Isolation and Purification of Propionicin PLG-1, a Bacteriocin Produced by a Strain of Propionibacterium thoenii†

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Production of propionicin PLG-1 by Propionibacterium thoenii P127 was pH dependent, with maximal activity detected in supernatants of cultures grown at pH 7.0. Propionibacterium PLG-1 was purified by ion-exchange chromatography and isoelectric focusing. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of propionicin PLG-1 purified through isoelectric focusing resolved a protein band with a molecular weight of 10,000. Propionibacterium PLG-1 was bactericidal to sensitive cells, demonstrating single-hit kinetics. The producing strain harbored a single plasmid (pLG1) with an approximate size of 250 kb. Preliminary data indicate that both Propionibacterium PLG-1 and immunity to the bacteriocin are encoded on the chromosome. Exposure of strain P127 to acriflavine or to N-methyl-N'-nitro-N-nitrosoguanidine yielded isolates that no longer produced bacteriocin activity and isolates that were cured of the plasmid. However, loss of bacteriocin production was not correlated with loss of the plasmid. Isolates cured of the plasmid were phenotypically identical to plasmid-bearing cells in fermentation patterns, pigment production, and growth characteristics.

Bacteriocins, which are produced by a heterogeneous group of microorganisms, have a wide range of chemical properties, modes of action, and antibacterial spectra. Numerous bacteriocins have been isolated from different genera of bacteria (5, 21).

Propionibacterium species were first described in 1909 by Orla-Jensen, who divided them into two principal groups: the classical or dairy propionibacteria and the cutaneous propionibacteria (17). The former are used in dairy fermentations and may contribute to natural fermentations of silage and olives (3, 19). As dairy starter cultures, the propionibacteria are used to produce the characteristic eyes and flavor of Swiss-type cheeses (2, 11, 17).

Although numerous bacteriocins from gram-positive organisms have been isolated, characterized, and purified, few propionibacterial bacteriocins have been identified (5, 8, 9, 22). Two bacteriocins in the P. acnes group (6, 18) and three in the classical propionibacteria (8, 13) have been reported. We reported previously that a bacteriocin produced by Propionibacterium thoenii P127 was active against closely related species and exhibited a broad range of activity against some gram-positive and some gram-negative bacteria and some molds and yeasts (13). This bacteriocin, designated propionicin PLG-1, was partially purified and shown to be heat labile, sensitive to several proteolytic enzymes, and stable at pH 3 through 9. In this paper, we describe the production, isolation, and purification of propionicin PLG-1.

MATERIALS AND METHODS

Cultures and media. Bacteriocin-producing strain P. thoenii P127 and sensitive indicator strain Propionibacterium acidipropionici P5 were propagated and media were prepared as described previously (13). Agrobacterium tumefaciens A281 was propagated in nutrient broth (Difco, Detroit, Mich.) with vigorous agitation at 25°C.

Production studies at controlled pH. A 1% (vol/vol) inoculum of an 18-h broth culture of P. thoenii P127 was used to inoculate a Biostat M fermentor (1.5-liter working volume; B. Braun Biotech, Allentown, Pa.). Cultures were grown at 32°C for 14 days and agitated at 150 rpm without aeration. The initial pH was adjusted with 3.0 M HCl to the specified pH and during fermentation was controlled within the pH range of 7.0 ± 0.05 by the addition of 3.0 M NaOH and 3.0 M HCl. At 12-h intervals, 25-ml samples were removed from the fermentor and evaluated for cell viability, turbidity (A490), and acid production. Periodically, 300-ml samples were removed and the bacteriocin was partially purified by ammonium sulfate precipitation as described below.

Bacteriocin assays. Propionibacterium PLG-1 activity was assayed by both the critical dilution method and the well diffusion method as described previously (15, 22). Activity of purified bacteriocin preparations was measured by adding 200 μl of purified propionicin PLG-1 to a well cut in a sodium lactate agar (NLB) plate or spotting 100 μl onto cell lawns prepared as described previously (1). Activity was defined as the reciprocal of the highest dilution causing complete inhibition of the indicator lawn and was expressed as arbitrary units (AU) per milliliters. Known amounts of bacteriocin activity (AU per milliliter), previously determined from critical dilution assays, were added to each well and allowed to diffuse out into the agar. Approximately 107 cells of indicator organisms were added to soft agar overlays, and plates were incubated at 32°C for 2 days. The diameters (millimeters) of the zones of inhibition were measured and noted for each concentration of bacteriocin used. The zones of inhibition and their respective concentrations of bacteriocin (AU per milliliter) were used to construct a standard curve. All assays were performed in duplicate, and the results presented are the means of duplicate trials.

Bactericidal action of propionicin PLG-1. Cells from 18-h sodium lactate broth (NLB) cultures of strains P127 and P5 were harvested by centrifugation (9,000 × g), washed in 0.05 M phosphate buffer, and added at final concentrations of 3.8 × 106 and 1.2 × 108 CFU/ml, respectively, to a purified bacteriocin preparation (500 ml; 32 AU/ml). After 60 min of

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incubation, cultures were serially diluted in 0.2% peptone, and survivors were counted on NLA. Survivor ratios were determined as described by Mayr-Harting et al. (15) with the following equation: \( \log R_s \) (survivor ratio) = (CFU/milliliter at given time)/(CFU/milliliter at time 0).

**Purification of propionicin PLG-1.**

(i) **Initial steps.** A 14-day culture of *P. thoenii* from the fermentor (2,500 ml) was centrifuged at 12,000 × g for 30 min at 4°C. Optimal conditions for ammonium sulfate precipitation of the bacteriocin from the supernatant were previously determined to be between 40 and 60% saturation at 4°C. Therefore, some of the undesirable proteins were precipitated by adding ammonium sulfate to 40% saturation at 4°C, the suspension was slowly stirred for 30 min, and precipitated proteins were collected by centrifugation at 29,000 × g for 30 min at 4°C. The supernatant was decanted, and ammonium sulfate was added until 60% saturation was reached. Precipitated proteins were pelleted by centrifugation (29,000 × g), resuspended in 5 ml of 20 mM bis(2-hydroxyethyl)imino(trimethylene)methane (BS-Tris [pH 7.0]; Sigma Chemical Co., St. Louis, Mo.), and dialyzed against 2 liters of the same buffer for 18 h in Spectra-Por no. 3 dialysis tubing (molecular weight cutoff, 3,500; Spectrum Medical Industries, Los Angeles, Calif.). This preparation was designated partially purified bacteriocin.

(ii) **Ion-exchange chromatography.** Partially purified bacteriocin (5 ml, 100 AU/ml) was applied to both a descending DEAE-Sepharose column (2.5 by 37 cm; Sigma) equilibrated with 20 mM Bis-Tris (pH 7.0) at 4°C and a descending carboxymethyl Sepharose column (2.5 by 35 cm; Sigma) equilibrated with 20 mM 2-(N-morpholino)ethanesulfonic acid (MES [pH 6.5]); Sigma) at 4°C. Proteins were eluted from the columns with a linear salt gradient (0 to 1.0 M NaCl, 500 ml) in their respective buffers. Fractions (4 ml) were collected, and their refractive indices were determined. Fractions containing protein were concentrated fivefold by dialysis against a Bio-Gel concentrator (Bio-Rad Laboratories, Richmond, Calif.) and assayed for bacteriocin activity by the well diffusion method.

(iii) **Rotofer isoelectric focusing.** A Rotofer isoelectric focusing chamber (Bio-Rad) was assembled as described by the manufacturer. Propionin PLG-1 was eluted from a carboxymethyl Sepharose column with MES (pH 6.5) containing 1 M NaCl. Fractions were assayed for propionin PLG-1 activity by the well diffusion method, and the fraction containing activity was dialyzed overnight against a 5% glycerol solution containing 1 mM phenylmethylsulfonyl fluoride (Sigma), 1 mM EDTA, and 100 mM KCl. The Rotofer cell was prefocused with 40 ml of 5% glycerol in distilled water containing 2% (vol/vol) Bio-Lyte ampholytes (1:1 ratio of pH 3 to 10 and pH 3 to 7; Bio-Rad), 1 mM EDTA, and 100 mM KCl with 12 W of constant power at 4°C for 1 h to establish the pH gradient. The initial prefocus conditions were 300 V and 32 mA; at pH equilibrium, the values were 989 V and 22 mA.

After the Rotofer cell was prefocused, the dialyzed bacteriocin sample was injected near the middle of the focusing chamber; the sample was focused for another 3 h, at which time the final voltage was 848 V. Twenty 1.5-ml fractions were harvested as described by the manufacturer of the cell, and the pH values of the fractions were measured. Ampholytes were removed by dialyzing (Spectra-Por no. 3 dialysis tubing) against 100 volumes of 1 M NaCl containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride. Individual fractions were assayed for bacteriocin activity by the dilution method (16), and the fraction containing activity was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on an 18% polyacrylamide tube as described below. Buffer alone was assayed for potential inhibitory activity against the indicator strain.

**Mutant selection and plasmid analysis.** Producer strain *P. thoenii* P127 was grown for 18 h at 32°C in NLAB containing various concentrations of acriflavine (5 to 50 mg/ml; Sigma) and N-methyl-N'-nitro-N-nitrosoguanidine (100 to 500 mg/ml; Sigma) as described previously (7). The percentage of kill resulting from exposure to mutagens was calculated as \( \frac{\text{[initial cell count} - \text{number of survivors}/\text{initial cell count}] \times 100}{\text{After mutagenesis, cultures were serially diluted and plated onto NLA. After incubation for 1 week at 32°C, 500 isolated colonies were transferred with sterile toothpicks onto duplicate master plates of NLA (7 colonies per plate) and reinoculated for 7 days. One plate was overlaid with soft NLA (5 ml, 0.5% agar) containing 100 ml of the indicator strain P5 (10⁸ CFU/ml). After development of the overlay lawn, individual P127 colonies were evaluated for bacteriocin production, and selected colonies were transferred from the second master plate to 10 ml of NLB and grown for 24 h at 32°C. These cultures were then inoculated at 1% into 1,000 ml of NLB and incubated at 32°C for 24 h to provide cultures to be screened for the presence of plasmid DNA.

Plasmid DNA was isolated from mutants by using the procedure described by Rehberger (20) with certain modifications. Cells were harvested by centrifugation at 10,000 × g, washed in 50 mM Tris-HCl–10 mM Na₂EDTA (pH 8.0), pelleted by centrifugation, and resuspended in 25 ml of 15% sucrose in 50 mM Tris-HCl–50 mM Na₂EDTA (pH 8.0). The cell suspension was transferred to a 250-ml centrifuge bottle, to which was added 6.25 ml of freshly prepared lysozyme (100 mg/ml in 50 mM Tris-HCl–50 mM Na₂EDTA [pH 8.0]). Contents were mixed by inversion and incubated at 32°C for 1 h. Pronase E (Sigma) (10 mg/ml in 50 mM Tris-HCl–5 mM Na₂EDTA–50 mM NaCl [pH 8.0]) was preincubated at 37°C for 1 h, and 3.25 ml was added to the tube and mixed by inversion. Then 11.5 ml of 25 mM Na₂EDTA (pH 8.0) was added and mixed by inversion, and the tube was placed on ice for 15 min. After incubation, 11.5 ml of 20% (wt/vol) SDS in 50 mM Tris-HCl–25 mM Na₂EDTA (pH 8.0) was added and gently mixed by inversion. A 5 M NaCl solution was added to a final concentration of 1 M NaCl and mixed by inversion, and the mixture was placed on ice for 4 h. Cell debris was pelleted by centrifugation at 12,000 × g for 15 min. An aliquot of 13% polyethylene glycol (molecular weight, 8,000; Sigma) in 50 mM Tris-HCl–10 mM Na₂EDTA (pH 8.0) equal to one-half the volume of the supernatant was added and gently mixed by inversion. The tube was placed on ice for 18 h, and then precipitated plasmid DNA was pelleted by centrifugation at 12,000 × g for 30 min. The DNA pellet was resuspended in 1.0 ml of 10 mM Tris-HCl–1 mM Na₂EDTA (pH 8.0), extracted with 1.0 ml of phenol–chloroform–isoamyl alcohol (25:24:1), and centrifuged at 12,000 × g for 5 min. The upper phase was transferred to a fresh centrifuge tube and extracted with 1.0 ml of chloroform–isoamyl alcohol (24:1), and the upper phase was transferred to a fresh tube. Residual amounts of phenol were removed by extracting twice with 1.0 ml of H₂O-saturated diethyl ether. The lower DNA phase was retained, and residual ether was evaporated in a hood. The plasmid DNA was purified by two consecutive cesium chloride–ethidium bromide density gradient centrifugations as described by Manatis et al. (14). DNA samples were extracted with isopropanol saturated with 5 M NaCl to remove the ethidium.
bromide, desalted, and concentrated in 10 mM Tris-HCl-1 mM Na₂EDTA (pH 7.2) by using a Centricon-30 microconcentrator as described by the manufacturer (Amicon Corp., Danvers, Mass.).

Plasmid preparations were analyzed by electrophoresis in 0.4% agarose gels. Mobility reference plasmids were isolated from A. tumefaciens A281 by the method of Casse et al. (4).

SDS-PAGE. PAGE in the presence of 0.1% SDS was performed as described by Laemmli (10). The polyacrylamide and N,N'-methylenebisacrylamide (Sigma) concentrations were 5 and 0.15%, respectively, in the stacking gel (1 ml) and 18 and 0.5%, respectively, in the separating gel (10 ml). The gels were stained with Coomassie brilliant blue R (Sigma), as described by Merrill et al. (16). Protein standards and their molecular weights were as follows: ovalbumin, 43,000; carbonic anhydrase, 29,000; β-lactoglobulin, 18,400; lysozyme, 14,300; bovine trypsin inhibitor 6,200; α and β insulin, 3,000 (all from Bethesda Research Laboratories, Gaithersburg, Md.).

**Protein determinations.** Protein was determined by a modification of the method of Lowry et al. (12), according to the specifications of the manufacturer of the reagents (Sigma). Bovine serum albumin was used to construct a standard curve.

**RESULTS**

**Effect of pH on propionicin production.** Propionicin PLG-1 was initially isolated from semisolid media because attempts to isolate it from cell supernatants of broth cultures were unsatisfactory (13). Since then, we have been able to detect propionicin PLG-1 in culture supernatants by allowing cultures to grow into the late-stationary phase. Propionicin PLG-1 was produced in a very low concentration in the culture supernatant (0.83 AU/ml); therefore, large volumes of culture supernatant (1,000 ml) were fractionated with ammonium sulfate and dialyzed before bacteriocin activity was assayed. After the conditions required for propionicin PLG-1 isolation were optimized, the production of propionicin PLG-1 was evaluated during anaerobic growth of *P. thoenii* P127 in NLB at controlled pH (Fig. 1). At pH 7.0, maximal production of propionicin (50 AU/ml) was observed at 180 h. Bacteriocin production was much lower in cultures grown at pH 6.0, 6.5, and 7.5, although culture growth at each pH was comparable to growth at pH 7.0. Growth at pH 8.0 was poor, and the maximal bacteriocin activity was only 5 AU/ml at 180 h (data not shown).

**Purification of propionicin.** Propionicin PLG-1 (0.83 AU/ml, 1.29 mg of protein per ml), which was found in the supernatant during the late-exponential phase of growth,
TABLE 1. Purification of propionicin PLG-1

<table>
<thead>
<tr>
<th>Purification stage</th>
<th>Total propionicin activity* (AU)</th>
<th>Sp act$^b$ (AU/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture supernatant</td>
<td>2,075</td>
<td>0.64</td>
</tr>
<tr>
<td>Ammonium sulfate fractionation</td>
<td>500</td>
<td>388</td>
</tr>
<tr>
<td>Carboxymethyl Sepharose eluent, pooled and concentrated</td>
<td>250</td>
<td>794</td>
</tr>
<tr>
<td>Isoelectric focusing</td>
<td>160</td>
<td>3,678</td>
</tr>
</tbody>
</table>

* Determined by the critical-dilution method.
$^b$ Protein concentration determined by the method of Lowry et al. (12).

was concentrated approximately 600-fold from the culture medium by ammonium sulfate precipitation. The specific activity increased from 0.64 AU/mg of protein in the culture supernatant to 388 AU/mg of protein after ammonium sulfate fractionation (Table 1). When partially purified propionicin was applied to a descending DEAE-Sepharose column previously equilibrated with Bis-Tris (pH 6.5), all bacteriocin activity was recovered in the void volume (data not shown). After partially purified bacteriocin (50 AU/ml; 129 μg of protein per ml) was applied to a carboxymethyl Sepharose column and eluted with a salt gradient, proteins were eluted in four peaks (Fig. 2). The first two peaks were eluted between 0 and 0.2 M NaCl, and the third peak was eluted between 0.4 and 0.6 M NaCl. A single fraction eluted within the third peak contained bacteriocin activity (50 AU/ml; 63 μg of protein per ml). After passage through the carboxymethyl Sepharose column, 12% of the propionicin PLG-1 activity was recovered and the specific activity was increased 1,200-fold (Table 1).

The active fraction was applied to the Rotofor for isoelectric focusing. Twenty fractions were collected from the Rotofor, and their pH values were determined. Propionicin PLG-1 activity (32 AU/ml, 8.7 μg/ml) was found in a single fraction (fraction 17), centering at approximately pH 8.8. After isoelectric focusing, 7% of propionicin PLG-1 activity was recovered and the specific activity was approximately 5,700-fold higher than that of the culture supernatant (Table 1).

SDS-PAGE. Propionicin PLG-1 preparations at various stages of purification were analyzed by SDS-PAGE in 18% polyacrylamide tube gels (Fig. 3). Several contaminating proteins were detected in the preparation after ammonium sulfate precipitation and dialysis. Propionicin preparation eluted from a descending carboxymethyl Sepharose column contained fewer proteins than did partially purified bacteriocin, but several proteins of both low and high molecular weight were still present. The single active fraction obtained after isoelectric focusing yielded in SDS-PAGE a single protein band with an apparent molecular weight of approximately 10,000.

Effect of propionicin PLG-1 on sensitive and nonsensitive cells. Crude preparations of propionicin PLG-1 adsorbed to sensitive cells of P5 and were bactericidal (13). Purified propionicin PLG-1 obtained after isoelectric focusing was reevaluated for its bactericidal activity against indicator strain P5. The viable counts of indicator strain P5 were reduced by 99.6% after exposure to purified propionicin, but the viable counts of strain P127 were unchanged (Fig. 4). Residual inhibitory activity was not detected in the supernatant of preparations mixed with cells of strain P5 but was unchanged in preparations mixed with P127 cells (data not shown). The absorbancies of both suspensions were stable throughout the experiment. Propionicin PLG-1 seemed to induce cell death without detectable cell lysis of the sensitive strain.

Plasmid involvement. Producer strain P. thoenii harbors a single large plasmid (250 kb), designated pLG1 (Fig. 5). To isolate the intact large plasmid, significant changes had to be made in the plasmid isolation procedure of Reuberger (20), as described in Materials and Methods. These changes include SDS-NaCl precipitation of denatured chromosomal
DNA, polyethylene glycol precipitation of the plasmid, and removal of single-stranded DNA by phenol extraction. Efforts were made to determine whether the plasmid carried genetic determinants responsible for propionicin production (Prp+) and host immunity (Imm+) to the bacteriocin. Mutants deficient in propionicin production (Prp-) and immunity (Imm-) were obtained by acriflavine and N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis. The percentage of kill in all experiments was within the range of 90 to 99%. When strain P127 was grown in the presence of 10 μg of acriflavine per ml, 5% of the surviving colonies were Prp-. After growth of strain P127 with 200 μg of N-methyl-N'-nitro-N-nitrosoguanidine per ml, 1% of surviving colonies were Prp-. The carbohydrate fermentation patterns, Gram stain reaction, pigment production, and growth characteristics exhibited by the Prp- mutants were identical to those of the parent culture, *P. thoennii* P127 (data not shown). All Prp- mutants had immunity to propionicin PLG-1, except for one mutant that was Prp- Imm-.

Agarose gel electrophoresis of plasmid DNA isolated from selected mutagenized isolates revealed that three Prp+ Imm+ isolates contained plasmid pLG1, whereas four Prp- Imm+ isolates were cured of pLG1 (Fig. 5). The single Prp- Imm- mutant isolated contained plasmid pLG1. We also examined several mutagenized Prp+ isolates. Of these, two Prp+ Imm+ isolates contained plasmid pLG1, whereas two Prp+ Imm+ isolates were cured of plasmid pLG1.

**FIG. 4.** Survival of producer strain P127 and indicator strain P5 after incubation at 32°C for up to 60 min with purified propionicin (32 AU/ml). Points are the averages of duplicate plates not differing by more than ±10 CFU/ml. Symbols: □, producer strain P127; ○, indicator strain P5.

**FIG. 5.** Agarose gel electrophoresis of plasmid DNA from *A. tumefaciens* A281 (250 kb; lane E), wild-type *P. thoennii* P127 (lane A), Prp- Imm+ mutants (lanes B, D, F, J, K, L, and M), Prp- Imm- mutant (lane I), and Prp+ Imm+ mutagenized isolates (lanes C, G, H, and N).

**DISCUSSION**

Tagg et al. (21) described bacteriocin action as a single-hit mechanism in which the bacteriocin adsorbs to, penetrates, and kills sensitive cells in an irreversible process. Purified propionicin PLG-1 added to sensitive cells of strain P5 was bactericidal; however, the bacteriocin did not inhibit the producer strain P127. More extensive testing with purified propionicin PLG-1 against a wider spectrum of microorganisms is needed. The targets of propionicin PLG-1 activity and its mode of action on sensitive cells have not been determined.

The accumulation of propionicin during the stationary phase is characteristic of a secondary metabolite. Maximal production of propionicin was observed at pH 7.0. Possible factors for poor production of propionicin at pH 6.0, 6.5, 7.5, and 8.0 include (i) the low cell density obtained at pH 8.0 and (ii) the possibility that the reaction(s) necessary for active propionicin synthesis is dependent upon one or more pH-sensitive enzymes. Propionicin PLG-1 showed many similarities to the lactacin B produced by a strain of *Lactobacillus acidophilus* (1).Maximum production of both bacteriocins occurred during the stationary growth phase of producer cultures. Both were originally isolated as high-molecular-weight protein aggregates. The initial molecular-weight estimates were 150,000 (13) and 100,000 (1) for propionicin PLG-1 and lactacin B, respectively.

Propionicin PLG-1 (molecular weight, 10,000) was isolated from contaminating proteins by ion-exchange chromatography and isoelectric focusing. Numerous contaminating proteins were removed by application of partially purified bacteriocin to a carboxymethyl Sepharose column. A single fraction within a peak of absorbance contained bacteriocin activity but also contained contaminating proteins. The bacteriocin within this fraction was then purified to homogeneity by isoelectric focusing. Evidence for homogeneity of propionicin PLG-1 was provided by the absence of contaminating proteins on an SDS-PAGE electropherogram.

*P. thoennii* P127 harbored a single large plasmid that seemed not to encode genetic determinants for propionicin PLG-1 production or host cell immunity. The wild-type parent, a Prp- Imm- mutant, some Prp+ Imm+ isolates exposed to mutagens, and some Prp- Imm+ mutants harbored the plasmid. In contrast, other Prp- Imm+ and Prp+ Imm- mutants lost the plasmid during curing experiments. No Prp- Imm- isolates were found. This phenotype probably would not be viable, because loss of the immunity protein would allow the bacteriocin to kill the producer cells. Whether no bacteriocin or an inactive bacteriocin molecule is produced remains to be determined.

If plasmid pLG1 does not carry the genes for bacteriocin activity and host immunity, these genetic determinants may be located within the chromosome. It is also possible that an unstable or undetected integrated plasmid is responsible for propionicin PLG-1 production. Although bacteriocin production is frequently plasmid associated (21), there is precedent for this trait to be found on the chromosome. For example, Joerger and Klaenhammer (9) found that helveticin J production and immunity in *Lactobacillus helveticus* 481 were chromosomally mediated.

No phenotypic changes in isolates cured of pLG1 were detected. In addition to bacteriocin production and sensitivity, the characteristics examined included pigment production, growth rates, and fermentation of arabinoose, fructose, glucose, lactose, maltose, mannose, mannitol, rhamnose, starch, sucrose, trehalose, and xylose. Thus, plasmid pLG1...
remains cryptic. The Prp− Imm− mutant was phenotypically identical to the wild type in all other characteristics. Elucidation of the amino acid sequence of propionicin PLG-1 should facilitate cloning of the genetic determinants for this bacteriocin. Further characterization of propionicin PLG-1 production and its mode of action is in progress.

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REFERENCES