ANTIBACTERIAL EFFECTIVENESS OF A PEDIOCIN AcH-BASED BIOPRESERVATIVE AGAINST SPOILAGE AND PATHOGENIC BACTERIA FROM VACUUM PACKAGED REFRIGERATED MEAT.

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SUMMARY

Bacteriocins of lactic acid bacteria differ greatly in bacteriocidal efficiency against Gram-positive bacteria. Even broad host range bacteriocins like pediocin AcH and nisin are not effective against many Gram-positive bacteria. However, in combination they have much higher bacteriocidal efficiency. Also both pediocin AcH and nisin became effective against sublethally injured Gram-negative bacteria. Bacteriocin-based biopreservatives have high bacteriocidal property against many bacteria associated with food spoilage and food-borne diseases. Treatment of vacuum-packaged meat products with such biopreservative preparations greatly controlled spoilage and pathogenic bacteria during extended refrigeration storage.

INTRODUCTION

Several psychrotrophic bacterial species have been predominantly involved in spoilage of vacuum packaged refrigerated fresh and processed meat products. These include strict anaerobe *Clostridium laramie* (KALCHAYANAND et al., 1989), *Leuconostoc carnosum, Leuconostoc gelidum* (SHAW and HARDING, 1989), *Carnobacterium* and *Brochothrix thermosphacta* (COLLINS et al., 1987), *Lactobacillus sake* (EGAN et al., 1989), and *Lactobacillus curvatus* (GRANT and PATTERSON, 1991). Anaerobic and facultative anaerobic psychrotrophic pathogens such as *Clostridium botulinum* type B (non-proteolytic), *Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophila* can also grow in such products (Anonymous, 1988; RAY, 1992a). Some bacteriocins of lactic acid bacteria such as nisin and pediocin AcH, are bacteriocidal to many Gram-positive bacteria including those involved in food spoilage and food-borne diseases and thus could be used as food biopreservatives (RAY, 1992 b,c). However, even these broad host range bacteriocins have limitations. They are not effective against Gram-negative bacteria and not all strains of Gram-positive bacteria. An effective bacteriocin-based biopreservative for use in refrigerated vacuum packaged meat products, should be bacteriocidal against both Gram-positive and Gram-negative spoilage and pathogenic bacteria and against most cells in a population. We present here some evidence by which this objective can be achieved.

MATERIALS AND METHODS

Bacteria strains.

Pediococcus acidilactici H for pediocin AcH and *Lactococcus lactis* subsp. *lactis* ATCC 11454 for nisin were grown in TGE broth for 16 to 18 h at 37°C and 30°C, respectively, (BISWAS et al., 1991). For testing bacteriocidal effectiveness either an ammonium sulphate precipitate or a culture broth preparation were used (BHUNIA et al., 1988; BISWAS et al., 1991). Potency was determined against *Lactobacillus plantarum* NCDO 955 and expressed as activity unit (AU)/ml of a preparation. A bacteriocin-based biopreservative (BP) was prepared to test in meat.

Bacteriological analysis.

Spoilage and pathogenic bacteria were grown in tryptic soy broth or in MRS broth overnight at about 20 to 22°C. The cultures were diluted in 0.1% peptone water to obtain desired cell concentrations. The cell suspensions were exposed to bacteriocin preparations and at selected intervals were enumerated for colony forming units (cfu)/ml by pour plating in tryptic soy agar or in TGE agar. For food inoculation study, fresh beef, beef by-products, roast beef, and frankfurters were inoculated with spoilage and pathogenic bacteria, treated with bacteriocin-based biopreservative, vacuum packaged, stored at 3°C for 4 wk and enumerated for cfu as described above.

RESULTS AND DISCUSSION

Cell suspensions (about 10^6 cells/ml) of three sensitive strains of Gram-positive bacteria were treated with either pediocin AcH

or nisin or a combination of the two in equal volume at 1600 AU/ml for 1 h at 3°C and enumerated for cfu using untreated samples as controls. Viability loss for each strain was calculated from the differences in cfu between the treated and the control samples. The results presented in Table 1 indicated that the three strains lost viability ranging from 51 to >99.9% by three treatments. However, in each strain the highest viability loss occurred when the two bacteriocins were used in combination. In a strain there were some cells that were resistant either to pediocin AcH or nisin but not to both; a preparation containing both bacteriocins killed more cells.

Cell suspensions of four strains of Gram-negative bacteria (about 10^3 cells/ml), that are normally resistant to both pediocin AcH or nisin, were sublethally injured either by freezing at -20°C for 2 h or by heating at 55°C for 15 min in the presence of the bacteriocins (4,000 Au/ml). Cell suspensions without bacteriocins were used as controls. Results in Table 2 indicated that the cells surviving the sublethal stresses (considered as 100%) became sensitive to both pediocin AcH and nisin, although sensitivity varied with species, kind of stress and type of bacteriocins. In the injured Gram-negative bacterial cells the barrier property of the lipopolysaccharide molecules in the outer membrane was impaired in such a way that bacteriocin molecules can enter inside, destabilize the inner membrane and kill the cells.

Based on the results presented in Tables 1 and 2 bacteriocin-based biopreservatives were prepared and used in pure culture and product inoculation studies. Spoilage and pathogenic bacteria in TS broth or TGE broth were treated with a biopreservative at 1% level. Controls were untreated cells. Samples were enumerated for cfu/ml after 2 h and 21 d of storage at 3° and the results were presented in Table 3. While all strains, including Gram-positive and Gram-negative bacteria, multiplied in the absence of biopreservative, the cfu of all except *Yersinia enterocolitica*, reduced greatly in its presence within 2 h; after 21 d the cfu remained very low. In *Yer. enterocolitica* the cfu reduced very little in the treated samples, but did not increase during storage. Four types of meat products were inoculated with the spoilage or pathogenic bacteria, then treated with the biopreservative at 1% level, vacuum packaged and stored at 3°C. Samples without biopreservative were used as controls. During storage up to 28 d the cfu increased in the control samples (Table 4). In the biopreservative treated samples the cfu remained very low (<1 or no cfu in 0.2 ml sample) for most samples, especially after 28 d. The reduction in cfu of the Gram-positive and Gram-negative spoilage and pathogenic bacteria, both in the pure culture and product inoculation studies, were the result of enhanced bacteriocidal property of more than one bacteriocin and the sublethal stress of the resistant strains.

CONCLUSION

(a) Bacteriocins of lactic acid bacteria, such as pediocin AcH and nisin, are more bacteriocidal against Gram-positive bacteria when used in combination. (b) Gram-negative bacteria, normally resistant to both pediocin AcH or nisin, became sensitive to the bacteriocins following sublethal treatment. (c) Bacteriocin-based biopreservatives can be prepared that are bacteriocidal against both Gram-positive and Gram-negative bacteria. (d) Treatment of biopreservatives in vacuum-packaged meat products, inoculated with spoilage and pathogenic Gram-positive and Gram-negative bacteria, greatly reduced their levels during refrigeration storage. (e) Such preparations can be used as a secondary barrier to enhance shelf-life and safety of refrigerated vacuum-packaged meat products.

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Table 1. Enhanced bacteriocidal activity of bacteriocins in combination.

Bacterial species	% viability loss following treatment with		
	Pediocin AcH	Nisin	Pediocin AcH + Nisin
List. monocytogenes	94	99	>99.9
Leuconostoc spp.	51	62	93
Lactobacillus spp.	57	71	85

Table 2. Bacteriocidal effect of bacteriocins against sublethally stressed Gram-negative bacteria.

Bacterial Species[a]	Sublethal stress	% viability loss of sublethally-injured cells by	
		Pediocin AcH	Nisin
Sal. typhimurium	Freezing	87	>90
	Heating	>90	34
Yer. enterocolitica	Freezing	90	>90
	Heating	>90	>90
Es. coli	Freezing	>90	58
	Heating	>90	84
Ps. fluorescens	Freezing	>90	>90
	Heating	>90	90

[a]See Table 3 for explanations

Table 3. **Bacteriocidal effectiveness of bacteriocin-based biopreservatives against pure cultures of Gram-positive and Gram-negative bacteria.**

Bacterial strains[a]	Days at 3°C	Log_{10} cfu/ml following treatment with None (control)	BP[b]
List. monocytogenes CA	0[c]	4.1	<1[d]
	21	7.7	<1
Lactobacillus spp.	0	3.0	2.2
	21	6.4	<1
Leuconostoc spp.	0	3.8	3.2
	21	4.1	<1
Yer. enterocolitica	0	3.7	3.6
	21	8.2	3.2
Ps. fluorescens	0	3.0	3.2
	21	9.1	<1

[a]Bacterial genera used: List, *Listeria*; Yer, *Yersinia*; Ps, *Pseudomonas*; *Lactobacillus* and *Leuconostoc* spp. were isolates from spoiled meat products. [b]BP: bacteriocin-based biopreservative used at 1% level. [c]0-d: Tested within 2 h after inoculation. [d]<1: No cfu in 0.2 ml of the sample.

Table 4. **Bacteriocidal effectiveness of bacteriocin-based biopreservative against Gram-positive and Gram-negative bacteria in inoculated meat products.**

Meat Products	Inoculated bacterial species[a]	Days at 3°C	Log_{10} cfu/g following treatment with None (control)	BP[b]
Fresh beef	*List. monocytogenes* CA	0[c]	2.9	1.2
		28	4.2	<1[d]
	Yer. enterocolitica	0	2.7	<1
		28	4.7	<1
Beef by-products (heart)	*List. monocytogenes* Scott A	0	3.3	<1
		28	5.4	<1
	Yer. enterocolitica	0	2.1	<1
		28	5.2	<1
Roast beef	*List. monocytogenes* CA	0	2.7	<1
		28	9.5	<1
	Yer. enterocolitica	0	2.6	<1
		28	9.0	<1
Frankfurter	*List. monocytogenes* Scott A	0	4.3	1.7
		28	10.5	<1
	Leuconostoc spp.	0	2.5	<1
		28	8.7	<1

[a]See Table 3 for explanations.