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Monoclonal Antibody-Colony Immunoblot Method Specific for Isolation of *Pediococcus acidilactici* from Foods and Correlation with Pediocin (Bacteriocin) Production†

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BALB/c mice were immunized with broken, heat-killed cells of *Pediococcus acidilactici* H. After murine cell fusions, one monoclonal antibody (MAb), Ped-2B2, was selected on the basis of its positive reaction with seven of seven strains tested in an enzyme-linked immunosorbent assay with whole cells of *P. acidilactici*. The MAb Ped-2B2 did not show any cross-reactions with other lactic-acid bacteria or other gram-positive or gram-negative organisms. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot (immunoblot) analysis of surface proteins of *P. acidilactici* indicated that Ped-2B2 reacted with a protein of 116 kDa. MAb Ped-2B2 was used as a probe to isolate *Pediococcus* species from fermented-meat products by colony immunoblotting. A total of 18 Ped-2B2-reactive *Pediococcus* spp. isolates were isolated from eight food samples and assayed for bacteriocin production. All of the isolates produced bacteriocins which were heat stable, proteinaceous, and inhibitory to *Lactobacillus plantarum* NCDO 955. Biochemical characterization of these isolates indicated that they were all *P. acidilactici*.

Pediococcus species are widely used for meat and vegetable fermentation (20). They are homofermentative, nonmotile, non-spore-forming spherical cells which form tetrads (10). Some *Pediococcus* species (mainly *Pediococcus acidilactici* and *Pediococcus pentosaceus*) are reported to produce antimicrobial peptides (pediocins) during their growth (3, 7–9, 11, 13). Pediocins, like other bacteriocins (24), are proteinaceous in nature and inhibit growth of closely related species as well as food-borne pathogens and food spoilage organisms (1, 4, 6, 9, 17, 18, 21, 26). Pediocin production is reported to be a plasmid-linked trait (8, 11, 13, 22). Among all of the pediocins, pediocin AcH (M_r, 2,700) was studied in detail and shown to be nonantigenic and nontoxic to mice (5). The mode of bactericidal action of pediocin AcH was shown to occur through initial binding to cell surface receptors and destabilization of the cytoplasmic membrane (6). Pediocin AcH inhibits growth of *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, and some spoilage *Lactobacillus*, *Leuconostoc*, and *Brocothrix* species associated with foods (4, 6, 18, 26). Bacteriocins of several other lactic-acid bacteria (LAB), including nisin and lactacins, have been reported in the literature (12, 14, 15). Nisin is used worldwide as a food preservative and is currently allowed as a biopreservative in pasteurized-processed cheeses and cheese spreads in the United States. The potential use of other bacteriocins as biopreservatives in foods is under investigation.

There are no suitable isolation methods available for *Pediococcus* species. However, some culture methods to isolate LAB in general have been described elsewhere (10). The purpose of the present study was to isolate *Pediococcus* species from fermented foods with a monoclonal antibody (MAb) probe and to screen these isolates for production of pediocins showing broader inhibitory spectra than previously described.

In this study, we describe the development and characterization of a MAb specific for *P. acidilactici*. This MAb was used as a probe in immunoblots to identify *Pediococcus* species colonies produced by organisms from fermented foods. These isolates were biochemically characterized and screened for bacteriocin production.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in this study are listed in Table 1. All of the LAB strains were maintained in MRS agar slants (Difco Laboratories, Detroit, Mich.), and the pathogens were maintained in Trypticase soy agar (Difco) slants with 0.5% yeast extract supplement. The fresh liquid cultures of LAB and pathogens were obtained by transferring slant cultures into casein glucose broth (CGB) (4) or Trypticase soy broth with 0.5% yeast extract supplement. Identifications of all of the *Pediococcus* cultures were verified by microscopic observation and carbohydrate fermentation patterns (10).

Antigen preparation and MAb production. *P. acidilactici* H was grown in 100 ml of CGB for 20 h at 32°C. The cells were harvested by centrifugation (9,800 × *g*, for 10 min) and washed once with 20 mM phosphate-buffered saline (PBS), pH 7.0. The cells were resuspended with PBS to obtain 10^{10} CFU/ml and were disrupted four times for 3 min each (70% Duty Cycle) by using a sonicator (Branson Sonic Power Co., Danbury, Conn.) at 4°C. The broken-cell mixture was then heated to 80°C for 20 min to kill any remaining viable cells.

A 0.25-ml portion of broken-cell mixture was injected intraperitoneally into each of three 6-week-old BALB/c mice. Immunizations were carried out over an 8-week period, with one injection per week. Spleen cells from one of the immunized mice were collected and fused with murine myeloma P3/Ns1/1-Ag4-1 (NS1) cells by methods described before (2, 16). The resulting hybridoma cells were screened

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TABLE 1. Bacterial strains tested with MAb Ped-2B2 in ELISA

Organism	Source[a]	ELISA[b]
Pediococcus spp.		
acidilactici 8042[c]	UMRL	+
acidilactici H[c]	B. Ray (UW)	+
acidilactici L[c]	B. Ray (UW)	+
acidilactici PC[c]	B. Ray (UW)	+
acidilactici PrF1[c]	B. Ray (UW)	+
acidilactici PD1[c]	B. Ray (UW)	+
acidilactici[c]	This laboratory	+
pentosaceus PT2	B. Ray (UW)	−
pentosaceus PI	B. Ray (UW)	−
pentosaceus PrL	B. Ray (UW)	−
pentosaceus PF3	B. Ray (UW)	−
pentosaceus Pr1	B. Ray (UW)	−
pentosaceus ATCC 43200	H. Fleming, USDA and NCSU	−
pentosaceus FBB39	H. Fleming, USDA and NCSU	−
damnosus 29358	ATCC	−
dextrinicus 33087	ATCC	−
parvulus 19371	ATCC	−
urinaeequi 29723	ATCC	−
halophilus 33315	ATCC	−
Lactobacillus spp.		
plantarum NCDO 955	B. Ray (UW)	−
acidophilus NRRL-B-1910	UMRL	−
acidophilus E4356	UMRL	−
casei	UMRL	−
leichmannii 7830	UMRL	−
Lactococcus lactis 29071	ATCC	−
Micrococcus spp.		
luteus 4698	UMRL	−
roseus	UMRL	−
Streptococcus spp.		
mutans 25175	ATCC	−
bovis Type II 49133	ATCC	−
Enterococcus faecalis 344	ATCC	−
Staphylococcus aureus 25923	ATCC	−
Corynebacterium glutamicum 31834	ATCC	−
Listeria spp.		
monocytogenes V7	USFDA	−
monocytogenes Scott A	USFDA	−
monocytogenes F4263	CDC	−
innocua F4248	CDC	−
ivanovii KC1714	CDC	−
welshimeri 35897	ATCC	−
Bacillus cereus 14597	ATCC	−
Pseudomonas spp.		
fluorescens 13525	ATCC	−
aeruginosa 10145	ATCC	−
Salmonella typhimurium 14028	ATCC	−
Escherichia coli 9002	ATCC	−

[a] UW, University of Wyoming; NCSU, North Carolina State University; UMRL, University Micro Reference Laboratory; USDA, U.S. Department of Agriculture; USFDA, U.S. Food and Drug Administration; CDC, Centers for Disease Control; ATCC, American Type Culture Collection.

[b] ELISA read at A_{490}. +, $A_{490} > 0.3$; −, $A_{490} < 0.05$.

[c] *P. acidilactici* H was positive for pediocin production when tested against *L. plantarum* (4). The other strains (UMRL 8042, L, PC, PrF1, and PD1) were negative for pediocin production.

for production of anti-*Pediococcus* antibodies by enzyme-linked immunosorbent assay (ELISA) (2). The hybridoma cells producing antibodies which reacted with the whole cells of *P. acidilactici* were cloned by limiting dilution. Aliquots of the selected monoclone (Ped-2B2) were injected into pristane-primed BALB/c mice for ascites production. The immunoglobulin isotypes of the MAbs produced in ascites fluid were determined by ELISA (Bio-Rad Laboratories, Richmond, Calif.).

Reactivity spectrum of MAb Ped-2B2. The reaction patterns of MAb Ped-2B2 with different bacterial strains were determined in ELISA. The organisms mentioned in Table 1 were grown in either CGB or Trypticase soy broth at 32 or 37°C for 18 to 20 h. The cells were harvested by centrifugation and resuspended with 0.05 M carbonate coating buffer, pH 9.6. Triplicate 0.1-ml portions of each culture (approximately 10^9 CFU/ml) were placed in wells of microtiter plates. After 12 h at 4°C, the plates were washed with PBS-Tween 20 (0.5%) for four times and immunoprobed with Ped-2B2 (1:2,000) as described before (2).

SDS-PAGE and Western blot (immunoblot). For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% acrylamide), the crude cell surface proteins from *P. acidilactici* H and UMRL 8042 and *P. pentosaceus* PT2 and PI were extracted by a rapid extraction method that used 4.6% SDS. A 100-ml volume of each culture was centrifuged, and the cell pellets were washed once with PBS and resuspended with 0.5 ml of sample buffer (25) containing 4.6% SDS, 10% β-mercaptoethanol, 20% glycerol, 1.5% Tris, and 1% bromophenol blue (pH 6.8) for 1 h at 37°C. The cell protein extracts were collected by centrifugation and heated to 60°C for 15 min. A 0.02-ml amount of each sample was loaded onto several wells of SDS-PAGE gels and electrophoresed at 25 mA for 4 h. After electrophoresis, the gels were either stained with Coomassie brilliant blue R 250 or transblotted to Immobilon P membranes (Millipore Corp., Bedford, Mass.). The membrane-transferred proteins were immunoprobed with MAb Ped-2B2 (2, 5).

Surface proteolysis. Surface proteolysis of *P. acidilactici* H and UMRL 8042 was achieved by using trypsin and pronase E (Sigma) treatments by the method described by Olson et al. (19). Briefly, the bacterial cultures (100 ml each) were grown in CGB for 20 h. The cells were harvested by centrifugation, washed once with PBS, and resuspended in 100 ml of PBS containing either trypsin (200 μg/ml) or pronase E (200 μg/ml) and incubated at 37°C for 1 h. The enzyme reaction was terminated by addition of either trypsin inhibitor (500 μg/ml) or 10 mM EDTA. The cells were centrifuged and washed three times with PBS, and the surface proteins were extracted with 4.6% SDS as mentioned before. A 0.02-ml amount of protein extract from each sample including controls (cells with no enzyme treatment) was directly loaded onto SDS-PAGE gels (12% acrylamide). After electrophoresis, the separated proteins were electroblotted onto Immobilon P membranes and immunoprobed with MAb Ped-2B2 (2).

Isolation of *Pediococcus* species by colony immunoblotting. Portions (10 g each) of several food samples (fermented and nonfermented sausages) were separately suspended in 25 ml of PBS and homogenized in a stomacher (Tekmar Co., Cincinnati, Ohio) for 2 min. A total of 25 0.02-ml volumes of each food suspension were spotted on each of 25 squares of prepoured MRS agar plates (100 by 100 mm) so that each plate received a total of 0.5 ml of food slurry. Each plate was air dried for 10 min, overlaid with an Immobilon P membrane, and incubated at 32°C for 48 h. The membranes were then peeled off from the agar surface and immunoprobed with MAb Ped-2B2. The colonies in the corresponding plates which matched locations with the blot-positive colonies (dark purple) on the membranes were picked, streaked on MRS plates, and incubated at 32°C for 24 h. The purified colonies were further propagated in CGB. After 18 to 22 h of incubation at 32°C, the cultures were checked under phase-contrast microscopy and assayed for bacteriocin production as described below.

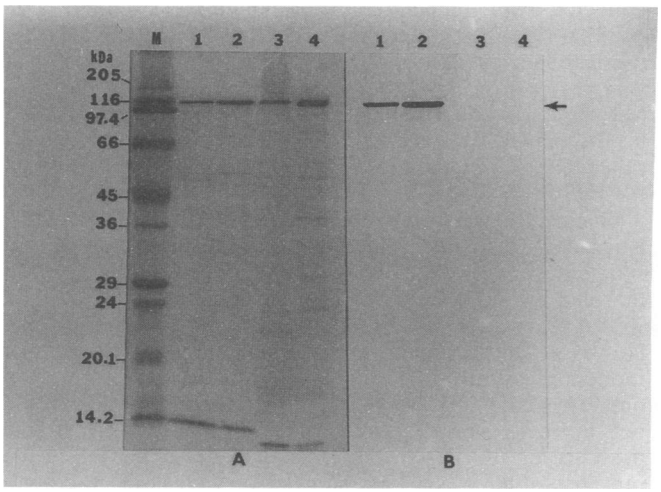

FIG. 1. SDS-PAGE and immunoblot of crude cell surface proteins from *P. acidilactici* and *P. pentosaceus*. (A) Coomassie blue-stained molecular mass standards (SDS-6H; Sigma) (lane M) and crude cell surface proteins from *P. acidilactici* H (lane 1) and UMRL 8042 (lane 2) and *P. pentosaceus* PT2 (lane 3) and PI (lane 4). (B) Immunoblot of proteins from panel A with MAb Ped-2B2. The 116-kDa protein from *P. acidilactici* H (lane 1) and UMRL 8042 (lane 2) reacted with Ped-2B2 (see arrow), whereas no reaction was noticed with *P. pentosaceus* PT2 (lane 3) and PI (lane 4).

Biochemical characterization of *Pediococcus* isolates. Characterizations of the carbohydrate fermentation patterns of *Pediococcus* isolates were carried out in microtiter plates (23). *Pediococcus* isolates were grown in CGB and washed twice in PBS, and 0.02 ml of each culture was inoculated into several wells of sterile microtiter plates (Corning Glass Works, Corning, N.Y.) containing maltose, lactose, ribose, sucrose, xylose, rhamnose, arbutin, glycerol, 6% NaCl, 10% NaCl, or 18% NaCl with dextrose. The carbohydrate concentration was 10 mg/ml of CHL medium (API Systems, Plainview, N.Y.), with bromocresol purple as a dye indicator. The plates were incubated at 32°C for 18 h, and the results were recorded. The isolates were also grown at 40 and 50°C for 20 h.

Bacteriocin assay. The *Pediococcus* cultures were centrifuged, and cell-free supernatants were checked for antimicrobial activity (4, 6). Briefly, a 0.1-ml volume of each culture supernatant was serially diluted in microtiter plates. A 5-μl volume of each diluted aliquot was placed in an MRS agar plate containing a lawn of *Lactobacillus plantarum* NCDO 955 (10^7 CFU/ml). The growth inhibition of indicator bacteria was detected after 18 h of incubation at 32°C. The bacteriocin activity was measured and expressed in arbitrary units (AU) per milliliter as described previously (6, 11).

Heat and enzyme treatment of bacteriocins. The cell-free culture supernatants of each isolate were either heated to 100°C for 30 min or treated with proteolytic enzymes (trypsin, chymotrypsin, protease types IV, XIV, or XXV, or proteinase K) at a concentration of 1 mg/ml at 37°C for 1 h. After heat and enzyme treatments, the samples were serially diluted in deionized water and bacteriocin activities were determined as described above.

RESULTS

MAbs. Several MAbs were obtained after murine cell fusions. Two MAbs, Ped-2B2 and Ped-2D5, were selected on the basis of their reactions with whole cells of *P. acidilactici* H in ELISA. Some other MAbs, namely Ped-3D8, Ped-3F10, Ped-4B8, Ped-4D8, and Ped-5D2, reacted with intracellular proteins of *P. acidilactici* H cells, as was evidenced by Western blotting (data not shown), and were not characterized further. The immunoglobulin isotypes of Ped-2B2 and Ped-2D5 were determined to be immunoglobulin G1 with κ light chains.

Reactivity spectrum of MAb Ped-2B2. Ped-2B2 reacted with whole cells of all seven *P. acidilactici* strains tested without any reaction with other *Pediococcus*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Staphylococcus*, *Listeria*, *Bacillus*, *Salmonella*, and *Escherichia* species in ELISA (Table 1).

SDS-PAGE and Western blot. SDS-PAGE analysis of the crude cell surface proteins of *P. acidilactici* H and UMRL 8042 and *P. pentosaceus* PT2 and PI indicated the presence of several protein bands upon Coomassie blue staining (Fig. 1A, lanes 1, 2, 3, and 4). Immunoblot results showed that MAb Ped-2B2 reacted with only one protein band of 116 kDa for both test strains of *P. acidilactici* (Fig. 1B, lanes 1 and 2) and showed no reaction with either test strain of *P. pentosaceus* (Fig. 1B, lanes 3 and 4).

Surface proteolysis. Trypsin or pronase treatment of whole cells of *P. acidilactici* H and UMRL 8042 led to the disappearance of the 116-kDa protein band in subsequent SDS-PAGE analyses (Fig. 2A, lanes TR and PR). Similarly, Western blots of the same protein preparations with Ped-2B2 indicated no reactions with samples from either trypsin- or pronase-treated cells (Fig. 2B, lanes TR and PR). However, control cells showed a reaction with Ped-2B2 (Fig. 2B, lane C), indicating that the 116-kDa protein is associated with the

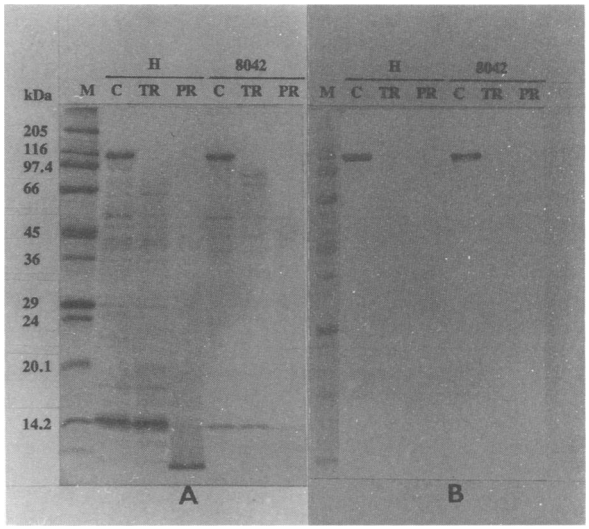

FIG. 2. Surface proteolysis of *P. acidilactici* H and UMRL 8042. (A) Coomassie blue-stained SDS-PAGE gels containing molecular mass standards (SDS-6H; Sigma) (lane M), *P. acidilactici* H and UMRL 8042 cell surface proteins (lane C), and cell surface proteins after trypsin treatment (lane TR) and after protease treatment (lane PR). (B) Immunoblot of proteins from panel A with MAb Ped-2B2. The protein extracts from cells without enzyme treatments showed reaction with Ped-2B2 (lane C). Protein extracts from cells after trypsin (lane TR) and protease treatment (lane PR) gave no reaction, indicating that enzyme treatment destroyed the Ped-2B2-reactive 116-kDa protein associated with the cell surface. Lane M contains a fast-green-stained molecular mass standard after transblotting.

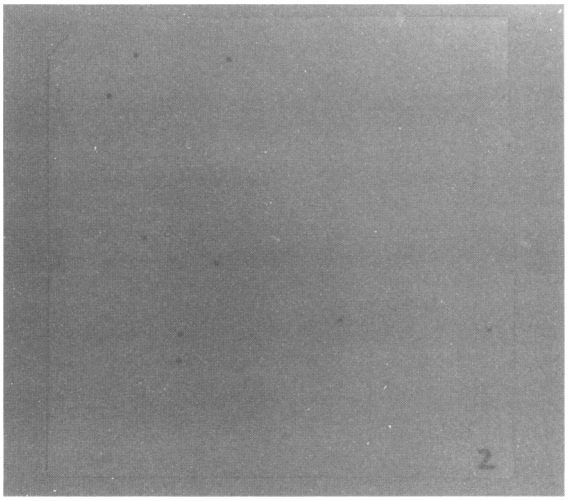

FIG. 3. Colony immunoblot of fermented-meat sample on Immobilon membrane. The membrane containing bacterial colonies was reacted with MAb Ped-2B2 (1:1,000). After immunoassay, the positive colonies were picked from the corresponding MRS agar plate, purified, and propagated in CGB for microscopic observation, biochemical characterization and bacteriocin assay.

cell surface of *P. acidilactici*. These cells, while enzyme treated, remained viable, since they formed colonies when plated on MRS agar.

Isolation of *Pediococcus* species. Colony immunoblots of food samples with MAb Ped-2B2 indicated the presence of 10 or more positive colonies per membrane (Fig. 3). The *Pediococcus* species-positive colonies arising from spotted slurries were calculated to be present at 20 to 40 CFU/ml of food slurry, and each food contained about 10^4 CFU of background microflora per ml. Each positive colony was picked, purified on MRS agar, propagated in CGB, checked for proper biochemical fermentation patterns conforming to those of *P. acidilactici* (10), and assigned a strain number (Table 2). A total of 18 isolates of *P. acidilactici* were collected from eight different fermented-meat samples. We could not directly isolate any MAb Ped-2B2-reactive *Pediococcus* species isolates from two other fermented-meat samples or from six other nonfermented-sausage samples. Some of these negative samples, upon enrichment in MRS broth, yielded tetrad cells, presumably *Pediococcus* species other than *P. acidilactici*, which still did not react with this MAb.

Biochemical characterization. The physiological characteristics of the isolates were summarized in Table 2. Microscopic observations of all isolates showed the typical tetrad morphology of *Pediococcus* species. All of the isolates grew well at 40 and 50°C. All isolates fermented ribose and xylose. However, lactose, rhamnose, arbutin, and glycerol were not fermented by any of the isolates. Maltose was fermented only by isolate RS2. Some isolates (PH6, SB1, and SB4) did not ferment sucrose. None of the isolates grew in the presence of 18% NaCl. All of the isolates grew well in the presence of 6.5% NaCl but showed very weak or no growth in the presence of 10% NaCl (Table 2). These results indicated that all of the isolates were *P. acidilactici*, of which RS2 is an atypical strain.

Bacteriocin assay and heat and enzyme treatments. All 18 *P. acidilactici* isolates were positive for bacteriocin production when tested against the sensitive *L. plantarum* strain NCDO 955. The antimicrobial activities of isolates ranged from 800 to 6,400 AU/ml (Table 3).

Of the 18 bacteriocin preparations tested, 9 showed no reduction while the other 9 showed 50% reduction in antimicrobial activities after heat treatment compared with the unheated controls (Table 3). All of the bacteriocins lost their antimicrobial activities after chymotrypsin, trypsin, or pronase treatment (Table 3).

DISCUSSION

This report of the development of a MAb specific for *P. acidilactici* (Ped-2B2) is the first such report, to the best of our knowledge. This MAb did not react with any other *Pediococcus* species or other LAB tested. This antibody

TABLE 2. Physiological characteristics of the isolates

Characteristic	Result for strain[a]:																		
	H[b]	PH3	PH4	PH5	PH6	SB1	SB2	SB3	SB4	SB5	SG1	SG2	RS2	AB1	AB2	AB3	AB4	AB5	AB6
Growth at:																			
40°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid production from:																			
Maltose	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Xylose	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Sucrose	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+
Rhamnose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Arbutin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glycerol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth in:																			
6.5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10% NaCl	−	−	±	±	−	±	±	±	±	±	−	±	−	−	−	±	−	−	−
18% NaCl	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Morphology[c]	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T

[a] +, positive; ±, weakly positive; −, negative; T, tetrad.
[b] *P. acidilactici* H was used as a reference control (4).
[c] Microscopic observation showed typical morphology of *Pediococcus* species (10).

TABLE 3. Properties of bacteriocins from *Pediococcus* isolates

Isolate[a]	Bacteriocin activity (AU/ml)[b]		
	Control	Heat-treated bacteriocins[c]	Enzyme-treated bacteriocins[d]
H[e]	3,200	3,200	0
PH3	3,200	3,200	0
PH4	3,200	3,200	0
PH5	3,200	3,200	0
PH6	3,200	1,600	0
SB1	3,200	1,600	0
SB2	3,200	3,200	0
SB3	3,200	3,200	0
SB4	6,400	3,200	0
SB5	3,200	1,600	0
SG1	800	400	0
SG2	1,600	800	0
RS2	3,200	3,200	0
AB1	3,200	1,600	0
AB2	3,200	3,200	0
AB3	3,200	3,200	0
AB4	6,400	3,200	0
AB5	6,400	3,200	0
AB6	6,400	3,200	0

[a] All of the isolates were identified as *P. acidilactici*.
[b] Bacteriocin activity was expressed as AU per milliliter; 5 μl of the highest dilution, showing zone of growth inhibition of *L. plantarum* cells, was defined as AU per milliliter (6).
[c] Heated at 100°C for 30 min.
[d] The enzymes trypsin, chymotrypsin, protease types IV, XIV, and XXV, and proteinase K were used at concentrations of 1 mg/ml.
[e] *P. acidilactici* H was used as a reference control (4).

also did not react with other indigenous bacteria present in the fermented meats we examined.

The MAb Ped-2B2 reacted in Western blots with the 116-kDa protein from *P. acidilactici* but not with that from *P. pentosaceus* (Fig. 1). This indicates that Ped-2B2 reacts with some specific epitopes present in the *P. acidilactici* 116-kDa proteins which are not shared with similar proteins from the surfaces of cells of *P. pentosaceus*.

The MAb Ped-2B2-reactive protein is a 116-kDa surface protein, since the proteolytic enzyme treatment of whole cells destroyed the antibody-binding sites of the protein. Other evidence that it is a surface protein is that the MAb reacted with the live cells in ELISA.

The Ped-2B2 MAb probe was useful in isolating low numbers of *Pediococcus* species cells from foods in the presence of 10,000-fold-greater numbers of other microorganisms. This antibody effectively detected in a colony immunoblot assay as few as 20 to 40 CFU of *P. acidilactici* per ml of food slurries which contained about 10^4 CFU of background microflora per ml.

Microscopic observation indicated that all of the isolates were *Pediococcus* species. Furthermore, physiological characteristics indicated that all of the isolates were *P. acidilactici*. However, isolate RS2 fermented maltose, which does not agree with the results of Garvie (10). However, this isolate possesses all of the other properties of *P. acidilactici*. It is possible that RS2 is a maltose-positive *P. acidilactici*. Further studies are necessary to confirm the identity of this organism. Garvie (10) reported that sucrose was not typically fermented by *P. acidilactici* strains. Conversely, studies by Gonzalez and Kunka (11) showed that their strains of *P. acidilactici* did ferment sucrose and that this trait was plasmid linked. In agreement with the latter authors, our sucrose fermentation data showed that 16 of 19 isolates fermented sucrose.

The bacteriocins of *P. acidilactici* isolates are shown to be heat stable. They appear to be proteinaceous in nature, since the antimicrobial activities were destroyed by proteolytic enzymes. Further characterizations of these pediocins as to their molecular weights, amino acid sequences, and inhibitory spectra and of the plasmid profiles of the pediocin-producing cells are under way.

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REFERENCES

1. **Berry, E. D., M. B. Liewen, R. W. Mandigo, and R. W. Hutkins.** 1990. Inhibition of *Listeria monocytogenes* by bacteriocin producing *Pediococcus* during the manufacture of fermented semidry sausage. J. Food Prot. **53**:194–197.
2. **Bhunia, A. K., P. H. Ball, A. T. Faud, B. W. Kurz, J. W. Emerson, and M. G. Johnson.** 1991. Development and characterization of a monoclonal antibody specific for *Listeria monocytogenes* and *Listeria innocua*. Infect. Immun. **59**:3176–3184.
3. **Bhunia, A. K., M. C. Johnson, and B. Ray.** 1987. Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. J. Ind. Microbiol. **2**:319–322.
4. **Bhunia, A. K., M. C. Johnson, and B. Ray.** 1988. Purification, characterization, and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. J. Appl. Bacteriol. **65**: 261–268.
5. **Bhunia, A. K., M. C. Johnson, B. Ray, and E. L. Belden.** 1990. Antigenic property of pediocin AcH produced by *Pediococcus acidilactici* H. J. Appl. Bacteriol. **69**:211–215.
6. **Bhunia, A. K., M. C. Johnson, B. Ray, and N. Kalchayanand.** 1991. Mode of action of pediocin AcH from *Pediococcus acidilactici* H on sensitive bacterial strains. J. Appl. Bacteriol. **70**:25–33.
7. **Biswas, S. R., P. Ray, M. C. Johnson, and B. Ray.** 1991. Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. Appl. Environ. Microbiol. **57**:1265–1267.
8. **Daeschell, M. A., and T. R. Klaenhammer.** 1985. Association of 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl. Environ. Microbiol. **50**:1528–1541.
9. **Fleming, H. P., J. L. Etchells, and R. N. Costilow.** 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. Appl. Microbiol. **30**:1040–1042.
10. **Garvie, E. I.** 1986. Genus *Pediococcus* Claussen 1903, 68^{AL}, p. 1075–1079. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
11. **Gonzalez, C. F., and B. S. Kunka.** 1987. Plasmid associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. Appl. Environ. Microbiol. **53**:2534–2538.
12. **Hansen, J. N., S. Banerjee, and G. W. Buchman.** 1989. Potential of small ribosomally synthesized bacteriocins in design of new food preservatives. J. Food Saf. **10**:119–130.
13. **Hoover, D. G., P. M. Walsh, K. M. Kolaetis, and M. M. Daly.** 1988. A bacteriocin produced by *Pediococcus* species associated with a 5.5-megadalton plasmid. J. Food Prot. **51**:29–31.
14. **Juven, B. J., R. J. Meinersmann, and N. J. Stern.** 1991. Antagonistic effects of lactobacilli and pediococci to control intestinal colonization by human enteropathogens in live poultry. J. Appl. Bacteriol. **70**:95–103.
15. **Klaenhammer, T. R.** 1988. Bacteriocins of lactic acid bacteria. Biochimie **70**:337–349.
16. **Kohler, G., and C. Milstein.** 1975. Continuous cultures of fused

cells secreting antibody of predefined specificity. Nature (London) **256:**495–497.
17. **Lewus, C. B., A. Kaiser, and T. J. Montville.** 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. **57:**1683–1688.
18. **Motlagh, A. M., M. C. Johnson, and B. Ray.** 1991. Viability loss of foodborne pathogens by starter culture metabolites. J. Food Prot. **54:**873–878.
19. **Olson, L. D., S. W. Shane, A. A. Karpas, T. M. Cunningham, P. S. Probst, and M. F. Barile.** 1991. Monoclonal antibodies to surface antigens of a pathogenic *Mycoplasma hominis* strain. Infect. Immun. **59:**1683–1689.
20. **Pederson, C. S.** 1949. The genus *Pediococcus*. Bacteriol. Rev. **13:**225–232.
21. **Pucci, M. J., E. R. Vedamuthu, B. S. Kunka, and P. A. Vandenbergh.** 1988. Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC1.0. Appl. Environ. Microbiol. **54:**2349–2353.
22. **Ray, S. K., W. J. Kim, M. C. Johnson, and B. Ray.** 1989. Conjugal transfer of a plasmid encoding bacteriocin production and immunity in *Pediococcus acidilactici* H. J. Appl. Bacteriol. **66:**393–399.
23. **Siragusa, G. R., and J. W. Nielson.** 1991. A modified microtiter plate method for biochemical characterization of *Listeria spp.* J. Food Prot. **54:**121–123.
24. **Tagg, J. R., A. S. Dajani, and L. W. Wannamaker.** 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev. **40:**722–756.
25. **Walker, J. M.** 1984. Gradient SDS polyacrylamide gel electrophoresis, p. 57–61. *In* J. M. Walker (ed.), Methods in molecular biology, vol. 1. Proteins. Humana Press, Clifton, N.J.
26. **Yousef, A. E., J. B. Luchansky, A. J. Degnan, and M. P. Doyle.** 1991. Behavior of *Listeria monocytogenes* in wiener exudates in the presence of *Pediococcus acidilactici* H or pediocin AcH during storage at 4 or 25°C. Appl. Environ. Microbiol. **57:**1461–1467.