Site-Directed Disruption
of the *fimA* and *fimF* Fimbrial Genes of *Xylella fastidiosa*

Helene Feil, William S. Feil, John C. Detter, Alexander H. Purcell, and Steven E. Lindow

First and fifth authors: Department of Plant and Microbial Biology, University of California, Berkeley 94720; second and fourth authors: Department of Environmental Science, Policy, and Management, University of California, Berkeley 94729; and third author: Department of Energy, Joint Genome Institute, Walnut Creek, CA 94598.
Accepted for publication 10 January 2003.

ABSTRACT

Feil, H., Feil, W. S., Detter, J. C., Purcell, A. H., and Lindow, S. E. 2003. Site-directed disruption of the *fimA* and *fimF* fimbrial genes of *Xylella fastidiosa*. Phytopathology 93:675-682.

Xylella fastidiosa causes Pierce's disease, a serious disease of grape, citrus variegated chlorosis, almond and oleander leaf scorches, and many other similar diseases. Although the complete genome sequences of several strains of this organism are now available, the function of most genes in this organism, especially those conferring virulence, is lacking. Attachment of *X. fastidiosa* to xylem vessels and insect vectors may be required for virulence and transmission; therefore, we disrupted *fimA* and *fimF*, genes encoding the major fimbrial protein FimA and a homolog of the fimbrial adhesin MrkD, to determine their role in the attachment process. Disruption of the *fimA* and *fimF* genes in Temecula1 and STL grape strains of *X. fastidiosa* was obtained by homologous recombination using plasmids pFAK and pFFK, respectively. These vectors contained a kanamycin resistance gene cloned into either the *fimA* or *fimF* genes of *X. fastidiosa* grape strains Temecula1 or STL. Efficiency of transformation was sufficiently high (≈600 transformants per µg of pFFK DNA) to enable selection of rare recombination events. Polymerase chain reaction and Southern blot analyses of the mutants indicated that a double cross-over event had occurred exclusively within the *fimA* and *fimF* genes, replacing the chromosomal gene with the disrupted gene and abolishing production of the corresponding proteins, FimA or FimF. Scanning electron microscopy revealed that fimbriae size and number, cell aggregation, and cell size were reduced for the FimA⁻ or FimF⁻ mutants of *X. fastidiosa* when compared with the parental strain. FimA⁻ or FimF⁻ mutants of *X. fastidiosa* remained pathogenic to grapevines, with bacterial populations slightly reduced compared with those of the wild-type *X. fastidiosa* cells. These mutants maintained their resistance to kanamycin in planta for at least 6 months in the greenhouse.

Xylella fastidiosa is a gram-negative bacterium which causes serious diseases of plants, such as Pierce's disease (PD) of grape (*Vitis vinifera* L.), citrus variegated chlorosis (CVC), and almond leaf scorch; it colonizes many other plant hosts as well as insect vectors (28). *X. fastidiosa* colonizes exclusively the xylem vessels of plants and, in species like grape, it multiplies rapidly, blocking the xylem, which results in the water stress symptoms characteristic of PD (8).

A striking feature of *X. fastidiosa* is its polar attachment to xylem cells via the production of fimbriae (3,8,20,29,30). Fimbriae are hair-like appendages that extend from the bacterial surface (36) and can serve as an anchor to substrata as well as a means of cell–cell aggregation. Mechanisms for fimbrial assembly of several gram-negative bacteria are diverse, typified by the P and type I pili systems of *Escherichia coli* (6,36).

Fimbriae- and pili-mediated attachment of bacteria to host tissues is important for bacterial colonization and pathogenicity (19). Virulence and adhesion of *Pseudomonas syringae* to leaves were facilitated by the presence of fimbriae (32). Similarly, fimbriae aided the adhesion of *X. campestris* pv. *hyacinthi* to plants and enhanced entry of the pathogen into bean leaves (39). The role of fimbriae in the virulence of *X. fastidiosa* to plants such as grape could be complex. Colonization of plants by *X. fastidiosa* probably involves both attached and mobile phases in the population of pathogen cells. Movement of the pathogen within the plant after inoculation by sucking insects such as sharpshooters (*Cicadelli-*

dae: subfamily *Cicadellinae*) likely requires that it degrade pit membranes to allow passage between xylem vessels. Fimbriae production might allow bacteria to attach to xylem vessels and for cell aggregates to form after attachment to such a surface. Local production of plant cell wall-degrading enzymes then could remain concentrated to the site of production rather than become diluted by the xylem sap. In contrast, movement along xylem vessels and between cells could be inhibited if adhesion was too strong or irreversible, keeping the bacterial cells tightly bound to one location. Thus, a careful examination of cells of *X. fastidiosa* altered in fimbriae production should provide insight into the colonization process and events that lead to symptom development.

In the P, type I, and type III pili systems of *E. coli* and of *Klebsiella pneumoniae*, the fimbrial genes are clustered in operons (11,23). Within the operons, the genes have similar placement and share the same functions in the fimbriae assembly (11,23). The fimbrial operon of *X. fastidiosa* is homologous to the type I fimbrial operon of *E. coli* (4). The first gene of the *X. fastidiosa* fimbrial operon is homologous to the *fimA* gene of the type I system of *E. coli* or to the *papA* gene of P pili of *E. coli* (4). This operon contains several genes responsible for the assembly of fimbriae and genes, such as *fimA*, which are responsible for the production of proteins called fimbrins. These fimbrins make up the core of the fimbrial rod and the fibrillar tip of the fimbriae. The last three genes of the *X. fastidiosa* fimbrial operon have homology to the *mrkD* gene of the type III fimbriae of *K. pneumoniae* (4). The MrkD gene product was named for mannose-resistant *Klebsiella*, as described by Gerlach et al. (11), and has been shown to mediate binding of *K. pneumoniae* to eukaryotic cells (34,37,38). Among the mrkD-homologs, we arbitrarily chose the last gene of the *X. fastidiosa* fimbrial operon, *fimF*, for disruption. We hypothesized

that both FimA and FimF are important in the adhesion of *X. fastidiosa* to xylem vessels and that the virulence to grape of FimA⁻ and FimF⁻ mutants of *X. fastidiosa* would be affected.

Efficient gene replacement strategies as well as the availability of genome sequences for several *X. fastidiosa* strains will facilitate analysis of virulence determinants in this pathogen. Genome sequences of the *X. fastidiosa* citrus strain 9a5c (35), almond strain Dixon, oleander strain Ann1 (Joint Genome Institute, Walnut Creek, CA), and grape strain Temecula1 (Onsena, Unicamp, Brazil) now are available. Two annotations of the *X. fastidiosa* citrus strain 9a5c genome (4,35) now are completed. In addition, the annotations of the gapped sequences of *X. fastidiosa* almond Dixon and oleander Ann1 strains have been completed by Integrated Genomics (4).

Transformation of the *X. fastidiosa* citrus strain with *oriC* plasmids has been demonstrated (27) and random transposon mutagenesis of *X. fastidiosa* has been described (13). Recently, site-directed gene disruption of an *X. fastidiosa* citrus strain using a modified Ori vector was described (7). One drawback of this approach to obtain gene replacements was the need to subculture *X. fastidiosa* for many generations in the laboratory, conditions which have been shown to reduce the virulence of the pathogen (17). In this report, we described a site-directed gene disruption method, which does not require subculturing of *X. fastidiosa*. We describe two narrow host range replicons for *X. fastidiosa* derived from pUC18 and demonstrate their utility in disrupting the *fimA* and *fimF* genes of *X. fastidiosa* grape strain Temecula1.

MATERIALS AND METHODS

Bacterial strains. Electrocompetent *E. coli* DH10B (Invitrogen, Carlsbad, CA) cells were used for electroporation and propagation of plasmids. *X. fastidiosa* grape strains Temecula 1 (ATCC 700964) and STL (ATCC 700963), isolated from a diseased grapevine (*Vitis vinifera*) in Temecula and in Napa, CA, respectively, were used in all experiments.

Map of the genomic region encompassing the *fimA* of *X. fastidiosa*. The names of the genes of the *X. fastidiosa* fimbrial operon were derived from the names of their conserved homologs of the fimbrial operons of *E. coli*. The first three genes are conserved and were named *fimA*, *ecpD*, and *fimC*, like the first three genes of the type I fimbrial operon of *E. coli*. The last three genes of the *X. fastidiosa* fimbrial operon share homology to the *mrkD* gene of the type III fimbrial operon of *K. pneumoniae*; however, because these genes were not named in the annotation of the citrus, al-

mond, or oleander sequences, we chose to name them *fimD*, *fimE*, and *fimF*. The sequence of the *X. fastidiosa* grape Temecula1 strain was used to determine the positions of *fimA* and *fimF* relative to neighboring genes. The annotation of the 11.4-kb region of the grape strain Temecula1 of *X. fastidiosa* depicted in Figure 1 was determined by comparing the annotated sequences of citrus, almond, and oleander strains of *X. fastidiosa* of Integrated Genomics (Integrated Genomics website) to the *X. fastidiosa* Temecula1 sequence (Onsona, Unicamp website). The identification numbers of the *fimA* and *fimF* homologs to the *X. fastidiosa* Temecula1 grape strain genes chosen for the study and located in the genomes of the *X. fastidiosa* citrus strain 9a5c, almond strain Dixon, and oleander strain Ann1 are presented in Table 1.

Polymerase chain reaction. The list of plasmids and primers used in the study are presented in Table 2. The primers for the genes used in the study were designed prior to the availability of the *X. fastidiosa* grape strain sequence and were based on the conserved regions between the citrus (35), almond, and oleander *X. fastidiosa* strains. Polymerase chain reactions (PCRs) were conducted using Taq DNA polymerase (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), except for isolation of *kan*, which was amplified with High Fidelity DNA polymerase (Roche Biodiagnostics, Indianapolis, IN), according to the manufacturer's instructions (Amersham Pharmacia Biotech, Inc.) in a thermal cycler (GeneAmp PCR System 9700; Perkin-Elmer Applied Biosystems, Foster City, CA). λ DNA/*Hin*dIII number 2 and φX174 DNA/*Bsu*RI (*Hae*III) (MBI, Fermentas, Hanover, MD) were used as molecular size standards in gel electrophoresis.

Vector construction. A 1,197-bp fragment containing a portion of *fimA* and the 5′ flanking region was obtained by PCR amplification of genomic DNA from *X. fastidiosa* Temecula1 or STL with primer pairs FA1 and FA2 (Table 2). This PCR product was digested with *Sac*I and ligated into the dephosphorylated, *Sac*I-linearized pUC18 to yield pFA or pSA. The EZ:TN (KAN-2) transposon was inserted into pUC19/3.4 according to the manufacturer's instructions (Epicentre, Madison, WI) to generate pEZ19. A 965-bp fragment composing the *kan* gene along with its 5′ and 3′ flanking regions was amplified from pUCEZ:TNK with primer pairs KAN1 and KAN2 (Table 2). This fragment was ligated into the dephosphorylated, *Mfe*I-linearized pFA or pSA to yield pFAK or pSAK, respectively.

To determine the transformation efficiency as a function of target gene size, primers internal to the *fimA* PCR product were designed. A 1,520-bp fragment containing *fimA* with the *kan* gene inserted at the *Mfe*I restriction site was obtained by PCR ampli-
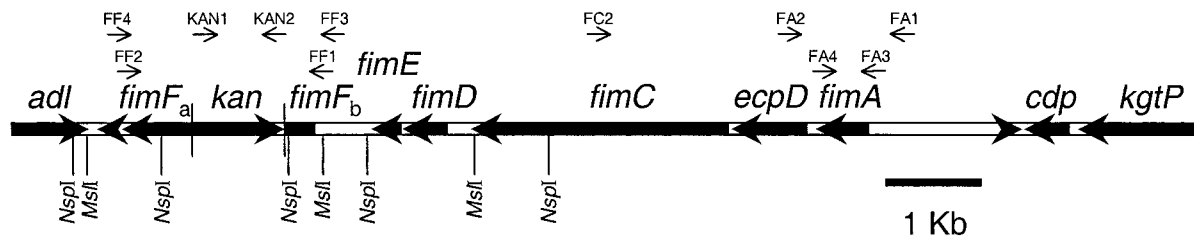

Fig. 1. Fimbrial operon within a portion of the genome of the *Xylella fastidiosa* grape strain Temecula1, including the location of the *kan* gene inserted into the *fimF* gene in the FimF⁻ mutant strain. The fimbrial operon starts with *fimA* and finishes with *fimF*. *fimF*ₐ and *fimF*ᵦ are the two fragments of the *fimF* gene that were separated by the *kan* gene insertion. *adl*, 4-amino-4-deoxychorismate lyase; *cdp*, competence-damage protein; *kgtP*, α-ketoglutarate permease. Thick arrows indicate the orientation of transcription. Small arrows indicate position and orientation of primers. Restriction sites used for the Southern blot analysis are marked below the disrupted region of the operon.

TABLE 1. Identification numbers for the *fimA* and *fimF* genes in the sequences of the citrus, almond, and oleander strains of *Xylella fastidiosa*

Gene name	Citrus strain 9a5c	Almond strain Dixon	Oleander strain Ann1
fimA	RXFA00083[a]	RXFX00292	RXFY00112
fimF	RXFA00077	RXFX00812	RXFY00292

[a] Based on the annotation from Integrated Genomics, Chicago.

fication of pFAK with primer pairs FA3 and FA4 (Table 2). This product was digested with *Sac*I and ligated into dephosphorylated, *Sac*I-linearized pUC18 to yield pFA2.

A vector similar to pFAK in which *fimF* was replaced by *fimA* was constructed. The *fimF* gene from the chromosome of *X. fastidiosa* Temecula1 or STL was amplified using primers FF1 and FF2 (Table 2). A 1,097-bp fragment containing a portion of *fimF* and sequences flanking the 5′ region of *fimF* was obtained by PCR amplification of genomic DNA from *X. fastidiosa* Temecula1 or STL with primer pairs FF1 and FF2 (Table 2). The *fimF* PCR product was digested with *Sac*I and ligated into dephosphorylated, *Sac*I-linearized pUC18 to yield pFF or pSF. The 965-bp fragment composing the *kan* gene described above was digested with *Mfe*I and ligated into the dephosphorylated, *Mfe*I-linearized pFF to yield pFFK (Fig. 2) or pSFK.

Electroporation. Cells of *X. fastidiosa* grape strains Temecula1 or STL grown on periwinkle wilt medium with gelrite (PWG) solid medium (16) for 7 days at 28°C were resuspended in 1 ml of PW broth and made electrocompetent as described by Ausubel et al. (2). To determine the optimal cell concentration needed for successful gene replacement, the cell suspension was serially diluted and aliquots plated on PWG to determine the cell concentrations (Table 3). An aliquot of 50 µl of each cell suspension was mixed with 5 µl of pFAK, pFA2, pFFK, pSAK, or pSFK (≈1.25 µg of DNA) and placed in a 0.1-cm cuvette. The electroporation parameters were 1.6 kV, 200 Ω, and 25 µF using a BioRad GenePulser II (BioRad, Hercules, CA). The time constant was approximately 4.5 milliseconds. Electroporated cells were resuspended in 1 ml of PW broth and incubated at 28°C for 24 h without agitation. The cells then were plated on PWG supplemented with kanamycin at 50 µg/ml (PWG-kan) and grown at 28°C for 8 days. Twenty-four of these kanamycin-resistant colonies were selected and restreaked three times on PWG-kan.

Sequence analysis of cloned genes. Aliquots (1 µl) of template DNA at 100 ng/µl were cycle sequenced with vector-specific primers PU1 and PU2 and gene-specific primers KAN1, KAN2, FA1, FA2, FF1, and FF2 (Table 2). The reactions contained 1 µl of template DNA, 4 pmoles primer, 5 µl of dH$_2$O, and 4 µl of DYEnamic ET terminator sequencing kit (Amersham Biosciences).

Cycle sequencing conditions were 30 rounds of 95°C for 25 s, 50°C for 10 s, 60°C for 2 min, hold at 4°C. The reaction products then were ethanol precipitated or purified by a magnetic bead protocol as described. Sequences were obtained on a MegaBACE 1000 (Amersham Biosciences) capillary DNA sequencer. The sequence of the *kan* gene in pFAK and in pFFK was compared to the sequence provided by Epicentre and was found to be identical. The sequences of the *fimA* and *fimF* portions included in pFAK and pFFK respectively, also were the same as the sequence of their respective region of *X. fastidiosa* version 4 sequence given by the Organization for Nucleotide Sequencing and Analysis in Brazil. The rest of the sequence of pFAK and of pFFK was identical to that of pUC18.

Confirmation of gene replacement by PCR. We arbitrarily selected two putative kanamycin-resistant FimA⁻ mutants of *X. fastidiosa* to characterize the genetic change that had occurred. We amplified the entire *fimA* gene and flanking *fimC* regions using primers FA1 and FC2 (Table 1) from the DNA of the FimA⁻ mutant of *X. fastidiosa* to determine if that region of the chromosome contained the *kan* gene used to disrupt *fimA* in vitro. We also used primers pairs FA1 and FA2 or KAN1 and KAN2 (Table 2) to determine the presence of *fimA* and *kan*, respectively, in putative mutant and wild-type cells. To determine the orientation of the *kan* gene within *fimA*, we used a combination of *fimA*- and *kan*-specific primers in PCR amplification.

A similar PCR-based strategy was used to confirm that putative FimF⁻ mutants were the result of a double crossover event. To amplify the entire *fimF* gene and flanking regions from the DNA of two FimF⁻ mutants of *X. fastidiosa*, we used primers FF3 and FF4 (Table 2). To determine the orientation of the *kan* gene within *fimF*, we used a combination of *fimF*- and *kan*-specific primers in PCR amplification. pUC18-specific primers were used in PCR to determine if pFAK or pFFK remained in the FimA⁻ or FimF⁻ mutants. Similar PCR were conducted on the FimA⁻ and FimF⁻ mutants of the *X. fastidiosa* grape strain STL.

Southern blot. The presence of a *kan* insertion in *fimF* in the FimF⁻ *X. fastidiosa* mutants was confirmed by Southern blotting with digoxygenin-labeled *fimF* as a probe as per the manufacturer of the kit (Roche Molecular Biochemicals). Total genomic DNA

TABLE 2. Plasmids and primers used in the study

Plasmids, primers	Relevant characteristics or primer sequence	Source or reference
Plasmids		
pUC18	pMB1 derivative, *rep* (pMB1), *bla* (Ap^R), *lacZ*	Roche
pUC19/3.4	pUC19 containing a 3.4-kb *Hpa*II fragment	Epicentre
pEZ19	pUC19/3.4 containing EZ:TN™ <KAN-2> transposon insertion	This work
pFA	pUC18 containing 1,197-bp *fimA* fragment of Temecula1	This work
pSA	pUC18 containing 1,197-bp *fimA* fragment of STL	This work
pFAK	pFA containing 965-bp fragment with *kan* gene	This work
pSAK	pSA containing 965-bp fragment with *kan* gene	This work
pFA2	pUC18 containing 1,520-bp *fimA/kan* fragment of pFAK	This work
pFF	pUC18 containing 1,097-bp *fimF* fragment of Temecula1	This work
pSF	pUC18 containing 1,097-bp *fimF* fragment of STL	This work
pFFK	pFF containing 965-bp fragment with *kan* gene	This work
pSFK	pSF containing 965-bp fragment with *kan* gene	This work
Primers		
PUC1	5′-CTTTACACTTTATGCTTCCGG-3′	…
PUC2	5′-GCAAGGCGATTAAGTTGG-3′	…
KAN1	5′-CTAGCAATTGCTGTCTCTTATACACATCTCAACCA-3′	…
KAN2	5′-TTGCCAATTGAACCAATTCTGATTAGAAAAACTCA-3′	…
FA1	5′-GTCTGAGCTCCAACATCAAGTCGACCAAGTG-3′	…
FA2	5′-TATGGAGCTCCGACTCGACTGATAGGAGGC-3′	…
FA3	5′-AGAGGGATCCCATGAAAATCATGAAAAATTTCGTACTCT-3′	…
FA4	5′-TGACGGATCCTTATTGGTAGATGATGGTGTACTGAATG-3′	…
FC1	5′-GGAAGCTGTCACTTGCATGA-3′	…
FC2	5′-ATGCACCAACTGGGGTAGAG-3′	…
FF1	5′-GTCTGAGCTCCAACTTTGGAAACCAGATGTCG-3′	…
FF2	5′-TATGGAGCTCACTGGTATTCAATCGTGAAGGTT-3′	…
FF3	5′-TCATCCAGATGGCATAACCA-3′	…
FF4	5′-TTTGCTTGGCGATTTTCTTT-3′	…

from wild-type and FimF⁻ *X. fastidiosa* strains, digested with either *Nsp*I or *Msl*I (New England Biolabs), and DNA of pFFK were used as target DNA on these blots. The absence of plasmid DNA sequences in the FimF⁻ *X. fastidiosa* mutants was confirmed by Southern blotting with digoxygenin-labeled pUC18 as probe.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. To determine the expression of fimbrial genes in wild-type *X. fastidiosa* Temecula1 and FimA⁻ and FimF⁻ mutants, extracellular proteins were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Bacteria grown on a modified PWG (bovine serum albumen was replaced with 0.5% fructose) for 7 days at 28°C were resuspended in 200 μl of double distilled water by gently pipetting for 5 min. The suspensions then were centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant of each cell suspension was mixed with 8× protein sample buffer according to the protocol outlined by Bollag and Edelstein (5). After denaturation, the proteins were separated on a 4 to 20% ready mix SDS-PAGE gradient gel (Bio-Rad), and then stained with Coomassie brilliant blue. A band with a mobility corresponding to approximately 15 kDa present in wild-type but not in the FimA⁻ mutant then was eluted, and digested using standard protocols described by Bollag and Edelstein (5). The digested protein was subjected to sequence analysis using a Voyager Biospectrometry Workstation (Perspective Biosystems, Framingham, MA) according to the method described by Hack and Benner (15). No band was analyzed for the FimF⁻ mutant.

Test for plasmid linearization. We observed 100% double crossover events using our mutagenesis method; therefore, we tested the hypothesis that linearization of pFFK occurred upon entry into *X. fastidiosa* cells. To test whether *X. fastidiosa* grape strain Temecula1 had a restriction enzyme that would linearize pFFK, we incubated pFFK with the supernatant of *X. fastidiosa* Temecula1 cell lysate under conditions typical for restriction digestion (2).

Scanning electron microscopy. We have examined *X. fastidiosa* Temecula1 wild-type, FimA⁻, or FimF⁻ cells grown on PW liquid broth. Suspensions of cells were suspended, lightly centrifuged, and washed in Na-Cacodylate buffer. The cells were again centrifuged, suspended in 2% glutaraldehyde in Na-Cacodylate buffer, and filtered through 0.45-μm silver filters (Millipore, Bedford, MA). Buffer, osmium tetroxide, buffer, and then ethanol (35, 50, 70, 95, and 100%) rinses of the cells were done by passing the solutions through the filter. After fixation, the filters were then critical point dried and coated with a 2-nm film of platinum stabilized by a second coating of 12 nm of carbon in a Balzers 301 freeze fracture unit (Balzers, Balzers, Lieschtenstein). Samples were viewed with a Hitachi S5000 SEM (Hitachi, Japan) at the Electron Microscopy Laboratory, University of California, Berkeley.

Pathogenicity tests. Grapevines of the cv. Cabernet sauvignon, which is moderately susceptible to PD (12), were rooted in the greenhouse from cuttings provided by the Foundation Plant Material Service, University of California, Davis. These grapevines were needle inoculated with FimA⁻, FimF⁻ mutants, or with wild-type *X. fastidiosa* Temecula1 in the leaf petiole according to the method of Hopkins and Adlerz (18). The inoculated grapevines were placed in a greenhouse and visualized for symptoms weekly. Infection of vines by *X. fastidiosa* was confirmed by reculturing of the pathogen from symptomatic vines by the method of Hill and Purcell (16). After 2 months, three infected petioles per infected vine per inoculation with either FimA⁻, FimF⁻ mutants, or wild-type cells of *X. fastidiosa* Temecula1 grape strain were tested for number of bacterial cells per gram of petiole tissue by the method of Hill and Purcell (16).

RESULTS

Transformation of *X. fastidiosa* with pFAK, pFFK, pSAK, and pFAK. The chromosomal *fimF* gene of *X. fastidiosa* grape strain Temecula1 was disrupted after introduction of plasmid pFFK harboring *fimF* containing an insertion of the *kan* gene. Gene replacement apparently occurred by a double crossover event mechanism (Fig. 2). Because this strategy involved use of a nonreplicating vector, high transformation efficiencies were needed to observe recombinational rescue of marker genes. Maximum electroporation efficiencies (≈600 transformants/μg of pFFK DNA) were observed when 3.0×10^8 to 1.5×10^9 cells in a 50-μl suspension were treated with 1 μg of plasmid DNA (pFFK) (Table 2). Electroporation efficiency decreased when using either higher or lower cell concentrations (Table 3). Similar recombination efficiencies were found when a disrupted *fimA* fragment was substituted for *fimF* (data not shown). Efficiency of recovery of putative FimA⁻ mutants was reduced by ≈10-fold when the DNA region containing the *kan*-disrupted *fimA* gene in pFA2 was reduced by approximately 50%. Apparent gene replacement using a similar strategy with cloned *fimA* and *fimF* homologs of *X. fastidiosa* grape strain STL occurred at a frequency similar to that in strain Temecula1. The efficiency of recovery of FimA⁻ and FimF⁻ mutants of strain STL was ≈10-fold lower when the *fimA* or *fimF* gene from Temecula1 (pFAK and pFFK, respectively) were used compared with the genes from strain STL itself (pSAK and pSFK, respectively).

Confirmation of gene replacement by PCR. The amplified fragment containing the *fimA/fimC* region using primers FA1 and FC2 from the parental strain was approximately 3.0 kb in size, while that for the putative marker exchange mutant was 4.0 kb, consistent with the presence of an inserted *kan* gene within *fimA*. The PCR fragment containing *fimA* obtained using primers FA1 and FA2 was approximately 1.0 kb in the wild-type strain but 2.0 kb in the transformants. Similarly, primers FA3 and FA4, internal to *fimA*, yielded a fragment of approximately 500 bp in
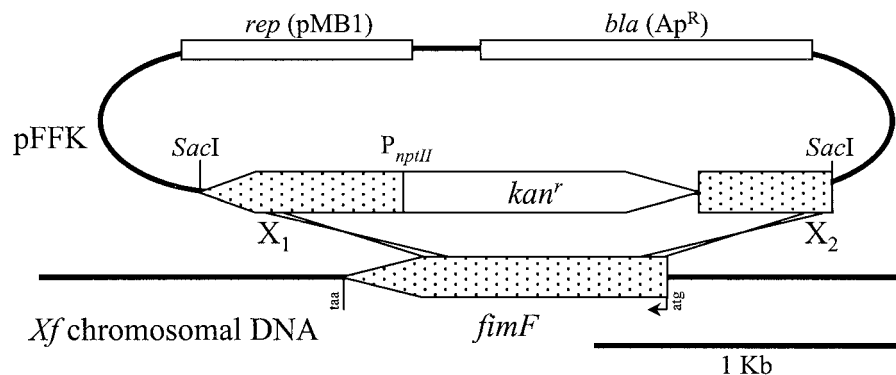

Fig. 2. Schematic representation of the double crossover event between the plasmid-borne disrupted *fimF* gene on pFFK and the *Xylella fastidiosa* chromosomal *fimF* gene. *kan*, kanamycin resistance gene; P*nptII*, *kan* promoter; *bla* (Ap^R), ampicillin resistance gene; *rep* (pMB1), origin of replication of pMB1; *Sac*I, restriction site. The textured rectangle represents the *fimF* sequences.

the wild-type strain but 1.5 kb in the transformant, consistent with the presence of an inserted *kan* gene within the internal portions of *fimA* in the mutants. The *kan* gene could be recovered by PCR amplification using the *kan* primers only from the putative FimA⁻ mutants.

Similarly, the amplified fragment containing *fimF* using primers FF3 and FF4 was approximately 1.2 kb in the parental Temecula1 strain but 2.2 kb in the two putative FimF⁻ mutants of *X. fastidiosa*. This difference in size is consistent with the presence of an inserted 1.0-kb *kan* gene within *fimF* in the mutants. The amplified fragment containing the *fimF* gene using primers FF1 and FF2 in the wild-type *X. fastidiosa* Temecula1 yielded a fragment of approximately 1.0-kb, and a fragment of approximately 2.0-kb in the FimF⁻ mutants. The *kan* gene could be recovered by PCR amplification only from the putative FimF⁻ mutants. PCR analysis revealed that the *kan* gene is oriented in the opposite orientation from that of the transcription of *fimA* or *fimF*, suggesting that the mutations would be polar. We could not amplify a 3.0-kb fragment corresponding to pUC18 amplified using primers PUC1 and PUC2 from either parental strain or FimA⁻ or FimF⁻ mutants, indicating that the plasmid vector had been evicted from the mutants, presumably as a result of a double crossover event. Similar PCR on the FimA⁻ or FimF⁻ mutants of *X. fastidiosa* grape strain STL indicated that the mutation occurred via a double crossover event.

Confirmation of transformation by Southern blots. To confirm the insertion of the *kan* gene into the *fimF* gene of putative FimF⁻ mutants, Southern blot hybridizations were performed using digoxygenin-labeled *fimF* gene as the probe (Fig. 3). Diges-

tion of total genomic DNA from FimF⁻ *X. fastidiosa* cells with *Msl*I yielded a 2.46-kb fragment with homology to *fimF*, which is consistent with the insertion of the *kan* gene into *fimF* (Fig. 3). Based on sequence alignment, there is a 308-bp portion of the *fimF* gene with 80% homology to *fimD*, which can explain the hybridization of the *fimD* gene to the digoxygenin-labeled *fimF* probe. This may explain the 1.58-kb *Msl*I fragment in the parental strain, which includes both the *fimD* and *fimE* genes with homology to *fimF*, while a 1.50-kb fragment corresponds to the *fimF* gene itself (Fig. 3). Similar results using Southern blotting were obtained when we used the restriction enzyme *Nsp*I to digest the DNA of wild-type or mutant strains (data not shown). The absence of pFFK DNA in both the parental and FimF⁻ mutants of *X. fastidiosa* was confirmed by the lack of hybridization of total genomic DNA from parental and transformed *X. fastidiosa* cells with digoxygenin-labeled pUC18 DNA (data not shown).

Analysis of fimbrial gene expression using SDS-PAGE. To determine if disruption of *fimA* and *fimF* resulted in altered fimbriae production, extracellular proteins, which should include fimbrial components, were recovered from cells grown on agar media and subjected to SDS-PAGE. A 15-kDa protein, observed in cultures of both parental and FimF⁻ mutant strains (Fig. 4), was identified as FimA by protein sequencing. This band was greatly reduced in cultures of the FimA⁻ mutant of *X. fastidiosa* (Fig. 4). Although there is some protein present corresponding to the FimA protein band in the FimA⁻ mutant of *X. fastidiosa*, this probably was due to residual proteins other than FimA but of the same size. A protein with a mass of approximately 14 kDa was present in both parental and FimA⁻ mutant strains but was missing in the FimF⁻ mutant strain. The abundance of FimA was much greater than that of the putative FimF (Fig. 4).

Scanning electron microscopy. Wild-type cells of *X. fastidiosa* Temecula1 had an extensive network of fimbriae serving to attach bacterial cells to the surface of the silver filter and to each other (Fig. 5A). These cells also formed large aggregates and most cells had fimbriae measuring approximately 3 nm in diameter (because the carbon/platinum coat was 17 nm thick) and ranging in length

TABLE 3. Efficiency of recovery of FimF⁻ mutants of *Xylella fastidiosa* strain Temecula1 that acquired a kanamycin resistance gene after electroporation of different concentrations of bacterial cells

Number of cells electroporated[a]	Number of transformants per microgram of plasmid DNA[b]
1.5×10^{10}	166.00 ± 38.66
3.0×10^9	400.67 ± 44.31
1.5×10^9	470.67 ± 148.96
3.0×10^8	599.33 ± 33.00
1.5×10^8	380.00 ± 62.23
1.5×10^7	42.00 ± 4.71

[a] Numbers are cell counts per 50 µl of cell suspension; 50 µl of cell suspension plus 5 µl of vector plasmid DNA (≈1.25 µg of DNA) was added to a 0.1-cm cuvette for electroporation at 16 kV/cm and 25 ohms for ≈4.5 milliseconds.

[b] Mean numbers of transformants ± standard deviation of two separate experiments.
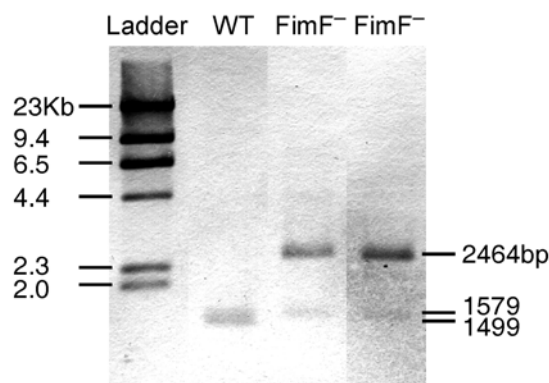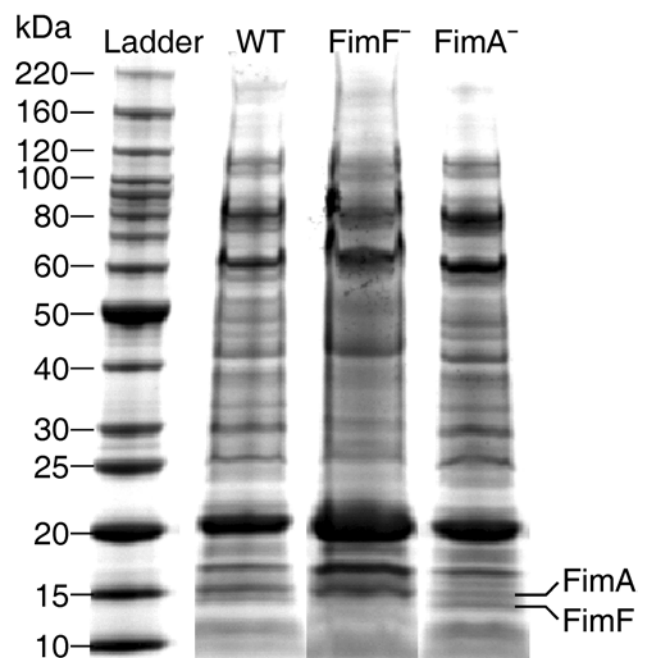

Fig. 3. Southern blot analyses of two putative FimF⁻ mutants of *Xylella fastidiosa* using a digoxygenin-labeled *fimF* probe. DNA of the parental strain (lane 2) and two FimF⁻ mutants (lanes 3 and 4) were digested with *Msl*I before hybridization with the probe. Lane 1 represents the molecular size standard digoxygenin-labeled λ DNA/*Hin*dIII number 2.

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins recovered from the supernatant of cultures of *Xylella fastidiosa*. Lane 1, molecular size markers; lane 2, grape parental strain Temecula1; lane 3, FimF⁻ mutant of strain Temecula1; lane 4, FimA⁻ mutant of strain Temecula1. The arrows indicate the 15-kDa protein identified as FimA and the putative 14-kDa FimF protein.

from 0.2 to 2 μm. Cells of the FimA⁻ mutant of *X. fastidiosa* Temecula1 were, on average, half the length of wild-type cells and appeared to have a much reduced number of fimbriae (Fig. 5B). The appendages seen on a few cells appeared different (thicker and sho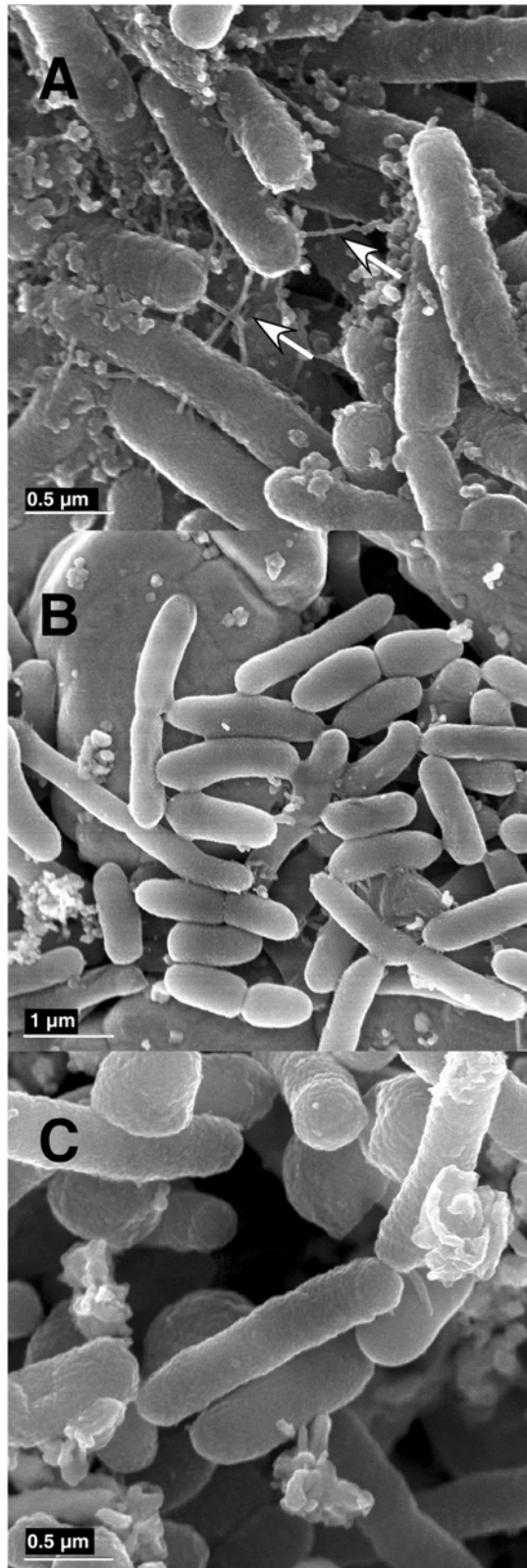rter) than those on the parental strain. The mutant cells aggregated much less than wild-type cells. Cells of the FimF⁻ mutant of *X. fastidiosa* were, on average, ≈80% the length of wild-type cells and appeared to have only a few, very short fimbriae (Fig. 5C). Large cell aggregates of FimF⁻ mutants did not occur.

Pathogenicity of the FimA⁻ and FimF⁻ mutants of *X. fastidiosa*. Disease onset and severity of grapevines inoculated with FimA⁻ or FimF⁻ mutants of the *X. fastidiosa* grape strain Temecula 1 were similar to that of the plants inoculated with the parental strain. Visible symptoms in vines inoculated with the parental strain or with the mutants appeared 4 weeks after inoculation. We were able to reisolate the FimA⁻ or FimF⁻ mutants of *X. fastidiosa* from infected grape on PWG-Kan 2 and 6 months after inoculation, indicating that the cells retained the kanamycin resistance gene in planta without selection. After 2 months, the average *X. fastidiosa* population in infected petioles was $9.6 \pm 9.8 \times 10^7$, $1.6 \pm 1.7 \times 10^8$, and $2.2 \pm 2.3 \times 10^8$ CFU/g of petiole tissue for the vines inoculated with FimA⁻ or FimF⁻ mutants and wild-type cells of the *X. fastidiosa* grape strain Temecula 1, respectively.

DISCUSSION

This study confirms that site-directed gene disruption is possible in the pathogen *X. fastidiosa*, an organism previously not amenable to molecular techniques. Marker-exchange mutagenesis in this species occurs at a low frequency compared with that of other phytopathogenic bacteria that have been studied, such as *Clavibacter michiganense* subsp. *michiganense* (25) or *Agrobacterium tumefaciens* (26). However, a gene replacement process similar to that used in other species, after optimization, yielded knock-out mutants appropriate for further analysis. Given that the genome sequence of this pathogen is now available, the ability to disrupt specific genes will be a useful technique for determining the role of specific genes in this pathogen.

Transformation of *X. fastidiosa* pv. *citrus* 9a5c occurred only when plasmids containing both the *oriC* and an *rRNA* gene of *X. fastidiosa* were used in electroporation (27). Although quite unstable, this vector did allow limited replication, thus facilitating recombination between genes on the vector and the chromosome. This resulted exclusively in single crossover events in which the entire plasmid integrated into the chromosome of the citrus strain of *X. fastidiosa* at the site of the homologous *rRNA* gene (27). Recently, site-directed gene disruption in this same *X. fastidiosa* strain was achieved using a semistable hybrid plasmid containing sequences from pXF1.3, a native *X. fastidiosa* plasmid (7). This vector is maintained stably in *X. fastidiosa*, necessitating extensive culturing of the cells on laboratory media to accumulate cells in which the marker gene is rescued by integration into the chromosome. However, this method required several passages of the culture, which may result in the loss of virulence determinants (17). We did not include the *oriC* of the *X. fastidiosa* grape strain Temecula1 or the origin of replication of an *X. fastidiosa* plasmid in our plasmid vectors. Omitting the origin of replication in the plasmid vector may have facilitated double crossover events in our mutants without the need for serial subculturing. Initially, we were surprised to find that our putative mutants were exclusively double recombinants rather than cis-merodiploids. In other studies, similar allelic exchange mutagenesis usually resulted in single rather than in double crossover homologous recombination events even though double crossover events have been found at a high frequency in some systems (9,22,24). We can only speculate as to why double recombination events occurred at such a high frequency in both *X. fastidiosa* Temecula1 and STL using our vector system but not in the vector systems used for the citrus strain of this species (7,27). The semireplicative vectors used for the citrus strain differed substantially from the pUC18-based suicide vector used in our study. It is possible that sequences on pUC18, or even the *fimA* or *fimF* genes themselves, somehow conferred counterselection of cis-merodiploids that may have formed, thus favoring

Fig. 5. Scanning electron micrographs of *Xylella fastidiosa* grape strain Temecula1 cells grown in PW broth and placed on a silver filter. **A,** Wild-type cells; **B,** FimA⁻ mutant cells; **C,** FimF⁻ mutant cells. Arrows indicate fimbriae.

retention only of strains in which a subsequent second recombination event had occurred. The later explanation seems unlikely because double recombination events were observed in both *fimA* and *fimF* knockout mutants as well as in several other genes we have investigated using this vector (K. L. Newman and S. E. Lindow, *unpublished data*). The origin of replication of pUC18 is very active, normally leading to very high plasmid contents in cells; thus, this locus might have interfered with normal chromosomal replication, leading to counter-selection of strains retaining such sequences. Alternatively, *X. fastidiosa* Temecula1 might contain restriction endonucleases capable of linearizing the plasmid DNA that was acquired during electroporation. Two crossovers are required to integrate linear DNA into the chromosome, and, although less frequent than single crossover events, such a phenomenon might explain both the low frequency of gene replacement as well as the exclusivity of double recombination events in our studies. However, we found no evidence of such an endonuclease in *X. fastidiosa* Temecula1 when we exposed plasmid DNA to cell lysates in vitro. It is significant that both Temecula1 and STL PD strains exhibited the high frequency of double recombination events that distinguished them from the citrus strain of *X. fastidiosa*.

In the present study, we chose the *fimA* and *fimF* genes as target genes because of their central role in the process of fimbriae production and adhesion. The *X. fastidiosa* fimbrial operon containing these genes is similar to the *pap* and type I fimbrial operons typical of the chaperone-usher pathway (36). By analogy to this pathway, the EcpD chaperone of *X. fastidiosa* is probably a periplasmic immunoglobulin-like chaperone and FimC an usher protein located in the outer membrane. Chaperones are known to form periplasmic preassembly complexes with each pilus or fimbriae subunit, such as the major subunit FimA of *X. fastidiosa*, before their incorporation to form a pilus or fimbriae (36). Once formed, the chaperone complexes are targeted to the outer membrane usher, which would correspond to FimC (a PapC homolog) in the case of *X. fastidiosa*. PapC, a typical outer membrane pore-forming protein, translocates pilus subunits across the outer membrane (36). Based on sequence homology, the *X. fastidiosa* FimF and FimG located downstream from FimC on the fimbrial operon are presumed to be, respectively, the fimbrillin or major subunit of the tip fibrillum and an adaptor which attaches the adhesin FimF to FimD. The *X. fastidiosa* FimF was found to have highest homology to MrkD, an adhesin of *K. pneumonia* (1). In *K. pneumoniae*, MrkD, which mediates binding of the fimbriae to collagen molecules (33), was found to be unnecessary for efficient biofilm formation (21). In this study, both FimA⁻ and FimF⁻ mutants remained pathogenic to grape even though the *X. fastidiosa* populations in vines inoculated with the mutants were slightly lower than those in vines inoculated with the wild-type *X. fastidiosa*. It is possible that FimF⁻ mutants of *X. fastidiosa* were still able to form a biofilm in the grape xylem vessels, thereby enabling them to plug the vessels and cause disease, but further studies are needed to characterize the binding properties of such mutants. Scanning electron microscopy revealed that FimA⁻ and FimF⁻ mutant cells had fewer and smaller fimbriae than wild-type cells and that their cell size also was reduced compared with the wild-type cells. These findings are consistent with the results of the SDS-PAGE, which showed that FimA was reduced in FimA⁻ mutant cells' supernatant compared with the wild-type cells. Further examination may show differences between mutant and wild-type *X. fastidiosa* cells in vitro and in planta. Likewise, detailed analyses of the movement of these and other fimbriae mutants, the structure of bacterial assemblages inside xylem elements, and the relative adhesiveness of mutants to themselves and to plant and insect structures should shed light on the role of attachment in the process of PD initiation. As speculated earlier, attachment is unlikely to be a qualitative virulence factor and may contribute only quantitatively to virulence of *X. fastidiosa*. Now that site-directed disruptions of genes can be readily produced for *X. fastidiosa*, understanding the pathogenicity of this organism should progress rapidly.

LITERATURE CITED

1. Allen, B. L., Gerlach, G.-F., and Clegg, S. 1991. Nucleotide sequence and functions of mrk determinants necessary for expression of Type 3 fimbriae in *Klebsiella pneumoniae*. J. Bacteriol. 173:916-920.
2. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds. 1987. Current Protocols in Molecular Biology. John Wiley & Sons, Inc., Canada.
3. Backus, E. A. 1985. Anatomical and sensory mechanisms of planthopper and leafhopper feeding. Pages 163-194 in: The Leafhoppers and Planthoppers. L. R. Nault and J. G. Rodriguez, eds. Wiley Interscience, New York.
4. Bhattacharyya, A, Stilwagen, S., Ivanova, N., D'Souza, M., Bernal, A., Lykidis, A., Kapatral, V., Anderson, I., Larsen, N., Tamara, L., Reznik, G., Selkov, E., Walunas, T., Feil, H., Feil, W. S., Purcell, A. H., Hawkins, T., Haselkorn, R., Overbeek, R. Predki, P. F., and Kyrpides, N. C. 2002. Whole-genome comparative analysis of three phytopathogenic *Xylella fastidiosa* strains. Proc. Natl. Acad. Sci. USA 99:12403-12408.
5. Bollag, D. M., and Edelstein, S. J. 1991. Protein Methods. Wiley-Liss, Inc., New York.
6. Clegg, S., and Gerlach, G. F. 1987. Enterobacterial fimbriae. J. Bacteriol. 169:934-938.
7. Da Silva Neto, J. F., Koide, T., Gomes, S. L., and Marques, M. V. 2002. Site-directed gene disruption in *Xylella fastidiosa*. FEMS Microbiol. Lett. 10432:1-6.
8. Davis, M. J., Whitcomb, R. F., and Gillaspie, A. G., Jr. 1981. Fastidious bacteria of plant vascular tissue and invertebrates (including so called rickettsia-like bacteria). Pages 2172-2188 in: The Prokaryotes: A Handbook on Habits, Isolation, and Identification of Bacteria. M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel, eds. Springer-Verlag, Heidelberg, Germany.
9. Dhandayuthapani, S., Rasmussen, W. G., and Baseman, J. B. 1999. Disruption of gene mg218 of *Mycoplasma genitalium* through homologous recombination leads to an adherence-deficient phenotype. Proc. Natl. Acad. Sci. USA 96:5227-5232.
10. Fernandez, L. A., and Berenguer, J. 2000. Secretion and assembly of regular surface structures in Gram-negative bacteria. FEMS Microbiol. Rev. 24:21-44.
11. Gerlach, G.-F., Bradley, B. L., and Clegg, S. 1988. Molecular characterization of the type 3 (MR/K) fimbriae of *Klebsiella pneumoniae*. J. Bacteriol. 170:3547-3553.
12. Goodwin, P., and Purcell, A. H. 1992. Pierce's disease. Pages 76-84 in: Grape Pest Management, 2nd ed. R. C. Pearson and A. C. Goheen, eds. Division of Agriculture and Natural Resources, University of California, Oakland.
13. Guilhabert, M. R., Hoffman, L. M., Mills, D. A., and Kirkpatrick, B. C. 2001. Transposon mutagenesis of *Xylella fastidiosa* by electroporation of Tn5 synaptic complexes. Mol. Plant-Microbe Interact. 14:701-706.
14. Gutterson, N. I., and Koshland, D. E. 1983. Replacement and amplification of bacterial genes with sequences altered *in vitro*. Proc. Natl. Acad. Sci. USA 80:4894-4898.
15. Hack, C. A., and Benner, W. H. 2002. A simple algorithm improves mass accuracy to 50-100 ppm for delayed extraction linear matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 16:1-9.
16. Hill, B. L., and Purcell, A. H. 1995. Multiplication and movement of *Xylella fastidiosa* within grape and four other plants. Phytopathology 85:1368-1372.
17. Hopkins, D. L. 1985. Physiological and pathological characteristics of virulent and avirulent strains of the bacterium that causes Pierce's disease of grapevine. Phytopathology 75:713-717.
18. Hopkins, D. L., and Adlerz, W. C. 1988. Natural hosts of *Xylella fastidiosa* in Florida. Plant Dis. 72:429:431.
19. Hultgren, S. J., Jones, C. H., and Normark, S. 1996. Bacterial adhesions and their assembly. Pages 2730-2756 in: *Escherichia coli* and *Salmonella typhiurium* Cellular and Molecular Biology. F. C. Neidhardt and R.

Curtiss, eds. ASM Press, Washington, DC.

20. Kitajima, E. W., Bakarcic, M., and Fernandez-Valiela, M. V. 1975. Association of rickettsia-like bacteria with plum leaf scald disease. Phytopathology 65:476-479.

21. Langstraat, J., Bohse, M., and Clegg, S. 2001. Type 3 fimbrial shaft (MrkA) of *Klebsiella pneumoniae*, but not the fimbrial adhesin (MrkD), facilitates biofilm formation. Infect. Immun. 69:5805-5812.

22. Leer, R. J., Christiaens, H., Verstraete, W., Peters, L., Posno, M., and Pouwels, P. H. 1993. Gene disruption in *Lactobacillus plantarum* strain 80 by site-specific recombination: Isolation of a mutant strain deficient in conjugated bile salt hydrolase activity. Mol. Gen. Genet. 239:269-272.

23. Low, D., Braaten, B., and Van der Woude, M. 2002. Fimbriae. Chapter 11 in: *Escherichia coli* and *Salmonella*, Cellular and Molecular Biology. 2nd ed. F. C. Neidhardt, R. Curtiss, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger, eds. ASM Press, Washington, DC.

24. Mahenthiralingam, E., Marklund, B.-I., Brooks, L. A., Smith, D. A., Bancroft, G. J., and Stokes, R. W. 1998. Site-directed mutagenesis of the 19-kilodalton lipoprotein antigen reveals no essential role for the protein in the growth and virulence of *Mycobacterium intracellulare*. Infect. Immun. 66:3626-3634.

25. Meletzus, D., and Eichenlaub, R. 1991. Transformation of the phytopathogenic bacterium *Clavibacter michiganense* subsp. *michiganense* by electroporation and development of a cloning vector. J. Bacteriol. 173:184-190.

26. Mersereau, M., Pazour, G. J., and Das, A. 1990. Efficient transformation of *Agrobacterium tumefaciens* by electroporation. Gene 90:149-151.

27. Monteiro, P. B., Teixeira, D. C., Palma, R. R., Garnier, M., Bove, J.-M., and Renaudin, J. 2001. Stable transformation of *Xylella fastidiosa* CVC strain with oriC plasmids. Appl. Environ. Microbiol. 67:2263-2269.

28. Purcell, A. H. 1997. *Xylella fastidiosa*, a regional problem or global threat? J. Plant Pathol. 79:99-105.

29. Purcell, A. H., Finlay, A. H., and McClean, D. L. 1979. Pierce's disease bacterium: Mechanism of transmission by leafhopper vectors. Science 206:839-841.

30. Purcell, A. H., and Suslow, K. G. 1988. Occurrence of *Clavibacter xyli* subsp. *cynodontis* in California and recovery from xylem-feeding leafhoppers. (Abstr.) Phytopathology 78:1541.

31. Romantschuk, M. 1992. Attachment of plant pathogenic bacteria to plant surfaces. Annu. Rev. Phytopathol. 30:225-243.

32. Romantschuk, M., and Bamford, D. 1986. The causal agent of halo blight in bean, *Pseudomonas syringae* pv. *phaseolicola*, attaches to stomata via its pili. Microb. Pathog. 1:139-148.

33. Schurtz Sebghati, T. A., Korhonen, T. K., Hornick, D. B., and Clegg, S. 1998. Characterization of the type 3 fimbrial adhesions of *Klebsiella* strains. Infect. Immun. 66:2887-2894.

34. Sebghati, T. A., and Clegg, S. 1999. Construction and characterization of mutations within the *Klebsiella mrkD*$_{1P}$ gene that affect binding to collagen type V. Infect. Immun. 67:1672-1676.

35. Simpson, A. J. G., Reinach, F. C., Arruda, P., Abreu, F. A., Acencio, M., Alvarenga, R., Alves, L. M. C., Araya, J. E., Baia, G. S., Baia, C. S., Baptista, C. S., Barros, M. H., Bonaccorsi, E. D., Bordin, S., Bove, J. M., Briones, M. R. S., Bueno, M. R. P., Camargo, A. A., Camargo, L. E. A., Carraro, D. M., Carrer, H., Colauto, N. B., Colombo, C., Costa, F. F., Costa, M. C. R., Costa-Neto, C. M., Coutinho, L. L., Cristofani, M., Dias-Neto, E., Docena, C., El-Dorry, H., Facincani, A. P., Ferreira, A. J. S., Ferreira, V. C. A., Ferro, J. A., Fraga, J. S., Franca, S. C., Franco, M. C., Frohme, M., Furlan, L. R., Garnier, M., Goldman, G. H., Goldman, M. H. S., Gomes, S. L., Gruber, A., Ho, P. L., Hoheisel, J. D., Junqueira, M. L., Kemper, E. L., Kitajima, J. P., Krieger, J. E., Kuramae, E. E., Laigret, F., Lambais, M. R., Leite, L. C. C., Lemos, E. G. M., Lemos, M. V. F., Lopes, S. A., Lopes, C. R., Machado, J. A., Machado, M. A., Madeira, A. M. B. N., Madeira, H. M. F., Marino, C. L., Marques, M. V., Martins, E. A. L., Martins, E. M. F., Matsukuma, A. Y., Menck, C. F. M., Miracca, E. C., Miyaki, C. Y., Monteiro-Vitorello, C. B., Moon, D. H., Nagai, M. A., Nascimento, A. L. T. O., Netto, L. E. S., Nhani, A., Nobrega, F. G., Nunes, L. R., Oliveira, M. A., de Oliveira, M. C., de Oliveira, R. C., Palmieri, D. A., Paris, A., Peixoto, B. R., Pereira, G. A. G., Pereira, H. A., Pesquero, J. B., Quaggio, R. B., Roberto, P. G., Rodrigues, V., de M. Rosa, A. J., de Rosa, V. E., de Sa, R. G., Santelli, R. V., Sawasaki, H. E., da Silva, A. C. R., da Silva, A. M., da Silva, F. R., Silva, W. A., and da Silveira, J. F. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature 406:151-159.

36. Soto, G. E., and Hultgren, S. J. 1999. Bacterial adhesions: Common themes and variations in architecture and assembly. J. Bacteriol. 181:1059-1071.

37. Tarkkanen, A. M., Allen, B. L., Westerlund, B., Holthofer, H., Kuusela, P., Risleti, L., Clegg, S., and Korhonen, T. K. 1990. Type V collagen as the target for type-3 fimbriae, enterobacterial adherence organelles. Mol. Microbiol. 4:1353-1361.

38. Tarkkanen, A. M., Virkola, R., Clegg, S., and Korhonen, T. K. 1997. Binding of the type 3 fimbriae of *Klebsiella pneumoniae* to human endothelial and urinary bladder cells. Infect. Immun. 65:1546-1549.

39. van Doorn, J., Boonekamp, P. M., and Oudega, B. 1994. Partial characterization of fimbriae of *Xanthomonas campestris* pv. *hyacinthi*. Mol. Plant-Microbe Interact. 7:334-344.