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Research Article

Behaviour and Biological Control of Bacteriocin-Producing Leuconostocs Associated with Spoilage of Vacuum-Packaged Sucuk

Özlem OSMANAĞAOĞLU*
Department of Biology, Faculty of Arts and Science, Gazi University,
06500, Teknikokullar, Ankara - TURKEY

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Abstract: Non-motile, Gram-positive and catalase-negative anaerobic Leuconostoc sp. were found to be involved in the spoilage of vacuum-packaged refrigerated sucuk samples. The spoilage was associated with an accumulation of large quantities of foul-smelling gas and purge in the bag and loss of colour and texture in the meat. Many of these isolates produce bacteriocins, to which the predominant Gram-positive bacteria normally found in these products are sensitive. As the leuconostocs grew at low temperatures and produced bacteriocins, they inhibited the growth of associated sensitive bacteria. Attempts to inoculate purge from spoiled samples into fresh meat, vacuum packaging and refrigeration storage facilitated the growth of Leuconostocs and produced characteristic meat spoilage.

Key Words: Lactic acid bacteria (LAB), Leuconostoc sp., food-spoilage, bacteriocin

Vakum ile Paketlenen Sucuk Örneklerinde Bozulmaya Sebep Olan ve Bakteriyosin Üreten Leuconostoc Türlerinin Davranışları ve Biyolojik Kontrolleri

Özet: Hareketsiz, Gram-pozitif, katalaz negatif ve anaerob Leuconostoc türleri vakum ile paketlenerek buzdolabında saklanan sucuk örneklerinde bozulmalara sebep olmaktadır. Ürünün bozulması pakette büyük miktardaki kokulu gazın ve sıvının birikimi ile etin renginin ve yumuşaklık/sertlik derecesinin kaybı ile karakterize edilmektedir. Bu izolatların çoğu normalde bu ürünün florasına hakim olan Gram-pozitif bakterilerin hassas oldukları bakteriyosinleri üretmektedir. Leuconostoc suşları düşük sıcaklıklarda üreyerek bakteriyosin üretebildikleri için hassas bakterilerin üremelerine engel olmaktadır. Bozulmuş üründeki sıvının taze et örneğine aşılanarak vakum ile paketlenmesi ve buzdolabında saklama girişimleri Leuconostoc türlerinin bu ortamda büyümelerini hızlandırarak etin bozulma belirtisi olan karakteristik özelliklerin gelişmesine sebep olmaktadır.

Anahtar Sözcükler: Laktik asid bakterileri (LAB), Leuconostoc sp., gıda bozulması, bacteriyosin

Introduction

Many consumers today are concerned about the synthetic "chemicals" used as preservatives in food, and there is a resulting trend towards less processed food. Alternatives include vacuum packing and refrigeration of foods. However, there are concerns among the regulatory agencies and food processors that psychrotrophic food-borne pathogens and food-spoilage organisms, even when initially present in low numbers, can multiply during extended storage and make these foods unfit and unsafe for consumption under refrigeration and without oxygen (1-3). Although vacuum-packaging and refrigeration have been used to extend the shelf-life of fresh meat, those in low-oxygen permeable packing materials in particular contain Gram-positive psychrotrophic facultative anaerobic species of Leuconostoc, Lactobacillus, Carnobacterium, and Enterobacteriaceae, Brocothrix thermosphacta and Alteromonas putrefaciens (4-8). One common type of spoilage is characterized by an accumulation of gas and purge in the package with products having a sour or decayed odour. Treatments such as ionizing radiation can destroy undesirable organisms non-chemically, but may affect flavour and do not protect food against post-

* Correspondence author

treatment contamination. It is therefore important to develop strategies to prevent the survival of organisms that cause food-spoilage and food-related diseases. Naturally produced biopreservatives may be useful in such controls. One area that has generated much interest is the use of antimicrobial compounds of lactic acid bacteria (LAB) employed in food fermentation. Since LAB are commonly associated with meat and meat products, these organisms can inhibit the microflora of meat products, thereby improving both shelf-life stability and product safety. Some of the metabolites of these bacteria have an antimicrobial effect against many food-spoilage and pathogenic bacteria, and include bacteriocins, lactic acid, diacetyl and propionic acid. A few of these are already being used in foods (9-16). Among these, the use of bacteriocins as natural food preservatives has attracted great interest for extending the shelf-life of products since bacteriocin-producing LAB are frequently isolated from fermented food and feed, and are certainly already "in use". They will provide a means to control pathogenic and food-spoilage organisms in food products. By incorporating antimicrobial metabolites in vacuum-packaged fresh meats, the growth of bacteria associated with food-spoilage and food-borne disease can be reduced. However, to exploit the potential of bacteriocin-producing cultures, more research is required into both new and established bacteriocin activities, and the results of these studies could be beneficial in the use of these compounds to enhance the safety of minimally processed refrigerated foods. Bacteriocins are defined as "agents produced by certain bacteria that inhibit or kill closely related species [of bacteria]" (17). Currently, many studies are being completed on the production and use of these bacteriocins as "natural food biopreservatives to control spoilage and pathogenic bacteria" (18). However, before a bacteriocin is considered for application in food, its antimicrobial spectrum, biochemical and genetic characteristics, effectiveness in food systems and regulatory implications should be established.

The objectives of this study were to examine some Leuconostoc species associated with food-spoilage for the possible production of bacteriocins, and to study both the physicochemical characteristics of these bacteriocins and the sensitivity of the isolates to bacteriocins of some homofermentative LAB such as *Pediococcus pentosaceus* Pep1 and *Lactococcus lactis* subsp. *lactis* OZ1, which can be used to develop a possible biological control system against isolates.

Materials and Methods

Examination of commercial spoiled vacuum-packaged sucuk. A total of 10 commercially produced vacuum-packaged low-heat processed Turkish sucuk samples showing gas and fluid (purge) accumulation in the bags were collected from local supermarkets. The samples were examined for colour and texture changes and fluid accumulation. The bags were opened, the gas was examined for odour, and the purge was used for bacteriological examination and inoculation into fresh meat.

Isolation of bacteriocin-producing Leuconostocs. Each bag was opened, and 1 ml of the purge was taken and serially diluted. Samples from three to four consecutive dilutions were pour plated in APT agar media (Difco Laboratories Inc., Detroit, MI). The plates were incubated at room temperature (22 to 25 °C) for five days. Plates containing 50 to 100 colonies were overlaid with 5 ml TGE (sigma) soft agar seeded with one of the five indicator bacteria: *Lactobacillus plantarum* NCDO 955, Leuconostoc *mesenteroides* Ly, *Pediococcus acidilactici* LB-42, *Enterococcus faecalis* MB1 and *Lactococcus lactis* subsp. *lactis* OZ1 (19-21). The plates were further incubated at 30 °C for 24 h and examined for a zone of growth inhibition around the colony. Representative colonies showing such zones were purified and examined for morphology using a phase contrast microscope and Gram stain characteristics. They were again grown in TGE buffer broth for 24 h at 30 °C. The heat-treated supernatants, with and without catalase treatment, were examined for zones of growth inhibition on the lawn of the five indicator bacteria (19-21). Those inhibiting at least three of the indicators were assumed to have a wider antimicrobial spectrum and were used in this study (Table 1).

Biochemical tests for characterization of the isolated strains. The isolates were examined for carbohydrate fermentation patterns and other characteristics to determine their genera and species (6, 22). Carbohydrate fermentation tests were carried out in TGE buffer agar plates containing 0.02% chlorophenol red as an indicator. In TGE buffer agar, membrane-filtered carbohydrates at 1% level were supplemented in place of glucose. Each strain was grown in TGE buffer broth for 24 h at 30 °C. The cells were harvested and washed twice with sterile 5 mM phosphate buffer, pH 7.0. The cells were resuspended in the same buffer to

Table 1. Inhibitory spectrum of the bacterial isolates obtained from spoiled vacuum-packaged sucuk samples.

	Inhibitory zones against indicator[b]				
Isolates designation[a]	Lp	Lm	Ped	E	Lac
OZ-D1	+	+	+	+	+
OZ-D2	+	+	+	+	+
OZ-K1	+	+	+	+	+
OZ-K2	+	+	+	+	+
OZ-N1	+	+	+	+	+
OZ-N2	+	+	+	+	+
OZ-N3	-	+	+	+	+

[a]The first alphabetic designation (OZ) indicates that they are our isolates and the second alphabetic designation indicates products made by different producers. The numerical designation after the same letter indicates separate samples from the same producer. [b]Isolates showing presence (+) or absence (-) of zone of growth inhibition around the colonies against *Lactobacillus plantarum* NcDO955 (Lp), Leuconostoc *mesenteroides* Ly (Lm), *Pediococcus acidilactici* LB42 (Ped), *Enterococcus faecalis* MB1 (E) and *Lactococcus lactis* subsp. *lactis* OZ1 (Lac) as indicators. All indicator strains were propagated in TGE broth at their optimum temperature.

their original volume and 5 µl of each strain was inoculated by stabbing in the agar media in the plates. The plates were incubated under anaerobic conditions at 25 °C for up to five days and examined for change in indicator colour. Dextran formation and arginine hydrolysis were carried out according to the outlined procedure (18,22). To determine growth at different temperatures, TGE buffer broth tubes were inoculated with the isolates and incubated at 4 °C for two days, 25 °C for 32 h and 37 °C for 24 h. Growth was visually examined from the turbidity of the culture broths. Each test was done at least twice, and the species of each isolate was determined by comparing the reaction profiles with published data (6).

Partial purification of bacteriocin of Leuconostoc sp. by adsorption onto producer cells and the nature of antimicrobial materials. Leuconostocs were grown in TGE buffer broth at 30 °C for 32 h. Semi-purified bacteriocins were obtained and freeze-dried according to the outlined procedure (19,23,24). The retention of activity following exposure to heat, pH, organic solvents and enzymes were examined accoding to reported methods (19,23-25).

Antimicrobial activity of bacteriocin. Leuconostoc strains were grown in TGE buffer broth at 30 °C for 24 h, and the culture broths were centrifuged to remove the cells. The supernatants were adjusted to pH 5 and heated (95 °C for 5 min). Selected strains of Gram-positive bacteria, many of which are associated with food-spoilage and food-related health hazards, were used as indicators to determine their sensitivity or resistance to the bacteriocin-containing heated broth cultures. The disc assay method using 25 µl of broth per disc was used over a lawn of each indicator strain on agar plates (19,26). The plates were incubated for 24 h at 30 °C and examined for the presence or absence of zones of growth inhibition. In addition, the heated culture broths were assayed for the activity unit (AU)/ml using *L. mesenteroides* Ly as an indicator (19,26).

Production and stability of bacteriocins. The relationship between the cell mass and the culture broth pH on the level of bacteriocin production by *L. carnosum* OZ-P2 during 24 h incubation was studied. The samples were grown in TGE buffer broth at 30 °C for up to 24 h. At selected intervals up to 24 h, samples were tested for OD at 600 nm, pH and bacteriocin level (AU/ml) (27). The results were used to determine the rate of bacteriocin production during growth. The thermal stability of bacteriocins was determined by heating 2 ml (10 mg/ml) freeze-dried semi-purified bacteriocin preparations at 100 °C for 15, 30 and 60 min, or by autoclaving at 121 °C for 15 min, cooling and assaying for activity. For storage stability, freeze-dried samples were kept at 4, 25 or –20 °C; samples were withdrawn periodically and assayed for antimicrobial activity.

Sensitivity of the isolates to other bacteriocins. The isolates which were used as lawns on TGE buffer agar plates were tested for sensitivity to other bacteriocins: 5 µl of heated culture broths of all Leuconostocs were tested against the lawn of all isolates. The plates incubating at 30 °C for 24 h were examined for the presence or absence of the zone of growth inhibition. In addition, 5 µl of each semi-purified nisin, pediocin P, bacteriocin of *L. carnosum* as well as pH adjusted (pH 5) and heated (95 °C for 5 min) TGE culture broths of *L. lactis* subsp. *lactis* OZ1 were used (19).

Inoculation of fresh meat. Fresh beef semitendinosus muscles were trimmed of fat and cut aseptically into 50 or 100 g portions in our facility. The portions were heated at 100 °C for 15 min, and after cooling each portion was placed in an oxygen-impermeable sterile plastic bag. The samples were inoculated with 1 ml of

purge from a spoiled vacuum-packaged sucuk, and stored at 1 to 4 °C in darkness for up to 15 days following vacuum sealing. The controls were minced meat without the inoculum of the purge. The samples were then removed at selected intervals during storage and examined for gas accumulation, changes in colour, texture and odour and the predominant microflora (by phase contrast microscopy).

Results

The results of our studies indicate that the spoilage of low heat-processed vacuum-packaged refrigerated sucuk is quite common but varies greatly with different brands. The predominant spoilage characteristics observed are accumulation of gas and cloudy purge in the packages, creamy growth on meat surfaces, and a slight sour to decayed odour. Several brands of spoiled vacuum-packaged sucuk samples were collected from local supermarkets, and the purge as well as the creamy growth were examined. Following the pour plating of purge in APT agar medium, the colonies of isolates producing inhibitory zones against at least three indicators were purified and designated on the basis of sources and sample numbers (Table 1). Only seven out of 28 isolates which were found to be capable of producing zones of growth inhibition against all five indicators from five genera were selected and further examined. The rest of the isolates produced zones against only one or two indicators and were not included in this study. During phase contrast microscope study of the purge, the predominant morphological types of the isolates were found to have lenticular cells arranged either in diploid or short chains and were non-motile and non-sporulating. Subsequent observations of Gram staining and a comparison of the biochemical reaction profiles with published data suggested that six of the seven isolates (OZ-D1, D2, K1, K2, N1, N2) were *L. carnosum* and one (OZ-N3) was *L. mesenteroides* (Table 2) (7).

In order to determine the bacteriocin nature of the antimicrobial substances of the isolates, retention of the activity of semi-purified freeze-dried extracts of Leuconostocs was tested after several treatments (Table 3), and the antimicrobial activities of Leuconostocs were retained after treatment with many physical and chemical agents, but were destroyed following treatment under incubation at pH 10 and with proteolytic enzymes such as chymotrypsin, ficin, papain, proteinase K, proteinase IV, proteinaseXIV, proteinase XXIV and trypsin. Untreated samples, or samples containing heat-inactivated enzymes, showed no loss of activity. Inactivation of antimicrobial activity by proteolytic enzymes suggested that the substances could be antimicrobial peptides or bacteriocins. Treatment with RNase, lysozyme or catalase did not affect the activity. Treatment with lipase, amylase, dextranase, cellulase or organic solvents did not cause any apparent loss of bacteriocin activity, indicating that lipid and carbohydrate moieties were absent in the molecule and therefore not responsible for inhibitory activity. Bacteriocins of Leuconostocs retained their biological activity after exposure to pH values of 2 to 12. When a bacteriocin was kept at 25 °C for 2 h, it was found to be stable at pH levels between 2 and 12. However, its activity was lost at pH 10 and above when samples were held at 25 °C for 24 h. Bacteriocins of Leuconostocs seem to be more stable in acidic conditions as compared to basic ones. Bacteriocin preparations at an initial activity of 40,000 AU/ml retained much of their activity after high temperature treatments. The activities of bacteriocins were undiminished after 30 min at 65 °C or 10 min at 100 °C; however, bacteriocin titres decreased by about 25 or 50%, respectively, when the bacteriocin preparation was heated at 100 °C for 30 and 60 min, or sterilized at 121 °C for 15 min. Bacteriocin preparations at an initial activity of 40,000 AU/ml retained full activity when stored at –20 ºC for up to four months; but samples stored at 4 °C showed a decline from 40,000 to 30,000 AU/ml from one to four months. All strains were able to produce bacteriocin at both 4 ºC (refrigeration temp.) and 25 °C (abusive temp.). Production levels at 25 °C were two to three times higher than at 4 °C (data not shown).

Supernatants of the pH-adjusted and heated broth cultures of the isolates containing bacteriocins as well as their semi-purified bacteriocins were examined for growth inhibition against several bacterial strains, many of which are associated with food-spoilage and food-related health hazards (Table 4). The rate of bacteriocin production (AU/ml) of *L. carnosum* OZ-P2 began during the late stages of the exponential phase of the growth of the *L. carnosum* cultivated in TGE buffer broth at 30 °C as indicated by an increase in OD_{600nm} and decrease in pH (Figure). The activity reached a maximum at 16 h during the late logarithmic and early stationary phases, which

Table 2: Biochemical and physiological characteristics of the isolates.

Parameters	Reaction or characteristic profile of isolates[a]						
	OZ-D1	OZ-D2	OZ-K1	OZ-K2	OZ-N1	OZ-N2	OZ-N3
Morphology[b]	le	le	le	le	le	le	le
Gram reaction	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-
Growth at							
4 °C	+	+	+	+	+	+	+
25 °C	+	+	+	+	+	+	+
37 °C	-	-	+	-	-	-	+
Gas formation	+	+	+	+	+	+	+
Arginine hydrolisis	-	-	-	-	-	-	-
Dextran formation	-	-	-	-	-	-	+
Acid produced from							
Amygdalin	+	-	+	-	-	-	+
Arabinose	-	-	-	-	-	-	+
Arbutin	-	-	-	-	-	-	+
Cellobiose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Galactose	-	-	-	-	-	-	+
Lactose	-	-	-	-	-	-	+
Maltose	+	-	+	+	+	+	+
Mannose	+	-	+	-	+	+	+
Raffinose	-	-	-	-	-	-	+
Ribose	+	-	+	+	+	+	+
Salicin	-	+	+	-	-	+	+
Sucrose	+	+	+	+	+	+	+
Trehalose	+	-	+	+	+	+	+
Xylose	-	-	-	-	-	-	+
Genus and species[c]	Lc	Lc	Lc	Lc	Lc	Lc	Lm

[a] (+), able to or (-), unable to perform the functions. [b]all isolates have lenticular cells arranged in short chains. [c]Most likely species: Lc, Leuconostoc carnosum; Lm, Leuconostoc *mesenteroides*

suggests the dependence of bacteriocin production on cell number under these growth conditions. During extended incubation at the stationary phase, activity decreased after 24 h (data not shown). This may be due to the activity of endogenous extracellular proteases which is induced during this growth phase. The sensitivity of the isolates to semi-purified nisin (from *L. lactis* subsp. *lactis* OZ1), pediocin P (from *P. pentosaceus* Pep1), bacteriocin (from *L. carnosum*) and their own bacteriocins was tested. The isolates were found to be resistant to all the bacteriocins they produce. In addition, five and four out of seven bacteriocin-producing Leuconostoc isolates, including the *L. mesenteroides* OZ-M3 strain, were found to be sensitive to nisin and pediocin, respectively, and none were sensitive to bacteriocins of *L. carnosum*.

Minced meat was chosen as a model food system and was heated (100 °C, 15 min) before inoculation with purge to eliminate the normal microbial population in meat. The inoculated fresh meat samples showed accumulations of large quantities of gas and purge within two weeks at 1 to 4 °C, but the control samples did not

Table 3. Effect of pH, heat, organic solvents and enzymes on antimicrobial activity of Leuconostoc isolates.

Treatments	Retention of activity by Leuconostoc isolates*						
	OZ-D1	OZ-D2	OZ-K1	OZ-K2	OZ-N1	OZ-N2	OZ-N3
pH							
2	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+
10	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-
Heat							
65 °C 30 min	+	+	+	+	+	+	+
100 °C 10 min	+	+	+	+	+	+	+
Organic solvents							
Acetone	+	+	+	+	+	+	+
Acetonitrile	+	+	+	+	+	+	+
Chloroform	+	+	+	+	+	+	+
Ethanol	+	+	+	+	+	+	+
Formaldehyde	+	+	+	+	+	+	+
Hexane	+	+	+	+	+	+	+
Isopropanol	+	+	+	+	+	+	+
Enzymes							
Amylase	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
Cellulase	+	+	+	+	+	+	+
Chymotrypsin	-	-	-	-	-	-	-
Dextranase	+	+	+	+	+	+	+
Ficin	-	-	-	-	-	-	-
Lipase	+	+	+	+	+	+	+
Lysozyme	+	+	+	+	+	+	+
Papain	-	-	-	-	-	-	-
Peroxidase	+	+	+	+	+	+	+
Proteinase K	-	-	-	-	-	-	-
Proteinase IV	-	-	-	-	-	-	-
Proteinase XIV	-	-	-	-	-	-	-
Proteinase XXIV	-	-	-	-	-	-	-
Ribonuclease A	+	+	+	+	+	+	+
Trypsin	-	-	-	-	-	-	-

* Retention of activity was tested on TGE agar plates, incubating the plate for 30 °C for 16 h, examining for (+) presence or (-) absence of zone of inhibition.

show any gas even after eight weeks. The gas and the meat from the spoiled samples had an offensive foul odour resembling hydrogen sulphide. The spoiled beef had a very soft texture. This texture loss and purge accumulation could be the result of proteolysis of muscle tissue by the proteolytic enzyme(s) of this organism. Phase contrast microscopy of the purges from both commercial and inoculated spoiled meat predominantly

Table 4. Antimicrobial activity spectrum of bacteriocins of Leuconostoc isolates[a].

Indicator strains[b]	Growth conditions	Reaction to bacteriocin[c]
Lactobacillus sp.		
Lac1	TGE, 30 °C	+
Lac2	TGE, 30 °C	+
Lac3	TGE, 30 °C	+
L. sake	TGE, 30 °C	+
L. curvatus	TGE, 30 °C	+
Leuc. gelidium	TGE, 30 °C	-
Leuc. cremoris	TGE, 30 °C	+
Leuc. mesenteroides	TGE, 30 °C	+
Listeria monocytogenes NCTC 5105	TSB + 5%yeast extract,35 °C	+
L. monocytogenes	TSB + 5%yeast extract,35 °C	+
Listeria ivanovii	TSB + 5%yeast extract,35 °C	+
Listeria innocua	TSB + 5%yeast extract,35 °C	+
Yersinia enterocolitica	TSB + 5%yeast extract,35 °C	+
Bacillus subtilis	TSB + 5%yeast extract,35 °C	+
Bacillus cereus	TSB + 5%yeast extract,35 °C	+

[a]Bacteriocin preparations of Leuconostoc isolates were prepared by growing the cells in TGE-buffered broth for 32 h at 25 °C, adjusting the culture broth to pH 5.0, and heating to kill the cells. Assay was conducted in TSA plates for pathogenic organisms and TGE agar without Tween 80 for other strains. [b]All indicator strains are from our stock culture collection. [c]Since all Leuconostoc isolates showed the same response to indicator strains, a single row is used to represent the sensitivity (+) or resistance (-) of all Leuconostocs.

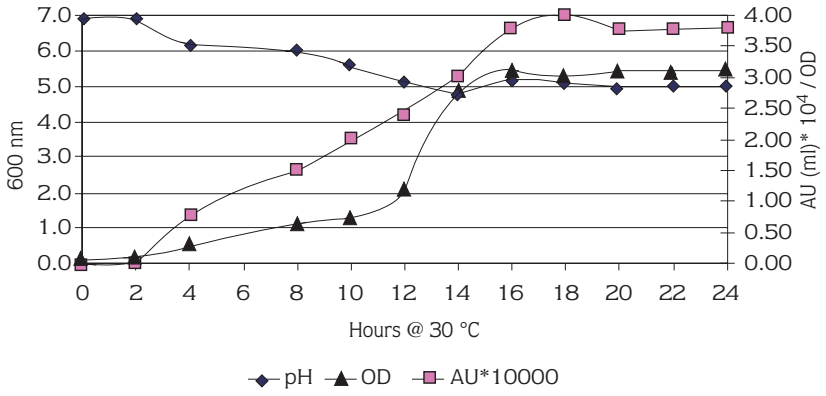

Figure. The rate of bacteriocin production (AU/ml) in relation to cell growth (OD @ 600nm) and acid production (reduction in pH) by Leuconostoc *carnosum* OZ-P2 in TGE buffered broth @ 30 °C.

showed the presence of non-motile, non-sporulating, lenticular cells arranged either in diploid or in short chains. Representative purified colonies from the plates were biochemically identified as Leuconostoc sp.

Discussion

Our studies indicate that heterofermentative leuconostocs are the predominant microflora of spoiled vacuum-packaged products. The antimicrobial activity of Leuconostocs reported here was not due to organic acids, hydrogen peroxide or bacteriophage as the heated cell-free media remained active following neutralization to pH 7.0 and treatment with catalase or peroxidase or autoclaving. The loss of antimicrobial activity following treatment with proteolytic enzymes indicated that the active component secreted extracellularly was proteinaceous. The wide spectrum of activity and

proteinaceous nature of the substance indicated that the antimicrobial substances produced by all Leuconostoc isolates were bacteriocins. In addition, the ability of Leuconostoc strains to retain their antimicrobial activity after treatment with formaldehyde or high temperature also indicates that the molecule is fairly small. Since they are heterofermentative, they do not produce large amounts of lactic acid. Therefore, by producing bacteriocins they are suspected of inhibiting the growth of other associated bacteria that are normally found in these products but sensitive to the bacteriocins. Since the sensitivity of many strains of Listeria, Lactobacillus, Leuconostoc and Carnobacteria sp. to bacteriocins produced by Leuconostoc sp. isolated from meat and meat products has been reported (18,19,28-32), an examination of the leuconostocs isolated from the spoiled products revealed that many produced bacteriocins and the ability of the heterofermentative Leuconostocs to produce bacteriocins, when combined with the storage conditions as well as product characteristics, might have given them growth advantages leading to the production of gas and other changes associated with spoilage of these products. Since leuconostocs are sensitive to heat used in processing of these products, their presence might be the result of post-heat contamination. The use of bacteriocins in combination with traditional methods of preservation in close conjunction with good manufacturing procedures and strict sanitation could be effective in controlling Leuconostocs in fresh meat, thus ensuring a safer product for consumption or further processing. It therefore would be advantageous to develop a procedure to inhibit growth of the heterofermentative leuconostocs to extend the shelf-life of these products. The results of this study have indicated that some strains of leuconostocs from commercial products showing spoilage characteristics of accumulations of gas and purge carry the bacteriocin-production trait. Except for one, *L. mesenteroides*, all of the bacteriocin-producing strains were *L. carnosum*. Evidence of the presence of these bacteria includes discoloration, decaying odours and taste, slime formation and package bloating and even rupture by CO_2 gas formation. In general, these anaerobic bacteria cause most of the characteristics of spoiled meat.

Because Leuconostocs sp. are known to cause food-spoilage in meats and meat products, more research is needed to develop measures to eliminate this organism from foods. The bacteriocins of our Leuconostoc isolates were effective against some of the spoilage bacteria found in vacuum-packaged refrigerated meats and the psychrotrophic pathogen *L. monocytogenes*. However, as the isolates are not sensitive to the bacteriocins that they themselves produce, other bacteriocins or bacteriocin-producing LAB strains will be necessary to control them. In addition, the sensitivity of these isolates to bacteriocins of homofermentative LAB such as *Pediococcus pentosaceus* Pep1 and *Lactococcus lactis* subsp. *lactis* OZ1 can be used to develop a possible biological control system against isolates, and the results of these studies could be beneficial in the use of these compounds to enhance the safety of minimally-processed refrigerated foods. The use of bacteriocin-producing starter LAB cultures has novel and potential applications in reducing the populations of Leuconostocs in fermented vegetable and dairy products as well as meats. In addition to their primary functions of improving the preservation quality and enhancing the flavour and texture of foods, bacteriocin-producing starter cultures could provide a natural and specific preservative effect targeted at particular pathogenic or spoilage organisms of concern. Since the occurrence of bacteriocin-producing LAB in foods is quite common and it has been consumed by human beings without any known adverse effects for thousands of years, the incorporation of a suitable bacteriocin or bacteriocin-producing lactic acid bacterium in foods in a purified form to extend shelf-life should pose no risk to consumers and may be regarded as safe. For this reason, a preparation containing nisin or pediocin can be used effectively to control these spoilage bacteria. Moreover, as *P. pentosaceus* Pep1 and *L. lactis* subsp. *lactis* OZ1 are homofermentative and capable of growing and producing bacteriocin against both *L. monocytogenes* and Leuconostoc sp. at low temperature, live cells of these strains in low numbers could probably be used in food systems, maybe along with some other suitable bacteriostatic agents, as a biopreservative in refrigerated foods to control the growth of the spoilage bacteria and increase the shelf-life of products.

Current studies are characterizing new bacteriocins produced by Leuconostoc and finding ways in which they may be put to use. One such study found that the reason the killing kinetics varied with pH is that the cells released the bacteriocins into solution at lower pHs. The bacteriocins were kept bound to the cell surface at higher

pH. This has shown that bacteriocins can be mass-produced on the cell surface and then released into solution for separation (19). Other studies have shown that these bacteriocins are heat resistant up to 121 °C in acidic environments (33) and are inactivated by a range of proteolytic enzymes but not catalase or lysozyme (34). These characteristics could allow the bacteriocins to be included in the packaging of foods. Further studies are continuing, including the use of recombinant DNA techniques to make novel synthetic bacteriocins and ways to implant bacteriocins into food or in packaging.

On the other hand, an anaerobic bacterium such as *L. carnosum*, which grows readily and dominates the bacteria population at refrigerated temperatures of 1-5 °C, causes food to spoil. However, the most thought provoking thing about this newly found creature is its possible beneficial uses as a natural preservative for meat and other similarly packaged foods due to its special inhibitory interaction with *L. monocytogenes*, a cause of listeriosis. *L. carnosum* could be the key to the eradication of this deadly disease from vacuum-packaged food, and one day the scourge of Listeria and the spoilage of food will be eliminated. It is amazing to think that *L. carnosum*, a cause of spoilage, could one day end up extending the shelf-life of many foods by weeks and eliminating the threat of Listeria.

Our studies will continue for an objective evaluation of the effectiveness of the bacteriocins in controlling the bacterial load of vacuum-packaged meat and its indirect relationship with bacteriological quality and spoilage potential.

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