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Jenseniin G, a Heat-Stable Bacteriocin Produced by *Propionibacterium jensenii* P126†

D. A. GRINSTEAD,[1] and S. F. BAREFOOT[1,2]*

Departments of Food Science[1] and Microbiology,[2] Clemson University, Clemson, South Carolina 29634-0371

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The genus *Propionibacterium* includes cutaneous species typically found on human skin and the dairy or classical species (*Propionibacterium freudenreichii*, *P. jensenii*, *P. thoenii*, and *P. acidipropionici*) used industrially for the production of Swiss cheese and propionic acid. Grinstead (1989, M.S. thesis, Iowa State University, Ames) has previously observed that some dairy propionibacteria inhibit other species in the classical grouping. We further investigated the inhibitor(s) produced by *P. jensenii* P126 (ATCC 4872). An antagonist(s) from anaerobic agar cultures of P126 strongly inhibited two closely related strains of propionibacteria, *P. acidipropionici* P5 and *P. jensenii* P54, and *Lactobacillus bulgaricus* NCDO 1489, *Lactobacillus delbrueckii* subsp. *lactis* ATCC 4797, *Lactococcus cremoris* NCDO 799, and *Lactococcus lactis* subsp. *lactis* C2. The inhibitor, designated jenseniin G, was active at pH 7.0; inactivated by treatment with pronase E, proteinase K, and type 14 protease; insensitive to catalase; and stable to freezing, cold storage (4°C, 3 days), and heat (100°C, 15 min). Classification of the inhibitor as a bacteriocin is supported by its proteinaceous nature and its bactericidal activity against *L. delbrueckii* subsp. *lactis* ATCC 4797. The lack of detectable plasmids suggests a chromosomal location for the determinant(s) of jenseniin G.

The genus *Propionibacterium* is divided into the cutaneous and the dairy or classical species. Classical species include *Propionibacterium jensenii*, *P. acidipropionici*, *P. freudenreichii* subsp. *freudenreichii* and *shermanii*, and *P. thoenii* (5). The industrial significance of this group lies in their contribution of the characteristic flavor, texture, and "eyes" of Swiss cheese, their prolific production of propionic acid, and their ability to synthesize vitamin B12.

The propionibacteria are well known for their production of inhibitory metabolites. During fermentative metabolism, they convert glucose and lactate to propionate, acetate, and carbon dioxide (9). The inhibitory effects of propionate and acetate are potentiated by the low pH encountered in Swiss cheese and other fermented products; the undissociated forms are effective against gram-negative bacteria (2). In addition, propionic acid and its salts are incorporated into bakery products to prevent mold growth and ropiness. Up to 6.12 ppm of the inhibitor diacetyl may be produced by strains of *P. freudenreichii* subsp. *shermanii* in milk (14). However, 172 to 344 ppm were required for inhibition of yeasts and non-lactic acid bacteria (12). Therefore, production of diacetyl probably does not contribute significantly to inhibition by propionibacteria.

Other inhibitors include the propionins, antiviral peptides obtained from cellular extracts of *P. freudenreichii* (20). Propionin A is a dialyzable peptide demonstrating in vitro activity against vaccinia viruses. Propionins B and C also are dialyzable; their sizes are approximately 1,000 to 2,000 Da, and they demonstrate both in vivo and in vitro activity against Columbia SK virus (21). No subsequent reports on propionins have been forthcoming.

The preservative Microgard is grade A skim milk that has been fermented by *P. freudenreichii* subsp. *shermanii* and pasteurized. It has been approved for food applications by the Food and Drug Administration and is reportedly used as a preservative in about 30% of the cottage cheese produced in the United States (6). Antimicrobial activity of Microgard against gram-negative bacteria, yeasts, and selected molds, but not gram-positive bacteria, has been well documented (1) and appears to parallel the actions of propionic acid. However, reports characterizing the inhibitory agents in Microgard are not available in the literature. A heat-stable polypeptide of 700 Da (6) has been reported to contribute significantly to the inhibitory effect of Microgard. No doubt, propionic acid also contributes to this antagonistic activity.

Production of bacteriocins in the propionibacteria has been described previously. Bacteriocins are bactericidal proteins with a narrow spectrum of activity targeted toward species related to the producer culture (23). Fujimura and Nakamura (7) have described a bacteriocinlike substance, termed acnecin, produced by the cutaneous species *P. acnes* CN-8. Sonication of *P. acnes* CN-8 cells yielded a 60,000-Da protein inhibitory to non-acnecin-producing strains of *P. acnes* and *Corynebacterium parvum*. Purified acnecin consisted of five subunits with a molecular size of 12,000 Da each. Because acnecin acted bacteriostatically against sensitive cells, the authors (7) termed it bacteriocinlike. More recently, Paul and Booth (19) have identified an inhibitory protein produced by *P. acnes* RTT 108. In many respects, the compound was similar to acnecin. It too was obtained from sonicated cells and was a large protein (78,000 Da). Like acnecin (7), its mode of action was bacteriostatic. In contrast, its spectrum of activity included gram-negative and gram-positive bacteria.

Specific inhibitory activity among the classical propionibacteria was first reported by Grinstead (8). An examination of 150 strains of dairy propionibacteria yielded several cultures that inhibited one or more cultures of 10 indicator propionibacteria. *P. jensenii* ATCC 4872 (P126) and *P. thoenii* ATCC 4874 (P127) were most inhibitory to the indicators, but the responsible antagonists were not identified. Strain P127 was examined further by Lyon and Glatz (16). The antagonist, designated propionicin PLG-1, was

* Corresponding author.
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TABLE 1. Effect of jenseniin G on selected indicator cultures[a]

Indicator organism	Sensitivity to jenseniin G[b]	Culture conditions	
		Temp (°C)	Medium[c]
Aspergillus carneus	–	25	PDA
Aspergillus niger	–	25	PDA
Aspergillus terreus	–	25	PDA
Cladiosporum sp.	–	25	PDA
Discula sp.	–	25	PDA
Penicillium sp.	–	25	PDA
Penicillium italicum	–	25	PDA
Ulodadium sp.	–	25	PDA
Bacillus cereus ATCC 232	–	37	BHI
Clostridium perfringens ATCC 3624	–	37	FTG[d]
Citrobacter freundii ATCC 8090	–	37	BHI
Enterobacter aerogenes ATCC 13048	–	37	BHI
Enterobacter cloacae ATCC 23355	–	37	BHI
Enterococcus faecalis ATCC 19433	–	37	M17
Escherichia coli ATCC 25922	–	37	BHI
Escherichia coli O:24 B:17	–	37	BHI
Klebsiella pneumoniae ATCC 13883	–	37	BHI
Listeria grayi ATCC 19120	–	37	BHI
Listeria innocua ATCC 33090	–	37	BHI
Listeria monocytogenes	–	37	BHI
Listeria murrayi ATCC 25402	–	37	BHI
Pseudomonas aeruginosa ATCC 27853	–	37	BHI
Salmonella typhimurium ATCC 14028	–	37	BHI
Seratia marcescens ATCC 8100	–	37	BHI
Shigella flexneri ATCC 12022	–	37	BHI
Shigella sonnei ATCC 25931	–	37	BHI
Staphylococcus aureus ATCC 25923	–	37	BHI
Staphylococcus epidermidis ATCC 12228	–	37	BHI

[a] Indicator cultures other than propionibacteria were incubated for 18 h prior to preparation of the lawn.
[b] Sensitivity (+) or insensitivity (–) of cultures to jenseniin G was determined by both deferred and agar plug assay methods.
[c] PDA, potato dextrose agar; BHI, brain heart infusion; FTG, fluid thioglycolate; M17, M-17 broth with 1% lactose. All media were obtained from Difco Laboratories.
[d] Broth cultures of *C. perfringens* were incubated in tightly closed screw-capped tubes.

TABLE 2. Effect of jenseniin G on selected lactic and propionic acid bacteria[a]

Indicator organism	Sensitivity to jenseniin G[b]	Culture conditions[c]	
		Temp (°C)	Medium[d]
Lactobacillus			
acidophilus ATCC 6032	–	37	MRS
acidophilus N2	–	37	MRS
bulgaricus NCDO 1489	+	37	MRS
casei ATCC 7469	–	37	MRS
delbrueckii subsp. *lactis* ATCC 4797	+	37	MRS
fermentum NCDO 1750	–	37	MRS
helveticus NCDO 87	+	37	MRS
lactis NCDO 970	–	37	MRS
plantarum NCDO 1752	–	37	MRS
viridescens ATCC 12706	–	37	MRS
Lactococcus			
lactis subsp. *cremoris* NCDO 799	+	32	M17
lactis subsp. *lactis* C2	+	32	M17
Propionibacterium			
acidipropionici P5	+	32	NLB
freudenreichii subsp. *shermanii* P38	–	32	NLB
jensenii P54	+	32	NLB
jensenii P63	–	32	NLB
freudenreichii subsp. *shermanii* P93	–	32	NLB
freudenreichii subsp. *shermanii* P100	–	32	NLB
freudenreichii subsp. *freudenreichii* P103	–	32	NLB
jensenii P126	–	32	NLB
thoenii P127	–	32	NLB

[a] Indicator cultures other than propionibacteria were incubated for 18 h prior to preparation of the lawn. Propionibacteria were held for 24 h.
[b] Sensitivity (+) or insensitivity (–) of cultures to jenseniin G was determined by both deferred and agar plug assay methods.
[c] All cultures were incubated under flowing CO_2 (0.4 liter/h).
[d] MRS, lactobacillus MRS broth (BBL); M17, M-17 broth with 1% lactose (Difco); NLB, sodium lactate broth.

isolated and partially purified from sodium lactate agar cultures; it was a 10,000-Da protein that displayed activity against other dairy propionibacteria, extending to gram-negative species including vibrios and pseudomonads.

Characteristics of the inhibitor produced by *P. jensenii* P126 are examined in this study. The inhibitor, jenseniin G, is a heat-stable protein with bactericidal action against some sensitive species and meets criteria (21) for classification as a bacteriocin.

MATERIALS AND METHODS

Strains. The producer culture *P. jensenii* P126, the sensitive indicator *P. acidipropionici* P5, and other indicator propionibacteria were obtained from B. A. Glatz (Iowa State University Department of Food and Human Nutrition, Ames). Other indicator cultures were obtained from the Clemson University Food Microbiology culture collection and are listed in Table 1.

Growth conditions. Propionibacteria were cultured in sodium lactate broth (NLB) that consisted of 1% Trypticase soy broth without dextrose (BBL Microbiology Systems, Cockeysville, Md.), 1% yeast extract (Difco Laboratories, Detroit, Mich.), and 1% sodium lactate syrup (60%; Fisher Scientific Co., Pittsburg, Pa.) in distilled water (10). Sodium lactate agar (NLA) was prepared from NLB by adding 1.8% agar (BBL); soft NLA was prepared by the addition of 0.7% agar.

Growth conditions for the indicator organisms employed in this study are presented in Tables 1 and 2. Stock cultures of *Clostridium perfringens* were maintained at room temperature in cooked-meat medium (Difco). Molds were stored on potato dextrose agar (Difco) plates at room temperature. All other cultures were maintained in the appropriate growth medium containing 10% glycerol and stored at −70°C.

All cultures were propagated twice in the appropriate medium and examined for purity by being streaked for isolation prior to experimentation. Gram stains and growth characteristics were used to confirm the identity of propionibacterial cultures.

Detection of inhibitory activity. Inhibitory activity was detected by two techniques. With the deferred method (24), the producer culture was spot inoculated to the center of NLA plates and held at 37°C under anaerobiosis (GasPak; BBL) for 7 to 10 days. Each plate was overlaid with tempered soft agar seeded with 10^4 to 10^5 cells of *P. acidipropionici* P5 or other appropriate indicators. Plates then were held under conditions optimal for growth of the indicator and observed for inhibition of the indicator lawn after 24 and 48 h.

The effects of growing cells were eliminated by detecting activity with an "agar plug" method. NLA plates were spot

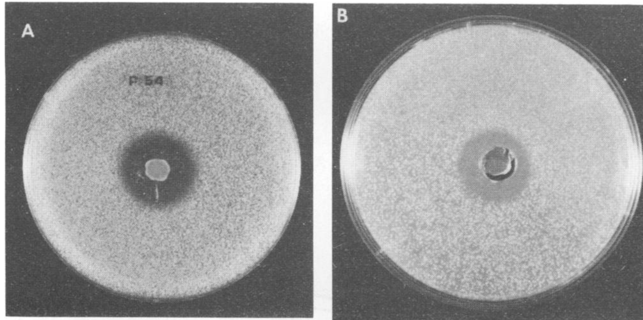

FIG. 1. Inhibition of indicator propionibacteria by *P. jensenii* P126. (A) Inhibition of *P. jensenii* P54 with the deferred method; (B) inhibition of *P. acidipropionici* P5 with the agar plug method.

inoculated with *P. jensenii* P126 and held for 7 to 10 days anaerobically or under flowing CO_2 (0.4 liter/h) at 32°C. Plugs (1.0-cm diameter) were cut from NLA agar next to producer colonies. Agar plugs were transferred aseptically to wells in sterile medium and overlaid with a soft-agar lawn of indicator cells, incubated appropriately, and observed for zones of inhibition.

Preparation of crude jenseniin G. Crude jenseniin G was prepared by two methods. Agar extracts containing activity were prepared by a modification of the method of Lyon and Glatz (16). P126 was spot inoculated to the surface of soft NLA and incubated at 32°C for 7 to 10 days under anaerobiosis. Producer colonies were removed with a sterile cork borer and discarded. The remaining agar was aseptically transferred to a sterile plastic bag, macerated by hand, and frozen overnight at −20°C. The bags then were held at 4°C for 24 h to permit diffusion of the inhibitor into the aqueous phase. Agar was removed by centrifugation (1 h, 4°C) at 9,000 rpm in a GSA JA-14 rotor (Beckman, Palo Alto, Calif.).

Crude jenseniin G also was obtained from broth cultures. NLB cultures of *P. jensenii* P126 were incubated at 32°C for 10 days under anerobiosis, and cells were removed by centrifugation as described above. The supernatant was passed sequentially through a 1.2-μm-pore-size membrane filter (Millipore, Bedford, Mass.) and a 0.45-μm-pore-size filter (Gelman, Ann Arbor, Mich.), transferred to dialysis tubing with a 10,000- to 12,000-molecular-weight cutoff (Spectrum Medical Industries, Inc., Los Angeles, Calif.), and concentrated to 1/50 to 1/100 of the original volume against polyethylene glycol 3,500 or 20,000 (Sigma). Alternatively, the supernatant was lyophilized in a freeze-drier (VirTis Inc., Gardiner, N.Y.) and resuspended in 1/50 to 1/100 of the original volume of sterile distilled water. Concentrated supernatant was dialyzed exhaustively against 0.1 M phosphate buffer (pH 6.8).

Activity of jenseniin G was assayed both by spotting 10- to 50-μl portions onto NLA plates containing an indicator lawn (10^4 to 10^5 CFU/ml), drying at 23°C for 120 min, and holding the plates at 32°C for 48 h and by the agar-well diffusion method of Tagg and McGiven (24). The titer of jenseniin G was defined as the reciprocal of the highest dilution inhibiting the indicator lawn and expressed as activity units (AU) per milliliter (3).

Jenseniin G was examined for adsorption to *P. acidipropionici* P5 and *Lactobacillus delbrueckii* subsp. *lactis* ATCC 4797. Typically, 1-ml aliquots of overnight cultures of *P. acidipropionici* P5 and *L. delbrueckii* subsp. *lactis* ATCC 4797 were pelleted by centrifugation. The cell pellets were washed twice in 0.1 M phosphate buffer (pH 6.8), resuspended in 0.1 ml of crude jenseniin G (20 or 1,600 AU/ml against *P. acidipropionici* P5 or *L. delbrueckii* subsp. *lactis* ATCC 4797, respectively), and held at 32, 37, and 4°C. Replicates were removed at 0, 1, 2, 3, 4, 5, 6, 12, and 24 h; cells were removed by centrifugation and filtration through a 0.45-μm-pore-size filter, and jenseniin G activity against *P. acidipropionici* P5 and *L. delbrueckii* subsp. *lactis* ATCC 4797 was determined. Viable cell counts were determined at each interval with standard methods (4).

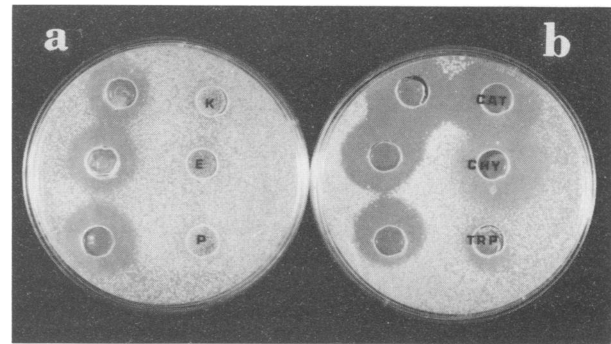

FIG. 2. Effect of enzymes on jenseniin G. Plugs from cultures of *P. jensenii* were untreated or were treated with 25 μl of a 20-mg/ml solution of proteinase K (K), pronase E (E), or type 14 protease (P) (a) and catalase (CAT), chymotrypsin (CHY), or trypsin (TRP) (b) for 1 h at 37°C. Plugs were added to sterile NLA plates, overlaid with a lawn of the sensitive indicator *P. acidipropionici* P5, and held at 32°C for 48 h under flowing CO_2 (0.4 liter/h).

Plasmids. Strain P126 was examined for plasmids by the procedure of Rehberger and Glatz (22).

RESULTS

Activity spectrum. *P. jensenii* P126 was examined by both deferred and agar plug methods (Fig. 1) for inhibition of molds, dairy propionibacteria, selected lactic acid bacteria, and other gram-positive and gram-negative indicator species (Tables 1 and 2). Inhibitory activity of *P. jensenii* P126 was confined to two dairy propionibacteria, *P. jensenii* P54 and *P. acidipropionici* P5, and selected lactic acid bacteria, *Lactobacillus bulgaricus* NCDO 1489, *Lactobacillus helveticus* NCDO 87, *L. delbrueckii* subsp. *lactis* (formerly *Lactobacillus leichmannii*) ATCC 4797, *Lactococcus lactis* subsp. *cremoris* NCDO 799, and *Lactococcus lactis* subsp. *lactis* C2. *P. jensenii* P126 did not inhibit itself or other strains examined in this study.

Enzymatic and temperature sensitivity. The effect of selected enzymes on the inhibitory agent produced by *P. jensenii* P126 was examined. Treatment of inhibitory agar cultures with proteinase K, pronase E, and type 14 protease (Fig. 2a) destroyed antagonistic activity, suggesting that the agent responsible for inhibition is a protein. Treatment with catalase had no effect (Fig. 2b), indicating that the inhibitor was not H_2O_2. The proteinaceous inhibitor produced by *P. jensenii* P126 was termed jenseniin G.

The temperature sensitivity of jenseniin G was examined. Inhibitory activity in agar plugs was not affected by heat treatment for 15, 30, 45, and 60 min at 50°C (Fig. 3) or 2 min at 100°C (Fig. 3). Longer treatments (5, 10, and 15 min) at 100°C diminished activity. Activity in agar plugs that had

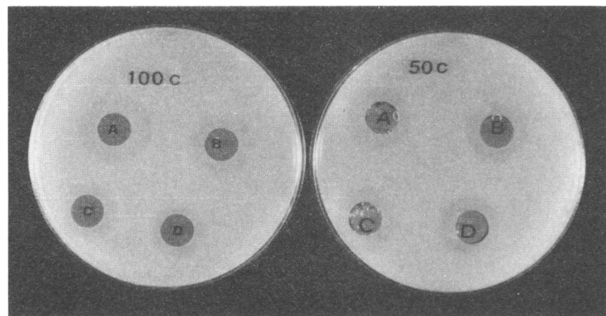

FIG. 3. Effect of heat treatment on activity of jenseniin G. Agar plugs were removed from NLA containing *P. jensenii* P126 colonies and heated to 50°C for 15 (A), 30 (B), 45 (C), or 60 (D) min or to 100°C for 2 (A), 5 (B), 10 (C), or 15 (D) min. Plugs were applied to sterile NLA plates and overlaid with a lawn of the sensitive indicator *P. acidipropionici* P5.

been frozen at −20°C for 16 to 18 h was indistinguishable from activity in unfrozen agar plugs.

Production of jenseniin G. Further characterization of jenseniin G required its production in a soluble form. No activity was detected in unconcentrated NLB cultures of *P. jensenii* P126 held at 32°C for 1 to 10 days. Adjustment of NLB to pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, or 9.0 prior to addition of the producer culture (1%) permitted growth but did not result in detectable production of jenseniin G prior to concentration. Jenseniin G activity was detected only in agar cultures of the producer *P. jensenii* P126 and concentrates (50× to 100×) of spent broth cultures. Producer cultures propagated on NLA, glucose agar (GA; NLA agar containing 0.6% glucose in lieu of sodium lactate), or lactose agar (LcA; NLA containing 0.6% lactose instead of sodium lactate) contained jenseniin G activity. More activity was detected in 24-h LcA producer cultures than in 24-h GA or NLA producer cultures (Fig. 4). However, continued producer growth on NLA resulted in more activity in 4- and 5-day NLA cultures than in the corresponding LcA or GA cultures. Anaerobic incubation (GasPak anaerobic system) and incubation under flowing CO_2 (0.4 liter/h) of NLA or LcA producer cultures resulted in equivalent production of jenseniin G. GA cultures held anaerobically produced more jenseniin G than those held under flowing CO_2 but not more than NLA producer cultures (Fig. 5). Therefore, for subsequent experiments, NLA or NLB cultures of *P. jensenii* P126 incubated under flowing CO_2 were used as the source of crude jenseniin G.

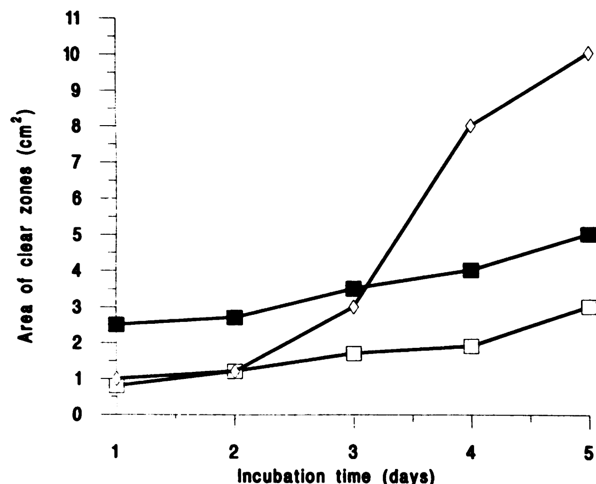

FIG. 4. Effect of medium on jenseniin G production. Producer strain *P. jensenii* P126 was spot inoculated onto NLA (◇), GA (□), or LcA (■); held for 24 to 120 h at 32°C under flowing CO_2 (0.4 liter/h); and overlaid with soft NLA seeded with the sensitive indicator *P. acidipropionici* P5. The plates were incubated at 32°C for 48 h under flowing CO_2 (0.4 liter/h), and the area of the zones of inhibition was determined.

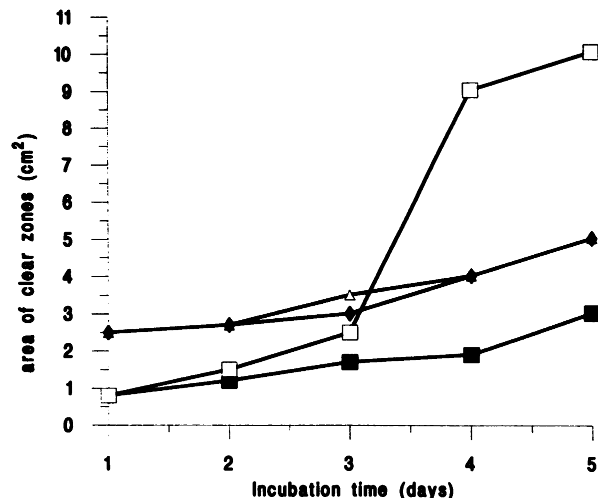

FIG. 5. Effect of atmosphere and medium on production of jenseniin G by P126. Producer strain *P. jensenii* P126 was spot inoculated onto GA or LcA, held for 24 to 120 h at 32°C under anaerobiosis (flowing CO_2, 0.4 liter/h) (■, GA; △, LcA) or in a GasPak anaerobic system (□, GA; ◆, LcA), and overlaid with soft NLA seeded with the sensitive indicator *P. acidipropionici* P5. The plates were incubated at 32°C for 48 h under flowing CO_2 (0.4 liter/h), and the area of the zones of inhibition was determined.

Like jenseniin G activity in agar plugs, activity in NLA producer culture extracts and concentrated NLB spent producer culture was sensitive to proteinase K and insensitive to catalase. In addition, the antagonist in culture extracts inhibited the same indicator species as did agar plugs containing jenseniin G. These data support the premise that agar plugs and culture extracts contained the same antagonist(s). The availability of jenseniin G in soluble form permitted its titration against sensitive indicator species. Crude jenseniin G from both sources was more inhibitory to *L. delbrueckii* subsp. *lactis* ATCC 4797 than to *P. acidipropionici* P5; titers of the same extract against these organisms were 400 and 20 AU/ml, respectively.

Bacteriocins typically exhibit bactericidal, rather than bacteriolytic, action (23). Addition of 20 AU of crude jenseniin G per ml to 1.9×10^7 cells of the sensitive indicator *P. acidipropionici* P5 stopped growth but did not result in cell death. Addition of lower concentrations (5 to 10 AU/ml) of jenseniin G had no effect on indicator cell growth (Table 3). Treatment of *P. acidipropionici* P5 with jenseniin G (20 AU/ml) for 1, 2, 3, 4, 5, 6, or 12 h resulted in decreased indicator populations (data not shown). Jenseniin G activity remained constant throughout the experiment. Exposure of *L. delbrueckii* subsp. *lactis* 4797 to jenseniin G decreased viable cell numbers by approximately 99% (Fig. 6). These results indicate a bacteriostatic action for jenseniin G toward

TABLE 3. Effect of jenseniin G on *P. acidipropionici* P5[a]

Activity (AU/ml)	Treatment temp (°C)	Population (CFU/ml) after 24 h
20	32	1.7×10^7
10	32	$>2.5 \times 10^{10}$
5	32	$>2.5 \times 10^{10}$
0	32	$>2.5 \times 10^{10}$
0	4	3.5×10^7

[a] Crude jenseniin G was obtained by freezing, thawing, and equilibrating (4°C, overnight) NLA cultures (32°C, 7 to 10 days) of *P. jensenii* P126, decanting the liquid, concentrating it 100-fold by lyophilization, and passing it through a microfilter (0.45-μm pore size) to remove cells. Crude jenseniin G was added to 1.9×10^7 CFU of *P. acidipropionici* P5 from an NLB culture (24 h, 32°C).

P. acidipropionici P5 and a bactericidal mode of action toward *L. delbrueckii* subsp. *lactis* 4797. Microscopic examination of treated lactobacilli revealed only intact cells. No cell fragments or lysed cells were detected.

Inhibitory effects of bacteriocins occur in a multistep process that begins with the binding of the bacteriocin to sensitive cells. Exposure of *L. delbrueckii* subsp. *lactis* 4797 to jenseniin G (1,600 AU/ml, 24 h at 4 or 37°C) reduced activity to 400 AU/ml. Treatment of *P. acidipropionici* P5 with jenseniin G (20 AU/ml) under the same conditions resulted in no decrease in activity. These data suggest that jenseniin G adsorbed to *L. delbrueckii* subsp. *lactis* 4797 but not to *P. acidipropionici* P5.

No plasmids were detected in *P. jensenii* P5.

DISCUSSION

To our knowledge, this report provides the first description of a heat-stable bacteriocin produced by the dairy or classical propionibacteria. The sensitivity of jenseniin G to proteases, its insensitivity to catalase, and its large size, as indicated by retention in dialysis tubing with a molecular size cutoff of 12,000 Da, provide support for the conclusion that jenseniin G is a protein. The heat stability of jenseniin G (15 min, 100°C) differs from the lability of propionicin PLG-1 to 85°C (16) but is somewhat common; other bacteriocins, including lactacin B (3), lactacin F (17), and nisin (11), are stable at 100°C for 30 to 60 min.

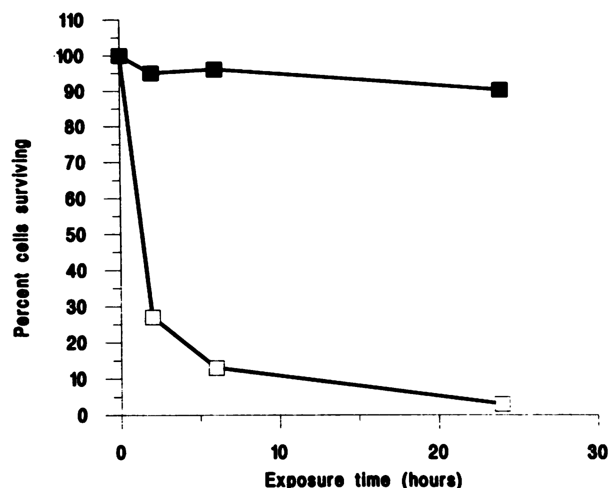

FIG. 6. Percent survival of *L. delbrueckii* subsp. *lactis* 4797 after treatment with jenseniin G. One-milliliter portions of *L. delbrueckii* subsp. *lactis* 4797 (typically 3×10^9 CFU/ml) were washed twice with phosphate buffer and resuspended in 0.1 ml of crude jenseniin G (□) or NLB (■). Surviving cells were determined at 2, 6, and 24 h and were expressed as a percentage of initial counts.

A common trait of bacteriocins is a narrow spectrum of activity (23). Jenseniin G is active against closely related classical propionibacteria and displays extended activity against lactococci and lactobacilli. This relatively narrow spectrum differs from that of propionicin PLG-1 (16); the latter inhibits some gram-negative bacteria, yeasts, and molds, in addition to propionibacteria and lactic acid bacteria. The narrow spectrum of activity displayed by jenseniin G may provide a partial explanation for bacteriocin production by *P. jensenii* P126. Classical propionibacteria are often found in environments such as silage and Swiss cheese fermentations in which they compete with other bacteria. The competitive flora of both products include lactic acid bacteria (15, 18). Production of a jenseniin G or other compounds that inhibit faster-growing lactic acid bacteria would provide a competitive advantage for propionibacteria.

The action of jenseniin G is bacteriostatic against *P. acidipropionici* P5 and bactericidal against *L. delbrueckii* subsp. *lactis* ATCC 4797. These results contrast with the bactericidal mode of action against propionibacteria identified for propionicin PLG-1 (16) and provide support for the conclusion that jenseniin G is a different bacteriocin.

That the lower concentration of crude jenseniin G did not inhibit broth cultures of *P. acidipropionici* P5 may be explained as follows. Agar lawns used for activity assays contained 10^4 to 10^5 CFU/ml; broth cultures contained 10^7 CFU/ml. The higher concentration of cells in broth may have diluted jenseniin G to below the level detectable by the methods employed.

Why jenseniin G inhibits some dairy lactobacilli more strongly than propionibacteria is not known at this time. The possibility that inhibition of propionibacteria and lactobacilli may be due to two different compounds cannot be discounted and awaits the purification of jenseniin G. Adsorption of jenseniin G to *L. delbrueckii* subsp. *lactis* suggests an alternative explanation; *L. delbrueckii* subsp. *lactis* 4797 may have more receptors for jenseniin G on its surface than does *P. acidipropionici* P5. Alternatively, the reduction of jenseniin G activity in crude extracts exposed to *L. delbrueckii* subsp. *lactis* might be the result of cell-associated proteases. However, enzymatic inactivation of jenseniin G would be expected to occur more slowly at 4°C than at 37°C, and exposure to cells caused the same reduction in jenseniin G activity at both temperatures.

Bacteriocins as defined by Tagg et al. (23) include proteins that have a bactericidal but not bacteriolytic action against sensitive organisms. Other common characteristics of bacteriocins include a narrow spectrum of activity and plasmid-borne determinants. Jenseniin G meets two primary criteria for bacteriocins; it is a protein and displays a bactericidal mode of action against *L. delbrueckii* subsp. *lactis* 4797. It displays a relatively narrow spectrum of activity with inhibitory action confined to propionibacteria and a few species of lactic acid bacteria. The absence of plasmids in the bacteriocin-producing strain P126 suggests a chromosomal locus; other bacteriocins produced by gram-positive organisms, including lactacin B (3), lactacin F (17), and helveticin J (13), are encoded chromosomally. Definitive identification of the locus encoding jenseniin G awaits purification and partial

sequencing of the protein, construction of a probe, and location of the genetic determinant(s).

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