Novel Lectin-Like Bacteriocins of Biocontrol Strain *Pseudomonas fluorescens* Pf-5

Annabel H. A. Parret, Koen Temmerman, and René De Mot*

Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, B-3001 Heverlee, Belgium

Received 30 November 2004/Accepted 1 April 2005

Bacteriocin LlpA, produced by *Pseudomonas* sp. strain BW11M1, is a peculiar antibacterial protein due to its homology to mannose-binding lectins mostly found in monocots (A. H. A. Parret, G. Schoofs, P. Proost, and R. De Mot, J. Bacteriol. 185:897–908, 2003). Biocontrol strain *Pseudomonas fluorescens* Pf-5 contains two *llpA*-like genes, named *llpA1*_{Pf-5} and *llpA2*_{Pf-5}. Recombinant *Escherichia coli* cells expressing *llpA1*_{Pf-5} or *llpA2*_{Pf-5} acquired bacteriocin activity and secreted a 31-kDa protein cross-reacting with LlpA_{BW11M1} antibodies. Antibacterial activity of the recombinant proteins was evidenced by gel overlay assays. Analysis of the antimicrobial spectrum indicated that LlpA1_{Pf-5} and LlpA2_{Pf-5} are able to inhibit *P. fluorescens* strains, as well as the related mushroom pathogen *Pseudomonas tolaasii*. LlpA-type bacteriocins are characterized by a domain structure consisting of tandem monocot mannose-binding lectin (MMBL) domains. Molecular phylogeny of these MMBL domains suggests that the individual MMBL domains within an LlpA protein have evolved separately toward a specific, as yet unknown, function or, alternatively, were acquired from different ancestral sources. Our observations are consistent with earlier observations, which hinted that MMBL-like bacteriocins represent a new family of antibacterial proteins, probably with a novel mode of action.

Bacteria living in a competitive environment are able to secrete proteinaceous toxins, known as bacteriocins, which can kill closely related bacterial competitors while causing little harm to the bacteriocinogenic cells. These bacterial inhibitors are produced by all major groups of *Bacteria* (53). Bacteriocin production has also been reported for *Halobacteriaceae*, a family of extremely halophilic *Archaea* (36). Bacteriocins constitute a structurally and functionally diverse group within the antimicrobials, ranging from small peptides, such as microcins of *Enterobacteriaceae* and antibiotics secreted by low-GC-content gram-positive bacteria (23, 31), to middle-sized polypeptides, such as colicins of *Escherichia coli* (34) and their counterparts in *Pseudomonas aeruginosa* (S pyocins) (39), to large phage tail-like multiprotein complexes, such as syringacin produced by *Pseudomonas syringae* (58) and R- and F-type pyocins of *P. aeruginosa* (39). Bacteriocin mode of killing can be either by membrane pore formation, nonspecific degradation of cellular DNA, cleavage of 16S rRNA or tRNA, or inhibition of peptidoglycan synthesis resulting in cell lysis (51).

Bacteriocins from lactic acid bacteria, such as nisin, have received considerable attention, given their potential as food preservatives (41, 42). Among bacteriocins of gram-negative bacteria, colicins are the most intensively studied. Colicins are produced by, and attack, strains of *E. coli*. These proteins, as well as the S pyocins, are organized in functional domains, namely, regions for cell attachment, translocation, and bactericidal activity. Colicins and S pyocins parasitize specific membrane-bound receptors on target cells, resulting in a rather narrow spectrum of activity. The host strain is protected from

its own bacteriocin through the action of a cognate immunity protein that is coproduced with the bacteriocin (39, 51).

It has been proposed that bacteriocins may play a key role in bacterial population dynamics (52, 53). In a recent study, Kirkup and Riley demonstrated that the production of colicins by *E. coli* colonizing the mouse colon gives the colicinogenic strains a competitive advantage by killing colicin-sensitive *E. coli* sharing the same ecological niche (33). Possible applications of bacteriocinogenic strains in agriculture include their use in biological control of soilborne or phyllosphere-inhabiting bacterial plant pathogens. In this way, heterologous production of the peptide bacteriocin trifolixotoxin by an avirulent *Agrobacterium* strain effectively enhances biological control of *Agrobacterium vitis* crown gall (24). Expression of the trifolixotoxin genes from *Rhizobium leguminosarum* bv. trifolii T24 in *Rhizobium etli* CE3 increases bean nodulation competitiveness of the recombinant strain in the presence of indigenous rhizobia under agricultural conditions (54). A phage tail-like bacteriocin (serracin P) produced by *Serratia plymuthicum* may be used for biological control of fire blight caused by *Erwinia amylovora* (30), while *Xanthomonas campestris* pv. glycines has antibacterial activity against phytopathogenic *Xanthomonas* species through the secretion of glycinecin A (26). Finally, a bacteriocin produced by *P. syringae* pv. ciccaronei inhibits *P. syringae* subsp. *savastanoi*, the causal agent of olive knot disease (35).

This study is part of an ongoing project examining bacteriocin production in rhizosphere-colonizing fluorescent *Pseudomonas* strains. Recently, we identified a novel type of *Pseudomonas* antibacterial protein (bacteriocin LlpA, lectin-like putidacin A) that is secreted by rhizosphere isolate *Pseudomonas* sp. BW11M1. LlpA is active against the rhizosphere-colonizing strain *Pseudomonas putida* GR12-2R3 as well as some phytopathogenic pseudomonads, including *P. syringae* pathovar strains (46). This novel bacteriocin exhibits remarkable

* Corresponding author. Mailing address: Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium. Phone: 32 16 329681. Fax: 32 16 321966. E-mail: rene.demot@biw.kuleuven.be.

similarity with a family of mannose-binding lectins, predominantly found in monocot plants. The sequence similarities of LlpA to these lectins are localized in two discrete regions, the so-called monocot mannose-binding lectin (MMBL) domains. Chen and coworkers identified such an MMBL domain in a 32-kDa bacteriocin (albusin B or AlbB) produced by the ruminal bacterium *Ruminococcus albus* strain 7. They showed that albusin B is a potent growth inhibitor of *Ruminococcus flavescentis* strains (11). Albusin B and LlpA_{BW11M1} are likely released from the bacteriocinogenic cells via a different route. LlpA_{BW11M1} secretion is signal peptide independent (46), while albusin B was shown to be produced as a prepeptide containing a 49-amino-acid signal peptide (11). The mechanism by which this type of bacteriocin inhibits the growth of sensitive bacteria is still under investigation. Despite the presence of MMBL domains, LlpA and albusin B are unable to bind mannose (11, 46).

In silico analysis indicated that the genome of *Pseudomonas fluorescens* Pf-5 contains two putative *llpA* homologues. This rhizosphere-inhabiting bacterium is a well-known biocontrol strain and produces an impressive array of secondary metabolites that inhibit fungal plant pathogens, including pyoluteorin, pyrrolnitrin, 2,4-diacetylphloroglucinol, and hydrogen cyanide (9, 40, 48). These properties have incited a genome sequencing project of this strain (<http://www.ars-grin.gov/hcrl/Pf5genome/index.htm>).

In this study, we show that both *llpA* homologues in strain Pf-5 are functional bacteriocin genes. As in the case for LlpA from *Pseudomonas* sp. BW11M1, these LlpA-like bacteriocins of Pf-5 have an antibacterial spectrum limited to *Pseudomonas* species.

MATERIALS AND METHODS

Bacterial strains, growth media, and culture conditions. Bacterial strains used and plasmids described in this study are listed in Table 1. *Pseudomonas* strains were routinely propagated in tryptic soy broth (BD Biosciences) at 30°C, with the exception of *P. aeruginosa*, which was cultured at 37°C. Luria-Bertani (LB) broth (55) was used for growing *E. coli* at 37°C. *Rhodococcus erythropolis* and *Streptomyces coelicolor* were grown in LB medium at 30°C. *Agrobacterium tumefaciens* and *Azospirillum brasilense* were cultivated at 30°C in YEP medium containing 1% Bacto peptone (BD Biosciences), 0.5% of NaCl, and 1% yeast extract (BD Biosciences). *Bacillus subtilis* was incubated at 30°C in TY medium (0.5% peptone and 0.3% yeast extract). Media were solidified with 1.5% agar (Invitrogen). Media were supplemented with antibiotics at the following concentrations: ampicillin, 100 µg/ml; tetracycline, 10 µg/ml; and kanamycin, 50 µg/ml. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside and isopropyl β-D-thiogalactoside (40 µg/ml) were added to detect the presence of insert DNA cloned in pUC18-derived plasmids in *E. coli*. Long-term storage of bacterial strains was at -80°C in the appropriate medium with 25% (wt/vol) glycerol added.

DNA and cellular manipulations. Standard methods were used for DNA electrophoresis, preparation of competent *E. coli* cells, and transformation of *E. coli* (55). Restriction enzymes were used as specified by the supplier (Roche Diagnostics). DNA fragments were recovered from agarose gels with a QIAquick Gel Extraction kit (QIAGEN). DNA ligations were performed using a Rapid DNA ligation kit (Roche Diagnostics). *Pseudomonas* total DNA was isolated from 5-ml early stationary phase cultures with a PureGene DNA purification kit (Gentra Systems). Plasmids were isolated using a QIAprep Spin Miniprep kit (QIAGEN). PCRs were performed in a Mastercycler Personal (Eppendorf) instrument.

Bacteriocin and antifungal assays. Standard bacteriocin plate assays based on deferred antagonism were performed as previously described (46). The effect of UV irradiation on bacteriocin production by strain Pf-5 and recombinant *E. coli* TOP10F' expressing *llpA*_{Pf-5} and *llpA*_{Pf-5} was examined as in Parret et al. (46). To analyze the effect of mannose (0.01 M and 0.1 M) on the activity of recombinant bacteriocins produced by *E. coli*, the assay was carried out in sugar-

supplemented LB medium. For detection of protein bands with antibacterial activity, a gel overlay assay was carried out (46) with the following modification. Instead of an overnight washing step for renaturation of bacteriocin bands, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were washed twice for 90 min at 4°C in 50 mM MES (2-morpholinoethanesulfonic acid)-NaOH (pH 6) containing 0.1% (wt/vol) Triton X-100. For detection of antifungal activity, an agar plug containing fungal mycelium was placed in the middle of potato dextrose (BD Biosciences) agar plates in duplicate, and fungal mycelium growth was initiated by incubation for 3 days at room temperature. The exact incubation time depended upon the growth rate of each fungus. Subsequently, a 3-µl drop of an overnight culture of the bacterial strain to be tested was spotted at one-third of the radius of the plates. The plates were further incubated at 25°C. Suppression of fungal growth was evaluated macroscopically after 2 days and again after 4 days. When evaluating antibacterial and antifungal activity of recombinant *E. coli* TOP10F' or *Pseudomonas* strains expressing *llpA* genes, *E. coli* TOP10F' carrying pUC18 or *Pseudomonas* sp. harboring pJB3Tc20 were included as a negative control.

Heterologous expression of *llpA*_{Pf-5} and *llpA*_{Pf-5}. The *llpA*_{Pf-5} and *llpA*_{Pf-5} genes were amplified by PCR with Platinum Pfx DNA polymerase (Invitrogen) using 100 ng of *P. fluorescens* Pf-5 genomic DNA as a template. The primers *llpA*_{Pf-5}-5'-BamHI and *llpA*_{Pf-5}-3'-SphI were utilized to amplify the *llpA*_{Pf-5} region as a 1,086-bp fragment ranging from position 394669 to 395754 of contig 3337 (7,075,370 bp) (<http://www.tigr.org/tdb/ufmg/>, version 15 November 2004). For amplification of *llpA*_{Pf-5}, primer *llpA*_{Pf-5}-5'-BamHI and *llpA*_{Pf-5}-3'-SphI were chosen to amplify a 1,236-bp region ranging from position 1343328 to 1344427 of contig 3337. PCR conditions used included 30 cycles of annealing at 61°C (30 s), polymerization at 68°C (1 min), and denaturation at 94°C (1 min). The resulting PCR products were cloned into the pCRII-TOPO vector (TOPO TA cloning Dual Promoter kit; Invitrogen). Amplicons were checked by sequencing, digested with BamHI and SphI, and ligated into the cognate sites of pUC18 under control of the *lacZ* promoter, resulting in plasmids pCMPG6052 (*llpA*_{Pf-5}) and pCMPG6053 (*llpA*_{Pf-5}), respectively. Ligation mixes were transformed to TOP10F' cells for expression in *E. coli*. For heterologous expression in *Pseudomonas*, the BamHI/SphI inserts from vectors pCMPG6052 and pCMPG6053 were recloned into the cognate sites of broad-host-range vector pJB3Tc20, in sense with the *lacZ* promoter, giving pCMPG6057 (*llpA*_{Pf-5}) and pCMPG6058 (*llpA*_{Pf-5}). Similarly, a third construct (pCMPG6060) was prepared consisting of an 897-bp fragment amplified by Pfx PCR using primers *llpA*_{BW11M1}-5'-KpnI and *llpA*_{BW11M1}-3'-BamHI using pCMPG6012 as a template. This fragment, containing *llpA* from *Pseudomonas* sp. BW11M1, was cut with KpnI and BamHI, followed by cloning into vector pJB3Tc20. The three resulting bacteriocin constructs were propagated in the nonmethylating *E. coli* strain GM2163, and plasmid DNA was extracted for electroporation of *Pseudomonas*. *Pseudomonas* transformants were then checked for the presence of *llpA* constructs by miniprep and restriction analysis.

Protein electrophoresis and immunodetection. *E. coli* TOP10F' cells harboring *llpA* constructs were grown to early stationary phase (optical density at 600 nm, 1.4). Proteins were phenol extracted from lyophilized cell-free culture supernatants as described by Parret et al. (46). Protein concentration was assayed using a 2D Quant kit (Amersham Biosciences) using bovine serum albumin (BSA; Sigma-Aldrich) as a standard. Protein fractions were separated on Criterion 12% ready gels (Bio-Rad) at 200 V for 60 min using a Bio-Rad mini-PROTEAN II electrophoresis apparatus. Proteins were visualized with Coomassie blue R-250. SDS-PAGE gels were dried with the DryEase Mini-Gel Drying System (Invitrogen) according to the manufacturer's instructions. Polyclonal rabbit antiserum against LlpA_{BW11M1} was obtained from two New Zealand White rabbits by a standard immunization protocol carried out in the facilities of Eurogentec (Seraing, Belgium) using 800 µg of bacteriocin purified as described previously (47). Upon delivery, rabbit serum was aliquoted and then stored at -20°C. Separated supernatant proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore) with an LKB Multiphor II blotting unit (Amersham Biosciences), according to the manufacturer's instructions. Western hybridization was carried out as described by Harlow and Lane (21) with minor modifications. Blocking of aspecific sites was carried out by incubation at room temperature for 2 h in Tris-buffered saline (TBS) (20 mM Tris base, 0.5 M NaCl [pH 7.5]) supplemented with 1% (wt/vol) BSA. Subsequently, the membrane was washed three times for 10 min with TBS buffer before incubation (at least 1 h) with primary antibody solution (rabbit antiserum diluted 1:500 in 1% BSA-TBS). Unbound polyclonal antibodies were removed by washing the membrane three times for 5 min in TBS buffer supplemented with 0.1% (wt/vol) Tween. Secondary antibody solution (3% goat anti-rabbit immunoglobulin G [IgG] horseradish peroxidase conjugate [Sigma-Aldrich] in 0.1% Tween-TBS) was then applied, and the membrane was incubated for 1 h. Unbound secondary

TABLE 1. Bacterial and fungal strains, plasmids and PCR primers used in this study

Strain, plasmid, or primer	Genotype, origin, or relevant characteristic ^a	Source or reference
Bacteria		
<i>Agrobacterium tumefaciens</i> LMG 187	Type strain	BCCM ^f
<i>Azospirillum brasilense</i> Sp245	Wheat rhizosphere isolate	2
<i>Bacillus subtilis</i> subsp. <i>subtilis</i> LMG 7135	Type strain	BCCM
<i>Clavibacter michiganensis</i> ^b LMG 2889	Pathovar reference strain	BCCM
<i>E. coli</i> strains		
GM2163	<i>ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 rpsL136 dam13::Tn9 xylA5 mtl-1 thi-1 mcrB1 hsdR2</i>	New England Biolabs
HB101	<i>ara-14 leuB6 lacY1 galK2 xyl-5 mtl-1 thi-1 hsdS20 supE44 recA13 proA2 rpsL20</i>	8
TOP10F'	F'[<i>lacI'</i> Tn10 (Tc ^r)] <i>mcrA</i> Δ(<i>mrr-hsdRMS-mcrBC</i>) φ80 <i>lacZ</i> ΔM15 Δ <i>lacZ</i> 74 <i>recA1 araD139</i> Δ(<i>ara-leu</i>)7697 <i>galU galK rpsL</i> (Str ^r) <i>endA1 nupG</i>	Invitrogen
<i>Pectobacterium carotovorum</i> LMG 2458 ^c	Tobacco isolate	BCCM
<i>Pseudomonas</i> sp. strain BW11M1	Banana rhizosphere isolate	68
<i>P. aeruginosa</i> PAO1	Clinical isolate	59
<i>P. agarici</i> LMG 2112	Mushroom isolate; type strain	BCCM
<i>P. aureofaciens</i> LMG 1245	Freshwater isolate; type strain	BCCM
<i>P. cichorii</i> LMG 2162	Cichory isolate; type strain	BCCM
<i>P. chlororaphis</i> LMG 5004	Type strain isolated from contaminated plate	BCCM
<i>P. fuscovaginae</i> LMG 2158	Rice isolate; type strain	BCCM
<i>P. fluorescens</i> strains		
2-79	Biocontrol strain isolated from wheat rhizosphere	69
13-79	Biocontrol strain isolated from wheat rhizosphere	69
A1-B	Biocontrol strain isolated from potato rhizosphere	M. N. Schroth ^g
BL915	Biocontrol strain	27
F113	Biocontrol strain isolated from sugar beet rhizosphere	56
LMG 1794	Freshwater isolate; type strain	BCCM
Pf0-1	Soil isolate	12
Pf-5	Biocontrol strain isolated from soil	28
OE 28.3	Wheat rhizosphere isolate	15
SBW25	Sugar beet isolate	50
WCS141	Wheat rhizosphere isolate	16
WCS365	Biocontrol strain isolated from potato rhizosphere; NaI ^r	19
<i>P. marginalis</i> pv. <i>marginalis</i> LMG 2210	Pathovar reference strain	BCCM
<i>P. mendocina</i> LMG 1223	Soil isolate; type strain	BCCM
<i>P. putida</i> strains		
GR12-2R3	Rifampicin-resistant derivative of strain GR12-2 isolated from grass rhizosphere	37
LMG 2257	Soil isolate; type strain	BCCM
KT2440	pWW0-cured, restriction-deficient derivative of <i>P. putida</i> mt-2	18
<i>P. resinovorans</i> LMG 2274	Soil isolate; type strain	BCCM
<i>P. stutzeri</i> LMG 11199	Clinical isolate; genomovar 1 reference strain	BCCM
<i>P. syringae</i> pv. <i>glycinea</i> LMG 5066	Soybean isolate; pathovar reference strain	BCCM
<i>P. syringae</i> pv. <i>syringae</i> LMG 1247	Lilac isolate; pathovar reference strain	BCCM
<i>P. syringae</i> pv. <i>tabaci</i> LMG 5192	Tobacco isolate	BCCM
<i>P. syringae</i> pv. <i>tomato</i> DC3000	Rifampicin-resistant derivative of strain DC52	14
<i>P. tolaasii</i> LMG 2344	Mushroom isolate	BCCM
<i>P. viridiflava</i> LMG 2352	Type strain	BCCM
<i>Rhodococcus erythropolis</i> N11	Soil isolate	20
<i>Streptomyces coelicolor</i> A3(2)	Soil isolate	5
Fungi		
<i>Alternaria brassicicola</i> CBS 762.84		CBS ^h
<i>Botrytis cinerea</i> JHCC 8973		JHCC ⁱ
<i>Fusarium culmorum</i> PGSF 15		PGSF ^j
<i>Fusarium graminearum</i> PGSF 35		PGSF
<i>Fusarium oxysporum</i> ^d CBS 873.95		CBS
<i>Neurospora crassa</i> FGSC 987		FGSC ^k
Plasmids		
pCMPG6012	pUC18 with 989-bp PCR-amplified fragment containing <i>llpA</i> _{BW11M1} gene cloned in KpnI/BamHI	43
pCMPG6023	pJB3Tc20 with KpnI/BamHI insert from pCMPG6012	This study
pCMPG6052	pUC18 with 1238-bp PCR-amplified fragment containing <i>llpA</i> _{2Pf-5} gene cloned in SphI/BamHI	This study
pCMPG6053	pUC18 with 1088-bp PCR-amplified fragment containing <i>llpA</i> _{1Pf-5} gene cloned in SphI/BamHI	This study

Continued on following page

TABLE 1—Continued

Strain, plasmid, or primer	Genotype, origin, or relevant characteristic ^a	Source or reference
pCMPG6057	pJB3Tc20 with SphI/BamHI insert from pCMPG6052	This study
pCMPG6058	pJB3Tc20 with SphI/BamHI insert from pCMPG6053	This study
pCMPG6060	pJB3Tc20 with 899-bp insert from pCMPG6012	This study
pCRII-TOPO	Direct cloning vector for PCR products; Ap ^r , Km ^r	Invitrogen
pJB3Tc20	Broad-host-range cloning vector; Ap ^r , Tc ^r	6
pUC18	ColE1 replicon, <i>lacZ</i> , cloning vector; Ap ^r	71
Primers^c		
llpA1 _{Pf-5} 5' BamHI	5'-TAGGATCCCCTCGAGCCTCGAGCAATCG-3'	This study
llpA1 _{Pf-5} 3' SphI	5'-TAGCATGCCGCACTAAGCTGATGCGGC-3'	This study
llpA2 _{Pf-5} 5' BamHI	5'-TAGGATCCCAGGTGTGAAAGCGGCGTCG-3'	This study
llpA2 _{Pf-5} 3' SphI	5'-TAGCATGCAGCTGTGCTTGGCCGTTTCG-3'	This study
llpA _{BW11M1} 5' KpnI	5'-TAGGTACCGCTCCCTTCAATCGATCCAGTAGG-3'	This study
llpA _{BW11M1} 3' BamHI	5'-TAGGATCCAAGCCGGGCGCCGTTAAGGC-3'	This study

^a Phenotype resistance abbreviations are as follows: Ap, ampicillin; Km, kanamycin; Nal, nalidixic; Str, streptomycin; Tc, tetracycline.

^b *Clavibacter michiganensis* subsp. *sepedonicus*.

^c *Erwinia carotovora* subsp. *carotovora*.

^d *Fusarium oxysporum* f. sp. *radicis-lycopersici*.

^e For primer oligonucleotide sequences, the restriction sites incorporated in primers are underlined: GGATCC, BamHI; GGTACC, KpnI; GCATGC, SphI.

^f BCM, Belgian Coordinated Collections of Microorganisms (<http://www.belspo.be/bccm/index.htm>).

^g M. N. Schroth, University of California, Berkeley, Calif.

^h CBS, Centraalbureau voor Schimmelculturen, Baarn, The Netherlands.

ⁱ JHCC, Jealott's Hill International Research Centre Culture Collection, Syngenta, Berkshire, United Kingdom.

^j PGSF, Public Good Science Fund, Wellington, New Zealand.

^k FGSC, Fungal Genetics Stock Center, Kansas City, KS.

antibodies were removed by washing the membrane three times for 10 min in 0.1% Tween-TBS. Binding of secondary antibody to the polyvinylidene difluoride membrane was visualized with nitroblue tetrazolium chloride and BCIP (5-bromo-4-chloro-3-indolylphosphate toluidine salt) by immersing the membrane in a diluted solution of 200 µl of nitroblue tetrazolium-BCIP stock solution (Roche Diagnostics) in 10 ml of 0.1 M Tris-HCl (pH 9.5), 0.1 M NaCl. All procedures were carried out at room temperature with gentle agitation, with the exception of the developing step, which was carried out without shaking.

DNA and protein sequence analysis. Nucleotide sequences were determined by the dideoxy chain terminator method using a BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) and universal M13 Forward and Reverse primers. Sequencing was carried out on an ABI 3100 Avant Genetic Analyzer (Applied Biosystems). The ClustalW program (62) was used to align DNA and amino acid sequences. The molecular weights and isoelectric points of proteins were estimated with the ProtParam tool available at the ExPASy molecular biology server (<http://us.expasy.org/tools/protparam.html>). Potential signal peptide cleavage sites were predicted using the SignalP World Wide Web Prediction Server (<http://www.cbs.dtu.dk/services/SignalP>).

Phylogenetic analysis of LlpA domains. Searches to compare protein sequences against the nonredundant protein sequence database (version 19 October 2004) at the National Center for Biotechnology Information (NCBI) were accomplished using the protein-BLAST (blastp) and translated-BLAST (tblastn) programs (version 2.2.10) (1). *llpA* homologues in unfinished genomes were identified using the tblastn program provided at NCBI (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). Detection of conserved protein domains was performed with the Pfam (Protein family database) search tool (version 15) on the Sanger Center Internet server (<http://www.sanger.ac.uk/Software/Pfam>). Known or predicted mannose-binding lectin domains (Pfam accession no. PF01453; B_Lectin) of similar protein sequences were truncated to the appropriate length (103 to 125 amino acids). The alignment was further adapted manually according to secondary structure predictions (Jpred2, <http://jura.ebi.ac.uk:8888/>) using the GeneDoc program (<http://www.psc.edu/biomed/genedoc/>). The wEMBOSS program clustaln provided by Belgian EMBnet Node was used to infer the tree topology by phylogenetic distance analysis (Kimura correction) with a neighbor-joining algorithm.

Sequence data for *P. fluorescens* Pf-5, *Mycobacterium smegmatis* mc²-155, and *R. albus* strain 8 were obtained from The Institute for Genomic Research website at <http://www.tigr.org> (version 15 November 2004). *Mycobacterium marinum* sequence data were produced by the *M. marinum* Sequencing Group at the Sanger Institute and were retrieved from <ftp://ftp.sanger.ac.uk/pub/pathogens/mm> (version 8 Nov. 2004). Sequence data for *Ralstonia metallidurans* strain

CH34 were produced by the U.S. Department of Energy Joint Genome Institute (<http://www.jgi.doe.gov/>, version 25 November 2003).

RESULTS

The *P. fluorescens* Pf-5 genome contains two *llpA* homologues. To identify possible homologues of the lectin-like bacteriocin LlpA produced by *Pseudomonas* sp. BW11M1 (LlpA_{BW11M1}) in other bacteria, the LlpA protein sequence was queried against the NCBI nonredundant database and against microbial genomes using both the blastp and tblastn programs. The tblastn search revealed two open reading frames in the unannotated genome of the rhizosphere strain *P. fluorescens* Pf-5 with significant sequence similarity to LlpA_{BW11M1}. These hypothetical genes, designated *llpA1*_{Pf-5} and *llpA2*_{Pf-5}, were located at positions 394751 to 395590 and 1343588 to 1344427 of the Pf-5 unfinished genome (contig 3337), respectively. Both genes would encode 280-amino-acid proteins with predicted isoelectric points of 8.74 and calculated molecular masses of 31,032 Da (LlpA1_{Pf-5}) and 31,104 Da (LlpA2_{Pf-5}). A SignalP scan did not indicate the presence of possible N-terminal signal peptides. A multiple protein alignment of LlpA1_{Pf-5}, LlpA2_{Pf-5}, and LlpA_{BW11M1} reveals strong homology between LlpA1_{Pf-5} and LlpA2_{Pf-5} (264 identical amino acids out of 280) and significant homology of each protein with LlpA_{BW11M1} (46% amino acid sequence identity). Analysis of the genomic contexts of the *llpA* paralogues of *P. fluorescens* Pf-5 revealed that *llpA1*_{Pf-5} is located about 300 bp upstream of the conserved gene cluster *ygdA-recA-recX* (on the same DNA strand). The *llpA2*_{Pf-5} downstream region contains, on the opposite strand, a gene encoding a hypothetical protein related to *P. aeruginosa* PA3270 and other acetyltransferases of the GNAT (GEN5-containing *N*-acetyltransferase) family (Pfam accession no. PF00583). Both *llpA* genes contain in their

TABLE 2. Antimicrobial activity of *P. fluorescens* Pf-5 and *E. coli* TOP10F' carrying pCMPG6053 (*llpA1*_{Pf-5}) or pCMPG6052 (*llpA2*_{Pf-5})

Indicator strain	Growth inhibition ^a by:			Indicator strain	Growth inhibition ^a by:		
	Pf-5	TOP10F' (<i>llpA1</i> _{Pf-5})	TOP10F' (<i>llpA2</i> _{Pf-5})		Pf-5	TOP10F' (<i>llpA1</i> _{Pf-5})	TOP10F' (<i>llpA2</i> _{Pf-5})
Gram-negative bacteria							
<i>Pseudomonas</i> sp.				<i>P. putida</i> LMG 2257	—	—	—
<i>P. aeruginosa</i> lineage				<i>P. putida</i> KT2440	—	—	—
<i>P. aeruginosa</i> PAO1	—	—	—	<i>P. resinovorans</i> lineage			
<i>P. stutzeri</i> LMG 11199	—	—	—	<i>P. resinovorans</i> LMG 2274	—	—	—
<i>P. agarici</i> lineage				<i>P. syringae</i> lineage			
<i>P. agarici</i> LMG 2112	+	—	—	<i>P. syringae</i> pv. <i>glycinea</i> LMG 5066	—	—	—
<i>P. cichorii</i> lineage				<i>P. syringae</i> pv. <i>syringae</i> LMG 1247	—	—	—
<i>P. cichorii</i> LMG 2162	—	—	—	<i>P. syringae</i> pv. <i>tabaci</i> LMG 5192	—	—	—
<i>P. fluorescens</i> lineage				Other lineages			
<i>P. aureofaciens</i> LMG 1245	—	—	—	<i>P. syringae</i> pv. <i>tomato</i> DC3000	—	—	—
<i>P. chlororaphis</i> LMG 5004	—	—	—	<i>P. fuscovaginae</i> LMG 2158	+	—	—
<i>P. fluorescens</i> 2-79	—	—	—	Non-pseudomonads			
<i>P. fluorescens</i> 13-79	—	—	—	<i>Agrobacterium tumefaciens</i>	+	—	—
<i>P. fluorescens</i> A1-B	—	—	—	<i>Azospirillum brasilense</i>	+	—	—
<i>P. fluorescens</i> F113	—	—	—	<i>Escherichia coli</i>	—	—	—
<i>P. fluorescens</i> LMG 1794	+	+	+ ^b	<i>Pectobacterium carotovorum</i>	+	—	—
<i>P. fluorescens</i> Pf0-1	+	+	+ ^b	Gram-positive bacteria			
<i>P. fluorescens</i> OE 28.3	—	—	—	<i>Bacillus subtilis</i>	+	—	—
<i>P. fluorescens</i> SBW25	—	—	—	<i>Clavibacter michiganensis</i>	+	—	—
<i>P. fluorescens</i> WCS141	—	—	—	<i>Rhodococcus erythropolis</i>	+	—	—
<i>P. fluorescens</i> WCS365	+	—	—	<i>Streptomyces coelicolor</i>	+	—	—
<i>P. marginalis</i> LMG 2210	—	—	—	Fungi			
<i>P. tolaasii</i> LMG 2344	+	+	+ ^b	<i>Botrytis cinerea</i>	+	—	—
<i>P. viridiflava</i> LMG 2352	—	—	—	<i>Fusarium culmorum</i>	+	—	—
<i>P. mendocina</i> lineage				<i>Fusarium graminearum</i>	+	—	—
<i>P. mendocina</i> LMG 1223	—	—	—	<i>Fusarium oxysporum</i>	+	—	—
<i>P. putida</i> lineage				<i>Neurospora crassa</i>	+	—	—
<i>P. putida</i> GR12-2R3	—	—	—				

^a —, no growth inhibition of indicator; +, growth inhibition.^b The diameter of the inhibition halo was reduced.

upstream region a gene with similarity to the bacteriophage Mu protein Com (22), but these paralogues are located at different intergenic distances (9 bp from *llpA1*_{Pf-5} and 233 bp from *llpA2*_{Pf-5}), suggesting that these genes do not constitute a conserved operon with *llpA*.

Heterologous expression of *llpA1*_{Pf-5} and *llpA2*_{Pf-5} confers bacteriocin activity to *E. coli* and *Pseudomonas*. To circumvent possible interference by other antagonistic compounds (including other bacteriocins) produced by *P. fluorescens* Pf-5, the bacteriocinogenic potential of the two *llpA* homologues was investigated using heterologous expression. The *llpA1*_{Pf-5} and *llpA2*_{Pf-5} open reading frames were amplified from *P. fluorescens* Pf-5 genomic DNA and cloned into pUC18, creating plasmids pCMPG6053 (*llpA1*_{Pf-5}) and pCMPG6052 (*llpA2*_{Pf-5}), which were used to transform *E. coli* TOP10F' cells. To allow expression from its own promoter, an upstream region of 260 bp was included for *llpA2*_{Pf-5}. The *llpA1*_{Pf-5} gene was expressed without the upstream region due to the presence of a predicted phage-like gene immediately upstream of the bacteriocin gene. The resulting *E. coli* transformants containing pCMPG6052 or pCMPG6053 were tested for growth-inhibitory activity against a set of strains representing all major groups of pseudomonads. Growth inhibition patterns obtained for each *E. coli* transformant were compared to the bacteriocin phenotype of *P. fluorescens* strain Pf-5 (Table 2). These bacteriocin activity assays indicated that *E. coli* TOP10F' cells carrying pCMPG6053 or,

to a lesser extent, TOP10F' cells carrying pCMPG6052 were able to inhibit the growth of some pseudomonads by expressing the *llpA1*_{Pf-5} and *llpA2*_{Pf-5} genes, respectively. No such growth inhibitory effects were observed in case of the control *E. coli* TOP10F' cells carrying pUC18 (Fig. 1). The apparent difference in expression levels of recombinant *llpA1*_{Pf-5} compared to *llpA2*_{Pf-5} could result from their vector-driven expression. Alternatively, lower expression of *llpA2*_{Pf-5} could be explained by the presence of some negative regulatory elements in its promoter region.

Both LlpA bacteriocins can inhibit the growth of *P. fluorescens* strains LMG 1794 and Pf0-1, as well as *Pseudomonas*

FIG. 1. Growth inhibition of *P. fluorescens* LMG 1794 by strain Pf-5 and by *E. coli* TOP10F' harboring pCMPG6052 (*llpA2*_{Pf-5}) and pCMPG6053 (*llpA1*_{Pf-5}). *E. coli* TOP10F' carrying pUC18 was included as a negative control.

tolaasii LMG 2344, while no inhibitory activity was observed against nonpseudomonad bacteria or toward a selection of fungi. Importantly, the exposure of *E. coli* cells expressing *llpA1*_{Pf-5} or *llpA2*_{Pf-5} to UV radiation did not result in larger inhibition halos (results not shown), whereas the production of LlpA_{BW11M1} was previously shown to be induced upon DNA-damaging treatment (46).

To explore possible expression of the *llpA* genes in other *Pseudomonas* species, the inserts from pCMPG6052 and pCMPG6053 were transferred to the broad-host-range vector pJB3Tc20. In this way, *llpA1*_{Pf-5} and *llpA2*_{Pf-5} were successfully expressed in *Pseudomonas* after electroporation of the constructs pCMPG6058 and pCMPG6057, respectively, into *P. aeruginosa* strain PA01, *P. putida* strain KT2440, and *P. fluorescens* strain OE 28.3.

Screening of 69 strains from an in-house collection of rhizosphere-colonizing fluorescent *Pseudomonas* isolates identified five additional rhizosphere strains that were inhibited by *E. coli* and *Pseudomonas* cells expressing *llpA1*_{Pf-5} or *llpA2*_{Pf-5}. These strains originated from the rhizosphere of wheat (*Pseudomonas* sp. strains OE 32.1, OE 39.2, and OE 45.2) and maize (*Pseudomonas* sp. strains OE 50.2 and PGSB 7716). These isolates had been tentatively characterized as belonging to the *P. fluorescens* species by metabolic profiling (68). Their tentative assignment to the genus *Pseudomonas sensu stricto* was confirmed by partial 16S rDNA sequencing (data not shown).

In view of the homology of LlpA proteins with MMBL-type mannose-binding lectins, the effect of mannose on bacteriocin activity was analyzed for recombinant LlpA1_{Pf-5} and LlpA2_{Pf-5}. Inclusion of mannose (up to 0.1 M) did not affect halo formation when *E. coli* TOP10F' cells carrying pCMPG6053 or pCMPG6052 were assayed against representative indicator strains (*P. fluorescens* OE 50.2 and LMG 1794). This lack of interference of mannose with activity of MMBL-like bacteriocins is consistent with previous reports in which no binding of mannose could be demonstrated (11, 46).

Expression of two *llpA* genes broadens the activity range of bacteriocinogenic *Pseudomonas* strains. A combinatory approach was performed in order to evaluate the effect of expression of several *llpA* genes in one *Pseudomonas* strain. To this end, *P. fluorescens* Pf-5 cells were electrotransformed with plasmid pCMPG6060 (*llpA*_{BW11M1}). Cells transformed with pJB3Tc20 plasmid DNA were included as a negative control. Bacteriocin plate assays showed that strain Pf-5 carrying pCMPG6060 also inhibited the growth of *Pseudomonas* sp. GR12-2R3, a strain routinely used as an indicator to detect LlpA_{BW11M1} activity (46). In addition, electrotransformation of *Pseudomonas* sp. BW11M1 was carried out with pCMPG6057 or pCMPG6058, respectively. The resulting transformants also showed growth inhibitory activity toward previously identified indicator strains for LlpA1_{Pf-5} and LlpA1_{Pf-5}.

Detection of LlpA1_{Pf-5} and LlpA2_{Pf-5} in culture supernatant of *E. coli* transformants. Culture supernatants of *E. coli* TOP10F' cells containing pCMPG6052 and pCMPG6053 were collected and concentrated 25-fold by lyophilization. Coomassie staining of an SDS-PAGE gel (Fig. 2) of phenol-extracted total supernatant proteins from *E. coli* TOP10F' harboring *llpA*_{Pf-5} constructs revealed the presence of many bands with

FIG. 2. Separation of *E. coli* supernatant proteins by SDS-PAGE (left panel) and gel overlay bacteriocin assay using *P. fluorescens* LMG 1794 as an indicator strain embedded in 0.5% tryptic soy agar (right panel). Total *E. coli* protein amounts were 20 μ g (SDS-PAGE) and 100 μ g (gel overlay). Lane 1, prestained low-range SDS PAGE protein markers; lane 2, *E. coli* TOP10F' carrying pUC18; lane 3, *E. coli* TOP10F' carrying pCMPG6052 and expressing *llpA2*_{Pf-5}; lane 4, *E. coli* TOP10F' carrying pCMPG6053 and expressing *llpA1*_{Pf-5}. The arrowheads indicate active bacteriocin bands. Due to the reproduction of the image, the vague inhibition zone (indicated by the dotted ellipse) generated by LlpA2_{Pf-5} is poorly visible.

no detectable difference compared to the negative control. This is probably due to low-level expression and/or comigration of the bacteriocins with an *E. coli* protein. We therefore attempted to demonstrate the presence of recombinant LlpA1_{Pf-5} and LlpA2_{Pf-5} proteins in culture supernatant by performing a gel overlay assay. An unstained SDS-PAGE gel run with concentrated supernatant proteins of recombinant *E. coli* was washed and then a lawn seeded with an indicator strain was applied. After overnight incubation, clearing zones indicative of bacteriocin activity were observed at positions corresponding to migration of the expected 31-kDa recombinant proteins (Fig. 2). The clearing zone was most obvious in the case of the protein sample from *E. coli* TOP10F' expressing *llpA1*_{Pf-5}. Pure recombinant LlpA_{BW11M1} protein (47) was used to generate polyclonal antibodies in New Zealand White rabbits. The cross-reaction of the anti-LlpA_{BW11M1} antibodies was tested against concentrated supernatant proteins obtained from overnight cultures of *E. coli* TOP10F' carrying pCMPG6053 (*llpA1*_{Pf-5}) or pCMPG6052 (*llpA2*_{Pf-5}). Immunoblotting revealed a single band of the expected size (31 kDa) per supernatant culture, while no band was visible in the control supernatant (fraction of *E. coli* TOP10F' carrying pUC18) (Fig. 3). The strongest immunoreactive band was observed for *E. coli* TOP10F' expressing *llpA1*_{Pf-5}.

Tandem domain structure of LlpA1_{Pf-5} and LlpA2_{Pf-5} bacteriocins. A Pfam search for conserved protein domains showed that LlpA1_{Pf-5} and LlpA2_{Pf-5} possess a domain structure consisting of two tandemly arranged MMBL domains linked by 10 amino acids, namely MMBL_1 ranging from amino acids 13 to 139 and MMBL_2 ranging from amino acids 147 to 255. MMBL_1 and MMBL_2 within each protein are quite dissimilar (16% identity and 28% similarity). A multiple protein alignment of each of the MMBL domains of LlpA1_{Pf-5}

FIG. 3. Immunodetection of recombinant LlpA homologues in culture supernatant from *E. coli*. Lane 1, MagicMark protein marker; lane 2, 10 ng of pure LlpA_{BW11M1}His₆; lane 3, 5 μ g of *E. coli* TOP10F' carrying pUC18; lane 4, 5 μ g of *E. coli* TOP10F' carrying pCMPG6052 and expressing *llpA2*_{Pf-5}; lane 5, 5 μ g of *E. coli* TOP10F' carrying pCMPG6053 and expressing *llpA1*_{Pf-5}. Proteins were detected using polyclonal antibodies raised against His₆-tagged LlpA_{BW11M1}.

and LlpA2_{Pf-5} with MMBL domains of the well-characterized mannose-binding garlic lectin ASAI (66) reveals the presence of two perfect or close matches to the characteristic mannose-binding motif Q-X-D-X-N-X-V-X-Y in each MMBL domain of the Pf-5 bacteriocins (Fig. 4). The equivalent of the third motif (in MMBL₂) and in particular the counterpart of the second motif (in MMBL₁) are poorly conserved in LlpA1_{Pf-5} and LlpA2_{Pf-5}. However, three highly conserved Trp (W) residues were detected in each MMBL domain. These hydrophobic residues are situated at the center of each of the monocot MMBL subunits, close to the threefold axis, and form a stabilizing hydrophobic core (10). The C-terminal MMBL domains of LlpA1_{Pf-5} and LlpA2_{Pf-5} display 30% amino acid identity to the N-terminal MMBL domain of albusin B (AlbB) (Fig. 4). The 290-amino-acid putative homologue of AlbB (AlbB2) was predicted from unfinished genome sequence data of *R. albus* strain 8 (contig 3858, retrieved from TIGR).

MMBL domains are found in diverse bacterial proteins. Following the identification of LlpA_{BW11M1} comprised of two MMBL domains, we described a similar tandem arrangement of MMBL domains in other bacterial MMBL proteins, namely, in the hypothetical protein XAC0868 of *Xanthomonas axonopodis* pv. citri and in a hypothetical protein from *R. metallidurans* (46). With the advent of more and more genome sequences, novel hypothetical bacterial MMBL-containing proteins emerge. The genome of *Chromobacterium violaceum* encodes a 113-amino-acid putative protein (protein CV3134 with an N-terminal extension of 34 amino acids) comprising a single MMBL domain. Additional MMBL domains were detected in the 212-amino-acid protein NFA49840 from *Nocardia farcinica* and in a putative 207-amino-acid protein from *M. marinum*. In both proteins, the MMBL domain is located at the N terminus and is connected to a putative LysM domain by a proline-rich linker sequence. A similar domain organization

was described for a putative protein from another actinomycete *M. smegmatis* (46). The LysM domain is one of the most common modules in bacterial cell surface proteins. It occurs most often in cell wall-degrading enzymes, where it probably acts to anchor the catalytic domains to their substrates (4). Additional MMBL domains were identified in putative proteins from environmental isolates from the Sargasso Sea (67). One of these protein sequences is truncated at the N terminus. Both putative proteins are composed of two C-terminally located tandemly organized MMBL domains. In the nontruncated protein, a Pfam search identified a peptidase domain as found in a large family of serine proteases (subtilisins). In *S. coelicolor* SCO3053, an MMBL domain is also fused to a protease domain (trypsin).

Phylogenetic analysis of MMBL domains. To further explore the apparent similarity of the Pf-5 LlpA-like bacteriocins with the superfamily of monocot mannose-binding lectins and other diverse proteins containing MMBL domains, we performed a molecular phylogenetic analysis of its MMBL domains. In addition to known and predicted MMBL domains from green plants and bacteria, we enclosed representative eukaryotic sequences from proteins that were identified from BLAST searches. Of particular interest are the pufflectins isolated from skin and intestinal mucus of the Japanese pufferfish *Takifugu rubripes* (63). Pufflectins are homodimers composed of two noncovalently associated subunits of 13 kDa. Each of these subunits contains one MMBL domain. Tsuitsui and co-workers demonstrated mannose-sensitive pufflectin binding to the surface of the parasitic trematode *Heterobothrium okamotoi*, suggesting a role of pufflectin in host defense mechanisms (63). The genome of another pufferfish, *Tetraodon nigroviridis*, encodes a 116-amino-acid pufflectin homologue (32). MMBL domains were also detected in fungal proteins, namely, in hypothetical proteins from *Aspergillus oryzae* and *Gibberella zeae*.

From a multiple alignment of MMBL₁ and MMBL₂ of each LlpA from Pf-5 with known or predicted MMBL domains, an unrooted phylogenetic tree was inferred by neighbor-joining distance analysis (Fig. 5). Four prominent clusters are indicated encompassing MMBL domains found in green plants, animal proteins, fungi, and hypothetical proteins from actinomycetes. MMBL domains of bacteriocins are found in two divergent clusters. The N-terminal MMBL domains from the LlpA bacteriocins are clustered with the N-terminal MMBL domain of a hypothetical bacteriocin from *X. axonopodis* pv. citri. On the other hand, C-terminal MMBL domains from *Pseudomonas* LlpA-type bacteriocins cluster with the C-terminal MMBL domains of the hypothetical bacteriocin from *X. axonopodis* pv. citri and the single N-terminal MMBL domain of bacteriocin AlbB from *R. albus*. It should be noted that tandem MMBL domains detected in multidomain proteins, such as the hypothetical proteins from *R. metallidurans* and environmental isolates from the Sargasso Sea, are much less divergent in comparison to tandem MMBL domains from bacteriocins.

DISCUSSION

The results described here show that, in addition to an arsenal of antifungal biosynthetic genes, the rhizosphere bacterium *P. fluorescens* Pf-5 is also equipped with at least two

FIG. 4. Multiple alignment of LlpA_{BW11M1} (LlpA), LlpA1_{Pf-5} (LlpA1), LlpA2_{Pf-5} (LlpA2), the N-terminal region (amino acids 50 to 200) of albumin B from *R. albus* strain 8 (AlbB_N), and its predicted homologue in *R. albus* strain 7 (AlbB2_N, amino acids 50 to 199) with mature ASAI, a mannose-specific lectin found in bulbs of garlic (*Allium sativum*). The signal peptides of albumin B (49 amino acids), as well as the predicted signal peptide of AlbB2 (49 amino acids), are not included in the alignment. Gaps used to optimize the alignment are indicated by dashes. Predicted MMBL domains are enclosed in boxes. Amino acids involved in mannose binding in ASAI are indicated by filled circles when present in LlpA1_{Pf-5} and LlpA2_{Pf-5} or by open circles when not conserved in LlpA1_{Pf-5} and LlpA2_{Pf-5}. The labeled tryptophan residues (asterisks) are part of a stabilizing hydrophobic core structure and are conserved in nearly all homologous proteins. Cysteine residues typical for plant MMBL domains are also marked (inverted open triangles).

functional antibacterial genes. Expression of each of these genes in *E. coli* and *Pseudomonas* results in the production of 31-kDa bacteriocins that are released in the growth medium. We named these bacteriocins LlpA1_{Pf-5} and LlpA2_{Pf-5} based on their similarity (chromosomally located structural gene, size, and domain structure) to LlpA_{BW11M1}, a plant lectin-like bacteriocin previously identified in rhizosphere isolate *Pseudomonas* sp. BW11M1 (46). Although displaying differences in activity spectrum, their killing activity is limited to fluorescent *Pseudomonas* strains. Finally, these LlpA-type bacteriocins can be produced in heterologous hosts (*E. coli* and other *Pseudomonas* species) without the need of a signal peptide for secretion or a specific immunity protein, in contrast to the “classical” bacteriocins of gram-negative bacteria such as pyocins (*P. aeruginosa*) and colicins (*Enterobacteriaceae*). It is very likely that these LlpA-type bacteriocins share a common mode of action that differs from those of previously characterized bacteriocins. We propose that LlpA-type bacteriocins interact with sensitive bacterial cells by binding to a specific glycosylated

outer membrane component, possibly lipopolysaccharide, resulting in cell death through a yet unknown mechanism.

Similar to LlpA_{BW11M1}, LlpA1_{Pf-5} and LlpA2_{Pf-5} are composed of two MMBL-like domains connected by a short linker sequence. These domains are conserved among mannose-specific lectins of monocots (57, 65). The in silico comparative analysis presented in this work, together with other data (11, 61), clearly establishes that MMBL domains have a much wider distribution than previously assumed. In this study, we identified MMBL domains not only in various (putative) proteins encoded by fungi and fish genomes but also in phylogenetically unrelated bacteria such as *C. violaceum* and several actinomycetes. In some cases, these bacterial MMBL domains were found to be linked to other protein domains with predicted bacterial cell wall-degrading or protease activity. However, the XAC0868 hypothetical protein from *X. axonopodis* pv. citri displays an identical tandem domain structure as the LlpA-type bacteriocins of *Pseudomonas* sp. BW11M1 and *P. fluorescens* Pf-5. Moreover, its domains constitute monophy-

FIG. 5. Molecular phylogeny of MMBL domains and domain structures of bacterial and selected eukaryotic proteins containing one or two MMBL domains. An unrooted neighbor-joining distance tree was constructed from a multiple alignment of amino acid sequences of known and inferred MMBL domains found in eukaryotic and bacterial proteins. The extensions “_1” and “_2” refer to tandem MMBL domains present in certain polypeptides. MMBL domains present in proteins containing another domain are indicated with an asterisk. The scale bar represents 0.2 substitutions per site. A selection of MMBL domains from lectins found in monocot plants belonging to 11 different families is included. Monocot lectins with a two-domain precursor are exemplified by lectins from *Allium sativum* (Allsat; GenBank accession no. S23495), *Crocus vernus* (Crover; P49329) and *Arum maculatum* (Arumac; AAC48997). Lectins from *Hyacinthoides hispanica* (Hyahis; AAD16403), *Galanthus nivalis* (Galniv; AAA33345), *Ananas comosus* (Anacom; AAM28277), *Tulipa* sp. (Tulipa; AAC49386), *Dioscorea polystachya* (Diopol; BAD67183), *Aloe arborescens* (Aloarb; P49329) and *Polygonatum multiflorum* (Polmul; AAC49413) represent proteins containing a single MMBL domain. Gastrodinanin, an antifungal protein from *Gastrodia elata* with mannose-binding capacity (Gasela; AAG52664) and the actin-binding mannose-specific protein from *Dictyostelium discoideum* (Dicdis; Q03380) are also included. Liverwort (*Marchantia polymorpha*) contains two MMBL-like proteins, Marpol-1 (Marpol1; AU08173) and Marpol-3 (Marpol3; C95861). MMBL domains were also detected in the MleA lectin of *Aspergillus oryzae* (Aspor; MleA lectin BAC56173) and in hypothetical proteins FG03689.1 (Gibzea1; EAA71502) and FG03828.1 (Gibzea2; EAA71530) from *Gibberella zeae*. Fish proteins containing MMBL domains are *T. rubripes* pufferlectins (Takrub1 from intestinal mucus, BAC57044; Takrub2 from skin mucus, BAC57043) and a hypothetical protein from *T. nigroviridis* (Tetvir; CAG10253). Bacterial proteins containing MMBL domains are LlpA1 and LlpA2 from *P. fluorescens* Pf-5, LlpA from *Pseudomonas* sp. BW11M1 (AAM95702), *R. albus* strain 7 bacterial inhibitor (Rumalb; AAL73547), a protein from *C. violaceum* (Chrvio; a truncated version of this protein, without the first 34 residues, is referred to in GenBank as a bulb-type mannose-specific lectin [AAQ60810]), *X. axonopodis* pv. citri hypothetical protein XAC0868 (Xanaxo; NP_641220), hypothetical protein NFA49840 from *N. farcinica* (Nocfar; YP_121200), the precursor of a putative antifungal protein from *M. smegmatis* strain mc²155 (Mycsme; GSMORF3668, retrieved from TIGR), putative secreted hydrolase SCO3053 from *S. coelicolor* (Strcoe; NP_627274), and two putative proteins encoded by environmental DNA samples isolated from the Sargasso Sea (Sargas1, EAK32154; Sargas2, EAD21447). Additional proteins with MMBL domains homologous to the LlpA MMBL domains were predicted from unfinished genome sequences of *R. albus* strain 8 (Rumalb2; contig 3859, positions 5864 to 4848; retrieved from TIGR), *M. marinum* (Mycmar, contig mar813f05.p2k1095, positions 653229 to 653849; retrieved from Sanger) and *R. metallidurans* strain CH34 (Ralmet, revised contig 67, gene 2519; retrieved from JGI; a truncated version of this protein, without the first 244 residues, is found in GenBank [ZP_00022141]).

letic groups with the cognate MMBL domains of the LlpA-type bacteriocins. This strongly suggests that this *Xanthomonas* protein is also an LlpA-type bacteriocin.

From the MMBL domain tree topology it is not obvious whether N-terminal and C-terminal MMBL domains in LlpA bacteriocins have evolved from a common ancestral protein by speciation or whether these MMBL domains have evolved toward specialized functions following a gene duplication event. Conceivably, one domain could be involved in target

binding, while the other might be responsible for the actual killing. As monocot mannose-binding lectins are the sole MMBL-containing proteins that have been crystallized and analyzed by X-ray diffraction (3, 25, 38, 70), we expect that ongoing studies to resolve the three-dimensional structure of recombinant LlpA_{BW11M1} crystals will ultimately provide more insight into the specific role of N- and C-terminal MMBL domains in LlpA-type bacteriocin activity (47).

Biological control of soilborne crop diseases by the intro-

duction of beneficial microorganisms into the rhizosphere has been proposed as an environmentally friendly alternative to the use of synthetic chemicals. Certain plant-associated bacteria, particularly fluorescent *Pseudomonas* spp., have been exploited for suppression of crop diseases by direct antagonism between the bacteria and soilborne pathogens (7, 49), by iron depletion through the production of siderophores (13), or by the induction of systemic resistance (29, 60). Exploiting fluorescent pseudomonads to improve crop yield has tremendous potential, but field trials yield inconsistent results. Introduction of these bacteria in the field often fails because the organisms are not able to recolonize the roots, or they colonize the roots but do not produce biocontrol metabolites in the new niche. This inconsistent biocontrol activity could be due, in part, to bacteriocins produced by endogenous pseudomonads active against the introduced strain. We have previously explored the potential of plant-associated *P. fluorescens* and *P. putida* to produce a large variety of pyocin S-like bacteriocins with nuclease activity (44, 45). While a similar in silico approach suggests that *P. fluorescens* Pf-5 does not produce S pyocins (data not shown), the current study hints that this strain does produce LlpA-like bacteriocins in the rhizosphere. Upon investigation of the antibacterial spectrum of LlpA_{Pf-5} and LlpA₂_{Pf-5} toward several fluorescent pseudomonads, no significant qualitative differences could be observed. However, this does not exclude the possibility that these bacteriocins act on different *Pseudomonas* strains in the rhizosphere. Alternatively, their expression may be triggered by different environmental signals. Various stress response pathways influence the expression of LlpA in *Pseudomonas* sp. BW11M1 (17). Bacteriocin production by biocontrol strains may contribute to their rhizosphere competence (64). If so, recombinant biocontrol strains equipped with (several) bacteriocin genes may perform better as plant growth-promoting inocula by outcompeting rhizosphere-inhabiting pseudomonads.

ACKNOWLEDGMENTS

A.P. is the recipient of a postdoctoral fellowship (PDM 03/197) from the Research Council of the Katholieke Universiteit Leuven. This work was supported by a grant (Onderzoeksproject G.0303.04 to R.D.M.) from the Fund for Scientific Research (F.W.O.-Vlaanderen).

The authors thank Joyce Loper (USDA-ARS-Horticultural Crops Research Unit, Corvallis, Oreg.) for providing a culture of *P. fluorescens* Pf-5.

ADDENDUM IN PROOF

In the published genome sequence of *P. fluorescens* Pf-5 (I. T. Paulsen et al., *Nature Biotechnol.* **23**:873–878, 2005), LlpA1 and LlpA2 correspond to the predicted proteins PFL1229 (gi:70728611) and PFL2127 (gi:70729496), respectively.

REFERENCES

- Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Baldani, V. L. D., M. A. Alvarze, J. I. Baldani, and J. Döbereiner. 1986. Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. *Plant Soil* **90**:35–46.
- Barre, A., Y. Bourne, E. J. M. Van Damme, W. J. Peumans, and P. Rougé. 2001. Mannose-binding plant lectins: different structural scaffolds for a common sugar-recognition process. *Biochimie* **83**:645–651.
- Bateman, A., and M. Bycroft. 2000. The structure of a LysM domain from *E. coli* membrane-bound lytic murein transglycosylase D (MltD). *J. Mol. Biol.* **299**:1113–1119.
- Bentley, S. D., K. F. Chater, A. M. Cerdeño-Tárraga, G. L. Challis, N. R. Thomson, K. D. James, D. E. Harris, M. A. Quail, H. Kieser, D. Harper, A. Bateman, S. Brown, G. Chandra, C. W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, S. Howarth, C.-H. Huang, T. Kieser, L. Larke, L. Murphy, K. Oliver, S. O'Neil, E. Rabinowitsch, M.-A. Rajandream, K. Rutherford, S. Rutter, K. Seeger, D. Saunders, S. Sharp, R. Squares, S. Squares, K. Taylor, T. Warren, A. Wietzorrek, J. Woodward, B. G. Barrell, J. Parkhill, and D. A. Hopwood. 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**:141–147.
- Blatny, J. M., T. Brautaset, H. C. Winther-Larsen, K. Haugan, and S. Valla. 1997. Construction and use of a versatile set of broad-host-range cloning and expression vectors based on the RK2 replicon. *Appl. Environ. Microbiol.* **63**:370–379.
- Bloemberg, G., and B. Lugtenberg. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol.* **4**:343–350.
- Boyer, H. W., and D. Roulland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* **41**:459.
- Brodhagen, M., M. D. Henkels, and J. E. Loper. 2004. Positive autoregulation and signaling properties of pyoluteorin, an antibiotic produced by the biological control organism *Pseudomonas fluorescens* Pf-5. *Appl. Environ. Microbiol.* **70**:1758–1766.
- Chandra, N. R., G. Ramachandiraiah, K. Bachhawat, T. K. Dam, A. Surlia, and M. Vijayan. 1999. Crystal structure of a dimeric mannose-specific agglutinin from garlic: quaternary association and carbohydrate specificity. *J. Mol. Biol.* **285**:1157–1168.
- Chen, J., D. M. Stevenson, and P. J. Weimer. 2004. Albusin B, a bacteriocin from the ruminal bacterium *Ruminococcus albus* 7 that inhibits growth of *Ruminococcus flavefaciens*. *Appl. Environ. Microbiol.* **70**:3167–3170.
- Compeau, G., B. J. Al-Achi, E. Platsouka, and S. B. Levy. 1988. Survival of rifampin-resistant mutants of *Pseudomonas fluorescens* and *Pseudomonas putida* in soil systems. *Appl. Environ. Microbiol.* **54**:2432–2438.
- Cornelis, P., and S. Matthijs. 2002. Diversity of siderophore-mediated iron uptake systems in fluorescent pseudomonads: not only pyoverdines. *Environ. Microbiol.* **4**:787–798.
- Cuppels, D. A. 1986. Generation and characterization of Tn5 insertion mutations in *Pseudomonas syringae* pv. tomato. *Appl. Environ. Microbiol.* **51**:323–327.
- De Mot, R., and J. Vanderleyden. 1991. Purification of root-adhesive outer membrane protein of root-colonizing *Pseudomonas fluorescens*. *FEMS Microbiol. Lett.* **81**:323–328.
- de Weger, L. A., R. van Bostel, B. van der Burg, R. A. Gruters, F. P. Geels, B. Schippers, and B. Lugtenberg. 1986. Siderophores and outer membrane proteins of antagonistic, plant-growth-stimulating, root-colonizing *Pseudomonas* spp. *J. Bacteriol.* **165**:585–594.
- Estrada de los Santos, P., A. H. A. Parret, and R. De Mot. 2005. Stress-related *Pseudomonas* genes involved in production of bacteriocin LlpA. *FEMS Microbiol. Lett.* **244**:243–250.
- Franklin, F. C., M. Bagdasarian, M. M. Bagdasarian, and K. N. Timmis. 1981. Molecular and functional analysis of the TOL plasmid pWWO from *Pseudomonas putida* and cloning of genes for the entire regulated aromatic ring meta cleavage pathway. *Proc. Natl. Acad. Sci. USA* **78**:7458–7462.
- Geels, F. P., and B. Schippers. 1983. Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. *Phytopathol. Z.* **108**:193–206.
- Goodfellow, M., E. G. Thomas, A. C. Ward, and A. L. James. 1990. Classification and identification of rhodococci. *Zentralbl. Bakteriol.* **274**:299–315.
- Harlow, E., and D. Lane. 1988. *Antibodies: a laboratory manual*, 1st ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Hattman, S. 1999. Unusual transcriptional and translational regulation of the bacteriophage Mu *mom* operon. *Pharmacol. Ther.* **84**:367–388.
- Hechard, Y., and H. G. Sahl. 2002. Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. *Biochimie* **84**:545–557.
- Herlache, T. C., and E. W. Triplett. 2002. Expression of a crown gall biological control phenotype in an avirulent strain of *Agrobacterium vitis* by addition of the trifolitoxin production and resistance genes. *BMC Biotechnol.* **2**:2.
- Hester, G., H. Kaku, I. J. Goldstein, and C. S. Wright. 1995. Structure of mannose-specific snowdrop (*Galanthus nivalis*) lectin is representative of a new plant lectin family. *Nat. Struct. Biol.* **2**:472–479.
- Heu, S., J. Oh, Y. Kang, S. Ryu, S. K. Cho, Y. Cho, and M. Cho. 2001. *gly* gene cloning and expression and purification of glycinecin A, a bacteriocin produced by *Xanthomonas campestris* pv. glycines 8ra. *Appl. Environ. Microbiol.* **67**:4105–4110.
- Hill, D. S., J. I. Stein, N. R. Torkewitz, A. M. Morse, C. R. Howell, J. P. Pachlatko, J. O. Becker, and J. M. Ligon. 1994. Cloning of genes involved in the synthesis of pyrrolnitrin from *Pseudomonas fluorescens* and role of pyr-

- rolnitrin synthesis in biological control of plant disease. *Appl. Environ. Microbiol.* **60**:78–85.
28. Howell, C. R., and R. D. Stipanovic. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology* **69**:480–482.
 29. Iavicoli, A., E. Boutet, A. Buchala, and J. P. Metraux. 2003. Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. *Mol. Plant Microbe Interact.* **16**:851–858.
 30. Jabrane, A., A. Sabri, P. Compère, P. Jacques, I. Vandenberghe, J. Van Beeumen, and P. Thonart. 2002. Characterization of serracin P, a phage-tail-like bacteriocin, and its activity against *Erwinia amylovora*, the fire blight pathogen. *Appl. Environ. Microbiol.* **68**:5704–5710.
 31. Jack, R. W., and G. Jung. 2000. Lantibiotics and microcins: polypeptides with unusual chemical diversity. *Curr. Opin. Chem. Biol.* **4**:310–317.
 32. Jaillon, O., J. M. Aury, F. Brunet, J. L. Petit, N. Stange-Thomann, E. Mauceli, L. Bouneau, C. Fischer, C. Ozouf-Costaz, A. Bernot, S. Nicaud, D. Jaffe, S. Fisher, G. Lutfalla, C. Dossat, B. Segurens, C. Dasilva, M. Salanoubat, M. Levy, N. Boudet, S. Castellano, V. Anthouard, C. Jubin, V. Castelli, M. Katinka, B. Vacherie, C. Biemont, Z. Skalli, L. Cattelico, J. Poulain, V. De Berardinis, C. Cruaud, S. Duprat, P. Brottier, J. P. Coutanceau, J. Gouzy, G. Parra, G. Lardier, C. Chapple, K. J. McKernan, P. McEwan, S. Bosak, M. Kellis, J. N. Volf, R. Guigo, M. C. Zody, J. Mesirov, K. Lindblad-Toh, B. Birren, C. Nusbaum, D. Kahn, M. Robinson-Rechavi, V. Laudet, V. Schachter, F. Quetier, W. Saurin, C. Scarpelli, P. Wincker, E. S. Lander, J. Weissenbach, and H. Roest Crollius. 2004. Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. *Nature* **431**:946–957.
 33. Kirkup, B. C., and M. A. Riley. 2004. Antibiotic-mediated antagonism leads to a bacterial game of rock-paper-scissors in vivo. *Nature* **428**:412–414.
 34. Lakey, J. H., and S. L. Slatin. 2001. Pore-forming colicins and their relatives. *Curr. Top. Microbiol. Immunol.* **257**:131–161.
 35. Lavermicocca, P., S. L. Lonigro, F. Valerio, A. Evidente, and A. Visconti. 2002. Reduction of olive knot disease by a bacteriocin from *Pseudomonas syringae* pv. *ciccaronei*. *Appl. Environ. Microbiol.* **68**:1403–1407.
 36. Li, Y., H. Xiang, J. Liu, M. Zhou, and H. Tan. 2003. Purification and biological characterization of halocin C8, a novel peptide antibiotic from *Halobacterium* strain AS7092. *Extremophiles* **7**:401–407.
 37. Lifshitz, R., J. W. Kloepper, M. Kozlowski, C. Simonson, J. Carlson, E. M. Tipping, and I. Zaleska. 1987. Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Can. J. Microbiol.* **33**:390–395.
 38. Liu, W., N. Yang, J. Ding, R. H. Huang, Z. Hu, and D. C. Wang. 2005. Structural mechanism governing the quaternary organization of monocoat mannose-binding lectin revealed by the novel monomeric structure of an orchid lectin. *J. Biol. Chem.* **280**:14865–14876.
 39. Michel-Briand, Y., and C. Baysse. 2002. The pyocins of *Pseudomonas aeruginosa*. *Biochimie* **84**:499–510.
 40. Nowak-Thompson, B., S. J. Gould, J. Kraus, and J. E. Loper. 1994. Production of 2,4-diacetylphloroglucinol by the biocontrol agent *Pseudomonas fluorescens* Pf-5. *Can. J. Microbiol.* **40**:1064–1066.
 41. O'Sullivan, L., R. P. Ross, and C. Hill. 2002. Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochimie* **84**:593–604.
 42. Papagianni, M. 2004. Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function, and applications. *Biotechnol. Adv.* **21**:465–499.
 43. Parret, A. H. A. 2002. Ph.D. thesis. Katholieke Universiteit Leuven, Heverlee, Belgium.
 44. Parret, A. H. A., and R. De Mot. 2001. Bacteriocin production by rhizosphere-colonizing fluorescent *Pseudomonas*. Proceedings of the 5th International PGPR Workshop. [Online.] <http://www.ag.auburn.edu/argentina/pdf/manuscripts/parret.pdf>.
 45. Parret, A. H. A., and R. De Mot. 2002. Bacteria killing their own kind: novel bacteriocins of *Pseudomonas* and other γ -proteobacteria. *Trends Microbiol.* **10**:107–112.
 46. Parret, A. H. A., G. Schoofs, P. Proost, and R. De Mot. 2003. A plant lectin-like bacteriocin from a rhizosphere-colonizing *Pseudomonas*. *J. Bacteriol.* **185**:897–908.
 47. Parret, A. H. A., L. Wyns, R. De Mot, and R. Loris. 2004. Overexpression, purification and crystallization of LlpA. *Acta Crystallogr. Sect. D* **60**:1922–1924.
 48. Pfender, W. F., J. Kraus, and J. E. Loper. 1993. A genomic region from *Pseudomonas fluorescens* Pf-5 required for pyrrolnitrin production and inhibition of *Pyrenophora tritici-repentis* in wheat straw. *Phytopathology* **83**:1223–1228.
 49. Raaijmakers, J. M., M. Vlami, and J. T. de Souza. 2002. Antibiotic production by bacterial biocontrol agents. *Antonie Leeuwenhoek* **81**:537–547.
 50. Rainey, P. B., and M. J. Bailey. 1996. Physical and genetic map of the *Pseudomonas fluorescens* SBW25 chromosome. *Mol. Microbiol.* **19**:521–533.
 51. Riley, M. A. 1998. Molecular mechanisms of bacteriocin evolution. *Annu. Rev. Genet.* **32**:255–278.
 52. Riley, M. A., and D. M. Gordon. 1999. The ecological role of bacteriocins in bacterial competition. *Trends Microbiol.* **7**:129–133.
 53. Riley, M. A., and J. E. Wertz. 2002. Bacteriocins: evolution, ecology, and application. *Annu. Rev. Microbiol.* **56**:117–137.
 54. Robledo, E. A., K. Kmiecik, E. S. Oplinger, J. Nienhuis, and E. W. Triplett. 1998. Trifolixotoxin production increases nodulation competitiveness of *Rhizobium etli* CE3 under agricultural conditions. *Appl. Environ. Microbiol.* **64**:2630–2633.
 55. Sambrook, J., and D. W. Russell. 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
 56. Shanahan, P., D. J. O'Sullivan, P. Simpson, J. D. Glennon, and F. O'Gara. 1992. Isolation and characterization of an antibiotic-like compound from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Appl. Environ. Microbiol.* **58**:353–358.
 57. Smeets, K., E. J. M. van Damme, P. Verhaert, A. Barre, P. Rouge, F. V. Leuven, and W. J. Peumans. 1997. Isolation, characterization and molecular cloning of the mannose-binding lectins from leaves and roots of garlic (*Allium sativum* L.). *Plant Mol. Biol.* **33**:223–234.
 58. Smidt, M. L., and A. K. Vidaver. 1982. Bacteriocin production by *Pseudomonas syringae* PsW-1 in plant tissue. *Can. J. Microbiol.* **28**:600–604.
 59. Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrenner, M. J. Hickey, F. S. L. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrook-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, S. K. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K.-S. Wong, Z. Wu, I. T. Paulsen, J. Reizer, M. H. Saier, R. E. W. Hancock, S. Lory, and M. V. Olson. 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**:959–964.
 60. Summermatter, K., L. Sticher, and J. P. Metraux. 1995. Systemic responses in *Arabidopsis thaliana* infected and challenged with *Pseudomonas syringae* pv. *syringae*. *Plant Physiol.* **108**:1379–1385.
 61. Suzuki, Y., S. Tasumi, S. Tsutsui, M. Okamoto, and H. Suetake. 2003. Molecular diversity of skin mucus lectins in fish. *Comp. Biochem. Physiol.* **136**:723–730.
 62. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**:4673–4680.
 63. Tsutsui, S., S. Tasumi, H. Suetake, and Y. Suzuki. 2003. Lectins homologous to those of monocotyledonous plants in the skin mucus and intestine of pufferfish, *Fugu rubripes*. *J. Biol. Chem.* **278**:20882–20889.
 64. Validov, S., O. Mavrodi, L. De La Fuente, A. Boronin, D. Weller, L. Thomashow, and D. Mavrodi. 2005. Antagonistic activity among 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. *FEMS Microbiol. Lett.* **242**:249–256.
 65. Van Damme, E. J. M., W. J. Peumans, A. Barre, and P. Rouge. 1998. Plant lectins: a composite of several distinct families of structurally and evolutionarily related proteins with diverse biological roles. *Crit. Rev. Plant Sci.* **17**:575–692.
 66. Van Damme, E. J. M., K. Smeets, S. Torrekens, F. Van Leuven, I. J. Goldstein, and W. J. Peumans. 1992. The closely related homomeric and heterodimeric mannose-binding lectins from garlic are encoded by one-domain and two-domain lectin genes, respectively. *Eur. J. Biochem.* **206**:413–420.
 67. Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealon, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkuch, Y. H. Rogers, and H. O. Smith. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**:66–74.
 68. Vlassak, K., L. Van Holm, L. Duchateau, J. Vanderleyden, and R. De Mot. 1992. Isolation and characterization of fluorescent *Pseudomonas* associated with the roots of rice and banana grown in Sri Lanka. *Plant Soil* **145**:51–63.
 69. Weller, D. M., and R. J. Cook. 1983. Suppression of Take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* **73**:463–469.
 70. Wright, L. M., E. J. M. Van Damme, A. Barre, A. K. Allen, F. Van Leuven, C. D. Reynolds, P. Rouge, and W. J. Peumans. 1999. Isolation, characterization, molecular cloning and molecular modelling of two lectins of different specificities from bluebell (*Scilla campanulata*) bulbs. *Biochem. J.* **340**:299–308.
 71. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**:103–119.