JOURNAL OF BACTERIOLOGY, Feb. 1997, p. 1280–1290 Vol. 179, No. 4
0021-9193/97/$04.00+0

Characterization of the *fimA* Gene Encoding Bundle-Forming Fimbriae of the Plant Pathogen *Xanthomonas campestris* pv. vesicatoria

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Received 16 October 1996/Accepted 9 December 1996

The *fimA* gene of *Xanthomonas campestris* pv. vesicatoria was identified and characterized. A 20-mer degenerate oligonucleotide complementary to the N-terminal amino acid sequence of the purified 15.5-kDa fimbrillin was used to locate *fimA* on a 2.6-kb *Sal*I fragment of the *X. campestris* pv. vesicatoria 3240 genome. The nucleotide sequence of a 1.4-kb fragment containing the *fimA* region revealed two open reading frames predicting highly homologous proteins FimA and FimB. FimA, which was composed of 136 amino acids and had a calculated molecular weight of 14,302, showed high sequence identity to the type IV fimbrillin precursors. *fimB* predicted a protein product of 135 amino acids and a molecular weight of 13,854. The open reading frame for *fimB* contained near the 5′ end a palindromic sequence with a terminator loop potential, and the expression level of *fimB* in vitro and in *Xanthomonas* was considerably lower than that of *fimA*. We detected an efficiently transcribed *fimA*-specific mRNA of 600 bases as well as two weakly expressed, longer mRNA species that reacted with both *fimA* and *fimB*. A homolog of *fimA* but not of *fimB* was detected by Southern hybridization in strains of *X. campestris* pv. vesicatoria, campestris, begoniae, translucens, and graminis. A *fimA*::Ω mutant of strain 3240 was not significantly reduced in virulence or adhesiveness to tomato leaves. However, the *fimA* mutant was dramatically reduced in cell aggregation in laboratory cultures and on infected tomato leaves. The *fimA* mutant strain also exhibited decreased tolerance to UV light.

Xanthomonas campestris is a plant pathogen divided into more than 140 pathovars on the basis of the host plants of *X. campestris* isolates (48). Commonly, the pathovars exhibit a high degree of host specificity in causing the disease, which makes *X. campestris* infections an interesting target for studies on bacterium-plant interactions and pathogenetic mechanisms of the infections. The ability of *X. campestris* isolates to cause disease is controlled by *hrp* (hypersensitive reaction and pathogenicity) genes, whose pathogenetic functions have not yet been characterized. Some of the identified *hrp* gene products of *X. campestris* exhibit sequence homology to proteins functioning in secretion of virulence factors of bacteria causing infections in animals (reviewed in reference 5), suggesting that secreted proteins play a role in pathogenesis of *X. campestris* infections. Isolates of *X. campestris* secrete plant cell wall-degrading enzymes whose function in the pathogenetic processes of *X. campestris* infections, however, has remained unclear (reviewed in reference 13).

In contrast to bacterial infections in mammals, the importance of fimbriae and bacterial adhesion to plant tissue in the pathogenetic processes of plant pathogens has remained controversial (reviewed in references 4 and 34). Fimbriae have been indicated to mediate adhesion of *Pseudomonas syringae* to bean leaves and to affect the virulence of *P. syringae* in bean (35). Recently, van Doorn et al. (47) isolated fimbriae from *X. campestris* pv. hyacinthi and showed that they bind to stomata of hyacinth leaves, suggesting a role for fimbriae in the entry of *X. campestris* pv. hyacinthi into its host plant. The N-terminal amino acid sequence of the *X. campestris* pv. hyacinthi fimbrillin shows identity to that of eubacterial type IV fimbrillins, suggesting that *X. campestris* isolates express this class of fimbrial filaments. Type IV fimbrial proteins have similarity to the extracellular protein secretory apparatus proteins. *X. campestris* expresses Xps proteins functioning in the secretion of degradative enzymes, some of which have sequence homology to N termini of type IV fimbrillins (14, 19). Type IV fimbriae are virulence factors of several human and animal pathogens and mediate bacterial adhesion to host epithelial cells (45). They also are involved in bacterial motility, the so-called twitching and social gliding motilities (45, 52), as well as in cell aggregation of human pathogens (8, 12, 17, 25, 42). As a step to analyze the role of fimbriae in *X. campestris* infections, we describe here the characterization of the *fimA* gene encoding bundle-forming fimbriae and cell aggregation of *X. campestris* pv. vesicatoria, the causal agent of spot disease of pepper and tomato plants, and show that a homologous gene exists in certain other *X. campestris* pathovars as well.

MATERIALS AND METHODS

Bacterial strains and media. The *X. campestris* pv. vesicatoria strain 3240, pv. campestris strains 528 and 1929, pv. pelargonii strains 2985 and 3119, pv. begoniae strains 2226 and 3021, pv. translucens strains 973 and 2929, and pv. graminis strains 2700 and 3041 have been described earlier (28); the strains were obtained from the National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom. Strains 75-3 and 85-10 of *X. campestris* pv. vesicatoria have been described previously (6). For fimbriae purification and microscopy, *X.*

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campestris pv. vesicatoria was cultured with shaking at 120 rpm to the early stationary phase in NYB medium (47); for counting viable cells, it was cultured on NYG agar (9) at 28°C. *Escherichia coli* DH5α (Bethesda Research Laboratories, Gaithersburg, Md.) was used as the host in all cloning experiments. *E. coli* cells were cultured in Luria broth at 37 or 28°C. Ampicillin (150 μg/ml), tetracycline (15 μg/ml), kanamycin (25 μg/ml), or spectinomycin (50 μg/ml) was added when appropriate.

Purification of proteins and amino acid sequencing. Fimbriae were isolated from strain 3240 of *X. campestris* pv. vesicatoria by use of a deoxycholate-sucrose density gradient as described by Korhonen et al. (22). The pathovar vesicatoria 3240 fimbriae behaved in the ultracentrifugation in deoxycholate buffer differently from enterobacterial fimbriae and sedimented to a density equalling approximately 48% sucrose. The fimbrial preparation was analyzed by polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate (SDS) in slab gels (15% acrylamide concentration) by the system of Laemmli (23). For N-terminal sequence analysis, the fimbriae were electrophoresed, blotted on a polyvinylidene difluoride membrane, and stained with Coomassie brilliant blue. The fimbrial polypeptide was then excised and subjected to Edman degradation in a gas-pulsed liquid-phase sequencer equipped with an on-line phenylthiohydantoin (PTH) amino acid analyzer.

DNA techniques. Standard recombinant DNA techniques were used (37). Enzymes were obtained from Promega or from New England Biolabs (Beverly, Mass.). Chromosomal DNA from *X. campestris* pv. vesicatoria 3240 was partially digested with *Sau*3A, and fragments of 25 to 30 kb were isolated and used to construct a genomic library in the wide-host-range cosmid pLAFR3 as described by Staskawicz et al. (44). The 20-mer oligonucleotide 5′-CCIATGTA(T/C)CA(A/G)GA(T/C)TA(T/C)GT-3′ complementary to the sequence 22-PMYQDYV-28 in the N-terminal amino acid sequence was used as a probe in Southern hybridization of DNA bound to Hybond-N nylon membranes (Amersham, Buckinghamshire, United Kingdom) as described in the manufacturer's instructions. Labelling of DNA fragments was performed by the random priming procedure (15) with [α-^{32}P]dCTP (Amersham), whereas oligonucleotides were end-labelled with [γ-^{32}P]dATP (Amersham). In subcloning of DNA fragments, hybridizations were also performed by the enhanced chemiluminescence 3′-oligolabelling and detection system (Amersham). The complete open reading frames (ORFs) for *fimA* and *fimB* were used to detect homologous genes in pathovars of *X. campestris*; hybridization was performed at 65°C in a buffer containing 5× SSPE (1× SSPE is 0.18 M NaCl, 0.01 M sodium phosphate, and 1 mM EDTA), 5× Denhardt's solution, and 0.5% (wt/vol) SDS, and the washes were done at 65°C in 1× SSPE containing 0.1% SDS (37). Two micrograms of digested DNA was analyzed in each Southern hybridization.

DNA sequencing on both strands of DNA fragments cloned in pBluescript (Stratagene, La Jolla, Calif.) was performed by the dideoxy chain termination method (38). The T7 sequencing kit (Pharmacia, Uppsala, Sweden) with [α-^{35}S]dATP (Amersham) and deaza-dGTP to resolve GC-rich regions was used. Subclones and a deletion series made with the Nested set deletion kit (Pharmacia) were used as templates with M13-specific, antisense, and synthetic sense oligonucleotides as primers for sequencing.

DNA and protein sequence analyses and homology searches were performed with the PC/Gene 6.7 package (IntelliGenetics Inc.) and the University of Wisconsin Genetics Computer Group 8 package (11) with programs BLAST (1), FSTNSCAN (24), and CLUSTAL (18).

Site-directed gene replacement using the omega fragment (Ω) (31) or kanamycin cassette from pUC4K (Pharmacia) was carried out as described before (6). The Ω fragment was inserted into a *Tth111*I site within *fimA*, and the kanamycin cassette was inserted into a *Pst*I site within *fimB*, both in a 2.6-kb *Sal*I fragment cloned into pLAFR3, a plasmid named pTMO7. Conjugation between *E. coli* and *Xanthomonas* was performed by a triparental mating with HB101(pRK2013) as a helper strain (16). The selected marker exchange mutants were analyzed by Southern hybridization of chromosomal DNA with the wild-type *fimA* or *fimB* and the antibiotic cassettes as probes.

Northern (RNA) blot analysis. RNA from log-phase cells was isolated as described by Ausubel et al. (3). RNA (10 μg per well) was separated in a 1% (wt/vol) agarose-formaldehyde gel (37) and transferred to a Hybond-N$^+$ nylon membrane. The probes and hybridization conditions were the same as in the Southern hybridizations described above.

Immunological and microscopic methods. Antifimbria antiserum was raised by immunization of rabbits by routine methods and used in immunoblotting essentially as adapted previously (49). For immunoelectron microscopy, bacterial cells were applied to copper grids coated with Pioloform and carbon and stained with the antifimbria antiserum (diluted 1/750 in phosphate-buffered saline [pH 7.1]) and protein A-gold particles (10-nm diameter; Janssen Biotech N.V., Olen, Belgium) as described in the manufacturer's instructions. The grids were stained with 1% (wt/vol) phosphotungstic acid adjusted with KOH to pH 6.5 and examined in an JEOL CX100 electron microscope at an operating voltage of 60 kV. For scanning electron microscopy, samples of infected tomato leaves were collected at 2, 24, and 48 h after bacteria were sprayed onto the leaves (see below). Cut leaf disks were processed for electron microscopy as detailed previously (36).

In vitro expression. For in vitro expression, *fimA* and *fimB* were amplified by PCR with a forward 5′ primer that contained an *Eco*RI restriction site and a reverse primer with a *Bam*HI restriction site after the stop codon. The oligonucleotides were 5′-GGGAATTCATGAAGAACAAAACGTTTTACG-3′ and 5′-GGGGATCCCATTAAGCACCCGGGCAACC-3′ for *fimA* and 5′-GGGAATTCATGGCGGAAAAATGGCTTT-3′ and 5′-GGGGATCCCTAACAACCAGCTGGCCGAT-3′ for *fimB*. The obtained fragments were cloned into the pTrc 99A expression vector (Pharmacia), giving plasmids pTMO5 and pTMO6, which were used in the *E. coli* S30-coupled transcription translation system (Promega) as described by Chen and Zubay (7). [^{35}S]methionine (Amersham) was incorporated into the polypeptides, and after the reactions, the polypeptides were precipitated with acetone, dried, and analyzed by SDS-PAGE. For immunoprecipitation, 10 to 20 μl of the reaction mixtures was treated with the antifimbria immunoglobulins and protein A-Sepharose (Pharmacia) as described before (33). After SDS-PAGE, the polypeptides in the precipitates were visualized by autoradiography.

Promoter fusions and assays for β-glucuronidase activity. Promoter fusions to the β-glucuronidase (*gusA*) gene were obtained after random mutagenesis of pTMO7 in *E. coli* with Tn*3-gus* (6) or cloning a fragment in front of the promoterless *gusA* gene in pLAFR6GUSB (21). For β-glucuronidase assays, bacterial cells grown in NYB medium to the exponential growth phase were harvested, and activity was measured as described previously (40).

Plant material and infections. Tomato plants (cultivar Moneymaker) were cultivated in peat moss-vermiculite at 24 ± 2°C under low humidity and with a photoperiod of 18 h. The pathogenicity of *X. campestris* pv. vesicatoria 3240 was tested by two procedures. First, young fully expanded leaves were inoculated by infiltrating a bacterial suspension (10^4 to 10^5 CFU/ml) with a plastic syringe into intercellular spaces of the leaves. The infiltration, pathogenicity assessment, and growth in planta were performed as detailed previously (6). Second, 80 ml of bacterial suspension (10^5 to 10^8 CFU/ml in 10 mM phosphate buffer [pH 7.2]) was sprayed onto the leaves. For estimation of bacterial growth in planta and for scanning electron microscopy, the infected leaves were air dried for 2 h and then washed by spraying with 200 ml of water. Plants were then enclosed in plastic bags (under conditions of >90% humidity) and cultivated for up to 4 days. The number of bacteria on tomato leaves was determined as viable counts from leaves treated in an Ultra-Turrax homogenizer (Janke & Kunkel KG, Breisgau, Germany). In the determination of bacterial numbers, 10 parallel independent samples were used. The adhesiveness of *Xanthomonas* to tomato leaves was evaluated by scanning electron microscopy as detailed previously (36).

Assessing the tolerance to UV light. The tolerance to UV light was measured as described before (50). Briefly, bacteria were harvested from NYB medium and suspended in phosphate buffer (10 mM, pH 7.2) to a concentration of 10^8 CFU/ml. Six milliliters of the suspension was placed on a plastic dish (5-cm diameter), and the dishes were placed on a shaker (100 rpm) at a distance of 20 cm beneath a UV lamp (Desaga UVIS, Heidelberg, Germany; 254-nm wavelength). Aliquots of 25 μl taken at 2-min intervals were plated on NYG plates to determine the number of viable cells.

Nucleotide sequence accession number. The nucleotide sequence described in this article will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Data Libraries under accession number Z48759.

RESULTS

Isolation and characterization of fimbriae from *X. campestris* pv. vesicatoria 3240. When cultured in liquid NYB medium, strain 3240 of *X. campestris* pv. vesicatoria produced fimbrial structures. Fimbrial filaments were purified from strain 3240 cells by deoxycholate solubilization. The resulting fimbrial preparation contained a major polypeptide, with an apparent molecular size of 15.5 kDa (Fig. 1A).

Antiserum was raised in rabbits against the isolated fimbriae of strain 3240 and used in immunoelectron microscopy of the bacteria. The immunoglobulin molecules reacted with fimbrial filaments on cells of strain 3240, and in crude extracts, the reacting filaments were mostly aggregated into 40- to 50-nm-thick bundles (Fig. 2). As a control, we also stained the cells of strain 3240 with an antiserum raised against the type IV fimbriae of *X. campestris* pv. hyacinthi (47). These immunoglobulins did not react with *X. campestris* pv. vesicatoria fimbriae (data not shown). It is noteworthy that the anti-3240-fimbria antiserum did not bind to flagellar filaments and that a fraction of the fimbria-like structures present on 3240 cells was not recognized by the anti-3240-fimbria immunoglobulins (Fig. 2). In Western blots using crude fimbrial extract as the antigen, the anti-3240 immunoglobulins reacted strongly with the 15.5-kDa protein of the strain and showed a weaker reactivity with diffuse material of larger molecular size, probably lipopolysaccharide present in the extract (Fig. 1A).

The 15.5-kDa protein was separated by SDS-PAGE, trans-

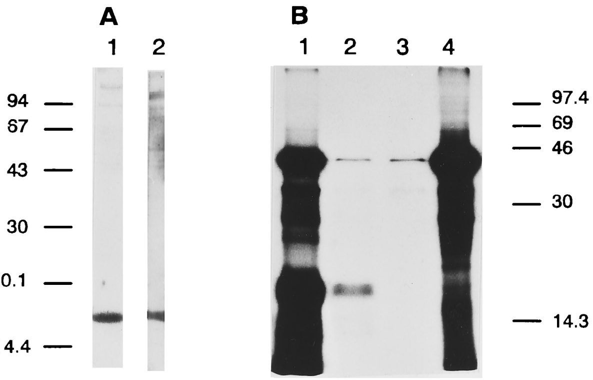

FIG. 1. Electrophoretic analyses of the *X. campestris* pv. vesicatoria 3240 fimbrial preparation (A) and the in vitro transcription and translation of *fimA* (B). (A) Lanes: 1, SDS-PAGE analysis of the purified 3240 fimbriae; 2, immunoblotting of a crude fimbrial extract with antiserum raised against the 3240 fimbrial preparation. It is important to note the intensive binding of the immunoglobulins to the FimA protein of 15.5 kDa. (B) Autoradiographic analysis of the polypeptides expressed by pTMO5 containing *fimA* in the expression vector pTrc 99A (lane 1), the immunoprecipitate obtained from the expression mixture with anti-3240-fimbria immunoglobulins (lane 2), the immunoprecipitate obtained from the expression mixture with plasmid pTrc 99A alone (lane 3), and the polypeptides expressed by pTrc 99A (lane 4). The migration distances of marker proteins (sizes in kilodaltons) are indicated on the left and the right.

ferred to a polyvinylidene difluoride membrane, and subjected to N-terminal sequence analysis. The obtained 30-residue sequence was XTLIELMIVIAIIAILAAIALPMYQDYVA, where X stands for an unidentified amino acid residue at the N terminus. The sequence is highly homologous to N-terminal sequences of type IV fimbrillins, which commonly carry *N*-methylphenylalanine as their N-terminal residue (45). The 15.5-kDa protein gave in the first sequencing cycle a residue, eluting slightly after PTH-leucine, at the suggested position of PTH-methylphenylalanine. Thus, it is probable that the 15.5-kDa polypeptide carries a *N*-methylphenylalanine at its N terminus. It was concluded that the 15.5-kDa protein was a fimbrial subunit, and the protein was named FimA.

Cloning and sequence analysis of the FimA-encoding DNA fragment. The oligonucleotide probe 5′-CCIATGTA(T/C)CA (A/G)GA(T/C)TA(T/C)GT-3′, deduced from the residues 22-PMYQDYV-28 of the N terminus of FimA, was used in Southern hybridization to locate *fimA* on a 4.4-kb *Kpn*I fragment of genomic DNA cloned into pBluescript, giving plasmid pTMO1. Sequence analysis of pTMO1 revealed an ORF with homology to the determined FimA sequence but no translational stop signals, indicating that the 3′ end of *fimA* was missing (see Fig. 3). To obtain the complete gene, a cosmid library of the *X. campestris* pv. vesicatoria 3240 genome was prepared in pLAFR3. A 607-base *Sal*I-*Kpn*I fragment from pTMO1 was used as a probe in colony hybridization to detect *fimA*-containing colonies. A colony reacting with the probe and containing a 26-kb insert in pLAFR3 was isolated and termed DH5α(pTMO3). pTMO3 was digested with *Sal*I, and a 2.6-kb DNA fragment reacting in Southern hybridization with the 607-base *fimA* probe was cloned into pBS SK, giving pTMO4. pTMO4 and its derivatives were used to obtain the nucleotide sequence of the complete *fimA* as well as of the flanking sequences shown in Fig. 3.

Both strands of the 1,463-bp DNA fragment starting at the 5′ end of the *Sal*I fragment in pTMO4 were sequenced. Two ORFs, one from nucleotides 258 to 665 and the other from nucleotides 857 to 1261, were identified (Fig. 3). The ORF1 encodes a polypeptide of 136 amino acid residues with a calculated molecular weight of 14,302. Residues 8 through 37 in the deduced N-terminal amino acid sequence of ORF1 perfectly match the determined N terminus of FimA, and since the calculated molecular weight is reasonably close to the one determined for FimA by SDS-PAGE analysis (Fig. 1A), ORF1 was concluded to represent the *fimA* gene. Residue 7 of the predicted amino acid sequence of *fimA* is phenylalanine, which in a methylated form, in analogy to other type IV fimbrillins (45), most likely was the anomalous N-terminal residue of the mature FimA peptide. The *fimA* gene thus encodes a leader sequence of 6 amino acid residues. The sequence contains a putative ribosome-binding site (41), AGGGGA, 7 bases upstream of the translational initiation codon ATG, and a sequence closely similar to the consensus sequence for RpoN (σ^{54})-dependent promoters (2) at nucleotides 151 to 165 was found. A potential transcriptional terminator hairpin loop (ΔG, −9.6 kcal) is present between nucleotides 772 and 792 (Fig. 3).

ORF2 (nucleotides 857 to 1261) predicts a polypeptide product of 135 amino acids with a molecular weight of 13,854. The deduced amino acid sequence of the gene product showed similarity to sequences of type IV fimbrillins (see below) but differed at five residues from the N-terminal sequence determined from the isolated FimA protein (Fig. 3). At the nucleotide level, the sequences of *fimA* and *fimB* are 58% identical. Due to the high homology of the predicted amino acid sequence with that of type IV prefimbrillins (see below), this ORF was named *fimB*. A weak potential ribosome-binding site, AGGGCG, is located 4 nucleotides upstream of the initiation codon ATG of *fimB*. A palindromic sequence capable of forming a transcriptional terminator loop structure (ΔG, −8.6 kcal) was identified close to the 5′ end of the *fimB* ORF (Fig. 3).

Homologs of FimA and FimB. The deduced amino acid sequences of FimA and FimB were used to conduct a search of the sequences in the GenBank database. The FimA and the FimB sequences showed highest homology to the type IV prefimbrillin sequences of *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Moraxella bovis* (10, 20, 26) as well as of a number of other type IV fimbria producers. Figure 4 shows that over their entire lengths, FimA and FimB of *X. campestris* pv. vesicatoria and the PilA sequences of *P. putida* and *P. aeruginosa* are 22% identical. The homology is highest in the 30 N-terminal amino acid residues. The predicted sequences of FimA and FimB are 49% identical to each other. As is typical for a number of type IV fimbrillins (reviewed in reference 32), the N termini of FimA and FimB also exhibited homology to the N termini of proteins active in eubacterial protein secretion, such as the Xps proteins of *X. campestris* pv. campestris (14, 19). The N terminus of FimA contains the consensus cleavage site for the *P. aeruginosa* type IV prefimbrillin peptidase PilD (45), GFTLIE. The corresponding sequence in FimB is GFSLIE (Fig. 4). Both predicted sequences also contain a 6-residue sequence very similar to the leader sequences found in *Pseudomonas* type IV prefimbrillin, and the leader sequence is followed by a stretch of 20 amino acids typical for type IV fimbrillins (Fig. 4).

In vitro expression of *fimA*. To confirm that *fimA* encodes the fimbrillin subunit, we tested the reactivity of the in vitro-synthesized FimA with the immunoglobulins raised against the isolated fimbriae. *fimA* and *fimB* were amplified by PCR and cloned into the IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible expression vector pTrc 99A, giving plasmids pTMO5 and pTMO6, respectively. Peptides encoded by pTMO5 and pTMO6 were expressed by use of the *E. coli* S30-coupled transcription translation system. Autoradiographic analyses

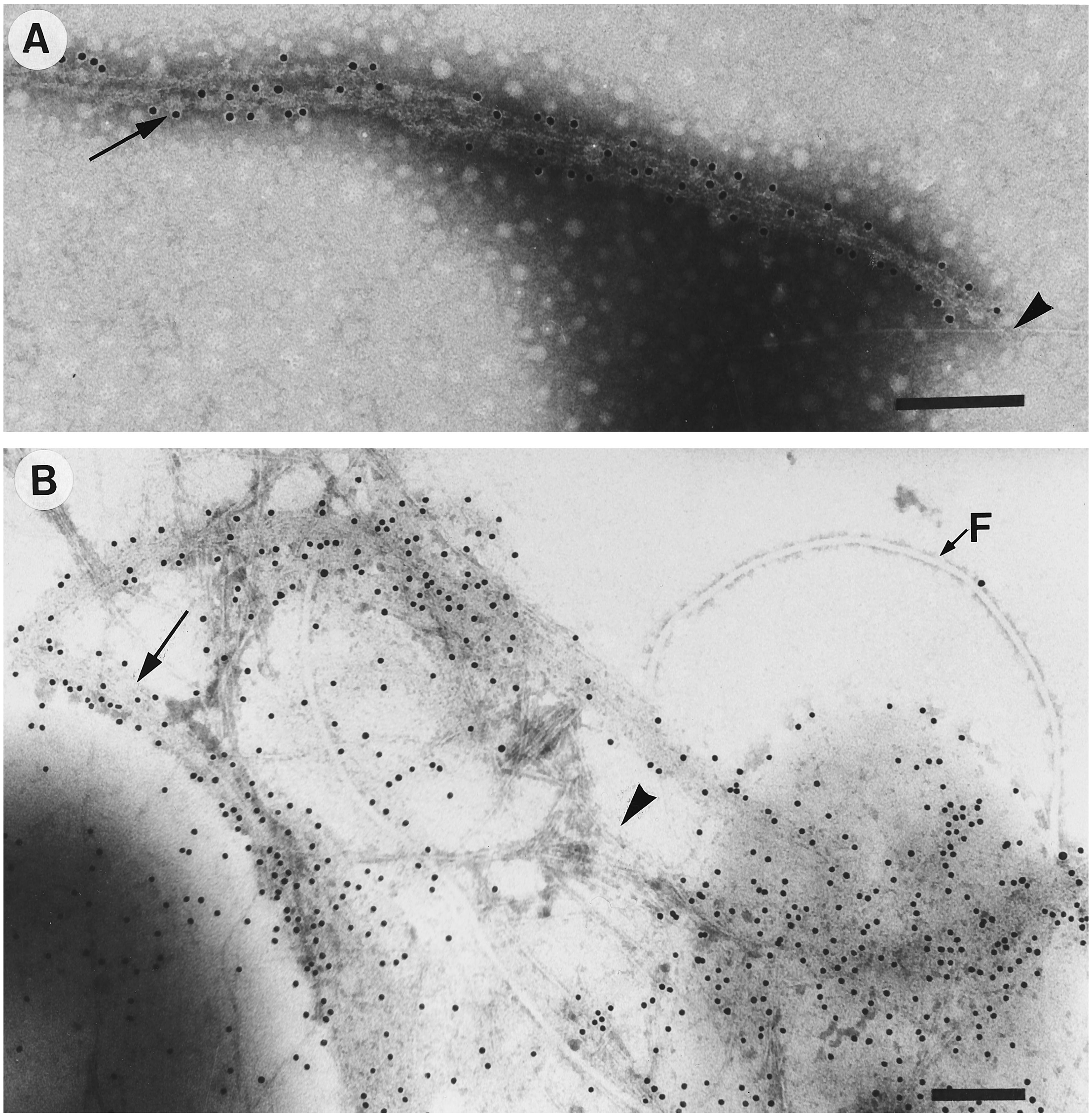

FIG. 2. Immunoelectron microscopy of bacterial samples treated with diluted anti-3240-fimbria serum and then with protein A-gold particles. (A) Reactivity with the immunoglobulins of fimbrial bundles (arrow) detached from cells; (B) reactivity of *X. campestris* pv. vesicatoria 3240 cells with the antiserum, showing the intense staining of fimbrial bundles (arrow) and the lack of reactivity of flagella. Portions of the fimbria-like structures in panels A and B were not reactive with the antiserum (arrowheads). Bars, 200 nm. F, flagellum.

showed that pTMO5 expressed a ca. 16.5-kDa peptide that was precipitated by the anti-3240-fimbria antibodies and not expressed by the vector pTrc 99A alone (Fig. 1B). The expressed FimA contained the leader sequence, which probably explains its high apparent molecular weight. No expression of *fimB* from pTMO6 was detected (data not shown). Various conditions for expression of peptides from pTMO6 were tested; these included different concentrations of template DNA (1 to 2 μg per reaction) and of inducer (0 to 2 mM IPTG) as well as different incubation temperatures (30 to 37°C). We also confirmed by nucleotide sequencing of the *fimB* insert and its flanking sequences in pTMO6 that *fimB* was correctly inserted in pTrc 99A and that the coding sequence did not contain any polymerase-induced changes.

Expression of *fimA* and *fimB* in *Xanthomonas*. The expression of *fimA* and *fimB* was studied by Northern blotting the total RNA from strain 3240. The main transcript shown in Fig. 5, lane 1, is approximately 600 nucleotides long and close to the predicted size of a transcript starting after the putative promoter at nucleotides 151 to 165 and extending to the putative termination loops (at nucleotides 772 to 792 and 893 to 910). *fimB* did not hybridize with this transcript (Fig. 5, lane 3). Both *fimA* and *fimB* gave a weaker signal with two minor transcripts about 1.5 and 2.5 kb in size (Fig. 5, lanes 2 and 3); these transcripts were visible only in overexposed autoradiograms. We constructed a site-specific mutant by introducing an Ω cassette into the *Tth111*I site of *fimA* (Fig. 3). The resulting mutant strain, designated 3240 *fimA*::Ω, did not produce FimA as assessed by immunoblotting and immunoelectron microscopy of whole cells with anti-3240-fimbria antibodies (details not shown). The total RNA of 3240 *fimA*::Ω was analyzed by Northern blotting with *fimA* and *fimB* as probes. *fimA* reacted with a transcript 300 bases in size; this size is to be expected for a transcript terminated at the Ω DNA. Neither of the two

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      SalI
     GTCGACTGCAGGGTGCCCCTAAGCGTCACCTTTTGACTCTGCTGGGCCGATCAAGCGTTGTGTCGCAGTTCG

73   GCAATTGAGGTGTTCCCTCCCTCAAAAGATCCGGCATCATTGTCTCAGCGGGCATCTGCAGGGTCGGTGGGG

145  AGAAGTTGGCACGTATCCTGCTTCGATTCATTTGCACGCGGCATTGCAACGCGGCGCCCAACGGGAGTGGCC

                                   rbs
217  CGCGGGCAAAGTAATTACACTTAACTCTAGGGGAAACCTTGATGAAGAAGCAAAACGGTTTTACGCTGATCG
                                               M  K  K  Q  N  G  F  T  L  I
                                                               △
                                                                       ●
289  AACTGATGATCGTGATTGCAATCATCGCAATTCTGGCTGCTATTGCACTGCCGATGTATCAAGACTATGTCG
     E  L  M  I  V  I  A  I  I  A  I  L  A  A  I  A  L  P  M  Y  Q  D  Y  V
                                                                      ◀
361  CCAAGTCGCAGGTCACCGCCGGCCTCGCTGAGATCAATCCTGGCAAGACGCAGTACGAAGTGGCACTTAACG
     A  K  S  Q  V  T  A  G  L  A  E  I  N  P  G  K  T  Q  Y  E  V  A  L  N

433  AAGGCAAAACCACCGTTGCCGACATCACCGAGTTGGGTCTGAAGTCTCCCTCTGAGCGCTGCACCATTGCGC
     E  G  K  T  T  V  A  D  I  T  E  L  G  L  K  S  P  S  E  R  C  T  I  A

505  CGATCACCGCTCTGAGCGCTACTGGCACCATCGAGTGTACTCTGAAGGGCAATACGCAGGTTGTGGGCAAGA
     P  I  T  A  L  S  A  T  G  T  I  E  C  T  L  K  G  N  T  Q  V  V  G  K

                                KpnI
577  AGGTGACATTGACGCGTGCAAATGATGGTACCTGGACCTGCAAGACGGATGCGCTGAAGAAGTACGCGCCTG
     K  V  T  L  T  R  A  N  D  G  T  W  T  C  K  T  D  A  L  K  K  Y  A  P

649  CCGGTTGCCCGGGTGCTTAATGTTTCCTAGCTGAAGAAGGAAGGCCTCGCTACAAGCGAGCTTCTGTTTTGA
     A  G  C  P  G  A

721  TGGGCTTTGATATTCATTGTGCTTGTACAGGTTTGATTGATTTTAAGGCCCATCACCTGGTCAAGAGGTGAT

                                                           rbs
793  TTTTCATTGTTTCAGCTACTCGCCGTCCGCTGTTTTGGCGAGCTGTTTACCGACAGGGCGCGATATGGCGCG
                                                                      M  A  R

865  GAAAAATGGCTTTTCTCTTATTGAGTTAATGATTGTTATAGCAATCATTGCCGTGTTGGCCGCGATAGCACT
       K  N  G  F  S  L  I  E  L  M  I  V  I  A  I  I  A  V  L  A  A  I  A  L

937  GCCTGTATATCAAGGTGCGGTTGCTAAAGCGCAATTGACAGCAGCTTTAGCTGAGCTGCGTCCTGGGAAGAC
       P  V  Y  Q  G  A  V  A  K  A  Q  L  T  A  A  L  A  E  L  R  P  G  K  T

                     ○
1009 CACTATTGAGGCTGCAGTCCAAGACGGTACAAATCCCTCGGTGATCGATGCGCCCTATATTGGATTATTGTC
       T  I  E  A  A  V  Q  D  G  T  N  P  S  V  I  D  A  P  Y  I  G  L  L  S

1081 TTCAACGCGATGTGCTCGCGTAAGTGCAGTCCTAAGCTCTACAGGTGTTGCTGAAATAAGCTGTACTTTGCA
       S  T  R  C  A  R  V  S  A  V  L  S  S  T  G  V  A  E  I  S  C  T  L  Q

1153 GGGTTCTGCATTAGTTTCAGGTATGGATCTTAAATTGAGGCGATCGGCCGACGGTGGTTGGATTTGTGATGG
       G  S  A  L  V  S  G  M  D  L  K  L  R  R  S  A  D  G  G  W  I  C  D  G
                                                               ▶
1225 CTCCGCTTTTGATGCAAAGTATCGGCCAGCTGGTTGTTAGAGTTAATGTCGTGTTTAAAGAGTGCGAGCGAC
       S  A  F  D  A  K  Y  R  P  A  G  C

1297 AGGGTGCGTGAAAATACTGGGGGCTGAAGTATGAGTGCTGTTTCGTCCGCTTTAAGTTGTTGGTATTACCGG

1369 AATCGCGCGTCGTTAGTACAAGATGGTGCGCTTGAAGAGACTGTAGCTGCCTCTGCGATGGATCAGGCAGCA

1441 GCGGCTAAGGTTCCATTACCTCA
```

FIG. 3. Nucleotide sequence of the 1,463-bp fragment from the *Sal*I insert in pTMO4 and predicted amino acid sequences of FimA and FimB. The *fimA* coding region starts at nucleotide 258 and ends at nucleotide 665. △, cleavage site for prefimbrillin peptidase; rbs, consensus sequence for a ribosome-binding site. A consensus RpoN-dependent promoter sequence is indicated by boldface type between nucleotides 151 and 165. Between nucleotides 772 and 792, a palindromic sequence with potential for a terminator hairpin loop is indicated by underlining. The *fimB* coding region starts at nucleotide 857 and ends at nucleotide 1261. Within the coding sequence, a palindromic potential terminator loop is underlined between nucleotides 892 and 913. The *Sal*I and *Kpn*I restriction sites used in cloning the genes are indicated, the *Tth111*I site used to insert the omega (Ω) cassette is indicated by the symbol ●, and the *Pst*I site used to insert the kanamycin cassette is indicated by the symbol ○. The symbols ◀ and ▶ indicate the Tn*3-gus* insertions and their orientations.

minor transcripts were detected. The total RNA from 3240 cells was also analyzed in dot blots, and the signals given by the *fimA* and the *fimB* probes were quantitated by counting the radioactivity hybridized onto the blots. The signal by *fimA* was over 10 times higher than that obtained by *fimB* (5,050 versus 420 cpm).

The expression levels of *fimA* and *fimB* in *Xanthomonas* were also compared by measuring the β-glucuronidase activity of *gus* fusions in both genes. *fimA* was fused into promoterless *gusA* by cloning the 4.4-kb *Kpn*I fragment from pTMO1 into pLAFRGUSB to obtain the plasmid pTMO33. In this construct, *gusA* is fused into the *Kpn*I site at nucleotide 608 (see

```
FimA      MKKQNGFTLIELMIVIAIIAILAAIALPMYQDYVAKSQVTAGLAE  45
FimB      MARKNGFSLIELMIVIAIIAVLAAIALPVYQGAVAKAQLTAALAE  45
PspPilA   MKGQRGITLIELMIVVAIIGILATIAIPMYTNHQSRTKAAAGLLE  45
PsaPilA   MKAQKGFTLIELMIVVAIIGILAAIAIPQYQNYVARSEGASALAS  45
          *  ..*..*******.***..**.**.* *  .  .... ...* .

FimA      INPGKTQYEVALN---------EG-KTTVADITELGLKSPSERCT-  80
FimB      LRPGKTTIEAAVQ---------DGTNPSVIDAPYIGLLSSST-RCAR  81
PspPilA   ISALKTAMDLRLN---------EGKD--VADVGALGGQPATAHCAI  80
PsaPilA   VNPLKTTVEEALSRGWSVKSGTGTEDATKKEVPLGVAADANKLGT  90
          ... **  .  .         .* .  . .   .*  . . . .

FimA      IA-PITALSATGTIE--CTL-KGNTQVVGKKVTLTR-ANDGTWTC  120
FimB      VS-AVLSSTGVAEIS--CTL-QGSALVSGMDLKLRR-SADGGWIC  121
PspPilA   TA-SGNAAAGTGSIV--CTLVDAPATVVGKALTLTR-SATG-WGC  120
PsaPilA   IALKPDPADGTADITLTFTMGGAGPKNKGKIITLTRTAADGLWKC  135
          ..    . .....*    *.  . .   *. ..* * ...* * *

FimA      KTDALK-KYAPAGCPGA   136
FimB      DGSAFDAKYRPAGC---   135
PspPilA   TTN-IEEDLAPSGCKGA   136
PsaPilA   TSDQ-DEQFIPKGCS-R   150
          ...  . .  * **
```

FIG. 4. Sequence alignment of FimA and FimB with PilA of *P. putida* (PspPilA) (10) and PilA of *P. aeruginosa* (PsaPilA) (20). An asterisk indicates identical amino acid residues; a period indicates similar amino acid residues.

Fig. 3). *fimB* fusion was obtained by a Tn*3-gus* insertion into pTMO7 16 bp downstream of the *fimB* stop codon, giving plasmid pTMO32 (Fig. 3). The β-glucuronidase activity of strain 3240(pTMO33) was 1×10^{-5} U/CFU, and the activity of the strain 3240(pTMO32) varied between 2×10^{-7} and 1×10^{-6} U/CFU in different experiments. A control strain containing the pTMO7 with Tn*3-gus* inserted in the *fimA* in the opposite 3′-5′ direction did not show detectable *gus* activity (Fig. 3).

Presence of *fimA* and *fimB* homologs in pathovars of *X. campestris*. We have earlier shown that surface antigens, lipopolysaccharides and outer membrane proteins, are conserved within *X. campestris* pv. graminis, begoniae, and pelargonii isolates but variable within *X. campestris* pv. campestris, translucens, and vesicatoria (28). We tested by hybridization the presence of *fimA*- and *fimB*-related DNA in 13 strains representing these six pathovars of *X. campestris* (Fig. 6). Under the conditions used, *fimA* did not hybridize to *fimB* and vice versa (Fig. 6A). *fimA* hybridized very weakly with total DNA from the two *X. campestris* pv. pelargonii strains but more strongly with DNA from the other pathovar strains (Fig. 6B). *fimB* hybridized only with DNA from the homologous *X. campestris* pv. vesicatoria strain 3240. We performed Southern hybridization of genomic DNAs digested with *Sal*I, *Kpn*I, or *Sac*II-*Sma*I with *fimA* as the probe; the results are shown for *Sal*I-digested genomic DNAs in Fig. 6C. The Southern hybridizations confirmed the presence of *fimA*-like DNA in pathovars translucens, begoniae, campestris, vesicatoria, and graminis, whereas only a poor signal was obtained with the two pathovar pelargonii isolates. In each hybridization, the signal was clearly strongest with DNA fragments from the homologous *X. campestris* pv. vesicatoria 3240 DNA. In the three hybridizations with DNA digested with different restriction enzymes, the isolates within the pathovar begoniae and those within the pathovar campestris carried the *fimA* homolog in fragments of similar sizes, whereas the three pathovar vesicatoria isolates each exhibited reactive fragments of different sizes. The pathovar vesicatoria genomes also differed from the others in exhibiting two reacting fragments.

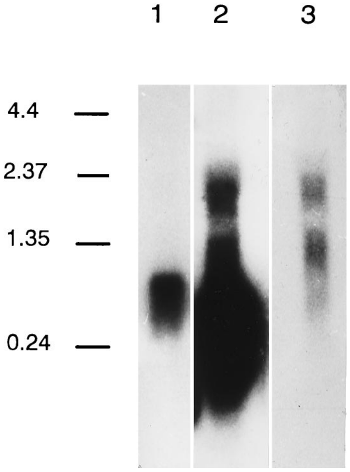

FIG. 5. Northern hybridization analysis of the total RNA from *X. campestris* pv. vesicatoria 3240 with *fimA* (with a short [lane 1] or long [lane 2] exposure time) or *fimB* (with a long exposure time; lane 3) as a probe. RNA size markers (in kilobases) are indicated on the left.

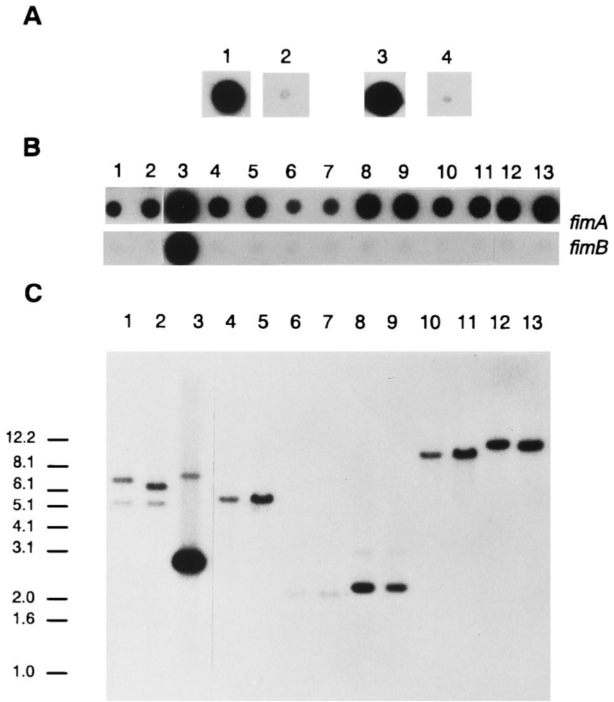

FIG. 6. DNA hybridizations with the coding region of *fimA* or *fimB* as a probe. (A) The lack of hybridization between *fimA* and *fimB* is shown. The target DNA was *fimA* (panels 1 and 4) and *fimB* (panels 2 and 3); the probe used was *fimA* (panels 1 and 2) or *fimB* (panels 3 and 4). (B) Reactivity in dot blot hybridization of *fimA* and *fimB* with total DNA from strains representing six pathovars of *X. campestris*. The strains represented are *X. campestris* pv. vesicatoria 75-3 (lane 1), 85-10 (lane 2), and 3240 (lane 3); *X. campestris* pv. campestris 528 (lane 4) and 1929 (lane 5); *X. campestris* pv. pelargonii 2985 (lane 6) and 3119 (lane 7); *X. campestris* pv. begoniae 2226 (lane 8) and 3021 (lane 9), *X. campestris* pv. translucens 973 (lane 10) and 2920 (lane 11); and *X. campestris* pv. graminis 2700 (lane 12) and 3041 (lane 13). It is important to note that *fimB* reacted only with the homologous pathovar vesicatoria strain 3240 and *fimA* reacted poorly with the two pathovar pelargonii DNAs. (C) Southern hybridization of the *Sal*I-digested target DNAs with *fimA* as a probe. The genomic DNAs in lanes 1 to 13 are the same as those described for panel B. The migration distances of standard DNA fragments (in kilobases) are indicated on the left.

Phenotype of the *fimA* mutant. Strains 3240 and 3240 *fimA*::Ω were compared for their infectiveness and pathogenicity on tomato plants under humid conditions by two procedures: infiltration or spraying of bacterial suspensions onto the tomato leaf surface. We did not observe any significant differ-

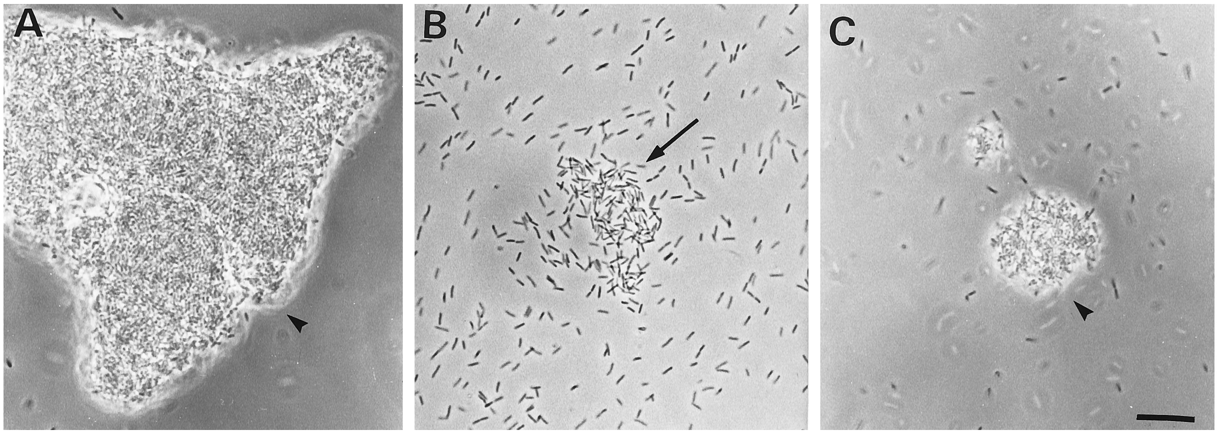

FIG. 7. Light microscopy of bacterial cultures. (A) *X. campestris* pv. vesicatoria 3240; (B) *X. campestris* pv. vesicatoria 3240 *fimA*::Ω; (C) *X. campestris* pv. vesicatoria 3240 *fimA*::Ω(pTMO3). Arrowheads (A and C) indicate the compact bacterial aggregates; an arrow (B) indicates the loose aggregates occasionally observed in cultures of the *fimA* mutant strain. Bar, 10 μm.

ence in the numbers of the two strains when measuring their initial colonization ability as viable counts from leaves washed at 30, 60, or 180 min after the infection (data not shown). Furthermore, no significant difference in bacterial multiplication (from 2 to 48 h after the infection) on tomato leaves was detected between the strains (data not shown). Neither were there any significant differences between the strains in the onset of disease symptoms on tomato leaves (data not shown).

However, a striking difference between the laboratory cultures of the strains was observed. The cells in cultures of the wild-type strain 3240 were strongly aggregated, whereas those of the strain 3240 *fimA*::Ω remained in suspension. Under the microscope, large and compact cell aggregates in cultures of strain 3240 were observed (Fig. 7A), whereas the cells of strain 3240 *fimA*::Ω remained individual. Occasionally, loose aggregates of 3240 *fimA*::Ω cells that were distinct phenotypically from those made by the strain 3240 were observed (Fig. 7B). Complementation of the *fimA*::Ω mutation with the plasmid pTMO3 or pTMO32 restored the formation of the compact cell aggregates (Fig. 7C). Unlike 3240 *fimA*::Ω, the complemented strain also reacted with anti-3240-fimbria antibodies in immunoblotting and immunoelectron microscopy of whole cells (details not shown). A *fimB* mutant constructed by inserting a kanamycin cassette into the *Pst*I site of *fimB* (Fig. 3) formed compact cell aggregates similar to those formed by the wild-type strain (data not shown). The *fimB* mutant formed fimbriae that reacted with the antiserum raised against the purified 3240 fimbriae (data not shown).

Adhesion to and colonization of tomato leaves by the wild-type and the *fimA* mutant strain were also assessed by scanning electron microscopy of leaves collected 30 min as well as 24 and 48 h after infection. We observed two differences between strain 3240 and its *fimA* mutant: 3240 colonized leaf surfaces in cell aggregates (Fig. 8A), whereas the *fimA* mutant strain did not (Fig. 9). Furthermore, strain 3240 adhered and efficiently colonized trichomes of tomato leaves (Fig. 8B), a phenomenon not observed with strain 3240 *fimA*::Ω (Fig. 9) during the 48-h tests. Both strains adhered and colonized other leaf surfaces with similar efficiencies.

Tolerance of bacteria to UV light. It has been proposed that aggregation of plant-associated bacteria affects their sensitivity to environmental factors such as drying or UV light (51). We therefore assessed the survival of the 3240 derivatives after exposure to UV light (Fig. 10). The *fimA*::Ω mutant exhibited an increased sensitivity to UV light. The complementation of the mutation by pTMO3 restored the tolerance to UV light close to the wild-type level.

DISCUSSION

The importance of fimbriae for bacterial pathogens infecting plants has remained controversial, in part due to the lack of well-characterized bacterial adhesins. In this communication, we report the characterization of a novel gene, *fimA*, encoding bundle-forming fimbriae and cell aggregation of the plant pathogen *X. campestris* pv. vesicatoria that infects pepper and tomato plants. We also show that a related gene is present in *X. campestris* isolates belonging to other pathovars and infecting other host plants. The predicted FimA and FimB proteins of *X. campestris* pv. vesicatoria exhibit several structural characteristics considered typical for type IV fimbrillins (reviewed in references 30 and 45). These involve their size of 136 (FimA) or 135 (FimB) amino acid residues and the presence of the basic leader sequence MKKQNG or MARKNG followed by a sequence [GF(T/S)LIE] homologous to the sequence recognized by PilD prefimbrillin leader peptidase as well as the high similarity of FimA and FimB primary structure to those of the previously described type IV fimbrillins. The *fimA* gene product here characterized is a determinant of cell aggregation and differs serologically and functionally from the recently described fimbriae of *X. campestris* pv. hyacinthi (47).

The *fimA* and *fimB* genes of *X. campestris* pv. vesicatoria 3240 appear to be complete and transcribed in the same direction. The genes are separated by 192 nucleotides. *fimA* is preceded by a sequence matching the consensus sequence for RpoN-dependent promoters (2). To date, only a few promoter sequences of *X. campestris* have been characterized, and it remains to be established whether such an activator-regulated promoter indeed functions in *fimA* transcription. It is likely that the hairpin loop structure with transcriptional terminator potential identified 104 nucleotides downstream of the *fimA* stop codon will significantly reduce expression of *fimB*. Indeed, the major *fimA* transcript detected in a Northern blot of strain 3240 RNA corresponded to a transcript of *fimA* only. Another putative terminator loop was identified within the *fimB* coding region close to the 5′ end. In contrast to *fimA*, no in vitro expression of *fimB* was detected when it was cloned under the control of *trc* promoter of *E. coli* in pTrc 99A. This gives

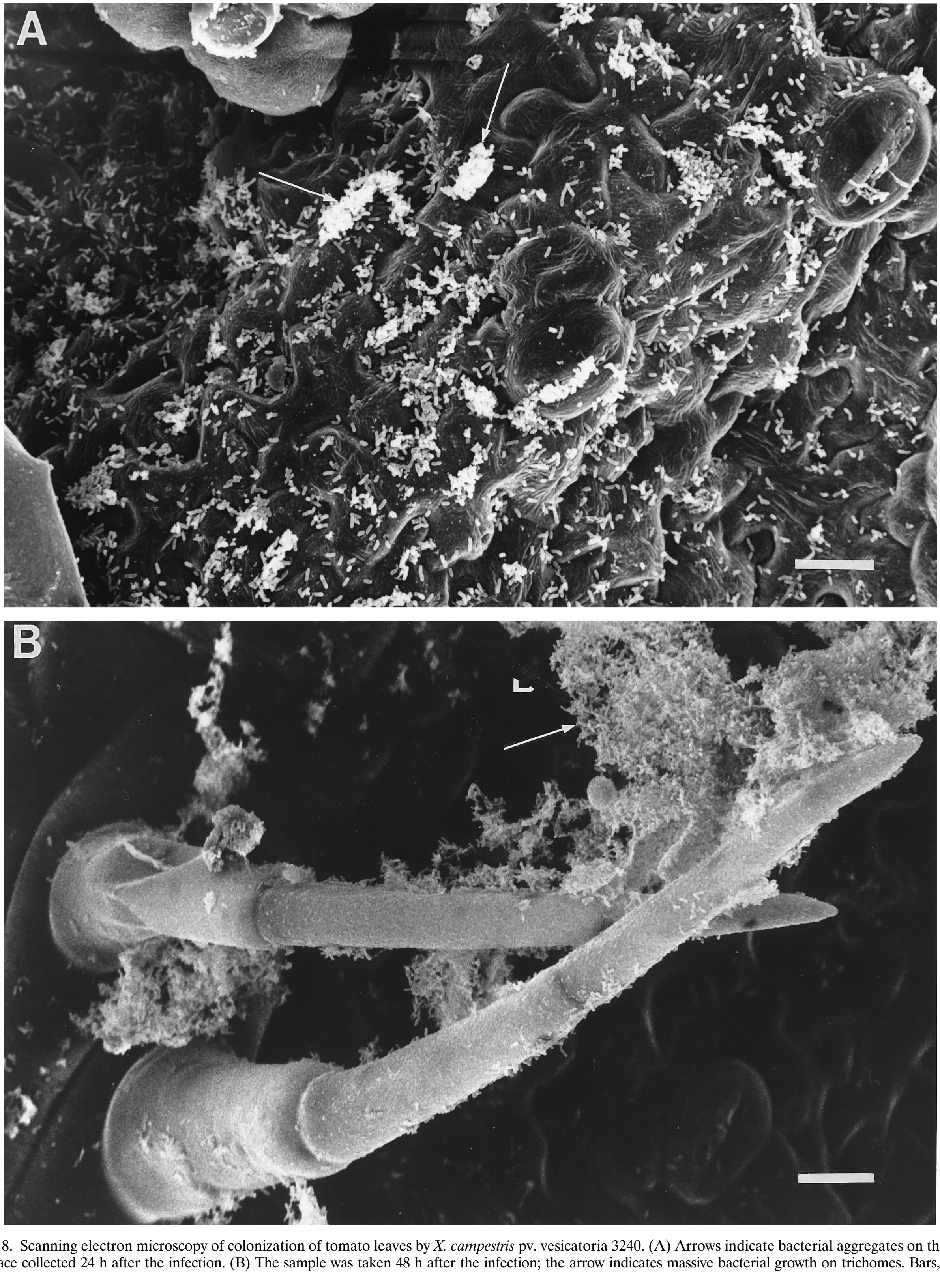

FIG. 8. Scanning electron microscopy of colonization of tomato leaves by *X. campestris* pv. vesicatoria 3240. (A) Arrows indicate bacterial aggregates on the tomato leaf surface collected 24 h after the infection. (B) The sample was taken 48 h after the infection; the arrow indicates massive bacterial growth on trichomes. Bars, 10 μm.

evidence that the hairpin loop has the capacity to function as a terminator structure. In addition, the amino acid sequencing of the purified fimbrillin from *X. campestris* pv. vesicatoria cells did not reveal any FimB-specific residues among those of the determined FimA sequence. In *Xanthomonas*, however, *fimB* was transcribed but at a low level compared to that of the expression of *fimA*. Comparison of the expression levels of *fimA* and *fimB* by using *gus* fusions gave a 10- to 50-fold

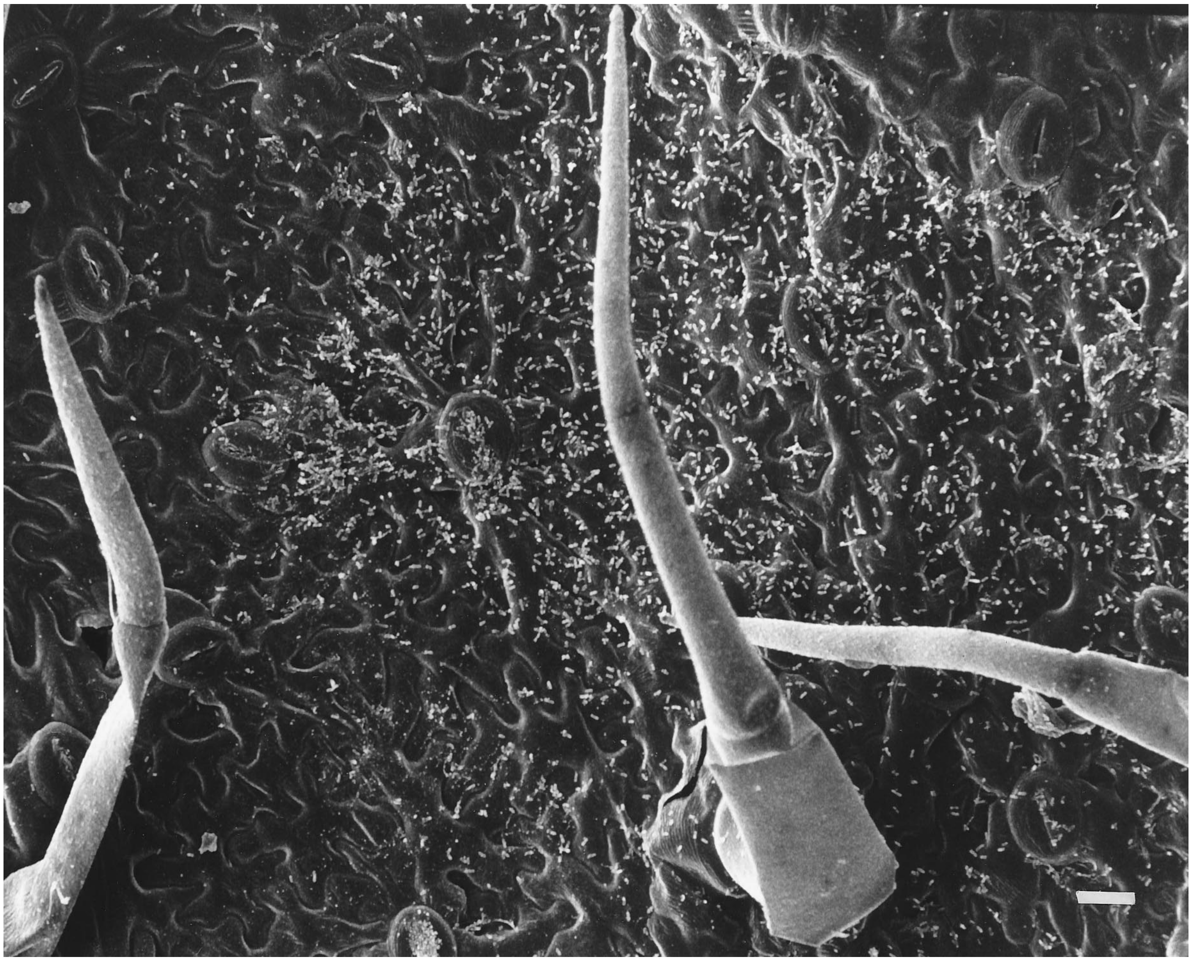

FIG. 9. Scanning electron microscopic analysis of the colonization of tomato leaf surface by *X. campestris* pv. vesicatoria 3240 *fimA*::Ω. It is important to note the even growth of bacteria on the leaf surface and the poor colonization of trichomes. The leaf sample was processed for microscopy 24 h after infection. Bar, 10 μm.

difference in their transcription activity. The day-to-day variability of the observed expression level of *fimB*::*gusA* probably resulted from cell aggregation in the suspensions used to measure the β-glucuronidase activity. The sizes of the two minor transcripts reacting with both the *fimB* and *fimA* probes are close to 1.5 and 2.5 kb. These transcripts were not detected in the *fimA*::Ω mutant. We detected only a single putative promoter site upstream of *fimA*, and our results suggest that the three transcripts are transcribed from the same promoter. This means that the long transcripts are terminated 0.5 to 1 kb downstream of the *fimB* stop codon. The nucleotide sequence obtained from one DNA strand revealed no additional *fim* homologs or potential termination loops 0.5 kb upstream or 0.5 kb downstream of the sequence shown in Fig. 3 (data not shown). We detected, upstream of *fimA*, a partial ORF encoding a product homologous to PilC which is needed for the biogenesis of type IV fimbriae in *P. aeruginosa* (27).

By using the complete *fimA* and *fimB* ORFs as probes in Southern and dot blot hybridizations, we detected a *fimA* homolog in *X. campestris* isolates, whereas *fimB* was present only in the homologous pathovar vesicatoria strain 3240. These hybridizations were performed under conditions where *fimA* and *fimB* did not hybridize with each other; *fimA* and *fimB* are 58% identical at the nucleotide level and show 55 to 61% identity with a number of genes encoding type IV prefimbrillins of other bacteria. It therefore seems that the DNA fragments hybridizing with *fimA* represent fimbria-encoding DNA that is more homologous to *fimA* of *X. campestris* pv. vesicatoria than to the previously described type IV fimbrillin genes from other bacteria, including the closely related genus *Pseudomonas*. Hybridization analysis with *fimA* as a probe also revealed that the DNA regions surrounding *fimA* are conserved in pathovars begoniae and campestris and variable in pathovar vesicatoria. This is compatible with the findings showing that isolates within the pathovar begoniae and those within the pathovar campestris share conserved phenotypic characteristics, whereas isolates within the pathovar vesicatoria exhibit variability (43, 48).

The Southern hybridizations with *fimA* as a probe revealed the presence of two hybridizing DNA fragments in the three pathovar vesicatoria isolates. This suggests that these strains possess at least two fimbrial variants. The expression of multiple fimbriae in *X. campestris* pv. vesicatoria 3240 is further suggested by our finding that a subset of fimbria-like structures that were observed by electron microscopy showed no reactivity with antibodies raised against the 3240 fimbriae. These structures were also produced by the 3240 *fimA*::Ω, indicating that they do not represent FimA or FimB (29).

Timmer et al. (46) described that *X. campestris* pv. vesicatoria colonizes tomato leaf surfaces in bacterial aggregates. Our

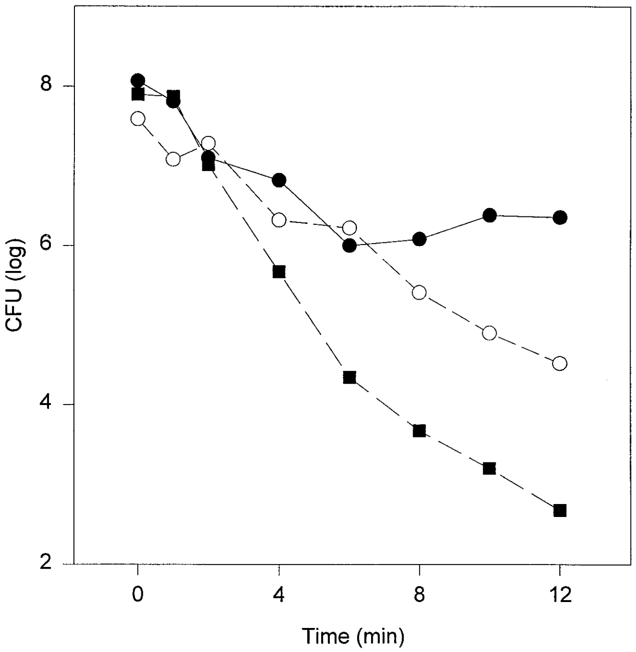

FIG. 10. Survival rate of *X. campestris* pv. vesicatoria 3240 (●), 3240 *fimA*::Ω (■), and 3240 *fimA*::Ω(pTMO3) (○) after in vitro exposure to UV light.

demonstration that *X. campestris* pv. vesicatoria 3240 forms compact aggregates, or microcolonies, on tomato leaf surfaces is in agreement with those findings. Furthermore, our results indicate that FimA of *X. campestris* pv. vesicatoria 3240 is involved in determining the cell-to-cell aggregation. We observed that the *fimA* mutant strain failed to form the compact aggregates that were formed efficiently by strain 3240 in vitro and in planta. The adhesion and the initial colonization of tomato leaves by strain 3240 were not significantly affected by the *fimA* mutation, suggesting that FimA is not a primary determinant of the initial colonization on tomato leaf surface. We observed, however, that in contrast to the wild-type strain 3240, the *fimA* mutant poorly colonized the trichomes of tomato leaves, suggesting that adhesiveness to these plant structures involves FimA. In two laboratory assays, i.e., involving infiltration of bacteria into or spraying of bacteria onto tomato leaves, we did not observe any effect by the *fimA* mutation on the pathogenicity of strain 3240. The lack of detectable virulence differences between 3240 and 3240 *fimA*::Ω suggests that such an adhesiveness to trichomes has no significant effect on the infectiveness of *X. campestris* under the conditions we used. It is interesting to note that *P. syringae* pv. tomato forms resident populations in the basals of the trichomes (39) and that the adhesiveness of *X. campestris* pv. vesicatoria to trichomes can have a function in the natural environment.

Wilson and Lindow (51) have provided evidence that high cell densities and aggregation of *P. syringae* on bean leaves increase the bacterial survival rate under field conditions. These authors concluded that agglomeration, or aggregation, of bacterial cells on the plant leaf surface is a mechanism to protect epiphytic bacteria against environmental stress, i.e., factors such as UV light or desiccation. We demonstrate here that the *fimA*-dependent cell aggregation indeed protects *X. campestris* pv. vesicatoria 3240 against UV light. Our results suggest a pathogenetic function for the *fimA*-encoded bundle-forming fimbriae of *X. campestris* pv. vesicatoria in creation of bacterial microcolonies on the leaf surface and in thus increasing epiphytic survival and stress tolerance of *Xanthomonas* under field conditions. Type IV fimbriae of certain human pathogens have been associated with cell aggregation and ability to form adherent colonies on the surface of the target epithelial cells, a phenomenon called localized adherence (17, 25, 42). The autoagglutination caused by a member of type IV family fimbriae, the toxin-coregulated pilus of *Vibrio cholera*, has been correlated with serum resistance and colonization ability (8). These results offer an intriguing example of how plant and human pathogenic bacteria may use similar surface structures to increase their colonization potential in different environments.

ACKNOWLEDGMENTS

This study was supported by the Finnish Cultural Foundation, the Academy of Finland, and the University of Helsinki.

We thank Maarit Lönnroth, Raili Lameranta, and Helmi Savolainen for technical assistance, Kristian Aspegren for the use of the fluorometry, and the Department of Electron Microscopy, University of Helsinki, for permission to use their electron microscopic facilities.

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