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Genetic Analysis of *Proteus mirabilis* Mutants Defective in Swarmer Cell Elongation†

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Swarmer cell differentiation is a complex process involving the activity of many gene products. In this report, we characterized the genetic locus of Tn*5* insertion in each of 12 mutants defective in swarmer cell elongation. The mutations fell into four categories affecting either flagellar biosynthesis or energetics, lipopolysaccharide and cell wall biosynthesis, cellular division, or proteolysis of peptides.

Proteus mirabilis swarmer cell differentiation and swarming motility are the end result of at least four separate processes (1, 6). First, the cells must sense cues from the environment, e.g., inhibition of flagellar rotation (6) and the concentration of glutamine (2), and respond accordingly by initiating the transcription of 25 to 50 genes whose expression ultimately produces the differentiated cell. Second, surface-induced transcription of genes whose products inhibit cell septation must produce an elongated cell. Third, the cell must synthesize vastly increased amounts of flagellin for new surface-induced flagella and must express the appropriate genes whose products will control the rotation of the flagella (7, 10). Fourth, multicellular interactions and signaling must occur between swarmer cells to allow them to move out on the agar surface in groups and to consolidate (dedifferentiate) in unison (6). If any one of these events is defective, the end result will be either complete loss of swarming motility or abnormal swarming behavior.

In this report, we concentrate on the second feature of swarmer cell differentiation, the surface-induced elongation of cell length. The process of elongation is believed to be due to an inhibition of the normal septation mechanism and takes place with only a slight increase in cell width, often resulting in nonseptated cells 40 to 80 μm in length. The overall goal of this research is to understand the molecular mechanism controlling surface-induced elongation of the *P. mirabilis* swarmer cell.

Transposon mutagenesis and phenotypic screening of mutants defective in swarmer cell elongation. Because cellular elongation is intimately involved in the process of swarming and differentiation, we examined a bank of 212 swarming null mutants (Swr^-) and crippled mutants (Swr^{cr}; those strains that produce aberrant swarming behavior) previously produced through Tn*5*-CM (15) mutagenesis (8, 9) for defects in swarmer cell elongation. Swarmer cell elongation was assessed by inoculating 5 μl of an overnight culture in the center of a petri dish containing L agar (8). After 4 to 6 h of incubation at 37°C, 100 μl of 1× phosphate-buffered saline (PBS; 20 mM sodium phosphate, 100 mM NaCl [pH 7.5]) was used to wash swarmer cells from the edge of the colony. The cells in this suspension were then diluted as required in 1× PBS and viewed by phase-contrast microscopy. Cell length was determined by taking photomicrographs of cells under inducing (growth on agar) and noninducing (liquid culture) conditions. The average length of 50 cells was digitally analyzed, and the mean cell length (±1 standard deviation from the mean) was recorded. Mutants defective in swarmer cell elongation accounted for 10% (7 of 70) of the Swr^- mutants and 18% (26 of 142) of the Swr^{cr} mutants. Of these 35 strains, 10 were chosen for more detailed analysis. As shown in Table 1, these strains included eight that failed to elongate under inducing conditions (Elo^-) and two that constitutively produced the elongated swarmer cells under all conditions (Elo^c). Swarming motility and behavior as well as swimming motility of these mutants were analyzed by previously described methods (9). BB2000 (wild type) served as a positive control in all cases.

Table 1 shows the results of the analysis of swarmer cell elongation of each of the 10 mutants and the wild-type strain. BB2000 typically showed >80% swarmer cells at an average length of 45 to 50 μm under inducing conditions. In contrast, Elo^- strains were markedly impaired in their ability to elongate under inducing conditions and could be divided into two groups. The first group (BB2066, BB2161, BB2182, and BB2217) was characterized as being unable to elongate under inducing conditions beyond the length of the swimmer cell (ca. 2 μm), while the second group (BB2005, BB2036, BB2045, and BB2063) produced swarmer cells that were less than half the length of the wild type. The two Elo^c mutants, BB2084 and BB2115, formed swarmer cells under all conditions, albeit the length of the swarmer cells was slightly greater under inducing conditions (Table 1). In all cases, swarming motility was dramatically affected by the mutation. Swimming motility was not affected in most of the elongation mutants, with the exception of BB2005 (presumed to be defective in chemotaxis [Che^-]) and BB2115, which was motile in semisolid agar but not in liquid media.

Cloning and nucleotide sequence analysis of the DNA flanking Tn*5*. To ascertain the gene or genes mutated by the insertion of the transposon, a cloning and sequencing strategy which afforded a quick way to identify the genetic locus affected was devised. A schematic representation of this strategy is shown in Fig. 1. Briefly, chromosomal DNA was isolated by the spooling method (32) and subjected to limited digestion with the restriction endonuclease *Hin*dIII and ligation to a suitable vector (e.g., pBluescript SK+ [Stratagene]). This approach permits selection of potential recombinant molecules containing the chloramphenicol (*cam*) gene of Tn*5*-CM and *P. mirabilis* flanking DNA from either or both sides of the transposon insertion point. Oligonucleotide primers synthesized to complement either the I or the O end of Tn*5* (15) were then used (in con-

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TABLE 1. Phenotypes of *P. mirabilis* mutants defective in swarmer cell elongation

Strain	Phenotype[a]	Avg cell length[b] (μm)		Motility[c]		Clone insert size (kb)
		Swimmer	Swarmer	Swimming	Swarming	
BB2000	Wild type, Elo^+	1.7 ± 0.3	47 ± 15	Mot^+	Swr^+	NA[d]
BB2005 (Tn*5*::2005)	Elo^-	1.5 ± 0.4	10 ± 4	Mot^+[e]	Swr^{cr}	2.1
BB2036 (Tn*5*::2036)	Elo^-	1.7 ± 0.4	21 ± 6	Mot^+	Swr^{cr}	6.0
BB2045 (Tn*5*::2045)	Elo^-	1.8 ± 0.3	8.5 ± 3.0	Mot^+	Swr^{cr}	1.05
BB2063 (Tn*5*::2063)	Elo^-	1.7 ± 0.2	10 ± 5	Mot^+	Swr^-	1.0
BB2066 (Tn*5*::2066)	Elo^-	1.7 ± 0.3	2.0 ± 0.4	Mot^+	Swr^{cr}	6.6
BB2084 (Tn*5*::2084)	Elo^c	21.6 ± 9	41 ± 9	Mot^+	Swr^{cr}	3.3
BB2115 (Tn*5*::2115)	Elo^c	25.2 ± 8	33 ± 8	Mot^+[f]	Swr^{cr}	1.95
BB2161 (Tn*5*::2161)	Elo^-	1.7 ± 0.5	2.3 ± 0.3	Mot^+	Swr^{cr}	11.0
BB2182 (Tn*5*::2182)	Elo^-	1.6 ± 0.4	1.8 ± 0.3	Mot^+	Swr^{cr}	2.2
BB2217 (Tn*5*::2217)	Elo^-	1.5 ± 0.2	1.7 ± 0.4	Mot^+[g]	Swr^{cr}	1.15

[a] Elo^+, wild-type swarmer cell elongation; Elo^c, constitutive swarmer cell elongation; Elo^-, defective swarmer cell elongation.
[b] Represented as the mean cell length in micrometers ± 1 standard deviation; $n = 50$ cell samples.
[c] Mot^+, wild-type swimming in liquid and semisolid media; Mot^-, no swimming motility; Swr^+, wild-type swarming behavior; Swr^{cr}, swarming crippled (non-wild-type swarming behavior); Swr^-, does not swarm on solid media.
[d] NA, not applicable.
[e] BB2005, Mot^+ in liquid but Mot^- in semisolid agar.
[f] BB2115, Mot^- in liquid but Mot^+ in semisolid agar.
[g] BB2217, originally isolated as Mot^- Swr^-, spontaneously converted to Mot^+ Swr^{cr}.

junction with the pBluescript −40 primer) to obtain the nucleotide sequence of the flanking *P. mirabilis* DNA by using Sequenase V2.0 (U.S. Biochemical-Amersham) enzyme and dideoxynucleotide sequencing methods (31). In most cases, clones were found that positioned the I or the O end opposite the pBluescript SK −40 primer site. Nucleotide sequencing involved using oligonucleotide primers directed to the Tn*5* I and O ends, the pBluescript SK −40 primer, and primers synthesized to internal regions within the *P. mirabilis* DNA (to obtain sequence information from both strands of DNA). This allowed both ends of the *P. mirabilis* flanking DNA to be sequenced and gave a better chance of identifying the region through DNA-DNA homology when computer databases were searched.

Table 1 lists the size (in kilobases) of each of the DNA fragments of *P. mirabilis* flanking DNA obtained from the mutants. The cloned *P. mirabilis* flanking DNA averaged ca. 3.5 kb, which facilitated identification of the genetic locus, usually to the gene level. Three clones (from strains BB2036, BB2066, and BB2161) were much larger than the average. These large inserts, ranging from 6.0 to 11 kb, imposed some limitations on our DNA-DNA homology search strategy but were useful in narrowing the mutation to a small section of the *P. mirabilis* chromosome. Nucleotide sequence ranging between 127 and 776 bases of DNA was obtained for both strands of the *P. mirabilis* clone. The minimum amount of nucleotide sequence obtained was determined by our ability to accurately locate an open reading frame in the deduced amino acid sequence. Nucleotide and peptide sequence databases were searched with the FASTA and TFASTA programs of the University of Wisconsin Genetics Computer Group (16) and the BLASTN and BLASTX programs developed by the National Center for Biotechnology Information (3, 18). Matches based on deduced amino acid sequence homology of *P. mirabilis* sequences were used for all sequences (in conjunction with supporting nucleotide sequence homology) and were considered significant only if >70% similarity was obtained within a minimum stretch of 20 amino acid residues. In many cases, >80% homology in amino acid sequence was found between the *P. mirabilis* sequences and those sequences of other enteric bacteria, such as *Escherichia coli* and *Salmonella typhimurium* (Fig. 2). The results of the computerized homology searches are shown in Fig. 2, listed in Table 2, and described in the following section.

Grouping of Elo^- and Elo^c mutants. (i) Flagellar defects. Inhibition of flagellar rotation is known to cause induction of swarmer cell differentiation (11, 25). It is therefore not surprising to find that mutations affecting flagellar genes represent the largest set of elongation mutants, composed of BB2066, BB2084, BB2115, and BB2217. For example, the Tn*5* insertion in BB2217 is at nucleotide 77 in *flaA*, one of three copies of flagellin-encoding genes present in *P. mirabilis* and the only copy expressed in the wild type (7, 10). This strain was originally isolated as a Mot^- Swr^- mutant but quickly reverted to Mot^+ Swr^{cr}, probably through a mechanism of flagellin gene conversion recently described by us (7). Its swarming motility is extremely reduced, indicative of impaired flagellar rotation that is also manifested in a reduced swimming speed in liquid media. Interestingly, BB2217 does not produce an elongated swarmer cell, suggesting that this mutation has prevented proper sensing of the inducing stimulus.

BB2115 has a Tn*5* insertion in *fliL* (Fig. 2G; 93% homology to *E. coli* FliL) which gives rise to an Elo^c phenotype and an unusual swimming motility active in semisolid media but not in liquid. In *E. coli*, the function of FliL is not known, although the protein does not appear to be essential for either the flagellar motor or the switch (30); however, the *fliL* homolog of *Caulobacter crescentus* is required for flagellar gene expression and normal cell division (34). Because of the Elo^c phenotype, *P. mirabilis* FliL may also be involved in some aspect of division or septation. Alternatively, the polar nature of the Tn*5* insertion may affect downstream genes in an operon, which has been shown to be the case for some *E. coli fliL* mutations (30).

The remaining two mutants, BB2066 and BB2084, are not well defined because nucleotide sequence analyses failed to show strong homology between the flanking DNA (directly adjacent to the Tn*5*) and any gene in the databases. However, it is very likely that the mutations in these strains are in genes associated with flagellar synthesis or energetics. The sequence of the cloned DNA distal to the Tn*5* insertion (at the opposite end of the cloned DNA) shows strong homology to *fliG* (Fig. 2E; a component of the flagellar switch), in the case of BB2066, and *flgH* (Fig. 2F; encoding the basal-body L ring), for BB2084 (20, 21). We, therefore, tentatively place the Tn*5* insertion in

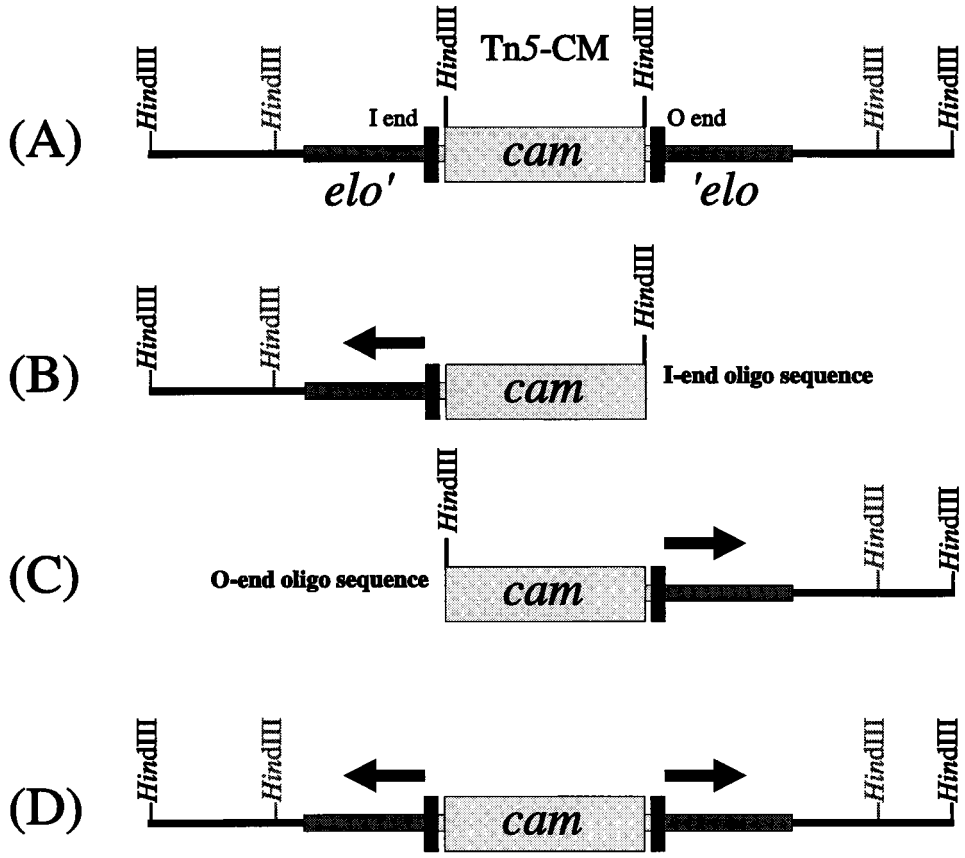

FIG. 1. Schematic representation of the cloning and nucleotide sequencing strategy used to characterize the mutation in *P. mirabilis* elongation mutants. (A to D) A chromosomal DNA locus containing a Tn*5*-CM (15) insertion in a gene essential for swarmer cell elongation (*elo*) results in either an Elo^- or Elo^c phenotype. Isolation and purification of this DNA, followed by partial cleavage with restriction endonuclease *Hin*dIII, are used to obtain recombinant clones in *E. coli* containing either (B) the *cam* gene, I end of Tn*5*, plus flanking *elo* DNA; or (C) the *cam* gene, O end of Tn*5*, plus flanking *elo* DNA; or (D) both ends of Tn*5*. Oligonucleotide primers complementary to either the I-end or O-end nucleotide sequence are then used to obtain partial DNA sequence of the cloned *elo* gene. Intact *Hin*dIII sites in the cloned fragment are indicated in gray.

BB2066 in flagellar gene region IIIb, whose genes in *E. coli* encode products necessary for the earliest stages of flagellar development, and the insertion in BB2084 in region I, involving the hook and basal-body structures (24). This placement assumes that *P. mirabilis* flagellar genes are arranged on the chromosome in an order similar to that of the flagellar genes of *E. coli*. Data from the *P. mirabilis flaD-flaA-flaB* locus, homologous to the *E. coli fliD-fliC* region, support this assumption (7, 10). Since both of these regions are composed of one or more large operons of related flagellar genes, it is not known whether the transposon has specifically inactivated the gene into which it inserted or a downstream gene. Interestingly, these mutations, while affecting both swarmer cell elongation (BB2066 is Elo^- while BB2084 is Elo^c) and swarming motility, do not cause severe problems with swimming motility. This suggests that subtle effects on the flagellar motor may cause major perturbations in swarming behavior.

(ii) LPS and peptidoglycan defects. The second class of mutations found to affect swarmer cell elongation was in genes responsible for O antigen (lipopolysaccharide [LPS]) and cell wall biosynthesis. As is shown in Fig. 2A, BB2036 (Elo^-) was found to have the Tn*5* inserted ca. 6 kb from an open reading frame similar to the hypothetical 43.3-kDa protein in the *E. coli* locus responsible for the chain-length determinant (*cld*) which confers a modal distribution of chain length on the O-antigen component of LPS (5). This protein is thought to have a dehydrogenase activity as indicated by the homology to UDP-glucose dehydrogenase from *Streptococcus pyogenes* and *Streptococcus pneumoniae* (Fig. 2A). Since *cld* comprises several open reading frames, it is possible that BB2036 is defective in maintaining the preferred O-antigen chain length. Alternatively, the mutation may be in the *rfb* gene cluster (O antigen), which is closely linked to *cld* in *E. coli* and *S. typhimurium* (5).

BB2063, also Elo^-, has a mutation in *rfaD* (Fig. 2C; 83% homology over 18 amino acids to *S. typhimurium* RfaD), encoding ADP-L-glycero-D-mannoheptose-6-epimerase. In *E. coli* and *S. typhimurium*, this mutation results in altered heptose (L-glycero-D-mannoheptose) and LPS biosynthesis and increased outer membrane permeability (29). The nucleotide sequence at the distal end of the clone insert is highly similar to that of *rfaC* (Fig. 2D; 88.5% homology to *S. typhimurium* RfaC over 26 residues), which encodes heptosyltransferase I (33). In *S. typhimurium*, this mutation leads to heptoseless LPS and rough colony phenotype (13, 33). Such rough mutants have, in other bacteria, been associated with defects in motility, which may explain the Mot^- phenotype of BB2063. *rfaC* is genetically linked to *rfaD*, supporting the idea that BB2063 is an *rfaD* mutation, and suggests that the mutation affects LPS structure and outer membrane permeability.

BB2161 is a *galU* mutation, affecting glucose-1-phosphate uridylyltransferase and cell wall synthesis. The DNA flanking the Tn*5* insertion is homologous to *galU* from other bacterial

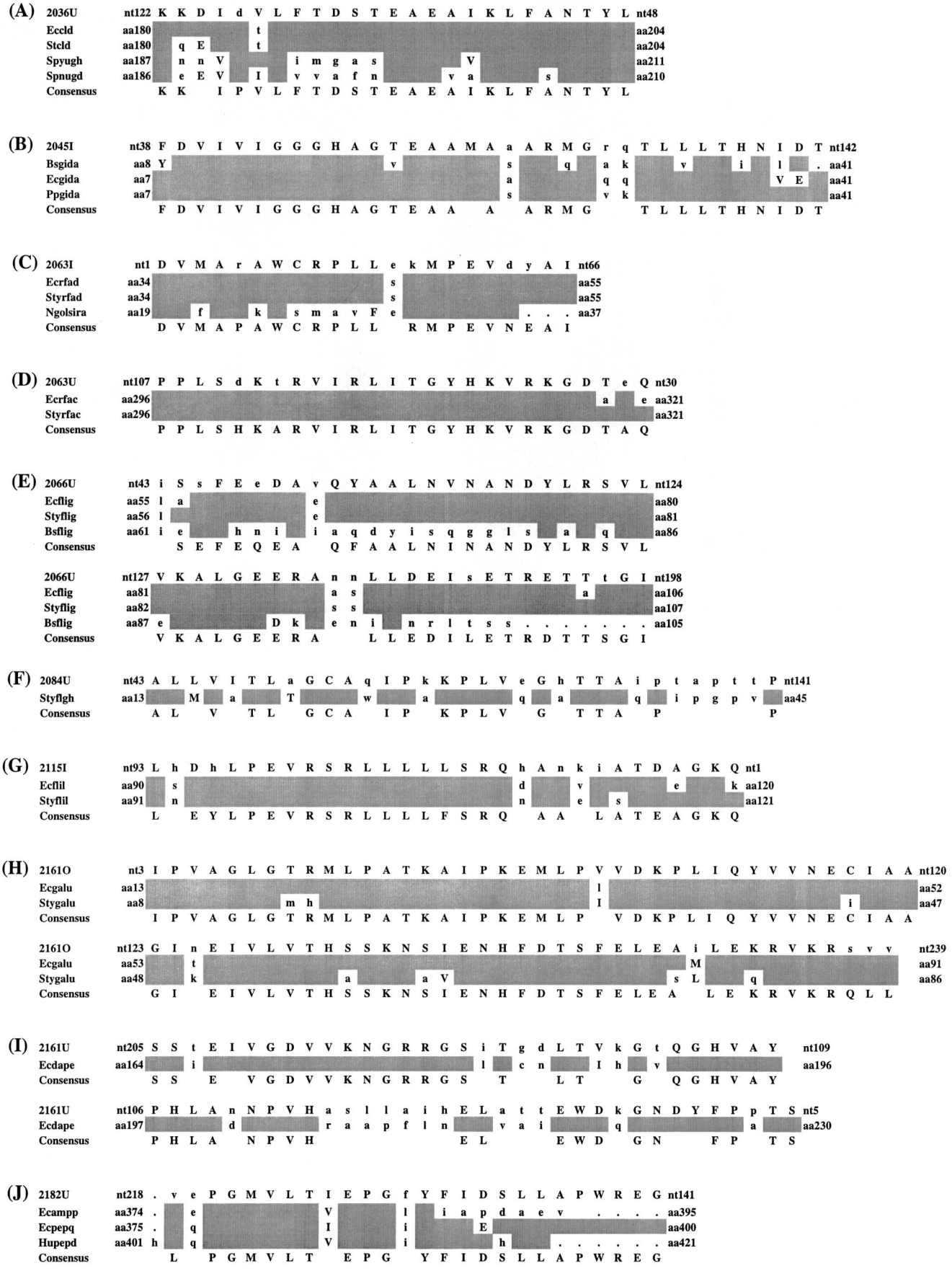
(A)
2036U nt122 K K D I d V L F T D S T E A E A I K L F A N T Y L nt48
Eccld aa180 t aa204
Stcld aa180 q E t aa204
Spyugh aa187 n n V i m g a s V aa211
Spnugd aa186 e E V I v v a f n v a s aa210
Consensus K K I P V L F T D S T E A E A I K L F A N T Y L
(B)
2045I nt38 F D V I V I G G G H A G T E A A M A a A R M G r q T L L L T H N I D T nt142
Bsgida aa8 Y v s q a k v i l . aa41
Ecgida aa7 a q q V E aa41
Ppgida aa7 s v k aa41
Consensus F D V I V I G G G H A G T E A A A A R M G T L L L T H N I D T
(C)
2063I nt1 D V M A r A W C R P L L e k M P E V d y A I nt66
Ecrfad aa34 s aa55
Styrfad aa34 s aa55
Ngolsira aa19 f k s m a v F e . . . aa37
Consensus D V M A P A W C R P L L R M P E V N E A I
(D)
2063U nt107 P P L S d K t R V I R L I T G Y H K V R K G D T e Q nt30
Ecrfac aa296 a e aa321
Styrfac aa296 aa321
Consensus P P L S H K A R V I R L I T G Y H K V R K G D T A Q
(E)
2066U nt43 i S s F E e D A v Q Y A A L N V N A N D Y L R S V L nt124
Ecflig aa55 l a e aa80
Styflig aa56 l e aa81
Bsflig aa61 i e h n i i a q d y i s q g g l s a q aa86
Consensus S E F E Q E A Q F A A L N I N A N D Y L R S V L
2066U nt127 V K A L G E E R A n n L L D E I s E T R E T T t G I nt198
Ecflig aa81 a s a aa106
Styflig aa82 s s aa107
Bsflig aa87 e D k e n i n r l t s s aa105
Consensus V K A L G E E R A L L E D I L E T R D T T S G I
(F)
2084U nt43 A L L V I T L a G C A q I P k K P L V e G h T T A i p t a p t t P nt141
Styflgh aa13 M a T w a q a q i p g p v aa45
Consensus A L V T L G C A I P K P L V G T T A P P
(G)
2115I nt93 L h D h L P E V R S R L L L L L L S R Q h A n k i A T D A G K Q nt1
Ecflil aa90 s d v e k aa120
Styflil aa91 n n e s aa121
Consensus L E Y L P E V R S R L L L L F S R Q A A L A T E A G K Q
(H)
2161O nt3 I P V A G L G T R M L P A T K A I P K E M L P V V D K P L I Q Y V V N E C I A A nt120
Ecgalu aa13 l aa52
Stygalu aa8 m h I i aa47
Consensus I P V A G L G T R M L P A T K A I P K E M L P V D K P L I Q Y V V N E C I A A
2161O nt123 G I n E I V L V T H S S K N S I E N H F D T S F E L E A i L E K R V K R s v v nt239
Ecgalu aa53 t M aa91
Stygalu aa48 k a a V s L q aa86
Consensus G I E I V L V T H S S K N S I E N H F D T S F E L E A L E K R V K R Q L L
(I)
2161U nt205 S S t E I V G D V V K N G R R G S i T g d L T V k G t Q G H V A Y nt109
Ecdape aa164 i l c n I h v aa196
Consensus S S E V G D V V K N G R R G S T L T G Q G H V A Y
2161U nt106 P H L A n N P V H a s l l a i h E L a t t E W D k G N D Y F P p T S nt5
Ecdape aa197 d r a a p f l n v a i q a aa230
Consensus P H L A N P V H E L E W D G N F P T S
(J)
2182U nt218 . v e P G M V L T I E P G f Y F I D S L L A P W R E G nt141
Ecampp aa374 . e V l i a p d a e v aa395
Ecpepq aa375 . q I i E aa400
Hupepd aa401 h q V i h aa421
Consensus L P G M V L T E P G Y F I D S L L A P W R E G

FIG. 2. Sequence alignment of deduced amino acid sequences from *P. mirabilis* Elo$^-$ and Elo^c mutants and their respective homologs. The letter after the strain number refers to the sequencing primer used: U, universal −40 pBluescript primer; I, Tn*5* I-end primer; and O, Tn*5* O-end primer. (A) BB2036; nucleotide sequence from ca. 6 kb away from the Tn*5* insertion point obtained with the universal −40 priming site on pBluescript SK. The *E. coli* hypothetical 43.3-kDa protein in *cld* is indicated by Eccld. The *S. typhimurium* hypothetical 43.6-kDa protein in *cld* is indicated by Stcld. The UDP-glucose dehydrogenases of *S. pyogenes* (Spyugh) and *S. pneumoniae* (Spnugd) are aligned below the *cld* sequences to emphasize the potential dehydrogenase activity of the *P. mirabilis* protein. (B) BB2045; nucleotide sequence of the DNA immediately adjacent to the Tn*5*. The deduced amino acid sequences for GidA from *Bacillus subtilis* (Bsgida), *E. coli* (Ecgida), and *Pseudomonas putida* (Ppgida) are shown, demonstrating the homology shared by the GidA proteins. (C) BB2063; nucleotide sequence of the DNA immediately flanking the transposon indicating homology to the RfaD protein of *E. coli* (Ecrfad) and *S. typhimurium* (Styrfad) and the LsiRA protein from *Neisseria gonorrhoeae* (Ngolsira). (D) BB2063; nucleotide sequence 1 kb away from the Tn*5* insertion indicating homology to the RfaC protein from *E. coli* (Ecrfac) and *S. typhimurium* (Styrfac). (E) BB2066U; nucleotide sequence obtained from ca. 6 kb away from Tn*5*. This deduced amino acid sequence has strong homology to the FliG protein of *E. coli* (Ecflig), *S. typhimurium* (Styflig), and *B. subtilis* (Bsflig). Such homology tentatively places the mutation in region IIIb of flagellar genes (24). (F) BB2084; nucleotide sequence obtained from 3.3 kb distal to the transposon insertion point. The *S. typhimurium* FlgH (Styflgh) (basal-body L ring) protein is similar to the *P. mirabilis* deduced amino acid, suggesting that the mutation lies in flagellar gene region I, involved in the hook and basal-body structures of the flagellum. (G) BB2115; nucleotide sequence from the *P. mirabilis* DNA flanking the Tn*5* insertion. BB2115 is *fliL*, as indicated by the close homology to both the *E. coli* (Ecflil) and *S. typhimurium* (Styflil) sequences. (H) BB2161; nucleotide sequence adjacent to the transposon indicating strong homology to the *E. coli* (Ecgalu) and *S. typhimurium* (Stygalu) GalU protein. (I) BB2161; nucleotide sequence obtained from the distal end of the cloned DNA located 11 kb away from Tn*5*. Computer-based alignments suggest that the *P. mirabilis* sequence is a homolog to *E. coli dapE* (Ecdape) deduced amino acid sequence. (J) BB2182; nucleotide sequence immediately adjacent to the transposon. The *P. mirabilis* deduced amino acid sequence has homology to the X-proline aminopeptidase (aminoacylproline aminopeptidase [Ecampp]) and the X-proline dipeptidase (Ecpepq) of *E. coli* and human X-proline dipeptidase (Hupepd). In all comparisons, the *P. mirabilis* deduced amino acid sequence is listed on the top line and is referenced relative to its respective nucleotide sequence (indicated by nucleotides at the beginning and end of the line). Homologous amino acid sequences are aligned under the *P. mirabilis* sequence and referenced directly to their amino acid residue position. The consensus sequence for all sequences in the set is displayed on the bottom line. For the *P. mirabilis* sequence, capital letters indicate direct matches to the consensus, while lowercase letters indicate a mismatch. Direct matches in the sequences are indicated by shading, capital letters indicate that the residue is similar (but not identical) in chemistry to the consensus, and lowercase letters indicate mismatch. Dots are used to indicate that gaps were introduced into the sequence for alignment purposes. Blanks in the consensus sequence indicate a lack of consensus among the set.

species (Fig. 2H; 93% homology to *E. coli*, *S. typhimurium*, and *Shigella flexneri* over 79 amino acids) (19, 22, 26, 35). The opposite end of the 11-kb clone from BB2161 is similar to *dapE* (Fig. 2I; 82% homology to *E. coli* DapE over 67 amino acids) (12). This gene encodes *N*-succinyl-L-diaminopimelic acid desuccinylase, an enzyme that catalyzes the synthesis of LL-diaminopimelic acid, one of the last steps in the diaminopimelic acid-lysine pathway leading to the development of peptidoglycan (12). Thus, it appears that, unlike in *E. coli*, *galU* and *dapE* are closely linked on the *P. mirabilis* chromosome.

All three of these mutations may affect swarmer cell elongation by preventing normal synthesis and rotation of the flagella. However, while swarming motility is affected by these mutations, swimming is not. Moreover, impairment of the flagella should result in the induction of transcription of the swarmer cell regulon, although each of these mutants is Elo$^-$. We are exploring these mutants in greater detail in an effort to understand more about the interaction of LPS and swarming.

(iii) Defects in cellular division (*gidA*). The mutation in BB2045 producing an Elo$^-$ Mot$^-$ Swr^{cr} phenotype is in *gidA* (glucose-inhibited division), a nonessential gene in *E. coli* very near *E. coli oriC* (28, 38). The homology between *P. mirabilis* GidA and its *E. coli* homolog is 96% over 28 amino acids (Fig. 2B), making this homology one of the strongest seen in this study. In *E. coli*, *gidA* mutations are silent on complex media; however, when grown on glucose-containing media, *E. coli gidA* strains produce long filamentous cells (36–38). Furthermore, there is evidence that *gidA* transcription is regulated by ppGpp and involved in initiation of chromosomal replication (4, 28). Thus, *gidA* may function to connect glucose metabolism, ribosome function, chromosome replication, and cell division. The function of *gidA* in swarmer cell differentiation and elongation is obscure but evidently essential for swarmer cell elongation and wild-type swarming behavior.

(iv) Defects in proline peptidase (*pepQ*). One of the Elo$^-$ mutants, BB2182, is the result of a mutation in the *P. mirabilis* homolog to *pepQ* (Fig. 2J; 81% homology to *E. coli* PepQ over 43 amino acids). This gene encodes X-proline dipeptidase (27)

TABLE 2. Tn*5* insertion sites of *P. mirabilis* mutants defective in swarmer cell elongation

Strain	Sequence[a]	Accession no.	bp	Tn*5* insertion site[b]
BB2005	2005I	L35157	275	Not homologous with published sequences
BB2036	2036I	L35158	127	Not homologous with published sequences
	2036U	L35159	222	Hypothetical 43.3-kDa protein for polysaccharide chain length determinant (*cld*)
BB2045	2045I	L35160	580	Glucose-inhibited division (*gidA*)
	2045U	L35162	174	Glucose-inhibited division (*gidA*)
BB2063	2063I	L35161	191	ADP-L-glycero-mannoheptose-6-epimerase (*rfaD*)
	2063U	L35164	173	LPS heptosyltransferase-1 (*rfaC*)
BB2066	2066I	L35163	188	Not homologous with published sequences
	2066U	L35165	178	Flagellar motor switch protein (*fliG*)
BB2084	2084I	L35170	255	Flagellar protein with partial homology to *fliL*
	2084U	L35166	147	Flagellar L ring (*flgH*)
BB2115	2115I	L35168	776	Flagellar protein *fliL*
	2115U	L35167	338	Not homologous with published sequences
BB2161	2161O	L35169	264	*galU*; UTP-glucose-1-phosphate uridylyltransferase
	2161U	L35171	228	*dapE*; succinyl-diaminopimelate desuccinylase
BB2182	2182U	L35172	267	Proline dipeptidase, *pepQ*
BB2217	2217I	L07270	247	nt 77 in *P. mirabilis flaA*

[a] The nucleotide sequence is designated by the strain number followed by I (Tn*5* I primer), O (Tn*5* O primer), or U (universal primer).
[b] Based on deduced amino acid sequence homology.

and shows strong identity to other proline dipeptidases as indicated in Fig. 2J. Insertion of the Tn*5* in *pepQ* may prevent normal uptake of important signal molecules used by *P. mirabilis* to control swarming behavior and consolidation (6).

(v) Unknown defects. Of the 10 mutants defective in elongation, no significant homology could be found to the nucleotide or deduced amino acid sequences of the flanking DNA from BB2005. This mutant is the only strain that is also Che^-, an interesting point since the closest homologies obtained were to a eukaryotic G protein and a histidine kinase (data not shown). Further nucleotide sequencing may help elucidate the nature of this mutation by yielding stronger homology to other bacterial genes.

Conclusions. This analysis is the first to our knowledge to characterize the genetic lesions resulting in defects in *P. mirabilis* swarmer cell elongation. At the onset, it was anticipated that many of the *P. mirabilis* elongation defects would occur in genes such as *ftsZAQ* and *minAB* which have been well characterized in *E. coli* as essential for septation (14, 17, 23). Instead, as this communication demonstrates, many of the elongation defects have occurred in genes that were not expected to play a central role in this aspect of swarmer cell differentiation. One possible explanation of our failure to obtain *ftsZ*, *-A*, and *-Q* and *minA* and *-B* is that Tn*5* insertion in these genes produces null mutants that are not recovered because the functions are essential for viability. Our current research is focused on determining the function of these genes in swarmer cell differentiation and swarming behavior.

Nucleotide sequence accession numbers. The nucleotide sequence data for the flanking DNA next to the Tn*5* transposon have been deposited in the GenBank database. The accession number for each sequence is listed in Table 2.

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