Identification and Characterization of a Novel Competence Gene, \textit{comC}, Required for DNA Binding and Uptake in \textit{Acinetobacter} sp. Strain BD413

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A gene (\textit{comC}) essential for natural transformation was identified in \textit{Acinetobacter} sp. strain BD413. \textit{ComC} has a typical leader sequence and is similar to different type IV pilus assembly factors. A \textit{comC} mutant (T308) is not able to bind or take up DNA but exhibits a piliation phenotype indistinguishable from the transformation wild type as revealed by electron microscopy.

Although natural transformation is a broadly distributed property among gram-negative soil bacteria, hardly anything is known about the components involved in DNA uptake and their assembly into the presumptive complex structures involved in DNA binding and uptake and the regulation of competence induction. \textit{Acinetobacter} spp. are ubiquitous in terrestrial and aquatic environments and have been the subject of many studies of the genetics of their broad catabolic capabilities (3–5). At least two \textit{Acinetobacter} sp. strains are highly competent for natural transformation (1, 11). We chose a miniencapsulated mutant of the highly competent \textit{Acinetobacter} sp. strain BD4 (11), designated \textit{Acinetobacter} sp. strain BD413, as a model microorganism to study natural transformation in gram-negative soil bacteria. We recently reported on the generation by random kanamycin marker insertion of five transformation-defective mutants completely defective in natural transformation from the highly transformable \textit{Acinetobacter} sp. strain BD413, as a model microorganism to study natural transformation in gram-negative soil bacteria. We recently reported on the generation by random kanamycin marker insertion of five \textit{Acinetobacter} mutants completely defective in natural transformation from the highly transformable \textit{Acinetobacter} sp. strain ADP239, a \textit{pobA} (the \textit{p}-hydroxybenzoate hydroxylase gene) mutant of strain BD413. Complementation studies of one such mutant, T205, led to the identification of the competence factor \textit{ComP} (14). To identify additional components of the natural transformation system in BD413 and to gain further insights into the biogenesis and the structure of the transformation system, we analyzed another transformation-defective mutant, T308.

Characterization of mutant T308. The strains and plasmids used in this study are shown in Table 1. Bacteria were grown in Luria-Bertani medium or in mineral medium (14). We previously reported a high proficiency of DNA repair in mutant T308 and therefore excluded the possibility that the transformation defect of T308 was the result of a RecA dysfunction (14). To characterize the mutant phenotype in more detail, we compared the DNA binding and uptake of ADP239 (the transformation wild type) and T308 essentially as described previously (14). These studies revealed that T308 exhibited a 66% reduced level of DNase-sensitive DNA. It is further evident from Fig. 1 that ADP239 took up DNA at a linear rate, reaching a maximum of 16,700 cpm after 120 min. In contrast, mutant T308 was completely defective in DNA uptake.

Cloning of T308 mutant allele and regeneration of T308 wild-type allele. All molecular procedures were standard techniques (20). Transformations of strain ADP239 and spot matings of the transformation-deficient recipients with recombinant \textit{Escherichia coli} S17-1 donor cells were performed as described recently (14). The mutated chromosomal locus from T308 was recovered on a 9.8-kb \textit{SfiI-XbaI} fragment (pRT308-2 [Fig. 2]). Transformation of the wild-type strain, ADP239, with this 9.8-kb \textit{SfiI-XbaI} fragment gave rise to mutants which had phenotypes indistinguishable from that of mutant T308. This result shows that pRT308-2 carries all of the sequence information required for competence induction.

**TABLE 1. Strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant genotype or phenotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Acinetobacter} sp.</td>
<td>Mutant strain BD413</td>
<td>11</td>
</tr>
<tr>
<td>ADP239</td>
<td>Spontaneous \textit{pobA} mutant of BD413</td>
<td>7</td>
</tr>
<tr>
<td>Transformation-defective mutants of \textit{Acinetobacter} sp. strain BD413</td>
<td>\textit{pobA comP::zptII} Km&lt;sup&gt;r&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>T205</td>
<td>\textit{pobA comP::zptII} Km&lt;sup&gt;r&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T308</td>
<td>\textit{pobA comP::zptII} Km&lt;sup&gt;r&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>\textit{E. coli} S17-1</td>
<td>Thi-p or \textit{hsdR17} (r&lt;sup&gt;K&lt;/sup&gt; m&lt;sup&gt;+&lt;/sup&gt;) \textit{recA1}</td>
<td>21</td>
</tr>
</tbody>
</table>

**Plasmids**

- pBluescriptII KS
- pRK415
- pUC4K
- pRT308-2
- pRT308
- pRT308
- pCL20
- pRK1
- pRK2
- pRK3
- pRK9
- pSE9
- pZFR408

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mation required to generate the transformation-defective phenotype of the mutant T308.

Southern hybridization experiments strongly suggest that the mutation in T308 is the result of an integration event giving rise to gene disruption. Thus, the deletion of the Km' marker from the recovered mutant locus should generate the wild-type allele. Deletion of the internal 1.3-kb BamHI fragment carrying the nptII gene resulted in plasmid pRT308, carrying a 8.5-kb SstI-XbaI insert (Fig. 2). pRT308 was introduced into mutant T308 by conjugation via E. coli S17-1. All transconjugants tested were able to take up DNA by natural transformation, with transformation frequencies of 3.9 × 10^{-3} to 4.1 × 10^{-3} transformants (based on viable counts) in the presence of saturating DNA concentrations. These frequencies are comparable to wild-type transformation frequencies, which is evidence for a complete restoration of the wild-type transformation phenotype by pRT308. All transconjugants still exhibited the nptII-encoded Km' phenotype, and plasmid pRT308 could be recovered from all of the transconjugants examined, which indicates that the transformation deficiency of T308 was complemented by providing the recombinant plasmid pRT308 in trans. Furthermore, these results indicate that the insertion of the nptII marker gene into the genome of mutant T308 does not cause any polar effects on the genes that may be located downstream of the marker-affected mutant locus. A variety of derivatives of the 8.5-kb SstI-XbaI fragment were constructed and tested for their ability to complement the mutation in T308. These studies identified the 4.6-kb ClaI-XbaI fragment (pRK9) as the smallest fragment able to restore the wild-type transformation phenotype (Fig. 2). To confirm that the fragment generated from the recovered mutant locus is identical to wild-type DNA, the wild-type gene was cloned from XbaI-digested chromosomal DNA of strain BD413 into pRK415. Plasmid pCL20 contained a 20.1-kb XbaI fragment and was found to restore the wild-type transformation phenotype of mutant T308 (Fig. 2). The results of restriction analysis, Southern hybridizations, and DNA sequencing revealed that the insert in pRK9 is identical to the DNA cloned from the wild type. These results show that the comC disruption in mutant T308 by nptII marker insertion was caused by an allelic replacement recombination of contiguous DNA fragments flanking the nptII gene, which resulted in a marker gene insertion without causing any deletion or duplication events.

**Identification and characterization of comC, a gene complementing the transformation defect of mutant T308.** The 4.6-kb ClaI-XbaI fragment of pRK9 was inserted into pBluescriptII KS, yielding pSE9 (Table 1), which was subjected to unidirectional deletions. The complete nucleotide sequence of the ClaI-XbaI fragment (4,584 bp) in pSE9 was determined for both strands, and sequence analysis revealed one complete open reading frame, designated comC, of 3,624 bp extending from nucleotide positions 717 (ATG) to 4340, spanning the BamHI site used to generate mutant T308 by marker insertion. comC is preceded by a well-conserved and well-placed Shine-Dalgarno sequence. The complementation of the transformation-defective mutant T308 was found to be independent of the insert orientation with respect to the lac promoter, as shown for complementation with pRK9 in Fig. 2. This indicates that comC is expressed under the control of its native promoter. A conserved ω70 promoter sequence (TTGCCGATA, TATTAA) was found within a region 141 to 172 bp upstream of comC. The 39.4% G+C content of comC is within the characteristic range of Escherichia coli.
of 37 to 45% for coding regions in *Acinetobacter* species, and the codon usage was much like that found in previously sequenced *Acinetobacter* genes (12, 24).

**Features of ComC and similarity to type IV pilus assembly and adhesion factors.** ComC contains 1,208 amino acids (aa), with a calculated molecular mass of 132 kDa. The N terminus exhibits structural features characteristic of the three domains of signal peptides (23): (i) a positively charged N terminus (Lys 8 and Arg 10), (ii) a hydrophobic core (Ala 11 to Ala 18), and (iii) a C-terminal domain containing small neutral residues (Lys 21 to Thr 24). These findings suggest that ComC is located, and acting, at the cell surface.

Database searches and sequence alignments revealed that ComC exhibits similarities to proteins involved in the assembly of type IV pili in pathogenic bacteria, such as PilC (1,037 aa; 21% identity) (19), PilC1 (1,038 aa; 22% identity), and PilC2 (1,048 aa; 22% identity) of *Neisseria meningitidis* (15); PilC1 (1,044 aa; 22% identity) and PilC2 (1,050 aa; 21% identity) of *Neisseria gonorrhoeae* (10); and PilY1 (1,161 aa; 21% identity) of *Pseudomonas aeruginosa* (2). The similarities of ComC to the type IV pilus biogenesis factors are in the same range as the similarities found for PilC2 to PilY1 and are not restricted to a certain region, whereas the similarities displayed by PilC2 to PilY1 are restricted to the C terminus. ComC and its homologs are also of similar size and possess large regions of hydrophilic amino acids.

*ComC is not essential for the biogenesis of pilus fibers and twitching motility.* PilC1 and PilC2 of *N. gonorrhoeae* and the
recently identified PilC of *N. meningitidis* exhibit dual functions in type IV pilus biogenesis and in transformation (16, 19). To address the question of whether ComC also displays such a dual function, the ultrastructures of ADP239, mutant T308, and transconjugant cells restored to natural transformation were analyzed. Electron micrographs confirmed our recent finding that cells of strain ADP239 possess two types of fimbrae (14): thin ones with a diameter of 3.5 nm, which appear in bundles, and thicker isolated fimbrae with a diameter of about 6 nm. The piliation phenotype of the transformation-defective mutant T308 was indistinguishable from the piliation of ADP239 cells (Fig. 3), indicating that ComC is not essential for the biogenesis of pili in *Acinetobacter* sp. strain BD413. The thick pilus of *Acinetobacter* sp. strains have been found to mediate a special kind of surface translocation, termed twitching motility (8). Since the presence of thick pili does not preclude a functional defect of the pili, such as a defect in twitching, T308 was analyzed for its ability to perform twitching motility, which was monitored by the appearance of spreading zones along the central streak of growth of the cells on agar plates as described previously (14). These studies revealed that T308 was not impaired in twitching (data not shown) and therefore provide substantial evidence that the transformation factor ComC is not essential for pilus biogenesis.

**Conclusions.** The PilC of *N. gonorrhoeae* and PilY1 of *P. aeruginosa* have been localized in both the outer membrane and the fibrillar fractions (2, 6), and PilC was found especially in the fibrillar tip of *N. gonorrhoeae* (18), which is also suggested for PilY1 of *P. aeruginosa* (2). The similarity of ComC to PilC and PilY1 and the DNA binding and uptake studies suggest that ComC is exported and acts in a cell surface protein complex required for DNA binding and uptake in *Acinetobacter* sp. strain BD413. Analogous to the function once predicted for the multifunctional gonococcal PilC2, ComC might represent a basement protein or a molecular usher, required for the correct subunit presentation of a growing oligomeric structure mediating DNA transfer through the outer membrane. Homologous sets of pil-like genes are found not only in DNA transfer systems but also in bacterial protein secretion systems (for a review, see reference 9). These homologies might reflect a common scheme of macromolecular transport.

**Nucleotide sequence accession number.** The sequence of the open reading frame designated *comC* has been deposited in the GenBank database under accession no. AF027189.

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**REFERENCES**


