SUPPLEMENTAL DATA

Construction of pSD5 – The PA103 *pilA* gene was cloned by ligating the *Bam*HI/*Sal*I fragment of PCR-amplified PA103 DNA with pUC18, which had been digested with the same enzymes, yielding pSAD100. The primers employed were 5′ – GCGCAGGATCCAGGTTCAGGCGTTAGGCC – 3′ and 5′ – GCGCAGTCGACCGACTGAGCTAATCCGGC – 3′. The cloned PA103 pilin gene was moved into the vector pMMB66EH by ligating the *Eco*RI/*Hin*dIII fragment of pSAD100 containing the PA103 *pilA* gene with pMMB66EH, which had been digested with the same enzymes, placing *pilA* under the control of a *tac* promoter. This plasmid was designated pSD5. Plasmid construction was conducted in *E. coli* HB101, and triparental mating was used (22) to mobilize this plasmid to strain 1244 where it could be tested for expression and glycosylation status.

Construction of pHYBRID and pHYB-O – Oligonucleotide primers, 5′ – CATGATTACGAATTCGAGCTCGGTACCCGG – 3′ and 5′ – GCAGACAGAAGCTTGCAAGGACACCGGTA ATTTCCCTGCCCGCATTCTTCGG – 3′ were used to amplify pSAD100 between the multicloning site and nucleotide 394 of the translated region of PA103 *pilA*. The amplified DNA was cut with *Eco*RI and *Hin*dIII and the 0.5 Kb fragment produced was ligated with pUC18, which had also been digested with *Eco*RI and *Hin*dIII, yielding the plasmid, pJAS58.

The final 370 translated base pairs of the PA 1244 *pilA* gene, the entire *pilO* gene, and the $tRNA^{thr}$ gene including the *Hin*dIII site in pUC46 were amplified using the primers 5′ – GCACGTAGGACCGGTTCGCGTAAAAATGA CGGAGTCTGG – 3′ and 5′ – CCAGTGCCAAGCTTCCGGCGGATCAACG – 3′. The amplified DNA was digested with *Age*I and *Hin*dIII, and the gel purified 1.9 Kb fragment was ligated with pJAS58 which had been digested with the same enzymes. This yielded the plasmid, pJAS75, which encoded residues 1-123 of PA103 pilin fused to the coding sequence of the 31 C-terminal residues of 1244 pilin. This protocol resulted in the conversion of Val 124 to Gly. The fusion junction was confirmed by nucleotide sequencing. The plasmid, pJAS75 was digested with *Eco*RI and *Hin*dIII, and the gel purified 2.4 Kb fragment was ligated with pMMB66EH digested with these same enzymes, which yielded pHYBRID. Construction of pHYBRID was conducted in *E. coli* DH5α, and this plasmid was moved into 1244.47 via triparental mating (22) to test for pilin expression and glycosylation status. Amino acid sequence of the relevant portion of pilin produced from the expression of pHYBRID is shown in Fig. 2.

The *Eco*RI/*Nhe*I pHYBRID fragment containing the hybrid pilin gene was ligated with pUC18 digested with *Eco*RI and *Xba*I. This yielded the plasmid, pUC HYB-O. This construct was digested with *Eco*RI and *Hin*dIII and the fragment containing the hybrid *pilA* was ligated with pMMB66EH which had been digested with the same enzymes. This yielded the plasmid pHYB-O which contained the chimeric *pilA* gene, but lacked a functional *pilO* gene. This plasmid was constructed in *E. coli* XL10-Gold and was moved to 1244.47 via triparental mating (22) to test for pilin expression and glycosylation status.

Mutagenesis of cloned pilA DNA – Site directed mutagenesis was accomplished by using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, Calif.) as previously described (14). Briefly, primers were designed that incorporated a single, double, or triple amino acid change into either 1244 or PA103 pilin. For primer sequences, see Table 3. The templates for these mutagenesis reactions were cloned *pilA* genes from the appropriate strains encoded within plasmids pUC18, pUC19 or pMMB66EH. Successful mutations were confirmed by nucleotide sequencing. When *pilA* encoded in pUC18 or pUC19 was used as a template, the *Eco*RI/*Hin*dIII fragments of the mutated plasmid were ligated with broad-host range vector pMMB66EH which had been digested with the same enzymes. Plasmid construction was conducted in *E. coli* XL1-Blue or XL10-Gold and triparental mating (22) was used to mobilize plasmids into appropriate *P. aeruginosa* strains for analysis. Relevant portions of the amino acid sequences of mutated pilins are shown in Fig. 2.

The ExSite mutagenesis kit (Stratagene, La Jolla, Calif.) was used to delete portions of the coding sequence of the DSL of 1244 pilin. The mutagenesis reactions were employed using appropriate primers, listed in Table, with pUC46 as a template. The deletions were confirmed by nucleotide sequencing. The 2.3 Kb gel purified *Eco*RI/*Hin*dIII fragment containing the *pilA* gene was ligated with the broad-host range vector, pMMB66EH, which had been digested with the same enzymes. The DSL structure of pilin produced by these plasmids (p1244Δ135-139, p1244Δ135-144, and p1244Δ128-144) is presented in Fig. 2. These plasmids were constructed in *E. coli* XL1-Blue and moved into *P. aeruginosa* 1244N3 by triparental mating (22) to test for expression of the mutant *pilA* gene and pilin glycosylation status.

The ExSite mutagenesis kit was used to insert the coding sequence for residues 135-139 of 1244 pilin (AWKPN) between the coding sequence of Met 135 and Phe 136 of PA103 pilin utilizing primers listed in Table 3, with pSAD100a as a template. The template gene was a mutant that coded for Ser at the 3′ codon. Proper insertion was confirmed by nucleotide sequencing. The 2.3 Kb *Eco*RI/*Hin*dIII fragment of this plasmid was ligated with the broad-host range vector, pMMB66EH, which was digested with the same enzymes, resulting in the plasmid, p103 INS136 (Fig. 2). This plasmid was constructed in *E. coli* XL1-Blue and moved into *P. aeruginosa* 1244.47 by triparental mating (22) to test for expression of the mutant *pilA* gene and pilin glycosylation status.

Construction of pUCP26pilO – This plasmid was constructed in order to present *pilO* in genetic environments lacking this gene. The *Bam*HI/*Hin*dIII fragment from the plasmid pPAL100 was ligated with pUCP26 which had been digested with the same enzymes. pUCP26*pilO* was constructed in *E. coli* HB101, and because this plasmid lacked *tra* sites, it was moved into *P. aeruginosa* 1244.47 via electroporation (44) rather than triparental mating. The pilin glycosylation ability of PilO produced by pUCP26*pilO* was confirmed by Western blot analysis of a cell extract of 1244.47 / pPAC24 / pUCP26*pilO* using an anti-1244 pilin monoclonal antibody, which revealed the presence of glycosylated strain 1244 pilin (data not shown).

Table 2. Intermediate plasmids used in this study.

Strains/plasmids	Description	Source or reference
Plasmids		
pRK2013	Km^R, helper plasmid for triparental mating	(22)
pUC18	2.7 Kb cloning vector; Ap^R	(45)
pUC19	2.7 Kb cloning vector; Ap^R	(45)
pPAL100	pMal-cRI with 1244 *pilO*, Ap^R	(46)
pUC46	pUC19 with 1244 *pilAO*	(14)
pSAD100	pUC18 with PA103 *pilA*	This study
pSAD100a	pSAD100 with coding sequence for Pro 144 of *pilA* mutated to Ser	This study
pSAD100b	pSAD100 with coding sequence for Asn 142, Glu 143, and Pro 144 of *pilA* mutated to Pro, Lys, and Ser respectively	This study
pJAS58	pUC18 with PA103 *pilA* that contains an *Age*I and *Hin*dIII site at the 5′ end of the gene	This study
pJAS75	pUC18 with a chimeric *pilA* coding for the first 123 residues of PA103 pilin, a Gly at residue 124, and the last 31 residues are those of 1244 pilin. This plasmid also contains *pilO* of PA1244.	This study
pUC HYB-O	pUC18 with the chimeric *pilA* from pHYBRID. This plasmid does not contain *pilO*.	This study
pUC135-139	pUC46 with coding sequence for residues 135-139 of *pilA* (AWKPN) deleted	This study
pUCRMD	pUC46 with coding sequence for residues 135-144 of *pilA* (AWKPNYAPAN) deleted	This study
pUC-loop	pUC46 with coding sequence for residues 128-144 of *pilA* (KITKTPTAWKPNYAPAN) deleted	This study
pUC+FIL	pSAD100a with coding sequence for residues 135-139 of 1244 *pilA* (AWKPN) inserted at amino acid positions 136-141 of PA103 *pilA* with Pro 144 (now Pro 149) mutated to Ser	This study
pUC S149	pUC46 with mutant *pilA* that had coding sequence for Ser 148 mutated to Ala and the addition of Ser 149 followed by a stop codon	This study
pUCNEAAS	pSAD100 with coding sequence for two Ala inserted into *pilA* at positions 144 and 145 terminating in Ser 146	This study

Table 3. Primers and templates used in ExSite and QuikChange mutagenesis.

Pilin type	Mutation	Primer Set[a]	Template
PA103	P 144 to S	5′ – CCAATCAACAAAAACAGCTATTAAGACTCATTACAA CCC– 3′and its complement	pSAD100
	N, E, P 142-144 to P, K, S	5′ – CCAATCAACAAAAACAGCTATTAAGACTTCGGACAA CCCTTAGG – 3′ and its complement	pSAD100a
1244	C 127 to A	5′ – GACGGAGTCTGGAACGCCAAAATCACCAAAACTCC – 3′ and its complement	pPAC46
	C 145 to A	5′ – CTACGCTCCGGCTAATGCGCCGAAATCCTAATCGG – 3′ and its complement	pPAC46
	Deletion of K 147	5′ – GCTCCGGCTAATTGCCCGTCCTAATAATCGGTTTTTT GAGTTG – 3′ and its complement	pPAC46
	Insertion of A at position 148 (before S148)	5′ – CGGCTAATTGCCCGAAAGCCTCCTAAGTTTTTTGAGT TGTTTTGAGTGTG – 3′ and its complement	pPAC46
	Insertion of A, A at positions 148 & 149 (before S148)	5′ – CGCTCCGGCTAATTGCCCGAAAGCCGCCTCCTAATC GGTTTTTTGAG – 3′ and its complement	p1244 S149
	S 148 to T	5′ – CCGGCTAATTGCCCGAAAACCTAATCGGTTTTTTGAG – 3′ and its complement	pPAC46
	Insertion of A at position 149 (beyond S148)	5′ – CGGCTAATTGCCCGAAATCCGCCTAAGTTTTTTGAGT TGTTTTGAGTGTG – 3′ and its complement	p1244 S149
	Deletion of residues 135-139	5′ – Phos TACGCTCCGGCTAATTGCCCGAAA – 3′ and 5′ – TGTAGGAGTTTTGGTGATTTTGCA – 3′	pUC46
	Deletion of residues 135-144	5′ – Phos TGCCCGAAATCCTAATCGGTTTTTTGAGTTGTTT TG – 3′ and 5′ – TGTAGGAGTTTTGGTGATTTTGCA – 3′	pUC46
	Deletion of residues 128-144	5′ – Phos TGCCCGAAATCCTAATCGGTTTTTTGAGTTGTTT TG – 3′ and 5′ – GCAGTTCCAGACTCCGTCATTTTTACG – 3′	pUC46
PA103 (P144S)	Insertion of 1244 pilin residues 135-139 to positions 136-141	5′ – GCCTGGAAGCCCAACTTTATTCCTAAGGGTTGTAATG AGTC – 3′ and 5′ – Phos CATCTCTTCCTGAGTAGAGGTGCA AGT – 3′	pSAD100a
	Insertion of A, A at positions 144 and 145 (before S144)	5′ – CTCAGGAAGAGATGTTTATTCCTAAGGGTTGTAATGAG GCGGCGTCTTAATAGCTGTTTTTGTTGATTGGTGTCGATCG GTATTG – 3′ and its complement	pSAD100a

[a]Phos, 5′ phosphorylated