BACTERIOCIN-BASED BIOPRESERVATIVES CONTROL GROWTH OF PATHOGENIC AND SPOILAGE BACTERIA IN VACUUM-PACKAGED REFRIGERATED LOW-HEAT PROCESSED MEAT PRODUCTS

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Background

Bactericidal effect of bacteriocins of lactic acid bacteria is enhanced against foodborne pathogenic and spoilage bacteria by combining two or more bacteriocins and by inducing sublethal injury in the target cells (Kalchayanand et al., 1992; Hanlin et al., 1993; Ray, 1993; Mulet-Powell et al., 1998). This observation was extended to increase viability loss and control growth of pathogenic and spoilage bacteria in vacuum-packaged refrigerated processed meat products which harbor survivors from low-heat treatment and the post-heat contaminants. During extended refrigerated storage with occasional temperature abuse, the psychrotrophic anaerobic and facultative anaerobic bacteria can grow in these products and even from a low initial population can reach to a high level to cause spoilage and foodborne diseases (Ray et al., 1995; Ray, 1996). To ensure the safety of consumers and reduce food and financial losses, new methods are being studied to control growth of bacteria in refrigerated vacuum-packaged processed meat products.

Objectives

We have examined the effectiveness of incorporating bacteriocin-based biopreservatives in vacuum-packaged processed meats to reduce survival and control growth of commonly occurring pathogenic and spoilage bacteria during refrigerated storage of the products.

Methods

Two bacteriocin-based biopreservatives (BP) were prepared from pediocin AcH, produced by *Pediococcus acidilactici* LB42-923 and nisin A, produced by *Lactococcus lactis* ssp. lactis ATCC11454 (Yang et al., 1994). BP1 had pediocin and nisin at 1:1 ratio and BP2 had BP1 + Na-lactate. They were used in the studies at a level of about 2000 AU (activity units) of bacteriocin/g of meat products and 0.3% lactic acid. The spoilage (isolated from spoiled meat) and pathogenic (involved in foodborne incidents) bacteria (listed in the Result section and Tables) were grown in suitable broths and used as cell or spore suspensions for inoculation. The products used were roast beef, hot dogs, hams and turkey rolls, either obtained from commercial sources or prepared in our laboratory as indicated in the Result section. Two types of studies were conducted. In one, a product (about 40 g) was put in a low oxygen permeable plastic bag, inoculated first with the cell suspension or spores of a test strain and then with a BP by spreading the cells (or spores) and BP on the surfaces. The bags were then vacuum-sealed and stored at 4°C. Triplicate samples for each study and controls (no BP) were then used for enumeration of viable cfu/g in selective media specific for a species. In studies with *Clo. botulinum*, spore samples were incubated at 4 and 25°C and the extract from each was assayed for botulin by injecting to mice. In the other study, processed meat products were prepared in our laboratory by mixing BP with fresh meat and other ingredients. Following cooking, the products were put in bags, inoculated with bacterial cell suspension, vacuum-sealed, stored at 4°C and enumerated (in triplicate) for cfu/g during storage.

Results and Discussion

Spoilage of heat processed vacuum-packaged refrigerated meat products mainly results from growth on the product surface of post-heat contaminating psychrotrophic facultative anaerobic lactic acid bacteria (Ray, 1996). To simulate this, hotdogs and sliced ham were inoculated with a *Leuconostoc* and a *Lactobacillus* strain with and without BP1 or BP2, and the cfu were enumerated during 9 weeks storage at 4°C. The results in Table 1 showed both BP1 and BP2 effectively controlled the two strains in hotdogs. In sliced ham both were controlled up to 6 weeks. After 9 weeks, they grew. This could be due to nonuniform spreading of the bacterial cells and BP over the surfaces of a ham slice. Similar nonuniformity has been observed in products with large surface areas (Kalchayanand et al., 1998). The growth of *Listeria monocytogenes* strains in all four products were effectively controlled by both BP during 4 to 8 weeks storage at 4°C (Table 2). The mesophilic pathogenic strains of *Salmonella* and *Escherichia coli*, although slowly died at 4°C in roast beef, the viability losses of both were faster in the presence of BP, especially BP2, containing both bacteriocins and lactic acid (Table 3). BP2, but not BP1, also controlled growth of psychrotrophic *Yersinia* strains. The Gram-negative cells, although not normally sensitive to bacteriocins, under a stressed environment (such as 4°C and/or lactate) are injured and become sensitive to bacteriocins (Ray, 1993). *Clo. botulinum* B spores, germinated, multiplied and produced toxin at 25°C in roast beef in the absence of BP; but in the presence of BP this was greatly inhibited (Table 4). Spores are not susceptible to bacteriocins; but following germination and outgrowth, they are killed by them. In the second study, low-fat turkey breast rolls and beef hotdogs were prepared along with BP1 and BP2 (5,000 AU/g and 1.3% lactic acid). The cooked products were contaminated with *Leuconostoc* cells, vacuum-packaged and enumerated for cfu. In the sliced turkey breast rolls, processed with BP1 and BP2 and contaminated with *Leuconostoc* cells, the cfu remained low during 9 weeks storage at 4°C (Table 5). In hotdogs, they showed some growth. This could be due to failure of bacteriocin molecules to be uniformly distributed on the surface along with the cells or bacteriocin molecules failed to come out on the cooked surface of hotdogs in sufficient concentration. Meat from both products tested positive for bacteriocins during storage.

Conclusion

These results showed that bacteriocin-based biopreservatives can be formulated to control foodborne spoilage and pathogenic bacteria in low-heat processed ready-to-eat meat products to enhance their shelf-life and safety.

Pertinent Literature

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Table 1. Growth of spoilage bacteria in vacuum-packaged processed meats, inoculated with BP inside the packages during storage at 4°C.

Products/Bacteria	BP used[a]	Log_{10} cfu/g after[b] 1d	3 wk	6 wk	9 wk
Hotdogs					
Leu. mesenteroides					
Ly	None	3.5	4.8	6.6	8.8
	BP1	<1.0	<1.0	<1.0	<1.0
	BP2	1.0	<1.0	<1.0	<1.0
Lab. viridescens					
23	None	2.7	6.8	7.8	8.4
	BP1	<1.0	<1.0	<1.0	1.8
	BP2	<1.0	<1.0	<1.0	1.7

Products/Bacteria	BP used[a]	Log_{10} cfu/g after[b] 1d	3 wk	6 wk	9 wk
Sliced Ham					
Leu. mesenteroides					
Ly	None	3.1	7.9	8.5	NT
	BP1	1.9	2.2	6.1	7.4
	BP2	2.0	2.5	6.0	7.2
Lab. viridescens					
23	None	2.7	6.7	9.5	NT
	BP 1	<1.0	<1.0	1.1	6.1
	BP2	<1.0	<1.0	1.5	5.5

[a]BP: Bacteriocin-based biopreservatives. BP1 is a mixture of pediocin AcH and nisin A; BP2 is BP1 + bacterial metabolites (organic acids).
[b]Each data is the average of triplicate samples. <1.0 indicates 0 to <10 cfu detected in 0.6 g of product from three samples; NT: not tested; wk: weeks. cfu: colony forming units; detected on MRS-agar medium with pH adjusted to 5.0 with lactic acid.

Table 2. Growth of *Listeria monocytogenes* in vacuum-packaged processed meats, inoculated with BP inside packages, during storage at 4°C.

Products/ strains	BP used[a]	Log_{10} cfu/g after 1d	2 wk	4 wk	6 wk	8 wk
Roast Beef						
Scott A	None	2.9	5.4	7.5	6.9	NT
	BP1	<1.0	<1.0	<1.0	<1.0	<1.0
	BP2	<1.0	<1.0	<1.0	<1.0	<1.0
CA	None	2.7	4.6	5.4	6.6	9.5
	BP1	<1.0	<1.0	<1.0	<1.0	<1.0
	BP2	<1.0	<1.0	<1.0	<1.0	<1.0
Hotdogs						
Scott A	None	3.4	NT	3.8	5.4	7.1
	BP1	1.4	NT	<1.0	1.4	1.5
	BP2	1.3	NT	<1.0	<1.0	<1.0

Products/ strains	BP used[a]	Log_{10} cfu/g after 1d	2 wk	4 wk	6 wk	8 wk
Sliced Ham						
Scott A	None	2.9	3.2	3.2	NT	NT
	BP1	<1.0	<1.0	<1.0	NT	NT
	BP2	<1.0	<1.0	<1.0	NT	NT
Chopped Ham						
Scott A	None	3.3	NT	NT	5.4	6.4
	BP1	<1.0	NT	NT	1.9	2.0
	BP2	1.0	NT	NT	1.8	1.9

[a]BP: See Table 1 footnote.
NT: not tested; wk: weeks. Cfu was determined on Modified Oxford agar medium.

Table 3. Growth of Gram-negative pathogens in vacuum-packaged roast beef, inoculated with BP inside the packages, during storage at 4°C.

Pathogens	BP used[a]	Log_{10} cfu/g after[b] 1d	2 wk	4 wk	6 wk	8 wk
Sal. typhimurium						
ATCC	None	2.7	2.5	2.0	1.7	2.0
	BP1	2.5	1.8	1.9	1.8	2.1
	BP2	2.3	1.8	1.3	<1.0	1.3
Entpath. *Esc. coli*						
SLR 503	None	3.2	3.0	2.6	2.5	2.2
	BP1	1.5	<1.0	<1.0	1.6	1.3
	BP2	1.4	<1.0	<1.0	<1.0	<1.0
Yer. enterocolitica	None	2.6	3.5	6.7	7.7	9.0
	BP1	2.5	3.3	6.1	7.6	8.9
	BP2	<1.0	<1.0	<1.0	<1.0	<1.0

[a]BP: See Table 1 footnote. Cfu was determined on xylose-lysine-deoxycholate agar for *Salmonella*, violet red bile agar for *Escherichia*, and MacConkey agar for *Yersinia*.

Table 4. Growth and toxin production of *Clostridium botulinum* B spores in foods, inoculated with BP, during storage at 25°C as detected by mice-assay.

Foods	BP used[a]	# Positive/# Tested after[b] 3 d	7 d	10 d	14 d
Beef broth	None	0/3	3/3	NT	NT
	BP1	0/3	0/3	0/3	0/3
	BP2	0/3	0/3	0/3	0/3
Roast beef	None	1/3	2/3	2/3	3/3
	BP1	0/3	0/3	0/3	0/3
	BP2	0/3	0/3	0/3	0/3

[a]BP: See Table 1 footnote.
[b]Positive: mouse injected with the 0.5 ml trypsin extract from a sample died. NT: not tested.

Table 5. Growth of Leuconostoc mesenteroides Ly in vacuum-packaged processed meat prepared with BP during storage at 4°C.

Products	BP used[a]	Log_{10} cfu/g after 1 d	1 wk	3 wk	6 wk	9 wk
Sliced Turkey						
Roll	None	3.8	3.8	4.1	4.3	7.9
	BP1	3.7	2.7	2.2	2.0	1.8
	BP2	3.4	2.8	1.8	1.5	1.8
Hotdogs	None	3.1	3.5	6.0	8.9	9.5
	BP1	3.1	2.3	5.9	8.0	8.7
	BP2	2.8	2.9	4.2	5.4	5.8

[a]BP: See Table 1 footnote.