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Isolation of *Lactobacillus salivarius* 1077 (NRRL B-50053) and Characterization of Its Bacteriocin, Including the Antimicrobial Activity Spectrum▽

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***Lactobacillus salivarius* 1077 (NRRL B-50053) was isolated from poultry intestinal materials, and *in vitro* anti-*Campylobacter jejuni* activity was demonstrated. The isolate was then used for bacteriocin production and its enrichment. The protein content of the cell-free supernatant from the spent medium was precipitated by ammonium sulfate and dialyzed to produce the crude antimicrobial preparation. A typical bacteriocin-like response of sensitivity to proteolytic enzymes and resistance to lysozyme, lipase, and 100°C was observed with this preparation. The polypeptide was further purified by gel filtration, ion-exchange, and hydrophobic-interaction chromatography. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), Edman degradation, and isoelectrofocusing were used to characterize its 3,454-Da molecular mass, the amino acid sequence of its 37 residue components, and the isoelectric point of pI 9.1 of the bacteriocin. Bacteriocin L-1077 contained the class IIa bacteriocin signature N-terminal sequence YGNGV. MICs of bacteriocin L-1077 against 33 bacterial isolates (both Gram negative and Gram positive) ranged from 0.09 to 1.5 μg/ml. Subsequently, the therapeutic benefit of bacteriocin L-1077 was demonstrated in market-age (40- to 43-day-old) broiler chickens colonized with both *C. jejuni* and *Salmonella enterica* serovar Enteritidis. Compared with untreated control birds, both *C. jejuni* and *S.* Enteritidis counts in colonized ceca were diminished by >4 $\log_{10}$ and *S.* Enteritidis counts in both the liver and the spleen of treated birds were reduced by 6 to 8 $\log_{10}$/g compared with those in the nontreated control birds. Bacteriocin L-1077 appears to hold promise in controlling *C. jejuni*/*S.* Enteritidis among commercial broiler chickens.**

Bacteriocins (BCNs) are low-molecular-weight proteins which are produced by bacterial ribosomes and possess antimicrobial properties (2, 5, 8, 14, 15). These proteins are primarily cationic, hydrophobic, or amphiphilic peptides, with molecular masses of 5 to 6 kDa. Mature bacteriocin antimicrobials are comprised of 20 to 60 amino acids (14, 15). Bacteriocins are produced by widely diverse microorganisms that belong to a variety of systematic groups and occupy various ecological niches. According to a widely accepted classification (8), subclass IIa carries pediocin-like anti-*Listeria* peptides, which have a conserved N-terminal sequence of YGNGV and one or two cysteine bridges.

Numerous aspects distinguish bacteriocins from antibiotics (2). (i) Bacteriocins are produced on the surface of ribosomes in microbial cells, while antibiotics are primarily secondary metabolites of the cell. (ii) Unlike producers of antibiotics, bacteriocin producers are insensitive to the bactericidal effects. (iii) Bacteriocin molecules may attach to the target cell wall anywhere on the surface, as no specific receptors on the target cell wall apparently exist. (iv) The mechanism of bacteriocin for lethality is versatile and is associated with the process of pore formation in the outer cell membrane. Bacteriocins bind with cell walls of susceptible microbes, cause ionic imbalances, and generate pores. Inorganic ions leak through the pores, thereby killing the target cell. Antibiotics, on the other hand, can inhibit synthesis of the subcellular processes (cell wall synthesis, intracellular protein production, and DNA and RNA replication). (v) Limited resistance of a target cell to bacteriocins develops when the target alters its cell membrane chemical composition.

Bacteriocins have advantages over antibiotics. (i) There is no information suggesting that bacteriocins are toxic for animals or accumulate in tissues. Bacteriocins are susceptible to proteases and should degrade in the host. (ii) Bacteriocins are effective against antibiotic-resistant pathogens (24). (iii) Bacteriocins are already produced in animal hosts by normal microflora and likely have an important role in the kinetics of the microbial ecosystem. (iv) Consumers desire naturally grown and processed foods. A European Union import ban on poultry treated with chlorinated water has been in place since 1997 and has limited imports of United States poultry meat, which is generally treated by this process. Application of bacteriocins would provide an alternative to the chemical disinfection approaches that are presently employed.

This paper describes the discovery, characterization, and effectiveness of a new bacteriocin, L-1077, produced by *Lactobacillus salivarius* L-1077 (NRRL B-50053). We also demonstrate one potential application of this bacteriocin as a thera-

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peutic agent administered to market-age broiler chickens in drinking water to effectively control both *Campylobacter jejuni* and *Salmonella* in the live animal.

MATERIALS AND METHODS

Microbiology. At a Russian slaughter/processing plant, healthy commercial broiler chickens served as donors of cecal content providing antagonistic flora. These materials were streaked onto lactic acid bacterial (de Man, Rogosa, and Sharpe [MRS]; Difco) agar and incubated within an anaerobic atmosphere overnight at 37°C. *Lactobacillus* colonies were isolated and identified with the API 50 CHL microtest system (bioMérieux, France). Isolates were grown anaerobically at 37°C overnight to confluence on MRS agar. Agar blocks (~0.5 cm^3) containing the growth were aseptically cut and transferred to brucella agar supplemented with 5% lysed blood seeded with *C. jejuni* NCTC 11168. Plates were incubated at 42°C under a microaerobic atmosphere for 24 to 48 h. Zones of growth inhibition were noted, and the diameters were measured.

Bacteriocin L-1077 purification. The isolate produced a zone of inhibition, and *Lactobacillus salivarius* L-1077 (NRRL B-50053) was isolated for further study. Consistent with methods previously described (21, 25), the BCN was produced and purified to homogeneity. The isolate was fermented overnight in 10% (vol/vol) brucella broth medium under quiescent aerobic conditions maintained at 37°C. The spent culture was centrifuged (10,000 × *g*, 10 min) to remove the cells. The supernatant was adjusted to pH 6.2 by adding 1 N NaOH and 130 U/ml catalase to remove the influence of organic acids and hydrogen peroxide. Proteins in the cell-free supernatant were precipitated with ammonium sulfate and then dialyzed as previously described (23). The crude preparation was filtered through a 0.22-μm filter (Millipore, Bedford, MA) and further purified by cation-exchange and hydrophobic-interaction chromatographic separations (23).

Bioassays and chemical analyses. Activities of the chromatographic fractions were initially screened against *C. jejuni* NCTC 11168. An additional 32 widely diverse, pathogenic bacterial isolates (see Table 2) were tested for MICs (μg/ml). Protein concentrations of the fractions were determined (11). Doubling dilutions of the preparations were performed, and 10 μl of each dilution was spotted onto plates of blood-supplemented brucella agar previously seeded with cells of *C. jejuni* NCTC 11168 or the additional bacterial isolates, as previously described (23). All assays were conducted in duplicate.

The amino acid sequence of the purified bacteriocin was determined by Edman degradation using a 491 cLC automatic sequencer (6) (Applied Biosystems). The bacteriocin molecular weight was determined by mass spectrometry (MS) using a Voyager-DERP mass spectrometer (Perkin-Elmer). The matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis was performed per the manufacturer's instructions. After the biochemical determination of the primary amino acid sequence, the physical characteristics were analyzed utilizing the Protean function of DNAStar (Madison, WI) (13). The primary amino acid sequence was entered into the BLAST database (1) to search for proteins with similar sequences.

Chicken challenges. Purified bacteriocin was diluted in tap water to provide 25, 12.5, or 6.25 mg or no bacteriocin L-1077/liter in the experimental bird drinking waters. We obtained commercial-age birds which had already been colonized by *C. jejuni* through field exposure. The 43-day-old birds were challenged with an 0.2-ml suspension of ~10^{10} to 10^{11} CFU of *Salmonella enterica* serovar Enteritidis 92 Rif^r. The rifampin-resistant *S.* Enteritidis isolate had been created by sequential passage on media containing increasing levels of rifampin. Strain *S.* Enteritidis 92 Rif^r was cultured overnight in Endo's medium containing 100 μg/ml rifampin. The birds were provided feed and water *ad libitum*. The three treated groups of broilers were provided one of the three levels of bacteriocin L-1077 per liter in drinking water for 3 days. In the second therapeutic trial, groups of 40-day-old broilers, commercially colonized with *C. jejuni* and challenged with *S.* Enteritidis 92 Rif^r, were treated for 1, 2, or 3 days with 12.5 mg bacteriocin L-1077/liter in their drinking water.

Microbiological sampling. At designated times (after day 1, 2, or 3 of treatment), groups of broilers were sacrificed and weighed. The average weights of all the birds within each group together with the amount of water that they consumed were used to estimate the total average amount of bacteriocin provided to the groups of broilers. The birds were dissected to aseptically remove the ceca, livers, and spleens of the animals. The chilled tissues were returned directly to the laboratory and were subjected to 10-fold serial dilutions and plated onto the following media and conditions: (i) MRS agar for 24 h at 37°C anaerobically, (ii) Campy-Cefex agar (19) for 48 h at 42°C microaerobically, or (iii) Endo agar plus 100 μg rifampin/ml for 24 h at 37°C aerobically, to estimate levels of lactic acid bacteria, *C. jejuni*, and *S.* Enteritidis, respectively.

TABLE 1. Biochemical purification of bacteriocin L-1077 using specified procedures

Sample purification step	Protein (mg/ml)	Sp act (AU/mg protein) against *C. jejuni*	Purity (%)
Culture supernatant	1.3	435	0
Gel filtration on Superose 12 HR 10/30	0.02	80,000	13.3
Binding to SP-Sepharose FF	0.015	107,000	76.4
Hydrophobic-interaction chromatography on Phenyl Sepharose 6 Fast Flow	0.007	229,000	95.7

RESULTS

After ammonium sulfate protein precipitation of the fermentation culture supernatant, bacteriocin L-1077 activity was low and the protein concentration was relatively high (Table 1). Each succeeding purification step increased the specific activity against *C. jejuni* and reduced the contaminating nonbacteriocin proteins. A 95.7% purity of the bacteriocin was ultimately achieved, and the specific activity was 229,000 absorbance units (AU)/mg of protein. This purified material was subjected to polyacrylamide gel electrophoresis (PAGE; data not shown) to further purify the molecule. The peptide band was then subjected to Edman degradation, and the characteristic YGNGV consensus sequence typical of class IIa bacteriocin was observed for bacteriocin L-1077. The MALDI-TOF mass spectrometry (MS) analysis indicated the molecular mass of bacteriocin L-1077 as 3,454 Da with an isoelectric point of pI 9.1. The chemically determined molecular mass and pI are in relatively close agreement with the predicted values of 4,000 Da and 9.8, respectively, based on the amino acid sequence of bacteriocin L-1077. The amino acid sequence is TNYGNGV GVPDAIMAGIIKLIFIFNIRQGYNFGKKAT. The bacteriocin is composed of 15 hydrophobic residues (A, I, L, F, and V) and 9 polar residues (N, Q, T, and Y) with one acidic (D) and four basic (K and R) amino acids. Bacteriocin L-1077 is predicted to have a charge of 2.91 at a pI of 7. The interior of the peptide from residues 9 through 25 is hydrophobic while the N-terminal and C-terminal regions are hydrophilic. No peptides similar to bacteriocin L-1077 were discovered following BLAST analyses.

The MIC values observed for the 32 pathogenic bacteria tested ranged from 0.09 to 1.5 μg/ml (Table 2). The wide genetic panorama among these tested susceptible isolates is significant. Many of the Gram-negative isolates tested approached the lower MIC values which are reported, but the Gram-positive *Listeria monocytogenes*, *Staphylococcus*, and *Clostridium perfringens* isolates also manifested sensitivities similar to that of bacteriocin L-1077. Each of the isolates tested represents consequential etiologic agents causing a variety of human diseases or pathological poultry and livestock infections (9, 17).

The therapeutic value of bacteriocin L-1077 in broilers infected with *C. jejuni* and *Salmonella* Enteritidis 92 Rif^r is rep-

TABLE 2. Antimicrobial activities of bacteriocin L-1077 against selected pathogens, as determined with a 10-μl spot test[a]

Strain no.	Test strain	MIC of L-1077 (μg/ml)
1	*Salmonella* Enteritidis 1	0.19
2	*Salmonella* Enteritidis 4	0.38
3	*Salmonella* Enteritidis 204	0.38
4	*Salmonella* Enteritidis 237	0.19
5	*Salmonella* Choleraesuis 434/4	0.38
6	*Salmonella* Choleraesuis 370	0.19
7	*Salmonella* Typhimurium 483/6	0.19
8	*Salmonella* Typhimurium 44/8	0.19
9	*Salmonella* Gallinarum-Pullorum	0.19
10	*Escherichia coli* HB 101	0.19
11	*E. coli*-600	0.19
12	*E. coli* O157:H7 Y-63	0.19
13	*E. coli* O157:H7 G-35	0.19
14	*E. coli* O157:H7 OD 30	0.19
15	*E. coli* O157:H7 T lab 39	0.19
16	*Yersinia enterocolitica* 03	0.76
17	*Yersinia enterocolitica* 09	0.76
18	*Yersinia enterocolitica* 11	0.76
19	*Citrobacter freundii*	0.76
20	*Klebsiella pneumoniae*	0.76
21	*Shigella dysenteriae*	0.76
22	*Staphylococcus aureus*	0.76
23	*Staphylococcus epidermidis*	0.76
24	*Yersinia pseudotuberculosis* 4	0.76
25	*Yersinia pseudotuberculosis* 99/14	0.76
26	*Pseudomonas aeruginosa* 508	0.38
27	*Proteus mirabilis*	0.38
28	*Morganella morganii*	0.38
29	*Listeria monocytogenes* A 9-72	0.19
30	*Proteus vulgaris* 7A	1.5
31	*Clostridium perfringens* 13124	0.7
32	*Campylobacter jejuni* L-4	0.09

[a] Test strains were surface plated onto agar plates, successive one-half-concentration dilutions were spotted onto the plates, and the plates were incubated to determine inhibitory concentrations.

resented in Tables 3 and 4. The data from the first experiment are presented in Table 3 and demonstrate the effectiveness of dosing birds with 5.5 to 21.3 mg/bird for 3 days to dramatically reduce both agents. The broilers weighed approximately 2 kg when the samples were taken. In this experiment, the nontreated control birds were intestinally colonized by *C. jejuni* in excess of 10^6 CFU/g and by *S.* Enteritidis 92 Rif^r in excess of 10^8 CFU/g of cecal content. Both the liver and spleen parenchymal organs of these birds were colonized by *S.* Enteritidis 92 Rif^r at $\geq 10^8$ CFU/g. The lactic acid bacteria were present at $\geq 10^{8.5}$ CFU/g of cecal content. In the group of birds provided 21 mg bacteriocin, *C. jejuni* was not detected in the ceca of any of the experimental broiler group. Likewise, the presence and levels of *S.* Enteritidis 92 Rif^r in the ceca, livers, and spleens of these treated birds were dramatically reduced compared with those in the positive-control group of broilers.

In the final experiment, commercial market-age birds already colonized with *C. jejuni* were also challenged and colonized with *S.* Enteritidis 92 Rif^r. Groups of broilers were treated with 12.5 mg bacteriocin L-1077/liter of drinking water over a 3-day study period (Table 4). A 4-$\log_{10}$ reduction in numbers of *C. jejuni* within the cecal contents was observed in each group over the 3 days of treatment. *Salmonella* Enteritidis was similarly reduced by >4 $\log_{10}$ in the ceca after 1 day of treatment and further reduced with two additional days of bacteriocin treatment to nearly nondetectable levels. Three- to 4-$\log_{10}$ *S.* Enteritidis reductions were observed in both the liver and the spleen samples after only 1 day of bacteriocin treatment, and further reductions occurred with each additional day of treatment, approaching nondetectable levels after 3 days of treatment. Finally, the lactic acid bacterial counts in the ceca of the treated birds were approximately the same as those observed in the control group or even had increases in cell densities of this general bacterial grouping within the tissue.

DISCUSSION

It is imperative to develop new innovative approaches for controlling food-borne bacterial disease-causing agents with the widely observed increase in antibiotic resistance among all bacterial groups (7, 12). Consequently, our research has focused on a search for new antimicrobials with emphasis on bacteriocins, antimicrobial peptides produced by generally recognized as safe (GRAS) bacterial entities (10, 21, 23). These bacteriocins could be utilized to control bacterial pathogens in poultry and potentially have positive physiological effects on avian gut morphology (3). The bacteriocin L-1077 can be classified as a class IIa pediocin-like antimicrobial peptide that contains the amino acid sequence YGNGV motif but lacks the XCXXXXVXV sequences with no C and V downstream residues. Consequently, L-1077 contains the hydrophilic, cationic N-terminal "pediocin box" region that is hypothesized to be the cell wall binding portion of the molecule (4). Although L-1077 has the YGNGV motif and a structure similar to those of other class II bacteriocins, it was not recognized as being similar to other peptides in the databases by BLAST analysis. Consequently, this is another novel peptide.

As with each of our four previously reported bacteriocins, substantial reductions (>4 $\log_{10}$ CFU) of *Salmonella* Enteritidis and/or *Campylobacter jejuni* in broiler ceca were therapeutically mediated by these four antimicrobial peptides (10, 20, 21, 23). The selected bacteriocin-producing organisms were produced by Gram-positive *Paenibacillus polymyxa*, *Lactobacillus salivarius*, *Enterococcus durans/faecium/hirae* group, and *Enterococcus faecium* isolates. Each isolate, derived from healthy adult broiler chicken intestinal material, was initially selected because of anti-*Campylobacter* activity. As *C. jejuni* is a Gram-negative organism which does not use carbohydrates as its energy source, the selective medium employed contained limited (0.1% glucose) amounts of sugar, and this precluded organic acid production as the mechanism for *Campylobacter* inhibition. Using this physiologic tool, our group has been able to select and identify unique bacteriocin-producing isolates. As directly fed, live bacteria, these isolates have been unable to therapeutically control *C. jejuni* in the gut, but the purified bacteriocin elicited the desired control mechanism required to mediate favorable control (22). We have learned that one mechanism for *C. jejuni* control in the live broiler is independent of the antagonistic cell and, rather, appears to be primarily dependent on the secreted and purified bacteriocin.

The MIC values listed in Table 2 indicated the high potency of bacteriocin L-1077. *Campylobacter jejuni* L-4 was the most sensitive isolate tested, with a MIC of 0.09 μg/ml, and *Proteus*

TABLE 3. Therapeutic effects of 3-day BCN L-1077 treatments, of various concentrations, against a mixed infection of *Campylobacter jejuni* and *Salmonella* Enteritidis 92 Rif^r in 43-day-old broilers[a]

Chicken group (conditions)	Broiler	BCN dose (mg/bird)	Log_{10} CFU/g organ homogenate (after treatment)				
			Lactic acid bacteria in cecal content	*C. jejuni*		*S.* Enteritidis	
				Liver	Spleen	Liver	Spleen
Control group (commercial feed; bacteriocin-free water)	1	0	6.79	4.86	8.65	8.60	8.60
	2		6.79	4.26	8.08	8.49	8.46
	3		8.57	4.80	8.56	7.90	8.28
	4		8.45	7.48	8.38	7.85	8.36
	5		8.11	5.26	8.46	8.52	8.59
	6		8.40	7.36	8.32	8.60	8.43
Avg (no. of birds averaged)			7.85 (6)	6.96 (6)	8.45 (6)	8.41 (6)	8.46 (6)
Exptl group 1 (commercial feed; 25 mg bacteriocin L-1077/liter)	7	21.3	8.15	ND[b]	ND	ND	4.61
	8		8.15	ND	4.15	4.23	ND
	9		8.76	ND	3.81	ND	ND
	10		7.56	ND	ND	ND	ND
	11		8.60	ND	ND	ND	ND
	12		7.30	ND	ND	ND	ND
	13		8.30	ND	2.76	ND	ND
	14		7.28	ND	2.60	ND	ND
Avg (no. of birds averaged)			8.28 (8)	ND (8)	ND (4), 2.69 (2), 3.81 (1), 4.15 (1)	ND (7), 4.23 (1)	ND (7), 4.61 (1)
Exptl group 2 (commercial feed and water with 12.5 mg BCN L-1077/liter)	15	10.9	8.46	ND	ND	3.43	ND
	16		7.60	ND	ND	ND	ND
	17		8.95	ND	ND	ND	ND
	18		8.81	ND	2.81	ND	ND
	19		8.04	ND	ND	ND	ND
	20		6.85	ND	ND	ND	ND
	21		7.77	ND	ND	ND	1.95
	22		8.08	ND	2.34	ND	ND
Avg (no. of birds averaged)			8.43 (8)	ND (8)	ND (6), 2.63 (2)	ND (7), 3.43 (1)	ND (7), 1.95 (1)
Exptl group 3 (commercial feed and water with 6.25 mg BCN L-1077/liter)	23	5.5	7.30	ND	ND	ND	ND
	24		8.11	ND	ND	ND	ND
	25		8.69	ND	ND	ND	ND
	26		6.52	ND	3.08	ND	ND
	27		8.60	ND	4.11	4.23	4.28
	28		9.26	ND	3.34	4.46	4.43
	29		8.30	ND	3.38	4.32	4.23
	30		7.83	ND	ND	4.32	4.15
Avg (no. of birds averaged)			8.59 (8)	ND (8)	ND (4), 3.28 (3), 4.11 (1)	ND (4), 4.34 (4)	ND (4), 4.28 (4)

[a] All birds were commercially colonized with nonspecified *C. jejuni*.
[b] ND, none detected; detection limit of >100 cells.

vulgaris 7A was the least sensitive of the isolates tested, at a highly acceptable MIC of 1.5 μg/ml. The pathogens tested manifest their virulence in both gastrointestinal and systemic infections. The Shiga-like toxin expressed by the isolates of *Escherichia coli* O157:H7 might initially be established within the intestinal tract, and the toxin might subsequently cause a more disseminated pathology. *Salmonella* serovar Enteritidis and *Salmonella enterica* serovar Choleraesuis will initially colonize the gastrointestinal (GI) tract of poultry and swine, respectively, but then translocate to infect the parenchymal organs of the host. The other pathogens have their unique mechanisms and sites of infection. Therefore, as observed with the *S.* Enteritidis 92 Rif^r strain in our chicken experiments, the BCN treatment dramatically reduced extraintestinal infections associated with the low MIC values. There appears to be an association of low MIC values with *in vivo* therapeutic control of bacterial infections with bacteriocin L-1077. Further application of bacteriocin to other bacterial infections *in vivo* remains to be explored. In our rudimentary attempts to extend BCN applications, we have shown modest therapeutic success against *Mycobacterium tuberculosis* using a mouse model (18). Further refinement in the delivery of bacteriocins to various sites of infection will be required.

Data were obtained with a young broiler chicken model. The data in Table 3 are derived from an experiment in which we provided different concentrations of bacteriocin L-1077 to infected broilers over a 3-day treatment. Table 4 provides data for a similar experiment in which a single fixed quantity of bacteriocin (25 mg/liter) was administered in drinking waters. These birds were sacrificed at daily intervals after 1 to 3 days of treatment. All levels of treatment greatly reduced the systemic *S.* Enteritidis infections (Table 3), as did all durations of treatment (Table 4). These observations are notable because bacteriocin was able to survive the acidic GI tract environment

TABLE 4. Therapeutic effects of 1 to 3 days of BCN L-1077 (12.5 mg/liter) treatment against a mixed infection of *Campylobacter jejuni* and *Salmonella* Enteritidis 92 Rif^r in 40- to 42-day-old broilers[a]

Chicken group (conditions)	Broiler	Bacteriocin dose (mg/bird)	Log_{10} CFU/g organ homogenate (after treatment)				
			Lactic acid bacteria in cecal content	*C. jejuni*		*S.* Enteritidis	
				Liver	Spleen	Liver	Spleen
C. jejuni- and *Salmonella* Enteritidis-colonized, untreated control birds	1	0	8.48	7.00	8.34	8.41	8.61
	2		8.36	6.04	8.08	8.66	8.73
	3		8.68	3.30	8.41	8.36	8.41
	4		8.13	6.30	8.26	8.40	8.41
	5		8.20	5.36	7.79	8.32	8.15
	6		8.30	8.08	7.78	8.59	8.32
Avg (no. of birds averaged)			8.53 (6)	7.15 (6)	8.18 (6)	8.48 (6)	8.48 (6)
C. jejuni and *Salmonella* Enteritidis colonized (treated for 1 day with bacteriocin L-1077)	7	3.75	8.85	3.89	ND[b]	6.43	6.48
	8		6.74	ND	ND	3.54	3.85
	9		7.68	ND	3.26	3.71	3.78
	10		9.00	3.81	3.57	6.54	6.89
	11		7.56	3.95	ND	6.81	6.45
	12		8.84	2.65	3.72	6.60	6.74
	13		8.76	3.74	ND	6.90	6.91
	14		8.34	ND	3.15	6.79	6.40
Avg (no. of birds averaged)			8.61 (8)	ND (3), 2.65 (1), 3.86 (4)	ND (4), 3.48 (4)	3.63 (2), 6.71 (6)	3.82 (2), 6.69 (6)
C. jejuni and *Salmonella* Enteritidis colonized (treated for 2 days with bacteriocin L-1077)	15	7.5	8.70	3.04	ND	ND	3.49
	16		8.91	3.57	ND	3.56	ND
	17		8.77	3.83	ND	3.28	3.40
	18		9.18	3.76	ND	3.40	3.23
	19		7.68	3.54	ND	3.62	3.46
	20		8.99	2.98	ND	3.30	3.32
	21		8.66	3.82	ND	3.71	3.43
	22		9.04	3.34	2.08	3.18	3.18
Avg (no. of birds averaged)			8.88	2.98 (1), 3.62 (7)	ND (7), 2.08 (1)	ND (1), 3.48 (7)	ND (1), 3.38 (7)
C. jejuni and *Salmonella* Enteritidis colonized (treated for 3 days with bacteriocin L-1077)	23	11.25	8.08	3.0	ND	ND	ND
	24		7.67	2.79	ND	ND	ND
	25		8.00	ND	ND	ND	ND
	26		8.65	3.60	ND	ND	3.23
	27		7.87	3.23	2.15	3.41	3.15
	28		8.75	3.43	ND	ND	ND
	29		9.32	3.40	ND	ND	ND
	30		8.81	3.18	ND	ND	ND
Avg (no. of birds averaged)			8.71 (8)	ND (1), 2.79 (1), 3.34 (6)	ND (7), 2.15 (1)	ND (7), 3.41 (1)	ND (6), 3.19 (2)

[a] Birds were challenged and colonized with 1.3×10^{10} CFU of *S.* Enteritidis 92 Rif^r. All birds were commercially colonized with nonspecified *C. jejuni*.
[b] ND, none detected; detection limit of >100 cells.

and protease activities within the intestinal tract and penetrate the intestinal wall following oral ingestion. Perhaps the bacteriocin was transported (likely through the blood) to the spleen and liver where the *S*. Enteritidis infection was prominent. The bacteriocin may also have triggered an immune response of the chicken to better control the pathogen. The orally administered bacteriocin was able to induce its bactericidal activity in those two organs as well as in the cecum. The bactericidal influence upon *C. jejuni* within the GI tract was less pronounced than what was seen for both *C. jejuni* L-4 and NCTC 11168 isolates under *in vitro* conditions. The *C. jejuni* infections acquired during commercial production contained isolates more resistant than the laboratory isolates. Thus, we observed only 4-log_{10} reductions as reported in Table 4 while the birds enrolled in the trial, reported in Table 3, showed reductions to nondetectable levels of *C. jejuni*. Reductions of 3 log_{10} on processed poultry are postulated to substantially reduce human health risks for *Campylobacter* (16). The lactic acid bacteria are widely considered to provide a beneficial component to the intestinal profile found in the healthy warm-blooded host, which may have also contributed to infection control. We did not explore whether the bacteriocin treatment killed individual isolates among the lactic acid bacterial category, but we did observe that quantitative influences of bacteriocin provided only increased levels of lactic acid bacteria. Even if some of these organisms were killed by the bacteriocin treatment, the resistant portion of this bacterial group was able to quickly replace that part of the microflora.

We describe the isolation of a *C. jejuni* antagonist, *L. salivarius* isolate L-1077, that produced bacteriocin L-1077. The bacteriocin was purified and characterized as an antimicrobial polypeptide 37 amino acids in length with a 3,454-Da molecular mass. The isoelectric point of bacteriocin L-1077 was pI 9.1, and its nitrogen-terminal amino acid sequence of YGNGV

was indicative of its class IIa bacteriocin assignment. MICs of bacteriocin L-1077 against 33 Gram-negative and Gram-positive bacterial isolates ranged from 0.09 to 1.5 μg/ml. The therapeutic benefit of bacteriocin L-1077 was demonstrated in market-age broiler chickens colonized with both *C. jejuni* and *Salmonella* Enteritidis. Compared with untreated control birds, both *C. jejuni* and *S*. Enteritidis counts in colonized ceca were diminished by >4 logs, and *S*. Enteritidis numbers in both the liver and the spleen of treated birds were reduced by 6 to 8 logs/g below those in the nontreated control birds. Bacteriocin L-1077 appears to hold promise to control *C. jejuni*/*S*. Enteritidis in commercial broiler chickens.

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