution factor was 125/175 and was based on the fact that a 50-μl inoculum of listeriae was added to 125 μl of TSY medium.

The activity of mesenterocin 5 obtained from cell-free supernatant concentrated by ammonium sulfate precipitation, dialyzed, and filter sterilized was 16,384 AU/ml, determined as described above. Strains to be tested were grown overnight at 30°C in TSY broth. Serial twofold dilutions of the bacteriocin preparation were made in 125 μl of TSY broth down to 1 AU/ml. Approximately 10^4 cells, corresponding to a volume of 50 μl of the indicator culture, were added to the bacteriocin dilutions, and the microplate was incubated for 18 h at 30°C. The MIC was defined as the lowest concentration of bacteriocin that resulted in no visible turbidity after 18 h of incubation. For each well showing no turbidity after 18 h, a 5-μl volume was subcultured on a surface of a TSY agar plate and incubated at 30°C. The MBC corresponded to the lowest concentration which, when plated onto TSY agar, showed no CFU.

Characterization of the bacteriocin. Cell-free culture medium of *L. mesenteroides* UL5, previously concentrated by ammonium sulfate precipitation, was subjected to heat treatment (100°C for 30 min), chloroform treatment (50 μl added to a 200-μl active sample and left at room temperature for 1 h), and protease treatment (100 μl of pronase E at 2 mg/ml added to 100 μl of bacteriocin solution and incubated at 37°C for 1 h). The remaining activity was measured by the agar well diffusion method against a sensitive indicator lawn. An untreated preparation of bacteriocin served as the control.

To test for lysozyme-like activity, a lawn of *Micrococcus lysodeikticus* cells was prepared on TSY agar by pouring 3 ml of TSY soft agar containing 0.5 g of lyophilized *M. lysodeikticus* cells (Sigma; M-3770) onto a TSY agar petri dish. Six-microliter samples of lysozyme (50 mg/ml), pronase (100 μl), and bacteriocin were spotted onto this lawn. After overnight incubation at 37°C, the inhibition zones were measured.

Kinetics of mesenterocin 5 production. A flask containing 200 ml of MRS broth was seeded with a 1% inoculum from a 24-h culture of *L. mesenteroides* UL5. The flask was incubated at 30°C with agitation at 150 rpm, and samples were taken at different time intervals to measure biomass by A_{650}, culture pH, cell growth by plate counts, and bacteriocin titer as described previously. Supernatants examined for inhibitory activity were directly dialyzed, filter sterilized, and frozen at −20°C.

Detection of mutants deficient in mesenterocin 5 production. Mutants deficient in mesenterocin 5 production were detected by growing cells in MRS broth containing various amounts of acriflavine (6, 15, or 25 μg/ml) or incubating them at 50°C with three consecutive transfers every 24 h. The traditional method for mutant detection (3, 27) consists of overlaying survivor colonies with a lawn of indicator organ-

isms. After development of the indicator lawn, mutant colonies are examined for surrounding zones of inhibition. In this work, another technique was employed to isolate the mutants. A 1-ml volume of an overnight culture of *L. monocytogenes* Scott A3 was mixed with 10 ml of molten MRS agar at 45°C and poured into a petri dish. After cooling, the plate was overlaid with 0.1 ml of treated culture appropriately diluted in order to obtain uncrowded colonies. Bacteriocin-negative mutants were detected by their inability to form inhibition zones after 48 h of incubation at 30°C.

SDS-PAGE analysis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) on a continuous gradient gel was used to separate low-molecular-weight proteins (5, 18). In the present study, *L. mesenteroides* strains were grown in MRS broth supplemented with 2% yeast extract. Fivefold-concentrated samples obtained by ultrafiltration, with or without antimicrobial activity (the latter corresponding to the mutant strain lacking a bacteriocin effect), were then mixed in a 1:1 ratio with sample buffer (4.6% SDS, 10% β-mercaptoethanol, 20% glycerol, 1.5% Tris base, 1% bromophenol blue) and heated at 100°C for 3 min. Forty-microliter samples were then subjected to SDS-PAGE on a 10 to 25% gradient gel using standard proteins as molecular weight markers (MW-SDS-6000SA; Bethesda Research Laboratories, Gaithersburg, Md.). After overnight electrophoresis at 90 V, the gel was removed and cut into two vertical parts. The first half, containing active and mutant samples and molecular weight standards, was fixed and stained with Coomassie blue R-250. The other half, containing only the active and mutant samples, was fixed immediately for 2 h in a solution of 20% isopropanol and 10% glacial acetic acid and washed in deionized water for 4 h. This gel was aseptically placed in a sterile petri dish, rinsed abundantly with sterile water, and overlaid with approximately 60 ml of TSY soft agar containing 5 ml of an overnight culture of *L. ivanovii* 28 diluted 100-fold. The plate was incubated at 30°C for 18 h and examined for zones of inhibition.

RESULTS

Nature of the antibacterial substance. The activity of the bacteriocin produced by *L. mesenteroides* UL5 was neutralized by protease (pronase) treatment but was resistant to heat (Fig. 1). The antibacterial compound was affected little after heating for 30 min at 100°C, clearly indicating that the active substance is a heat-stable protein. There was a partial reduction of bacteriocin activity after chloroform treatment (data not shown).

Mesenterocin 5 was tested to determine whether or not its activity was similar to that of lysozyme and whether it could lyse the target bacteria through lysozyme-like action. Mesenterocin 5 did not show lysozyme-like action on *M. lysodeikticus*, while lysozyme produced a prominent and clear zone of lysis (data not shown).

Bacteriocin production. The activity of mesenterocin 5 in MRS broth as determined by the micromethod was dependent upon the phase of bacterial growth (Fig. 2). No activity was detected during the first 5 h of incubation. It reached a maximum (2,048 AU/ml) at 8 h at a culture pH of 5.5 and a cell count of 8×10^8 CFU/ml. Bacteriocin titers decreased by more than 90% after 24 h of incubation.

The activity of the supernatant recovered from bacterial cell lysis was not detectable. It is possible that much greater force is required to disrupt *L. mesenteroides*, although *Lactobacillus casei* can be disrupted by this method, as shown by Arora et al. (2). Therefore, all of the assays for this experiment were performed by using such cell lysates.

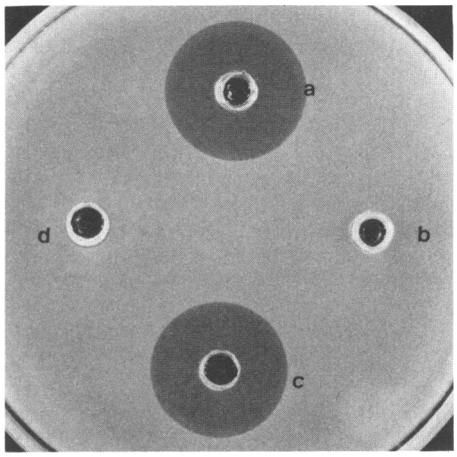

FIG. 1. Agar well diffusion showing bacteriocin activity from *L. mesenteroides* UL5 (a, c, and d) and UL5-A (b) against indicator *L. ivanovii* 28. Wells: a, untreated; c, sample after heating at 100°C for 30 min; d, sample treated with pronase E (1 mg/ml, 1 h, 37°C). Each well contained 100 μl of cell-free supernatant precipitated by ammonium sulfate and dialyzed.

Determination of MICs and MBCs. MICs and MBCs were determined against different *L. monocytogenes* strains and *L. ivanovii* 28, a strain previously used as a sensitive indicator of mesenterocin 5 activity. Average MICs and MBCs for two assays are given in Table 1. Among the *Listeria* spp. tested, *L. ivanovii* 28, *L. monocytogenes* 1089, and *L. monocytogenes* Lm13 were the most sensitive while *L. monocytogenes* Scott A3, *L. monocytogenes* Lm8, and *L. monocytogenes* Lm21 were more resistant.

Spectrum of inhibitory activity. The antibacterial effect of the cell-free medium from a culture of *L. mesenteroides* UL5, fivefold concentrated by ultrafiltration, was tested by the well diffusion method on several lactic acid bacteria commonly used in the food industry and on various gram-positive bacteria (Table 2). The concentrated supernatant was effective against all of the *Listeria* strains tested and also

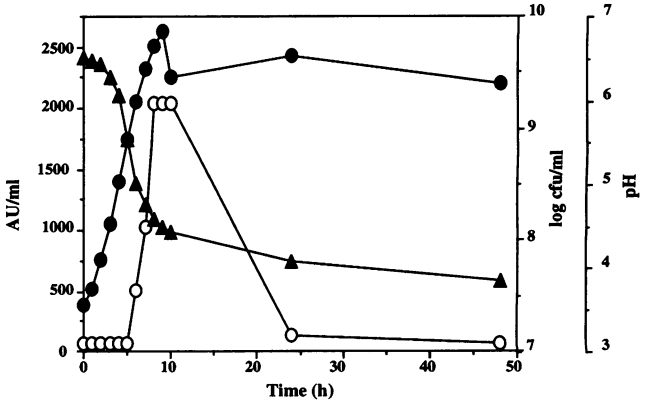

FIG. 2. Kinetics of production of mesenterocin 5 by *L. mesenteroides* UL5 in MRS broth at 30°C. At different time intervals, samples were taken and CFU per milliliter (●), culture pH (▲), and amount of bacteriocin active against *L. ivanovii* 28 (○) were determined.

TABLE 1. Determination of MICs and MBCs of mesenterocin 5 by the critical-dilution micromethod for different *Listeria* strains

Strain	MIC (AU/ml)	MBC (AU/ml)
L. ivanovii 28	6	>11,703
L. monocytogenes Scott A3	>11,703	>11,703
L. monocytogenes 1089	23	>11,703
L. monocytogenes Lm8	>11,703	>11,703
L. monocytogenes Lm13	11,703	>11,703
L. monocytogenes Lm21	>11,703	>11,703

against *Streptococcus faecalis*, *Brevibacterium linens*, and *Pediococcus pentosaceus*. Other gram-positive bacteria, including food-spoiling and food-borne pathogenic species, were not affected by fivefold-concentrated supernatant under the conditions of the experiments. These bacteria included *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Salmonella choleraesuis*, *S. typhimurium*, *Serratia marcescens*, and *Yersinia enterocolitica*.

Bacteriocin-negative mutants. Elevated temperature (50°C) and acriflavine treatments (at concentrations of 6 to 25 μg/ml of culture medium) were used to obtain mutants. High-temperature incubation failed to yield bacteriocin-negative mutants from cultures of *L. mesenteroides* UL5, since all of the colonies examined were still able to produce an inhibition zone on a lawn of *L. monocytogenes*. However, after three consecutive transfers every 24 h in the presence of acriflavine (15 μg/ml), 3 of 63 *L. mesenteroides* UL5 colonies were differentiated by absence of an inhibition zone. One of

TABLE 2. Activity spectrum of the bacteriocin produced by *L. mesenteroides* UL5 against gram-positve bacteria

Indicator species	Strain	Source[a]	Inhibition by fivefold-concentrated supernatant from *L. mesenteroides* UL5[b]
Brevibacterium linens	D2	Our strain collection	(+)
Brochotrix thermosphacta	11509	ATCC	−
Kurthia zopfii	3403	ATCC	−
Lactobacillus acidophilus	4356	ATCC	−
Lactobacillus delbrueckii subsp. *bulgaricus*	7994	ATCC	−
Lactobacillus brevis	D7	Our strain collection	−
Lactobacillus casei subsp. *casei*	L2A	Our strain collection	−
Lactobacillus casei subsp. *pseudoplantarum*	137	Our strain collection	−
Lactobacillus casei subsp. *pseudoplantarum*	138A	Our strain collection	−
Lactobacillus casei subsp. *rhamnosus*	L2F	Our strain collection	−
Lactobacillus fermentum	S23	Our strain collection	−
Lactobacillus helveticus	10797	ATCC	−
Lactobacillus plantarum	8014	ATCC	−
Lactobacillus lactis subsp. *lactis*	D8	Our strain collection	
Lactococcus cremoris	D15	Our strain collection	−
Lactococcus diacetylactis	11007	ATCC	−
Lactococcus lactis	7962	ATCC	−
Lactococcus lactis	14871	ATCC	−
Leuconostoc mesenteroides subsp. *cremoris*	D9	Our strain collection	−
Leuconostoc mesenteroides subsp. *dextranicum*	D10	Our strain collection	−
Leuconostoc lactis	19256	ATCC	−
Leuconostoc mesenteroides subsp. *mesenteroides*	23386	ATCC	−
Listeria denitrificans	31	HW	+
Listeria grayi	29	HW	+
Listeria innocua	13	HW	+
Listeria ivanovii	28	HW	+
Listeria monocytogenes	Scott A3	HW	+
Listeria monocytogenes	Lm8	FRDC	+
Listeria monocytogenes	Lm13	FRDC	+
Listeria monocytogenes	Lm21	FRDC	+
Listeria monocytogenes	1089	Our strain collection	+
Listeria murrayi	30	HW	+
Listeria seeligeri	62	HW	+
Listeria welshimeri	89	HW	+
Micrococcus flavus	D11	Our strain collection	(+)
Micrococcus varians	15936	ATCC	−
Micrococcus varians	D12	Our strain collection	−
Pediococcus cerevisiae	S39	Our strain collection	−
Pediococcus pentosaceus	D13	Our strain collection	(+)
Pediococcus parvulus	S50	Our strain collection	−
Staphylococcus aureus	D14	Our strain collection	−
Streptococcus faecalis	D16	Our strain collection	(+)
Streptococcus thermophilus	S73	Our strain collection	−

[a] Abbreviations: ATCC, American Type Culture Collection (Rockville, Md.); HW, Health and Welfare Canada (Ottawa, Ontario); FRDC, Food Research and Development Center (St.-Hyacinthe, Quebec, Canada).

[b] Symbols: −, no inhibition zone; +, inhibition zone; (+), small inhibition zone (0.5 to 2.0 mm).

these bacteriocin-negative mutants was isolated, and it was further observed that this mutant did not differ from the parent strain with respect to acid formation and carbohydrate fermented. For example, we have observed that the glucose, lactose, and lactic and acetic acids concentrations from overnight cultures of *L. mesenteroides* and its mutant incubated at 30°C, analyzed by high-performance liquid chromatography (Waters, Bedford, Mass.), were 0.75, 0, 0.77, and 0.11%, respectively. This mutant, designated *L. mesenteroides* UL5-A, was not inhibited by the bacteriocin produced by the parent strain.

Direct detection of bacteriocin by SDS-PAGE. The electrophoretogram of the gel stained with Coomassie blue shows several polypeptide bands for both *L. mesenteroides* UL5 and UL5-A, as measured against protein molecular weight standards ranging approximately from 3 to 43 kDa (Fig. 3A). The other half of the gel, overlaid with the indicator strain, showed a large inhibitory zone produced exclusively by bacteriocin from the parent, *L. mesenteroides* UL5 (Fig. 3B), corresponding to an apparent molecular mass of about 4.5 kDa. Proteins in the other bands exhibited no antimicrobial property. However, gradient SDS-PAGE did not permit identification of a specific protein band for the active bacteriocin of *L. mesenteroides* UL5, since *L. mesenteroides* UL5 and *L. mesenteroides* UL5-A gave similar protein profiles on the gel stained with Coomassie blue (Fig. 3A).

DISCUSSION

L. mesenteroides UL5 has been shown to produce a proteinaceous substance with antibacterial activity. This bacteriocin, named mesenterocin 5, demonstrated a bacteriostatic but not bactericidal effect on bacteria tested over an incubation period of 18 h. The micromethod was more rapid and economical than classical methods and was developed originally to test the antimicrobial activities of disinfectants (10). The micromethod is accurate and reproducible and could be used to evaluate the activities of other bacteriocins.

In comparison with ammonium sulfate precipitation, ultrafiltration was a more convenient and efficient method to concentrate samples and excluded nonspecific inhibition by organic acids.

Mesenterocin 5 was secreted from cells principally during the late exponential phase of growth. The activity dropped sharply during the early stationary phase. A similar activity pattern without pH control has been obtained for a bacteriocin produced by a strain of *Streptococcus thermophilus* (6), but no decline in activity was observed over a 24-h growth period in the cases of *Lactobacillus* sp. (30) and *Streptococcus lactis* (20). Preliminary experiments with the pH controlled at 5.0 did not show this large decrease in bacteriocin activity during the stationary phase, suggesting that pH has an effect on bacteriocin stability and activity.

SDS-PAGE was able to detect the bacteriocin activity produced by *L. mesenteroides* UL5, to determine its apparent molecular weight, and to demonstrate that the activity was absent in mutant strain UL5-A. This method allowed detection of the bacteriocin at about 4.5 kDa but did not reveal a specific band for the active bacteriocin with Coomassie blue staining. Additional studies should be undertaken to purify and characterize this compound.

Unlike other results (28), loss of the bacteriocin production gene in the mutant strain was not associated with loss of bacteriocin immunity. These results indicate that the genes responsible for production and immunity are not genetically linked in *L. mesenteroides* UL5.

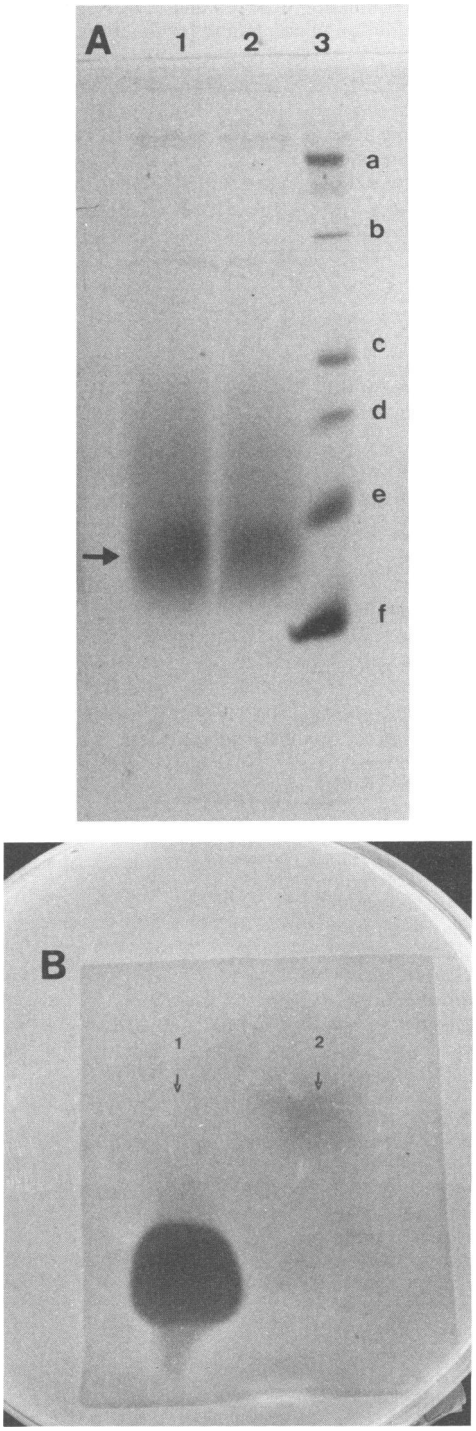

FIG. 3. (A) Stained half of the SDS-PAGE gel showing proteins present in the concentrated cell-free supernatants from *L. mesenteroides* UL5 (lane 1) and UL5-A (lane 2). Lane 3 contained the following low-molecular-weight standards (MW-SDS-6000-SA; Bethesda Research Laboratories): a, 43,000; b, 29,000; c, 18,400; d, 14,300; e, 6,200; f, 3,000. The arrow indicates the location of bacteriocin. (B) Other half of the gel, overlaid with *L. ivanovii* 28 to estimate the molecular weight of the antimicrobial compound. Lane 1, corresponding to *L. mesenteroides* UL5, shows a zone of inhibition located between the two lowermost bands (molecular weight, 4,500). Lane 2, corresponding to the sample of *L. mesenteroides* UL5-A, shows no antibacterial activity.

The mesenterocin reported in this study possesses several advantages over other bacteriocins. *L. mesenteroides* is already used in Swiss-type cheese (7), and a metabolite secreted by this bacterium should therefore be acceptable for use in other foods. The spectrum of target bacteria against which the bacteriocin is effective is interesting. Among the bacteria tested, the pathogen *L. monocytogenes* and other *Listeria* species were sensitive, as shown with the well diffusion assay. Most of the *L. monocytogenes* strains were, however, resistant to the bacteriocin in the critical-dilution micromethod. Important strain differences were observed in the degree of inhibition, showing that some *Listeria* strains are more sensitive to mesenterocin 5 than others. A majority of lactobacillus species were unaffected under the test conditions used. Since attempts have been made to use lactobacilli and lactococci to inhibit the growth of contaminant bacteria (1, 3, 6, 14), a combined treatment using mesenterocin 5 along with lactobacilli for a synergistic effect can be envisaged.

Bacteriocins have attracted the attention of the food industry as potential natural food preservatives against several spoilage bacteria and pathogens (8, 9), and a number of bacteriocins with a spectrum of activity covering several target bacteria have been cited, including some recent examples of bacteriocins produced by lactic acid bacteria that are effective against *L. monocytogenes* (4, 16, 17, 23). Bacteriocins elaborated by bacteria generally regarded as safe are thus becoming attractive as food additives.

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