Eikenella corrodens Phase Variation Involves a Posttranslational Event in Pilus Formation

MARIA T. VILLAR, JENNIFER T. HELBER, BECKY HOOD, MICHAEL R. SCHAEFER,* AND RONA L. HIRSCHBERG†

Division of Molecular Biology and Biochemistry, School of Biological Sciences, University of Missouri—Kansas City, Kansas City, Missouri 64110

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The human pathogen Eikenella corrodens synthesizes type IV pili and exhibits a phase variation involving the irreversible transition from piliated to nonpiliated variants. On solid medium, piliated variants form small (S-phase), corroding colonies whereas nonpiliated variants form large (L-phase), noncorroding colonies. We are studying the molecular basis of this phase variation in the clinical isolate E. corrodens VA1. A genomic fragment encoding the major type IV pilin was cloned from the S-phase variant of strain VA1. Sequence analysis of the fragment revealed four tandemly arranged potential open reading frames (ORFs), designated pilA1, pilA2, pilB, and hagA. Both pilA1 and pilA2 predict a type IV pilin. The protein predicted by pilB shares sequence identity with the Dichelobacter nodosus FimB fimbrial assembly protein. The protein predicted by hagA resembles a hemagglutinin. The region containing these four ORFs was designated the pilA locus. DNA hybridization and sequence analysis showed that the pilA locus of an L-phase variant of strain VA1 was identical to that of the S-phase variant. An abundant pilA1 transcript initiating upstream of pilA1 and terminating at a predicted hairpin structure between pilA1 and pilA2 was detected by several assays, as was a less abundant read-through transcript encompassing pilA1, pilA2, and pilB. Transcription from the pilA locus was nearly indistinguishable between S- and L-phase variants. Electron microscopy and immunochemical analysis showed that S-phase variants synthesize, export, and assemble pilin into pili. In contrast, L-phase variants synthesize pilin but do not export and assemble it into pili. These data suggest that a posttranslational event, possibly involving an alteration in pilin export and assembly, is responsible for phase variation in E. corrodens.

Eikenella corrodens is a gram-negative human pathogen (10) that can cause endocarditis (6, 16), a variety of soft tissue and wound infections (10, 15, 20, 30), and other opportunistic infections. This bacterium has also been associated with periodontal diseases (4, 33, 37), although a causal role has not been clearly established. Despite increasing recognition of its role in disease, as reflected in growing numbers of case studies and clinical reports, little is known about the molecular factors that contribute to E. corrodens pathogenicity and virulence.

Most strains of E. corrodens exhibit an irreversible phase variation that is reflected in colony morphology changes. On solid medium, small (S-phase), so-called corroding, and large (L-phase), so-called noncorroding colonies are observed (11, 23, 28, 46). The L-phase variants arise from S-phase variants at a frequency much greater than mutation rates (24). Colony morphology and phase variation correlates with the presence of pili on S-phase variants and the absence of pili on L-phase variants (22, 23). In terms of pilination, phase variation in E. corrodens resembles the phase variation exhibited by pathogens such as Neisseria gonorrhoeae (7, 36, 50, 51) and Moraxella bovis (18, 34). In the latter species, the phase variation typically involves genomic recombination or mutagenic events that directly affect pilin synthesis or pilus assembly. Given that pilin can be determinants of pathogenesis (1, 8, 31, 47), and that modulation of pilination may represent a mechanism to evade host immune response (9, 45, 49), the molecular basis of phase-antigenic variation is of considerable interest.

The pathogens mentioned above, as well as other gram-negative bacterial species, synthesize type IV pili (1, 14, 47). These pili are composed primarily of type IV pilin, a protein which ranges from 150 to 165 amino acids in length. Type IV pilin is synthesized as a precursor form (prepilin) that contains a basic leader sequence of variable length (4 to 25 residues). Following translation, the prepilin is cleaved at an atypical site by a cognate peptidase that simultaneously methylates the resultant amino-terminal amino acid, which is typically a phenylalanine residue. Following processing, the mature pilin is exported to the cell surface by a specific transport mechanism and assembled into pili. Class A type IV pili from different species share a highly conserved, 30- to 32-residue hydrophobic amino-terminal domain that functions in protein-protein interactions during pilus assembly (47). The remainder of the mature pilin protein is less conserved and contains the major antigenic determinants.

We are examining the molecular basis of the pilus-associated phase variation exhibited by E. corrodens. Recently, we characterized the major pilus protein of clinical isolate strain VA1 and confirmed that the observed pili were type IV (27). Although genes encoding type IV pilin have also been cloned from two other E. corrodens strains (39, 53), their expression and potential role in phase variation have not been defined. In this report, we document the structure and expression of the major type IV pilin gene in S- and L-phase variants of strain VA1. Analyses of pilin gene expression and pilin processing suggest that a posttranslational event, possibly involving an
alteration in pilin export and/or assembly, is responsible for phase variation in *E. corrodens*.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** The strains and plasmids used in this study are listed in Table 1. *E. corrodens* VA1 is a clinical isolate obtained from the Veterans Administration Medical Center, Kansas City, Mo. (12). Strain VA1 forms both S- and L-phase colonies on solid medium. Strains VA1-SI, VA1-S2, and VA1-S3 are independent S-phase isolates of strain VA1 that form S-phase colonies and exhibit typical frequencies of phase variation to L-phase colonies. Strain VA1-L2 is an L-phase isolate of VA1 that forms only large colonies. *E. corrodens* was cultured aerobically at 35°C on chocolate agar plates purchased from Remel (Lenexa, Kans.).

*Escherichia coli* DH5α was used as the host for general cloning vectors. *E. coli* BL21 (DE3) was used as the host for PET-22b-based protein expression vectors. Both strains were propagated in liquid or in solid Luria-Bertani medium with antibiotics at standard concentrations (5).

**DNA methods.** Restriction endonucleases and modifying enzymes were purchased from Promega (Madison, Wis.). [α-32P]ATP and [35S]dATP were purchased from Amersham (Arlington Heights, Ill.). DNA manipulations, including restriction digests, agarose gel electrophoresis, ligations, PCR amplifications, transformation of *E. coli*, and plasmid minipreparations, were performed by established protocols (5, 43). *E. corrodens* genomic DNA was prepared by the procedure described for *E. coli* in reference 43 or with a kit from Qiagen (Chatsworth, Calif.). For DNA hybridization analysis, digested DNA was transferred to a charged nylon membrane (Hybond-N; Amersham) by the method of Reed and Mann (41). A DNA probe encompassing the pilA locus of strain VA1 (3.9-kbp EcoRI fragment) from plasmid pEC114 was generated from the gel-purified fragment by random-primer labeling with a kit from Promega. DNA hybridizations were performed at 38°C as described by Sambrook et al. (43).

**Cloning and sequencing of the pilA locus.** Total DNA from strain VA1-S1 was partially digested with *Sna*AI, and the digestion products were size fractionated by NaCl gradient centrifugation and purified. To generate a subgenomic library, DNA fragments in the range of 15 to 20 kbp were ligated into the BamHI site of phage GEM11. The phage library was screened by hybridization to a probe for the pilEI gene (1.4-kbp Smal fragment from plasmid pVD203) from *N. gonorrhoeae* MS11 (7), which was kindly provided by M. Koooy. One strongly hybridizing clone was identified, isolated, and designated EP101. Mapping and DNA sequence analyses showed that a terminal 0.6-kbp EcoRI fragment from the EP101 genomic insert contained sequences predicting a type IV pilin.

**DNA hybridization analysis.** The 0.6-kbp fragment identified a 3.9-kbp EcoRI fragment of strain VA1-S1 total DNA. To clone the latter fragment, genomic DNA from strain VA1-S1 was digested with EcoRI and size fractionated on an agarose gel. Fragments in the range of 3.9 kbp were eluted from the gel and ligated into the EcoRI site of vector pGEM-3Zf(−) (Promega), and the ligation products were used to transform *E. coli* DH5α. The transformants were screened for plasmids containing pilin-like sequences by hybridization against the 0.6-kbp EcoRI fragment from EP101, using a colony screening method (5). Plasmid pEC114, which hybridized to the 0.6-kbp probe and contained the predicted 3.9-kbp insert, was chosen for further analysis.

**DNA sequence analysis.** Double-stranded DNA sequencing templates were isolated and purified with a kit from Promega. Double-stranded sequencing of the 3.9-kbp EcoRI fragment of pEC114 was performed by the dideoxynucleotide chain termination method (44), using both manual and automated procedures. Manual sequencing was performed with Sequenase version 2.0 modified T7 DNA polymerase purchased from United States Biochemical (Cleveland, Ohio). Automated sequencing was performed on an Applied Biosystems (Foster City, Calif.) model 377 sequencer with Amplitaq DNA polymerase. Sequencing reactions were primed with M13 universal primers or oligonucleotides synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer. A portion of the pilA locus of strain VA1-L2 was sequenced from a PCR amplification product obtained by using primers 105-R3 (5′-GCCAGCTATTGCAGAATA-3′) and 107-R7 (5′-TGGACCACTTCAAACCG-3′), corresponding to sequences determined from pEC114. DNA and protein sequences were analyzed and compared with sequences in the GenBank database by using MacVector (Oxford Molecular Group, Campbell, Calif.) and BLAST (2) sequence analysis programs.

**RNA methods.** Total RNA was isolated from S- and L-phase variants by using an RNeasy kit (Qiagen). Contaminating DNA was digested with RNase-free DNase, and the RNA was further purified by passage through an RNaseasy column. For hybridization analysis, RNA samples were denatured, separated by electrophoresis on 1.2% agarose gels (43), and transferred to Hybond-N membranes and hybridized to antisense RNA probes for pilA (generated from plasmid pEC203) or pilA2 (generated from plasmid pEC205), using the Riboprobe system (Promega). RNA hybridizations were performed at 42°C as described by Ausubel et al. (5).

**Reverse transcriptase PCR.** Single-stranded cDNA was synthesized from total RNA with an avian myeloblastosis virus reverse transcriptase system (Promega), using oligo(dT) or random primers. A negative control without reverse transcriptase was performed. An aliquot of the reaction volume was amplified by PCR using Taq polymerase (Promega) and the pilA locus specific primers 105-R1 (5′-TGTATGCGCCATTTAAGG-3′), 107-R3 (5′-CAATCTCTCCGACCTTGTG-3′), 107-F3 (5′-AGGAGCACTCGCTTACCC-3′), RH-2 (5′-GGCAACCTTGAGCAATAATCTAC-3′), 107-R6 (5′-GTGAAAGGGTIGTGTGGGTGCC-3′), RH-3 (5′-GGACCCCAACCATTTACAGG-3′), and 107-R7 (5′-TGACCCATCTACACACC-3′). The PCR amplification products were transferred to Hybond-N membranes and hybridized to antisense RNA probes for pilA1 and pilA2 as described above.

** Primer extension.** cDNA was synthesized with an avian myeloblastosis virus reverse transcriptase primer extension system (Promega) using primer 105-F1 (5′-CCAAAATACCGATAATGG-3′) complementary to nucleotides 538 to 555 at the 5′ end of pilA1, which was end labeled with [γ-32P]ATP by using T4

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**Table 1. Strains and plasmids used**

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<th>Strain or plasmid</th>
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<td>pVD203</td>
<td>Carries pilE1 gene from <em>N. gonorrhoeae</em></td>
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poly nucleotide kinase (5). The primer extension products were analyzed against a sequencing ladder on a denaturing polyacrylamide (8% acrylamide) gel.

**Results**

**Sequence analysis of the pilA locus.** Plasmid pEC114 contains the 3.9-kbp *EcoRI* fragment of strain VA1-S1 genomic DNA that was identified by DNA hybridization against the pilE1 gene from *N. gonorrhoeae* MS11. DNA sequence analysis revealed four potential open reading frames (ORFs) arranged in tandem on the pEC114 insert (Fig. 1). The first ORF (nucleotide positions 484 to 943) and second ORF (nucleotide positions 1013 to 1460) predict proteins of 153 and 149 residues, respectively. A BLAST search of the GenBank database showed that both ORFs predict obvious type IV pilins. On the basis of this identity, we designated the first ORF pilA1 and the second ORF pilA2. These and the subsequent gene designations are consistent with recommended bacterial gene nomenclature (17). The predicted PilA1 and PilA2 proteins respectively contain eight- and six-residue amino-terminal leader sequences upstream of the conserved phenylalanine that is presumed to represent the amino terminus of each mature protein. In comparison, the mature PilA1 and PilA2 proteins share 57% overall sequence identity and 87% sequence identity among their 32 amino-terminal residues. The predicted PilA1 amino-terminal sequence matched the amino terminus of each mature protein.

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FIG. 3. Expression of pilA genes in E. corrodens VA1-S1 and VA1-L2. Samples of total RNA (5 μg per lane) from strain VA1-S1 (lanes 1) or VA1-L2 (lanes 2) were subjected to blot hybridization against RNA probes specific for transcripts from pilA1 (A) or pilA2 (B). The positions of RNA molecular size standards are shown at the left.

other restriction enzymes. No hybridizing genomic fragments other than those originating from the pilA1 locus were observed. A similar analysis for other S- and L-phase variants yielded identical results (data not shown). In a related effort, the sequence of a 1.5-kbp region of strain VA1-L2 genomic DNA encompassing pilA and pilB was determined. When compared, the strain VA1-L2 sequence was found to be identical to that for strain VA1-S1 (data not shown).

Pilin gene expression in phase variants. To characterize differential pilination between S- and L-phase variants at the level of pilin gene expression, transcripts from pilA1 and pilA2 in both variants were examined by RNA hybridization analysis. Total RNA isolated from cells of strains VA1-S1 and VA1-L2 was hybridized to RNA probes specific for pilA1 or pilA2 (includes pilB sequences). Similar hybridization profiles were obtained for both variants: the pilA1 probe detected an abundant 0.6-kb transcript and a less abundant 1.5-kb transcript (Fig. 3A), while the pilA2 probe detected a 1.5-kb transcript (Fig. 3B). Transcripts from pilA1 and pilA2 were also examined in two additional S-phase variants (strains VA1-S2 and VA1-S3) and two additional L-phase variants (strains VA1-L1 and VA1-L3), yielding identical results (data not shown). The 0.6-kb transcript is predicted to include pilA1 sequences, whereas the 1.5-kb transcript is predicted to include pilA1, pilA2, and pilB sequences. For both probes, the relative hybridization signal intensity was lower for strain VA1-L2 (Fig. 3; compare lanes 1 and 2). Replicate experiments showed that the pilA1 and pilA1A2B transcript levels in L-phase variants ranged from 43 to 80% of those in S-phase variants. Despite this difference, these results support transcription of the pilA locus in strain VA1-L2 and, coupled with pilin localization studies (see below), suggest a posttranscriptional basis for the lack of pilination in L-phase variants.

Transcription of the pilA locus was also examined by reverse transcriptase PCR. The reverse transcriptase reactions were carried out with select primer pairs using total RNA from strains VA1-S1 and VA1-L2 as the template. The cDNAs were amplified by PCR, and the products were analyzed by hybridization against the RNA probes specific for pilA1 or pilA2. PCR products corresponding to transcripts encoding pilA1, pilA2, or pilB were equally detected in both phase variants (data not shown). In addition, PCR products corresponding to polycistronic transcripts encompassing pilA1 and pilA2, as well as pilA1 through pilB, were detected in both variants (data not shown). These data further support equivalent transcription of the pilA locus in S- and L-phase variants.

The 5′ end of the pilA1 transcript from strain VA1-S1 was mapped by primer extension. Using a primer complementary to a region in the 5′ end of pilA1 (nucleotides 538 to 555), we detected two products 200 and 130 nucleotides in length (data not shown). The larger product mapped to a guanine located 117 bases upstream (nucleotide 367) of the putative translation initiation codon, whereas the smaller product mapped to a cytosine located 53 bases upstream (nucleotide 431). Identical products were obtained for strain VA1-L2. An analysis of the DNA sequence in the vicinity of these sites suggests that the larger product reflects the native pilA1 transcription initiation site: two potential σ70 consensus promoter sequences and a potential σ24 consensus promoter sequence are located immediately upstream of the identified guanine (Fig. 4). Located further upstream is a 40-bp AT-rich region. A similar analysis of the region between pilA1 and pilA2 did not reveal potential promoter sequences; however, a 25-bp region (nucleotides 968 to 993) predicted to form a near-perfect hairpin was detected.

Pilin localization in phase variants. The experiments described above indicated that both S- and L-phase variants express intact pilA1 transcripts. To define a cellular basis for the lack of pilation among L-phase variants, we examined the synthesis and localization of PilA1 in both S- and L-phase variants. Surface, soluble, and insoluble protein fractions were isolated from strains VA1-S1 and VA1-L2 and analyzed for PilA1 content by using a PilA1-specific antiserum. The strain VA1-S1 surface fraction, which should include pilin from pili and pilin loosely associated with the cell surface, contained a considerable amount of mature PilA1 (Fig. 5, lane 1). In contrast, no PilA1 was detected in the surface fraction of strain VA1-L2 (lane 2). Comparable amounts of mature PilA1 were detected in the soluble fraction of both strains (compare lanes 3 and 4). Similarly, the insoluble fraction of both strains contained comparable amounts of mature PilA1 (compare lanes 5 and 6). In addition to mature pilin, the insoluble fraction of both strains contained a unidentified 19-kDa immunoreactive protein (lanes 5 and 6). Control assays with different pilA1 and pilA2 mutants indicated that the 19-kDa immunoreactive species is ubiquitous to the insoluble fraction and is not a form of pilin. No PilA1 was detected in the periplasmic fraction of
either strain VA1-S1 or strain VA1-L2 (data not shown). Collectively, these data suggest that the lack of piliation in L-phase variants is due to a posttranslational event, possibly involving PilA1 export and/or pilus assembly.

**Piliation of phase variants.** Previous reports have described differential piliation among S- and L-phase variants of *E. corrodens* (22, 23). However, this phenomenon was not well documented, and subsequent studies have questioned the presence of pili on *E. corrodens* (32, 38). To document their differential piliation, cells of strains VA1-S3 and VA1-L2 were examined by immunogold electron microscopy using a polyclonal antiserum raised against pilin purified from strain VA1-S1 (27). In an analysis of over 200 strain VA1 cells, we detected one or more immunoreactive pili on 47% of the strain VA1-S3 cells but on none of the strain VA1-L2 cells. Furthermore, no immunoreactive material was observed on the cell surface of either strain. A representative electron micrograph for strains VA1-S3 and VA1-S2 is presented in Fig. 6. In a similar analysis of over 200 *E. corrodens* ATCC 23834 cells, immunoreactive pili were detected on all of the S-phase cells and none of the L-phase cells.

**DISCUSSION**

The gram-negative pathogen *E. corrodens* elaborates type IV pili and exhibits a phase variation involving an irreversible transition from piliated to nonpiliated cells. To initiate an investigation into the molecular basis of this transition event, we have cloned and characterized the *pilA* locus from *E. corrodens* VA1. The *pilA* locus includes four putative genes arranged in tandem. The first two genes, designated *pilA1* and *pilA2*, each encode a type IV pilin. The third gene, designated *pilB*, encodes a potential pilus assembly protein, whereas the fourth gene, designated *hagA*, encodes a putative hemagglutinin. In terms of the pilin and hemagglutinin genes, the strain VA1 *pilA* locus structurally resembles the similar locus described for *E. corrodens* ATCC 23834 (39) (see below). However, the strain VA1 *pilA* locus differs from the strain ATCC 23834 locus by the presence of *pilB*. Given that DNA hybridization analyses of genomic DNA using heterologous and homologous probes failed to detect additional hybridizing fragments, *pilA1* and *pilA2* most likely represent the only type IV pilin genes present in strain VA1.

This study showed that S- and L-phase variants of *E. corrodens* are identical with respect to *pilA1* and *pilA2* structure and sequence. This differs from what is most commonly observed for the best-characterized bacterial pathogens exhibiting a similar phase and/or antigenic variation that involves expression of type IV pilin. Among these are *N. gonorrhoeae* and *N. meningitidis*, which possess one or two pilin (*pilE*) genes and a variable number of silent partial pilin (*pilS*) genes. For these strains, phase variation can be achieved by spontaneous mutations in *pilE*, which results in nonpiliated cells (7, 50, 51). In addition, irreversible intragenic recombination events involving the *pilE* and *pilS* genes result in the synthesis of structurally altered pilins, giving rise to antigenic variation (19, 36, 45, 52). Another example is *M. bovis*, in which two different pilin genes (*fimL* and *fimQ*) are alternatively expressed by means of a DNA inversion that links one or the other gene to a single common promoter (18, 34). Thus, the most common phase and/or antigenic variation exhibited by *N. gonorrhoeae* and *N. meningitidis* and *M. bovis* results from a genomic mutation or recombination events directly involving type IV pilin genes. In comparison, the phase variation exhibited by *E. corrodens* is unique in that it does not involve a pilin gene-associated mutation or genomic recombination event.

The RNA hybridization analysis indicated that transcripts from *pilA1*, but not *pilA2*, were abundant in both S- and L-phase variants of strain VA1, consistent with previous work showing that PilA1 was the major pilus protein for this strain (27). The 5′-end mapping and sequence analyses suggest that the abundant *pilA1* transcript originates from a σ70-type promoter located 200 bp upstream of the putative PilA1 translation initiation codon. If correct, the 0.6-kb *pilA1* transcript terminates at the *pilA1-pilA2* intergenic region, the sequence of which predicts a potential hairpin structure. A low-abundance 1.5-kbp transcript that hybridized to probes for both
pilA1 and pilA2 was also detected in the S- and L-phase variants. This larger transcript is most likely the product of transcription from the pilA1 promoter through the pilA1-pilA2 intergenic region, giving rise to a pilA1A2B polycistronic message. Presumably, the predicted hairpin structure between pilA1 and pilA2 represents the terminator component of a transcription attenuation mechanism. Such an attenuation mechanism would provide for controlled expression of pilA1, pilA2, and pilB as required for pilus formation and resembles a similar mechanism that has been proposed for D. nodosus (25).

The detection of transcripts from pilA1 and pilA2 in the L-phase variant of strain VA1 was somewhat unexpected. In the absence of a detectable pilin gene-associated recombination or mutagenesis event, it was hypothesized that the phase variation exhibited by strain VA1 might be achieved through differential expression of pilA1 or pilA2. The abundance of both the 0.6-kb pilA1 transcript and 1.5-kb pilA1A2B transcript was consistently lower in L-phase variants; however, this difference was deemed insufficient to account for their lack of piliation. We suspect that the decreased level of the pilA1 and pilA1A2B transcripts is related to factors associated with extraction of RNA from the morphologically distinct L-phase variants, as opposed to factors associated with transcription. Collectively, the pilA1 and pilA2 structure and transcription data support a posttranscriptional basis for the nonpiliated phenotype of the L-phase variants.

The pilin localization studies revealed that L-phase variants are not compromised in pilin biosynthesis but that they differ from S-phase variants with respect to the fate of synthesized pilin. For both S- and L-phase variants, similar levels of mature PilA1 were detected in the insoluble protein fraction. Because this fraction includes the cytoplasmic membrane and associated components, the presence of mature PilA1 is consistent with studies of Pseudomonas aeruginosa and other species showing that initial processing of type IV pilins (leader sequence cleavage and amino-terminal methylation) is accomplished on the cytoplasmic surface of the cytoplasmic membrane by membrane-associated cognate preplin peptidases (47). Moreover, the similarity in pilin composition of the insoluble protein fractions suggests that initial processing of PilA1 is not significantly altered in the L-phase variants. The S- and L-phase variants were also indistinguishable in the PilA1 composition of their soluble protein fractions, as similar levels of mature PilA1 were detected for both. In contrast to these results is the PilA1 composition of the surface protein fraction: whereas mature PilA1 was readily detected in the S-phase variant surface protein fraction, none was detected in the corresponding fraction from the L-phase variant. This lack of pilin in the L-phase variant surface fraction is consistent with the immunogold electron microscopic analysis showing that only S-phase variants possessed intact pilus. On the basis of these data, we conclude that an altered pilin posttranslational event, possibly involving one or more steps in pilin export and/or assembly, is responsible for the lack of piliation associated with the L-phase variants of E. corrodens VA1.

The specific stage or event in export or assembly of pilin that might be affected in the L-phase variants is not known. In general, the pathway for processing, export, and assembly of type IV pilins is not well defined. Perhaps the best-characterized type IV pilus biosynthetic pathway is that of P. aeruginosa, for which more than 22 genes involved in pilus expression and/or pilus assembly and function have been identified by transposon tagging (35). More than half of these genes appear to be directly involved in pilus assembly and function and are thought to represent a subset of a general system for the formation of surface-associated protein complexes (26). Of particular interest is the observation that mutations in the individual genes in this subset result in the lack of piliation.

Homologs to many of the putative pilus assembly genes have been identified in other type IV piliated bacteria, supporting a common mechanism for processing, export, and assembly of type IV pili. Presumably, E. corrodens shares this mechanism; if this is so, events that affect one or more of the corresponding genes might be involved in the transition from S- to L-phase variants. Experiments to examine this possibility are in progress.

A precedence for phase variation mediated by a type IV pilin-associated posttranslational event has been established for N. gonorrhoeae MS11. This strain, like most examined N. gonorrhoeae strains, contains two unlinked copies of the pilC gene (not a P. aeruginosa pilC homolog), designated pilC1 and pilC2, which encode a protein involved in pilus assembly (29).

Only pilC2 is expressed in piliated MS11 cells due to a translational frameshift in pilC1. Spontaneous phase variation of MS11 cells is achieved by frameshift mutations in a run of G residues within the region of pilC2 encoding the signal peptide of PilC, abolishing translation of the protein. Pilin synthesis is maintained in the absence of PilC, but no pili are assembled. In this regard, the strain MS11 pilC2 mutants and the L-phase variants of E. corrodens VA1 are phenotypically indistinguishable. Whether E. corrodens VA1 possesses a homolog to PilC from N. gonorrhoeae remains to be examined; however, we note that thus far, PilC appears to be unique to N. gonorrhoeae (47). A significant difference between the pilC2-based phase variation in N. gonorrhoeae MS11 and the phase variation in E. corrodens VA1 is that the former is reversible. Piliated revertants to strain MS11 pilC2 mutants are readily obtainable; the reversion involves mutations in pilC1 or pilC2 that result in translation of PilC. In contrast, no L- to S-phase revertants of E. corrodens VA1 have been observed. Given these data, we predict that the putative posttranslational basis of phase variation in E. corrodens VA1 differs mechanistically from pilC2-based phase variation in N. gonorrhoeae.

Genes encoding type IV pilins have been cloned from two other E. corrodens strains, ATCC 23834 (39) and ATCC 31745 (53). A feature common to these two strains and strain VA1 is the presence of two tandemly arranged type IV pilin genes separated by 70 to 80 bp. The strain ATCC 23834 pilin genes are designated ecpA and ecpB (39), whereas the strain ATCC 31745 pilin genes are designated ecpC and ecpD (53). For the strain VA1 pilin genes pilA1 and pilA2, we adopted the designations of the P. aeruginosa pilin gene system (1), which is consistent with recommendations for bacterial gene nomenclature (17). The six predicted pilins share 80 to 97% sequence identity at their amino termini (first 32 residues of the mature protein), which is typical for all examined type IV pilins (47). Overall, the six predicted pilins share 35 to 43% sequence identity, which is consistent with the reported decreased sequence conservation within the carboxyl regions of type IV pilins (47).

In general, the two pilins from a given strain share greater overall similarity within the carboxyl regions of type IV pilins (47). In particular, the six predicted pilins share 35 to 43% sequence identity, which is consistent with the reported decreased sequence conservation within the carboxyl regions of type IV pilins (47). Overall, the six predicted pilins share 35 to 43% sequence identity, which is consistent with the reported decreased sequence conservation within the carboxyl regions of type IV pilins (47). In general, the two pilins from a given strain share greater overall sequence identity with each other than with pilins from another strain, suggesting that for each strain, the pilin gene pairs arose by a duplication event. The G+C contents of pilA1 and pilA2 (47.9 and 40.1%, respectively) significantly differ from the genomic G+C contents (53 to 58%) reported for several different E. corrodens strains (13, 28, 42), suggesting that the pilin gene(s) may have been acquired by horizontal transfer. Since these are characteristics of a variety of virulence genes acquired by intra- and interspecies gene transfer in bacterial pathogens (21, 54), the pilA locus may represent an acquired region encoding pathogenicity-associated proteins.
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