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Production of Pediocin PA-1 by *Lactococcus lactis* Using the Lactococcin A Secretory Apparatus

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The class II bacteriocins pediocin PA-1, from *Pediococcus acidilactici*, and lactococcin A, from *Lactococcus lactis* subsp. *lactis* bv. diacetylactis WM4 have a number of features in common. They are produced as precursor peptides containing similar amino-terminal leader sequences with a conserved processing site (Gly-Gly at positions −1 and −2). Translocation of both bacteriocins occurs via a dedicated secretory system. Because of the strong antilisterial activity of pediocin PA-1, its production by lactic acid bacteria strains adapted to dairy environments would considerably extend its application in the dairy industry. In this study, the lactococcin A secretory system was adapted for the expression and secretion of pediocin PA-1. A vector containing an in-frame fusion of sequences encoding the *lcnA* promoter, the lactococcin A leader, and the mature pediocin PA-1, was introduced into *L. lactis* IL1403. This strain is resistant to pediocin PA-1 and encodes a lactococcin translocation apparatus. The resulting *L. lactis* strains secreted a bacteriocin with an antimicrobial activity of approximately 25% of that displayed by the parental pediocin-producing *P. acidilactici* 347. A noncompetitive indirect enzyme-linked immunosorbent assay with pediocin PA-1-specific antibodies and amino-terminal amino acid sequencing confirmed that pediocin PA-1 was being produced by the heterologous host.

Bacteriocins of lactic acid bacteria have received considerable attention in recent years due to their potential application in the food industry as natural preservatives. Most interest has focused on lantibiotics (class I bacteriocins), e.g., nisin, and small heat-stable non-lanthionine-containing bacteriocins (class II) (22, 23). A major subgroup of class II bacteriocins (IIa) has been given the generic name of pediocin family (28) after its most extensively studied member, pediocin PA-1. Members of this class have a number of features in common, including a very strong antimicrobial activity against *Listeria* species (28). The food-borne pathogen *Listeria monocytogenes* is a major concern in the dairy industry since it can grow in a variety of dairy products at low temperature and pH (13). Although a pediocin PA-1-producing *Lactobacillus plantarum* strain has recently been isolated (12), this bacteriocin is generally produced by *Pediococcus acidilactici* strains of meat origin (3, 16, 18, 29, 31). Because of its antilisterial activity, the expression of pediocin PA-1 in strains of dairy origin would be highly desirable.

Pediocin PA-1 production, immunity, and secretion are determined by an operon containing four genes (26). The structural gene, *pedA*, encodes the pediocin PA-1 precursor, *pedB* specifies immunity, and the *pedC* and *pedD* gene products are membrane-bound proteins required for secretion of the active peptide (39). Homologs of these genes have been described for related peptides. Biosynthesis of the well-characterized class II bacteriocin, lactococcin A, produced by strains of *Lactococcus lactis* also involves four genes (20, 36, 40). In addition to the structural gene (*lcnA*) and immunity gene (*lciA*), there are two genes (*lcnC* and *lcnD*) whose products together form a transport system dedicated to the translocation of lactococcin through the host membrane. The LcnC protein belongs to the family of ATP-binding cassette transporter proteins (40), and LcnD acts as an accessory protein (14). These two proteins have considerable homology to PedD and PedC, respectively (39), suggesting that the latter proteins play a similar role in the transport of active pediocin. The two bacteriocins also share the double glycine-processing site found in many lactic acid bacteria class II bacteriocins, some lantibiotics, and the *Escherichia coli* bacteriocin, colicin V (17).

Van Belkum et al. (38) have recently investigated the role of leader sequences of the class II bacteriocins in the recognition of the precursor peptide by the dedicated translocation machinery of the host organism. By constructing hybrid genes, they demonstrated that the leader peptides of leucocin A, lactococcin A, and colicin V, which are cleaved at the Gly-Gly (positions −2 and −1) site, can direct the secretion of the nonrelated bacteriocin divergicin A. Our studies have focused on the class II bacteriocins pediocin PA-1 and lactococcin A. Since these peptides have a number of features in common, it might be expected that a pediocin PA-1 precursor could be secreted and processed by using the lactococcin A translocation machinery. *L. lactis* IL1403 is a plasmid-free strain that does not produce bacteriocin but contains chromosomal copies of genes analogous to *lcnC* and *lcnD* (33, 40). In addition, the natural resistance of this strain to pediocin PA-1 (8) makes it an ideal candidate for a production host to investigate the expression of pediocin PA-1 in lactococci.

This paper describes the development of an expression system geared to the production of heterologous peptides in *L. lactis*. Testing the system with pediocin PA-1 involved the construction of a vector containing an in-frame fusion between sequences encoding the lactococcin A leader and the structural part of mature pediocin PA-1. The hybrid genes were introduced into *L. lactis* IL1403, and the ability of these strains to produce and secrete pediocin PA-1 was investigated.

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TABLE 1. Bacterial strains and plasmid used in this study

Strain	Relevant characteristics	Source or reference
L. lactis subsp. lactis bv. diacetylactis WM4	Lactococcin A producer	32
L. lactis IL1403	Plasmid-free; LcnA$^-$, LciA$^-$; containing *lcnC* and *lcnD* gene analogs; pediocin PA-1 resistant	40
L. lactis MG1614	Plasmid free; LcnA$^-$, LciA$^-$	15
L. lactis FI8817	IL1403(pFI2058)	This study
L. lactis FI9043	IL1403(pFI2126)	This study
P. acidilactici 347	Pediocin PA-1 producer	31
P. acidilactici 347-8	*P. acidilactici* 347-cured derivative	NBIII[a]
E. faecium P21	Pediocin PA-1-sensitive indicator	NBIII[a]
E. coli MC1022	Plasmid free	7

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MATERIALS AND METHODS

Microbiological techniques, strains, and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Lactococcal strains were routinely grown in M17 medium (34) supplemented with 0.5% (wt/vol) glucose (GM17 medium) at 30°C without agitation. *P. acidilactici* was grown in MRS medium (Oxoid, Unipath Ltd., Basingstoke, United Kingdom) at 30°C without agitation. *E. coli* was grown in L broth (24) at 37°C on an orbital shaker. Agar plates were made by the addition of 1.5% (wt/vol) agar to broth media. Antibiotics were added as selective agents when appropriate: chloramphenicol, 5 μg ml^{-1} for lactococci and 15 μg ml^{-1} for *E. coli*, and ampicillin, 200 μg ml^{-1}.

Antimicrobial activity in cultures was assayed by a plate diffusion bioassay performed as previously described (11) with *L. lactis* MG1614 and *Enterococcus faecium* P21 as indicator organisms sensitive to lactococcin A and pediocin PA-1, respectively. Pediocin PA-1 production was quantified with a series of pure pediocin PA-1 standards ranging from 0 to 20 μg ml^{-1}. The zones of inhibition were measured and plotted against the logarithm of their concentration to give a standard curve from which test supernatant concentrations were estimated.

Molecular techniques. Plasmid DNA isolation was carried out as described by Dodd et al. (10). Restriction enzymes and other DNA-modifying enzymes from various sources were used as specified by the suppliers. Recombinant plasmids were recovered by transformation of *E. coli* as described previously by Dodd et al. (10) or by electroporation of *L. lactis* by the method of Holo and Nes (19) with the modifications used by Dodd et al. (10). Conditions used for PCR were as described by Horn et al. (21), and the primers were synthesized on an Applied Biosystems DNA synthesizer (model 381A). Fragments generated for the construction of vectors were amplified with Dynazyme (Flowgen) and cloned into pCRII (Invitrogen) before nucleotide sequence confirmation. For routine PCR screening of recombinant clones, AmpliTaq DNA polymerase (Perkin-Elmer) was used. The nucleotide sequences of PCR-generated fragments were confirmed on purified plasmid DNA with an Applied Biosystems DNA sequencer (model 373A) and the manufacturer's Taq DyeDeoxy Terminator Cycle sequencing kit.

Construction of pFI2058 (containing *lcnA* and *lciA*). Lactococcin A genes were introduced into the shuttle vector pTG262 by PCR amplification of the relevant segment of DNA from *L. lactis* WM4 with flanking primers plcn1 (5′-CAATC AGTAGAGTTATTAACATTTG-3′) and plcn2 (5′-GATTTAAAAAGACATT CGATTATTAT-3′) (Fig. 1a). This generated a 770-bp PCR fragment containing the *lcnA* and *lciA* genes with the upstream promoter and downstream putative transcription terminator sites (33). The fragment was cloned into pCRII, and the nucleotide sequence of the inserted DNA was confirmed. The PCR-generated *lcnA* and *lciA* genes were recovered as an *Eco*RI fragment and cloned into the *Eco*RI site of pTG262 to generate pFI2058.

Construction of the *lcnA*/*pedA* hybrid gene. The technique of spliced overlap extension was used in the construction of in-frame fusions of sequences encoding the lactococcin A leader (Fig. 1a) and the structural portion of mature pediocin PA-1 (Fig. 1b). This initially involved the amplification of two DNA fragments.

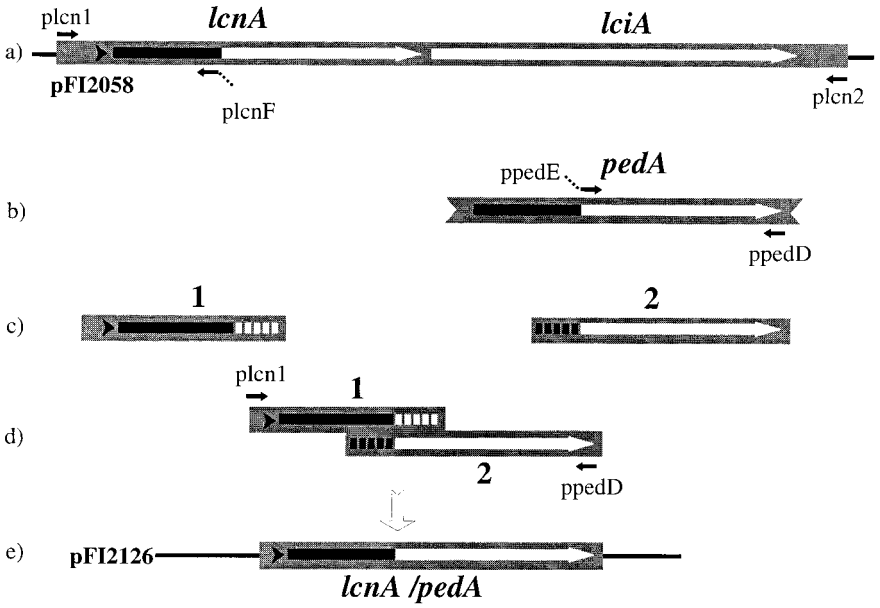

FIG. 1. PCR strategy used for splicing the *lacA* and *pedA* genes. (a) Region of the lactococcin A operon, containing *lcnA* and *lciA* genes, cloned into pFI2058. (b) Map of the *pedA* gene. The thick arrows show coding regions with the amino-terminal leaders indicated in black. The small arrows above and below the maps indicate the position and direction of primers used for PCR. Nonhomologous tails on primers plcnF and ppepE are represented by dashed lines. (c) Fragments generated by the first PCR step. (d) Homologous regions in fragments 1 and 2 annealed to provide a template for the second PCR step, involving primers plcn1 and ppepD. (e) Product of the second PCR step, cloned into pFI2126. An in-frame fusion resulted in the creation of a consensus amino-terminal cleavage site (vertical arrow) between two parts of the *lcnA*/*pedA* hybrid gene.

Primers plcn1 and plcnF (5′-CCATTACCGTAGTATTTTCCTCCGTTAGCTTC-3′) were used to amplify a 170-bp fragment, encoding the lactococcal A leader and promoter (fragment 1 [Fig. 1c]). DNA from colonies of *L. lactis* WM4 was used as the template. The 17 nucleotides forming a tail at the 5′ end of primer plcnF (underlined) are complementary to the amino-terminal sequences of mature pediocin PA-1, i.e., after cleavage at the Gly-Gly site of the leader peptide (26). Primers ppedD (5′-ACCCCGGGATTGATGCCAGCTC-3′) and ppedE (5′-GAAGCTAACGGAGGAAAATACTACGGTAATGG-3′) were used to amplify a 180-bp fragment 2 (Fig. 1c), comprising exclusively the part of the *pedA* gene that encodes mature pediocin PA-1 (26). The template was provided by DNA from colonies of *P. acidilactici* 347. Primer ppedE was designed with a 5′ tail corresponding to sequences within the lactococcin A leader. These 19 nucleotides (underlined) are complementary to the 3′ end of fragment 1 (Fig. 1c). Fragments 1 and 2 were diluted (1/200) in distilled water, and equal quantities were mixed. This mixture was used as the template to amplify a 312-bp fragment with primers plcn1 and ppedD (Fig. 1d). The fragment was cloned into pCRII, and nucleotide sequence analysis confirmed that it was composed of sequences corresponding precisely to the in-frame fusion of the lactococcin A leader and mature pediocin PA-1 (Fig. 1e). The hybrid gene and upstream promoter region was isolated as an *Eco*RI fragment and cloned into pTG262 to generate pFI2126. Transformation of *L. lactis* IL1403 with this recombinant plasmid generated strain FI9043.

Purification and amino acid sequencing of pediocin PA-1. The bacteriocin produced by *L. lactis* FI9043 was purified from a 1-liter culture grown in MRS broth at 30°C to late logarithmic phase. The procedure, involving ammonium sulfate precipitation and, successively, cation-exchange, hydrophobic interaction, and reverse-phase chromatography (PepRPC HR5/5 fast protein liquid chromatography system; Pharmacia LKB, Uppsala, Sweden) was essentially as previously described (9, 30) except that the fraction obtained after ammonium sulfate precipitation was applied to a Sephadex G-25 gel filtration column (Pharmacia) and equilibrated with 20 mM sodium phosphate buffer (pH 5.8). The fraction displaying activity was then applied to the cation-exchange column. The active fraction, obtained after hydrophobic interaction chromatography, was applied to the reverse-phase column, and the bacteriocin was eluted with a linear gradient ranging from 10 to 60% 2-propanol containing 0.1% trifluoroacetic acid. Purification steps were performed at room temperature, and the chromatographic equipment and reagents were obtained from Pharmacia and used as specified by the supplier. The microtiter plate assay system developed by Holo et al. (20) was used to quantify the bacteriocin activity during the purification process. One bacteriocin unit was defined as the reciprocal of the highest dilution causing 50% growth inhibition of the indicator organism, *Enterococcus faecium* P21.

The reverse-phase fraction containing the bacteriocin was desiccated by rotary evaporation and resuspended in an equivalent volume of deionized water. The concentration of pure bacteriocin was estimated by using the molar extinction coefficient of pediocin PA-1 (an absorbance at 280 nm of 3.1 corresponds to 1.0 mg ml^{-1}). The amino-terminal sequence of the purified bacteriocin was determined by Edman degradation with an automatic sequencer (model 47A; Applied Biosystems).

Specific detection of pediocin PA-1 by ELISA. The production of pediocin PA-1 by strains used in this study was assessed using a noncompetitive indirect enzyme-linked immunosorbent assay (NCI-ELISA), based on the method of Bubert et al. (6). Briefly, 100 μl of pure bacteriocin samples (100 μl) was serially diluted in 0.1 M sodium carbonate-bicarbonate buffer (pH 9.6) to give a range of concentrations from 0 to 2.5 μg ml^{-1}. Samples were incubated in 96-well polystyrene microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) for 3 h at 37°C. After the coated bacteriocin was washed with phosphate-buffered saline (PBS), wells were blocked for 1 h at 37°C with 0.01 M PBS (pH 7.2) containing 1% ovalbumin (OA). A 50-μl volume of antiserum, diluted 1:1,000 in PBS, was then added, and the plates were incubated for 1 h at 37°C. The antiserum contained rabbit antibodies raised against a synthetic peptide (PH2) composed of the carboxy-terminal 11 amino acids of pediocin PA-1 (25). For colorimetric reactions, horseradish peroxidase-conjugated goat anti-rabbit antibodies (Cappel Laboratories, West Chester, Pa.), diluted 1:500 in PBS, and the substrate 2,2′-azinobis(3′-ethylbenzothiazoline-6-sulfonic acid) (Sigma, St. Louis, Mo.) were used. The absorbance was read at 405 nm in a Labsystems iEMS reader with a built-in software package for data analysis (Labsystems, Helsinki, Finland). PH2 conjugated to OA by the glutaraldehyde method (OA-PH2) (5), pure nisin (Aplin and Barrett, Trowbridge, United Kingdom), pediocin PA-1 (produced by *P. acidilactici* 347 and purified by the same method cited above), and the protein fraction obtained from culture supernatants of *P. acidilactici* 347-8 (a pediocin PA-1 nonproducer) were used as controls at the equivalent concentrations.

RESULTS

Lactococcin A expression. The production of lactococcin A in *L. lactis* IL1403 involved cloning the structural gene (*lcnA*) and immunity gene (*lciA*) of *L. lactis* subsp. *diacetylactis* WM4 into the shuttle vector pTG262, to generate pFI2058 (Fig. 1a). To determine whether the host containing homologs of the *lcnC* and *lcnD* genes could complement the equivalent processing genes, missing from pFI2058, the recombinant plasmid was introduced into this strain and a bioassay was carried out on the transformants. Supernatants from *L. lactis* IL1403 cells harboring pFI2058 (strain FI8817) inhibited the growth of the indicator strain *L. lactis* MG1614, indicating that lactococcin A was being produced (data not shown). Cells carrying the vector alone showed no inhibitory effect. The level of antimicrobial activity displayed by FI8817 was approximately 80% of that of the lactococcin A-producing parental strain, *L. lactis* WM4.

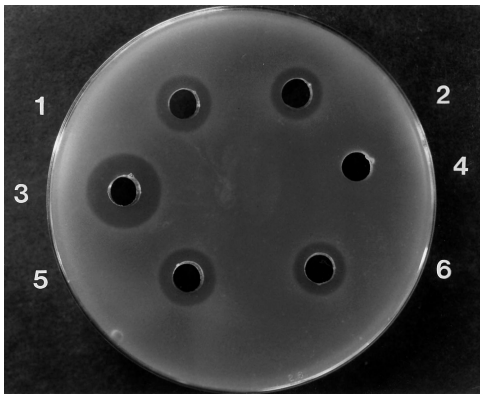

FIG. 2. Agar diffusion bioassay for detection of bacteriocin activity against *E. faecium* P21. 1 and 5, *L. lactis* FI9043 (MRS culture); 2 and 6, *L. lactis* FI9043 (GM17 culture); 3, *P. acidilactici* 347; 4, *L. lactis* IL1403.

***lcnA*/*pedA* hybrid gene.** To determine whether pediocin PA-1 could be expressed and secreted in *L. lactis* IL1403, using the translocation machinery of lactococcin A, a hybrid *lcnA*/*pedA* gene was constructed (Fig. 1d). Plasmid pFI2126 contains an in-frame fusion of sequences encoding the lactococcin A leader and the mature part of pediocin PA-1 and is preceded by the promoter-active region upstream of the *lcnA* gene (Fig. 1e). The downstream lactococcal sequences, including the *lciA* gene, were not included, nor was the pediocin PA-1 immunity gene (*pedB*) necessary because of the natural resistance of the lactococcal host to this bacteriocin.

Transformation of *L. lactis* IL1403 with pFI2126 generated strain FI9043, which, after growth in either MRS or GM17 broth, was tested for antimicrobial activity. In plate diffusion bioassays, inhibition of the pediocin-sensitive indicator organism, *E. faecium* P21, was detected (Fig. 2), with cultures grown in MRS broth (final pH 4.6) displaying slightly higher antimicrobial activity than those grown in GM17 broth (final pH 5.3). The bacteriocin production level of *L. lactis* FI9043 was lower than that of the natural pediocin PA-1 producer, *P. acidilactici* 347 (Fig. 2). The zones of inhibition displayed by the *L. lactis* IL1403 derivatives (~270 ng ml^{-1}) represent approximately one-quarter of the pediocin produced by the homologous host (~1,200 ng ml^{-1}).

Bacteriocin purification and characterization. Subsequent analysis of the bacteriocin produced by *L. lactis* FI9043 involved purification of the active peptide with the various stages of the recovery procedure summarized in Table 2. Fractions from the first run on the reverse-phase column which showed the highest activity were collected and rechromatographed. An absorbance peak, coincident with the activity peak, was observed (Fig. 3). The final specific activity of the pure bacteriocin was approximately 10^6-fold higher than that in the crude culture supernatant, and the recovery was 617%.

Further characterization of the bacteriocin was carried out by NCI-ELISAs with specific anti-pediocin PA-1 antibodies. *L. lactis* FI9043 crude culture supernatants did not cross-react

TABLE 2. Purification of pediocin PA-1 from *L. lactis* FI9043

Purification stage	Vol (ml)	Total A_{280}[a]	Total activity (BU)	Sp act[b]	Increase in sp act (fold)	Yield (%)
Culture supernatant	1,000	28,500	202,295	7	1	100
Fraction:						
I. Ammonium sulfate precipitation	100	1,490	92,494	62	8.7	46
II. Gel filtration chromatography	200	700	96,701	138	19.7	48
III. Cation-exchange chromatography	50	29.3	41,142	1,404	198	20
IV. Hydrophobic-interaction chromatography	10	12.6	1,316,750	104,090	14,681	650
V. Reverse-phase chromatography	0.7	0.16	1,248,026	7,849,220	1,107,083	617

[a] Total A_{280} equals the optical density at 280 nm multiplied by the volume (in milliliters).
[b] Specific activity is bacteriocin units (BU) per milliliter divided by the optical density at 280 nm.

with the antibodies despite exhibiting antimicrobial activity (Fig. 2). This is due to the calculated bacteriocin level in the supernatants being below the sensitivity level of the pediocin PA-1 immunoassay (25). However, a strong reactivity with the antibodies was observed when purified bacteriocin from *L. lactis* FI9043 was tested at concentrations greater than 500 ng ml^{-1}, indicating that this host strain was producing pediocin PA-1 (Fig. 4). Additional data supporting this result was supplied by amino-terminal sequence analysis of the purified peptide. The first 6 residues at the amino-terminal end of the secreted peptide were KYYGNG, which is the correct sequence for the amino terminus of pediocin PA-1 and one that distinguishes it from lactococcin A. Moreover, this result established that correct processing of the hybrid precursor peptide had occurred and was consistent with the heterologous production of pediocin from *L. lactis* FI9043.

DISCUSSION

An expression system for heterologous peptides was developed in *L. lactis* IL1403, based on the genes and transcription signals required for lactococcin A production. In this host, the translocation functions (*lcnC* and *lcnD*) necessary for processing and secretion of lactococcins are provided by chromosomal gene analogs (33, 40). Hence, expression of the *lcnA* and *lciA* genes is the minimum requirement for production of lactococcin A.

The flexibility of the translocatory apparatus of class II bacteriocins was recently demonstrated by van Belkum et al. (38). Gene fusions were generated in which sequences encoding the leader peptides of leucocin A, lactococcin A, and colicin V were fused to divergicin A, an alternative bacteriocin that is secreted via the general secretion pathway of the cells (42). The different leader peptides were able to direct the secretion of divergicin in *Leuconostoc gelidum*, *L. lactis*, and *E. coli*, respectively (i.e., the homologous hosts). Furthermore, certain host-vector combinations gave rise to the production of divergicin when the leader peptides were used in heterologous hosts. The same strategy was also used for the production of colicin V from *L. lactis* IL1403. In this case, the *E. coli* gene was fused to sequences encoding the leucocin A leader peptide (38). The various components of the class II translocatory apparatus are not universally interchangeable indicating that some leader peptides are poorly recognized by heterologous ATP-binding cassette transporter proteins (35, 38). Allison et al. (2) have shown that both peptides of the two-component lactacin F complex can use the secretion machinery of *Carnobacterium piscicola* LV17, a strain that produces carnobacteriocins A, BM1, and B2 (1). The fact that the amino-terminal leaders of these carnobacteriocins and lactacin F peptides have the highest degree of homology among class II bacteriocins may have facilitated the secretion of lactacin F peptides in this heterologous host. In contrast, the translocatory apparatus for lactococcin A was not able to bring about secretion of leucocin A in *L. lactis* (35).

Pediocin PA-1 and lactococcin A are both class II bacterio-

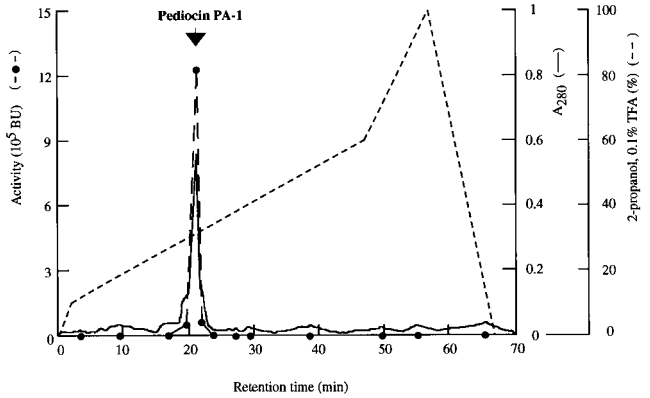

FIG. 3. Reverse-phase chromatography of pediocin PA-1. The amount applied to the column was obtained from a 1-liter culture of *L. lactis* FI9043. BU, bacteriocin units.

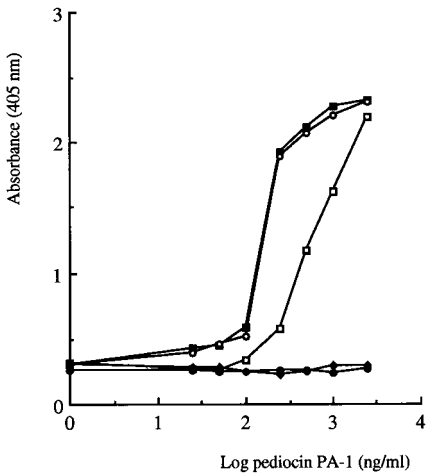

FIG. 4. Standard curves for pediocin PA-1 purified from *P. acidilactici* 347 (■) and *L. lactis* FI9043 (○), OA-PH2 (□), nisin (●), and the protein fraction obtained from culture supernatants of *P. acidilactici* 347-8, a *P. acidilactici* 347-cured derivative that does not produce pediocin PA-1 (◆), as determined by NCI-ELISA with anti-pediocin PA-1 rabbit antibodies. Each datum point represents the average value of triplicate determinations in a single microtiter plate.

cins and hence are likely candidates for expression and secretion via a heterologous translocatory apparatus. A *lcnA/pedA* hybrid gene was constructed by substituting the nucleotide sequences downstream of the lactococcin A Gly-Gly cleavage site with the equivalent region of the *pedA* gene. This gene, expressed in *L. lactis* FI9043, gave rise to antimicrobial activity against the pediocin-sensitive strain *E. faecium* P21 (Fig. 2). Confirmation that this strain was producing pediocin PA-1 came from amino-terminal sequencing of the purified product and also from immunoanalysis with antibodies which specifically recognize pediocin PA-1 (Fig. 4). This established that the lactococcin A leader peptide was capable of directing the secretion of pediocin PA-1 from *L. lactis* IL1403 and that correct processing of this leader peptide had occurred at the consensus cleavage site with release of mature pediocin into the growth medium.

Chikindas et al. (8) have described a similar IL1403 expression system in which the four *ped* determinants were cloned into a lactococcal vector. In this strain, secretion of pediocin PA-1, directed by its own *pedA*-encoded leader, was detected only when the *ped* operon was under the control of a lactococcal promoter. Under these conditions, the pediocin PA-1 yield was less than 1% of the production level by the parental *Pediococcus* strain. This suggests that in *L. lactis*, lactococcin A-directed secretion of pediocin PA-1 is more efficient than the equivalent process directed by the normal pediocin leader sequence. It was possible to increase the relative level of pediocin PA-1 production to approximately 50% when using its own dedicated PedCD translocatory machinery, by increasing the copy number of the *ped* operon, contained on the plasmid, in a specifically mutated lactococcal host (8).

The reduced level of pediocin PA-1 production in the *L. lactis* IL1403 derivative described here (~25% of that in the parental pediococcal strain) may be attributed to the low copy number of the chromosomal *lcnC* and *lcnD* gene analogs, resulting in less efficient secretion of the bacteriocin (33). Similar observations have been presented by van Belkum et al. (37) and Holo et al. (20), who both reported a reduction in the yield of lactococcin A expressed in an IL1403 derivative. The recent analysis of the IL1403 secretion system indicated that these genes are not identical to the equivalent lactococcin A translocatory machinery (40). This may result in only partial complementation of the *lcnC* and *lcnD* genes, with less efficient processing of the bacteriocin using this secretory system. It has been reported that when the dedicated *lcnC* and *lcnD* genes were included in equivalent lactococcal expression systems, bacteriocin production was increased at least 10-fold (38, 40). The possibility that the introduction of these plasmid genes from the lactococcin A-producing strain *L. lactis* WM4 (33) into FI9043 has a similar effect on pediocin PA-1 production is being investigated.

Culture pH may also play a role in the reduced yield of pediocin PA-1 from the heterologous lactococcal host. In pediocin PA-1 bioassays involving *L. lactis* FI9043, larger inhibition zones were generated from supernatants of cultures grown in MRS broth (final pH 4.6) than from those in GM17 broth (final pH 5.3) (Fig. 2). It has been reported that processing of the prepediocin to active pediocin PA-1 by *P. acidilactici* strains can take place efficiently only when the final pH of the culture medium is less than or equal to 5.0 (4, 12, 43). In contrast, Ennahar et al. (12) reported that the production of pediocin AcH-1 from *Lactobacillus plantarum* WHE 92 was not reduced when the pH was raised to 6.0. It was suggested that the efficiency of processing of prepediocin to pediocin may differ in *Lactobacillus* and *Pediococcus* species (12). This observation has important industrial implications, since a pH of 5.0 and above is often encountered in dairy products.

Pediococci are usually associated with vegetable and meat material and are used commercially in the fermentation of vegetables and meat. Pediocin PA-1 is a bacteriocin with a broad inhibitory spectrum and is particularly effective in combating the growth of *Listeria monocytogenes*. However, pediococci are poorly adapted for colonizing foods in which they do not naturally reside (27) and are therefore not the ideal organisms for controlling the growth of *L. monocytogenes* in dairy products. In this study, we have demonstrated heterologous expression of pediocin PA-1 in *L. lactis* IL1403 containing a fusion of the pediocin PA-1 structural gene (devoid of the sequence encoding its natural leader peptide) behind the sequence encoding the lactococcin A leader. Expression and secretion of this bacteriocin in lactococci provides a way in which the beneficial properties of pediocin PA-1 production can be applied to the dairy industry. This approach could be extended with the aim of expressing other bacteriocins, peptides, or proteins of interest (hybrid bacteriocin molecules with a broader antimicrobial spectrum, cecrapin, yeast killer toxin) in food-grade strains. A strategy involving a dedicated secretory system could also be used to investigate vaccine delivery vehicles in mucosal environments by using lactic acid bacteria (41).

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