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# Identification and Characterization of ComE and ComF, Two Novel Pilin-Like Competence Factors Involved in Natural Transformation of *Acinetobacter* sp. Strain BD413

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**Although the high level of competence for natural transformation of *Acinetobacter* sp. strain BD413 has been the subject of numerous studies, only two competence genes, *comC* and *comP*, have been identified to date. By chromosomal walking analysis we found two overlapping open reading frames, designated *comE* and *comF*, starting 61 bp downstream of *comC*. *comE* and *comF* are expressed as stable proteins in *Escherichia coli*, thus proving that they are indeed coding regions, but expression was successful only with 5′-deleted genes. ComE and ComF are similar to pilins and pilin-like components. Both genes were mutated, and the phenotypes of the mutants were analyzed. Natural transformation in *comF* mutants is 1,000-fold reduced, whereas *comE* mutants exhibit 10-fold-reduced transformation frequencies. This is clear evidence that *comE* and *comF* are involved in natural transformation. However, ComE and ComF are specific for DNA translocation, since *comE* and *comF* defects affected neither piliation nor lipase secretion. These results suggest that the type IV pili, the general protein secretion pathway, and the DNA translocation machinery in *Acinetobacter* sp. strain BD413 are evolutionary related but functionally distinct systems.**

Bacterial genetic competence for natural transformation has been defined as a physiological state that permits the uptake of exogenous DNA. This process can be dissected into the discrete, sequential steps of DNA binding, DNA translocation across the inner and outer membranes, and subsequent recombination with homologous counterparts in the genome or plasmid amplification. A broad range of bacterial species have been reported to undergo natural transformation (23). Despite the broad distribution of natural transformation among different taxonomic and trophic groups, knowledge about the DNA uptake machinery is very limited and is restricted to some model microorganisms, such as *Bacillus subtilis*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae* (7–9, 14, 27), and information about the molecular basis of natural transformation in gram-negative soil bacteria is scarce.

To obtain insights into the molecular basis of natural transformation in gram-negative soil bacteria, we chose to study the nutritionally versatile strain *Acinetobacter* sp. strain BD413. This strain, formerly designated *Acinetobacter calcoaceticus* BD413, is known for its extraordinarily high frequency of natural transformation (20, 30). Recently we identified and characterized two novel competence factors, ComP and ComC, which are both essential for binding and uptake of DNA in *Acinetobacter* sp. strain ADP239, a *pobA* (*p*-hydroxybenzoate hydroxylase) mutant strain of BD413 (22, 34). ComP is similar to prepilins of type IV pili and to pilin-like components of protein translocation machinery (19), whereas ComC is similar to various type IV pilus biogenesis factors.

The DNA-translocating structures involved in natural transformation in gram-negative bacteria known so far are predicted to be built by an oligomeric structure presumably comprising pilin or pilin-like subunits. We show in this report that *Acinetobacter* sp. strain BD413 has multiple pilin-like genes, all of which are involved in natural transformation but not in piliation or lipase excretion. These findings suggest a multisubunit oligomeric structure similar to that of type IV pili involved in DNA uptake in *Acinetobacter* sp. strain BD413. Furthermore, the DNA-translocating structure is proposed to be different from the type IV pili and the general secretory pathway (GSP) present in this strain.

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## MATERIALS AND METHODS

**Bacterial strains, plasmids, and DNA manipulations.** The bacterial strains and plasmids used in this study are listed in Table 1. *Acinetobacter* wild-type and mutant strains were grown in mineral medium (34). DNA manipulations were done in *Escherichia coli* DH5α cultured in Luria-Bertani (LB) medium (41). Antibiotics were added when appropriate (kanamycin at 20 μg/ml for both *E. coli* and *Acinetobacter* strains, ampicillin at 100 μg/ml, and tetracycline at 15 μg/ml). The molecular procedures used were standard techniques (41) or were performed as recommended by the manufacturers of the reagents. Conjugation, transformation, complementation experiments, and Southern hybridization experiments were performed as described previously (34).

**DNA sequencing and analysis.** Sequencing of DNA was done by the chain termination method (42) with an ALF sequencer (Pharmacia Biotech Europe GmbH). For each sequencing reaction, 100 fmol of DNA was labeled via cycle sequencing, using a SequiThermExcel DNA sequencing kit (Epicentre Technologies, Madison, Wis.) and fluorescein-labeled standard primers or primers generated from the derived sequence information. Sequence data were compiled and analyzed with the programs DNA Strider and MegAlign and by using the software package (version 8.1) of the Genetics Computer Group (University of Wisconsin Biotechnology Center).

**Transposon mutagenesis.** The insert of plasmid pRK13 (see Fig. 1) was subjected to transposon mutagenesis by the use of a genetically engineered derivative of Tn*10*, which will be referred to as mini-Tn*10*pLOF/Km (18). The *E. coli* S17-1(λpir)(pLOF/Km) donor strain and the *E. coli* DH5α(pRK13) recipient strain were cultured overnight. Matings between donor and recipient were performed by the filter mating technique as described elsewhere (34). Transconjugants were selected on LB plates containing kanamycin and tetracycline, resuspended in LB broth, and grown overnight. Plasmids were prepared and transformed into *E. coli* DH5α, and transconjugants were selected on LB plates containing kanamycin and tetracycline to identify plasmid DNA containing the transposon. The DNA of colonies was purified and subjected to restriction analysis. One of the recombinant plasmids was found to carry the kanamycin resistance gene of mini-Tn*10*pLOF/Km in *comF*. This plasmid was designated pRK13-8 (see Fig. 1).

**Disruption of *comE*.** To construct an insertional mutation in *comE*, a kanamycin resistance cassette was inserted into its *Xba*I site. A 5.2-kb *Bam*HI-*Kpn*I fragment from plasmid pRK13 (Fig. 1) covering *comE* and *comF* was subcloned

TABLE 1. Strains and plasmids used in this study

| Strain or plasmid | Relevant genotype and/or phenotype | Source or reference |
|---|---|---|
| *Acinetobacter* sp. strains | | |
| BD413 | Wild type | 20 |
| ADP239 | Spontaneous *pobA* mutant of BD413 | 16 |
| ADP501 | *pobA comF*::miniTn*10*; $Km^r$ | This study |
| ADP503 | *pobA comE*::*nptII*; $Km^r$ | This study |
| WH399 | *xcpR* mutant of BD413 | 31 |
| *E. coli* strains | | |
| DH5α | $F^-$ *lacZΔM15 recA1 endA1 hsdR17 supE44 (lacZYA argF)* | 15 |
| S17.1 (λpir) | *trp* $Sm^r$ *recA thi pro* $hsdM^+$ RP4-2-Tc::Mu::Km Tn*7* λpir; *hsdR* mutant | 43 |
| Plasmids | | |
| pBSK | $Ap^r$ | Stratagene |
| pRK415 | $Tc^r$; *lacPOZ′* | 21 |
| pUC4K | $Ap^r$, $Km^r$ | Pharmacia |
| pLOF/Km | $Ap^r$; Tn*10*-based delivery plasmid with $Km^r$ | 18 |
| pMAL-c2 | $Ap^r$ *lacPOZ′* oriR *malE-lacZα rrB* $lacI^q$ | |
| pSE17 | 15.9-kb *Eco*RI insert in pBSK; $Ap^r$ | This study |
| pRK13 | 12.1-kb *Bam*HI-*Pst*I fragment in pRK415; $Tc^r$ | This study |
| pRK13-8 | $Km^r$ gene of pLOF/Km inserted as a 1.7-kb fragment into the *comF* gene of pRK13; $Tc^r$ | This study |
| pSB13-2 | 5.2-kb *Bam*HI-*Kpn*I fragment of pSE17 in pRK415; $Ap^r$ | This study |
| pSB13-21 | Identical to pSB13-2 but the unique *Xba*I site in pBSK is deleted; $Ap^r$ | This study |
| pSB13-22 | *nptII* gene of pUC4K inserted as a 1.3-kb blunt-end fragment into the *comE* gene of pSB13-21; $Ap^r$ | This study |
| pCR5 | *comF* gene inserted as a 475-bp *Pst*I-*Bam*HI fragment into pRK415 | This study |

into the vector pBluescript II (Stratagene), resulting in plasmid pSE13-2. To delete the *Xba*I site, pSE13-2 was digested with *Bam*HI and *Not*I and then incubated with the Klenow fragment of DNA polymerase in the presence of deoxynucleoside triphosphates, and the resulting blunt ends were ligated, yielding plasmid pSE13-21 (Fig. 1). pSE13-21, which carries a unique *Xba*I site in *comE*, was digested with *Xba*I. The plasmid harboring the kanamycin resistance cassette, pUC4K (Pharmacia), was digested with *Eco*RI to yield a 1.3-kb *Eco*RI fragment containing the kanamycin resistance gene. Both the 1.3-kb *Eco*RI fragment from pUC4K and *Xba*I-linearized plasmid pSE13-21 were incubated with Klenow enzyme and deoxynucleoside triphosphates. After ligation, the plasmids were transformed into *E. coli* DH5α, and transformants were selected on medium containing kanamycin; the identities of the recombinant plasmids (pSE13-22 [Fig. 1]) were verified by DNA sequencing. pSE13-22 was digested with *Bst*XI and *Kpn*I, and the 4.8-kb *Bst*XI-*Kpn*I fragment (Fig. 1) carrying the marker-disrupted *comE* gene plus flanking DNA was isolated and transformed into *Acinetobacter* sp. strain ADP239, a *pobA* mutant of strain BD413 (16). Kanamycin-resistant colonies were purified, and the correct allelic replacement of chromosomal wild-type *comE* by the disrupted *comE* was verified by Southern blotting.

**Physiological studies.** The abilities of the mutants and transformants to take up DNA via natural transformation were analyzed by spot transformation, their piliation phenotypes were analyzed by electron microscopy, and their abilities to perform twitching were monitored on freshly prepared agar plates incubated in a humidified atmosphere as described recently (34). Growth on hexadecane was analyzed by spreading cells on mineral agar in the presence of hexadecane, which was delivered through the gas phase from droplets on three filter disks in the lids of the plates. The secretion of lipase was monitored on nutrient broth (NB) indicator plates containing 1.5% (vol/vol) egg yolk (Oxoid) according to the method of Owens (29). An *xcpR* mutant, strain WH399, that did not grow on hexadecane and was defective in lipase secretion was used as a negative control (31). Adherence to hexadecane was monitored according to the procedure of Neu and Poralla (28).

**Complementation studies.** To perform complementation studies with the transformation-deficient *comF* mutant (T501), a 475-bp fragment representing the entire *A. calcoaceticus* BD413 *comF* gene was amplified from plasmid pSE13 by PCR. The sequences of the 5′ and 3′ primers used were AAGCTCCTGCAGTACAGG and TTTGCATCTAGAGCCCAT, respectively. Both primers contained mutations introducing a *Pst*I site upstream of the start codon and a *Xba*I site downstream of the stop codon. (The *Pst*I site and the *Xba*I site are underlined.) The PCR product was cloned into the broad-host-range plasmid pRK415 (21) in the correct orientation with respect to the *lac* promoter. The *comF*-containing plasmid pCR5 was transformed into T501. Transconjugants were selected for growth on LB plates containing tetracycline, and single transconjugants were purified and analyzed for the presence of plasmid pCR5.

**Construction of *malE-comE* and *malE-comF* fusions and purification of fusion proteins.** *malE-comE* and *malE-comF* translational fusions were constructed by PCR amplification of 5′-deleted *comE* and *comF*, using primers E-*Eco*RI (5′TATCAG**GAATTC**ATACGT3′), E-*Bam*HI (5′ATCATG**GGATCC**ATCAAT3′), F-*Eco*RI (5′GTTTCACAG**GAATTC**CAA3′), and F-*Xba*I (5′TTTGCA**TCTAGA**GCCCAT3′). Bases altered from the wild-type sequence are in boldface, and the positions of the engineered *Eco*RI, *Bam*HI, and *Xba*I restriction sites in the primers are underlined. PCR products were digested with *Eco*RI and *Bam*HI or with *Eco*RI and *Xba*I and cloned into pMAL-c2 (New England Biolabs) to produce pME28 and pMF18, respectively. In pME28, ComE starts at residue 35, and in pMF18, ComF starts at residue 54. The identities of the PCR products were confirmed by sequence analysis.

The MalE-ComE and MalE-ComF fusion proteins were purified from *E. coli* grown at 37°C in LB medium containing ampicillin. Expression was induced at an optical density at 600 nm of 0.6 by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM. The fusion proteins were purified from French press-disrupted cells by affinity chromatography on an amylose column (New England BioLabs, Schwalbach, Germany) according to the manufacturer's instructions.

**SDS-PAGE, immunoblotting, and autoradiography.** Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% gels and transferred to nitrocellulose membranes (Sartorius, Göttingen, Germany) according to the procedure of Sambrook et al. (41). Membranes were incubated with anti-MalE antibodies diluted 1:10,000 followed by peroxidase-conjugated goat anti-rabbit immunoglobulin G (Boehringer Mannheim, Mannheim, Germany) diluted 3:10,000. The blots were developed on X-Omat AR film (Kodak, Stuttgart, Germany) for 1 to 10 min. Coomassie staining was performed according to the procedure of Weber and Osborne (47).

**Nucleotide sequence accession number.** The sequence data have been submitted to the GenBank database under accession no. AF027189.

## RESULTS

**Identification of two pilin-like protein open reading frames located downstream of the competence gene *comC*.** Since genes involved in DNA and protein translocation systems are often organized in clusters (19), we cloned a 15.9-kb *Eco*RI fragment spanning the previously described *comC* mutant locus (22) of mutant T308 and 11.2 kb of flanking DNA located downstream (pSE17 [Fig. 1]). The *nptII* marker gene in *comC* was deleted via subcloning of a 12.1-kb *Bam*HI-*Pst*I fragment into pRK415 (21), resulting in plasmid pRK13 (Fig. 1). The DNA sequence downstream of *comC* was determined. Eight base pairs downstream of *comC* is the start of a stem-loop structure which could act as a transcriptional terminator for *comC*. Sixty-one base pairs downstream of *comC* is the start of an open reading frame (*comE*) with 507 nucleotides. Overlapping the stop codon of *comE* is the start codon of another open reading frame (*comF*) with 432 nucleotides. Both genes start with an ATG codon and are preceded by well-conserved and well-placed Shine-Dalgarno sequences. Seventy and 154 bp upstream of the start codons of *comE* and *comF*, respectively, are

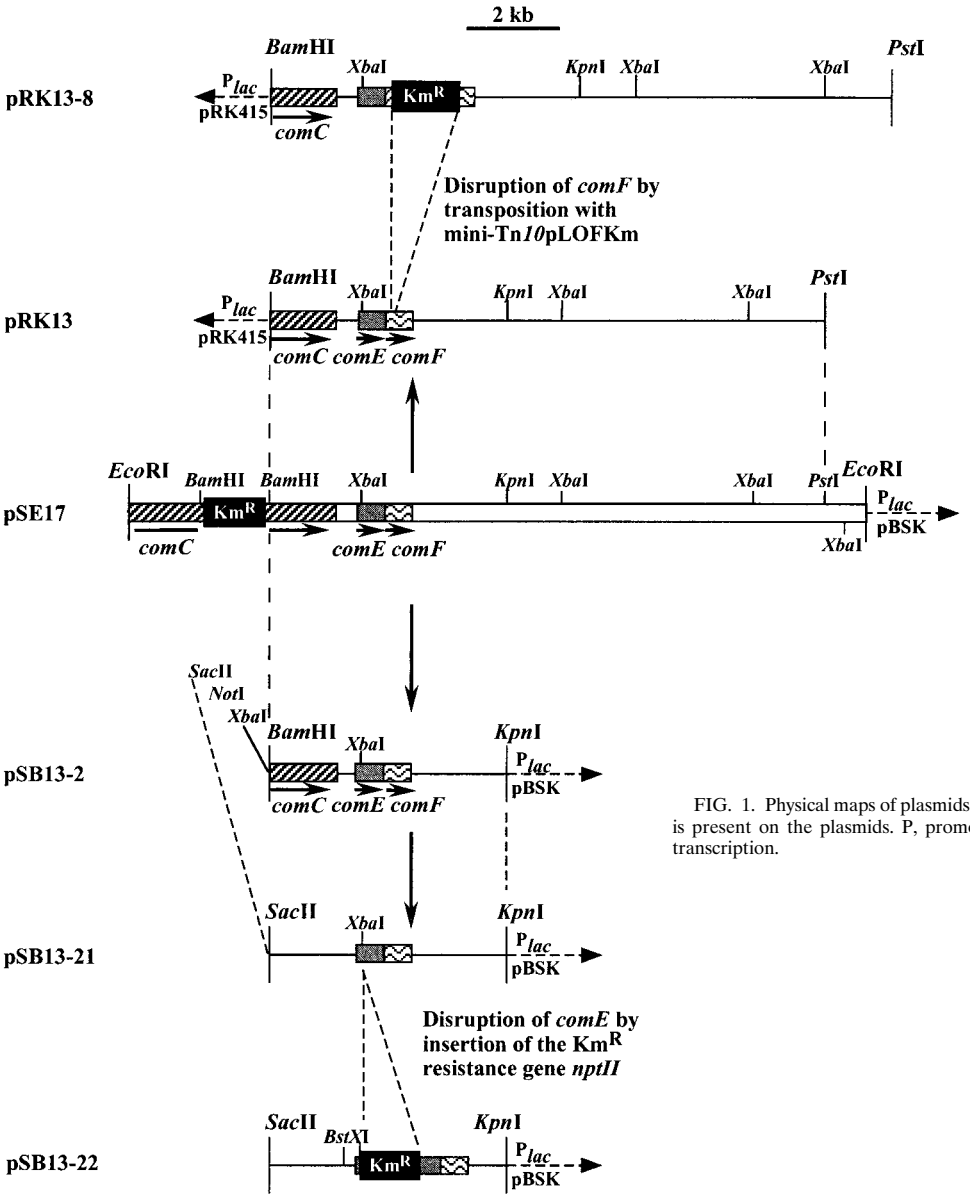



FIG. 1. Physical maps of plasmids used in this study. Only the 3′ end of *comC* is present on the plasmids. P, promoter. The arrows indicate the direction of transcription.

conserved −24(GG)/−12(GC) sites (2) for a presumptive $\sigma^{54}$ (RpoN)-dependent promoter. *comC*, *comE*, and *comF* are followed by inverted repeats which are predicted to act as rho-independent terminators of transcription (5), indicating that *comE* and *comF* are transcribed separately. One hundred forty-five base pairs downstream of *comF* is another open reading frame (*orf5*) whose deduced product is very similar to ribosomal protein S16 and, therefore, is probably not involved in natural transformation.

*comE* and *comF* encode polypeptides of 169 and 144 amino acids, respectively, with deduced molecular masses of 18.9 and 15.6 kDa, respectively. ComE and ComF have deduced isoelectric points of 9.23 and 9.33. Hydropathy analysis predicts a two-domain structure for both polypeptides, with a highly hydrophobic, α-helical (12), potentially membrane-spanning N-terminal region of ca. 30 residues and a hydrophilic C-terminal domain of ca. 100 residues. Database searches revealed that ComE and ComF are very similar to prepilins, the precursors of the structural subunits of type IV pili. Twenty-seven and 39% of the residues of ComE and ComF, respectively, are identical to residues in PilE from *Pseudomonas aeruginosa* (40), and 21 and 30% of their residues are conserved in ComP of *Acinetobacter* sp. strain BD413 (34). Thirteen to 25% of their residues are conserved in subunits of protein secretion systems, i.e., PulG of *Klebsiella pneumoniae* (37), OutG of *Erwinia carotovora* (36), and XcpT of *Pseudomonas* species (accession no. AF062532). Prepilins are characterized by a short leader peptide, which is removed upon maturation of the protein; an 8-amino-acid cleavage motif for an endopeptidase ($[KRHEQSTAG]_1$-$G_2$-$[FYLMIV]_3$-$[ST]_4$-$[LT]_5$-$[LIVP]_6$-$E_7$-$[LIVMFWSTAG]_8$); and a hydrophobic N terminus. These features are also present in ComE and ComF (Fig. 2). Taken together, this is clear evidence that *comE* and *comF* code for pilin-like proteins.

**Heterologous expression *E. coli*.** *comE* and *comF* were amplified by PCR and cloned as translational fusions to *malE*.

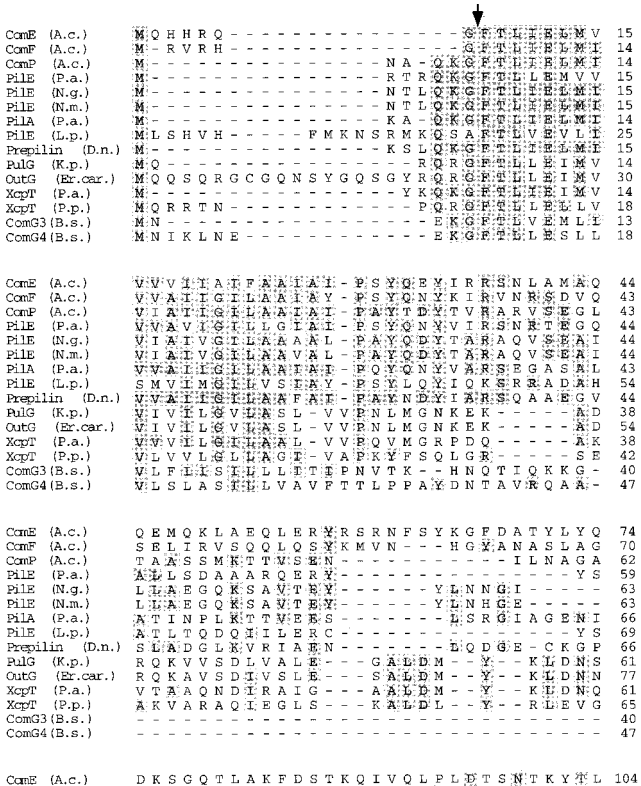
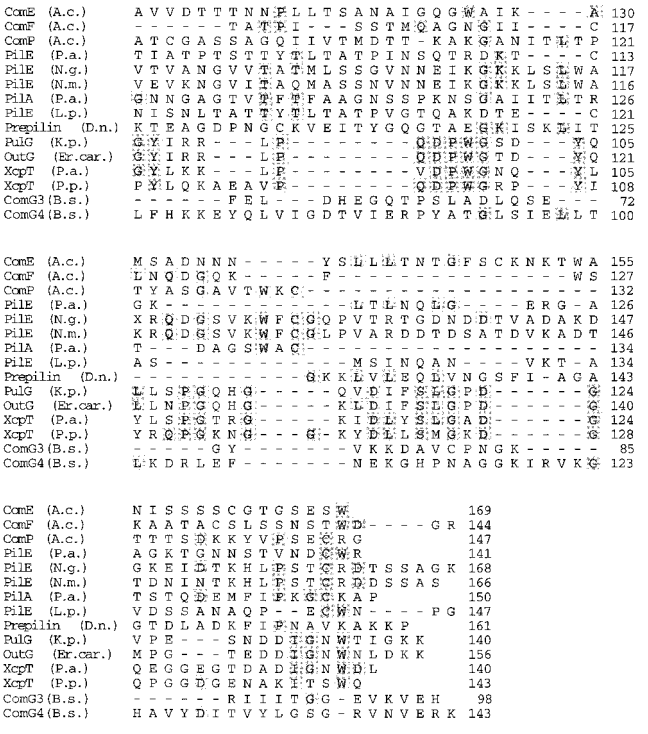


FIG. 2. Alignment of the deduced amino acid sequences of ComE and ComF with the sequences of pilins and pilin-like polypeptides. Residues identical in a minimum of 4 of the 15 proteins are indicated by gray boxes. The arrow denotes the endopeptidase cleavage site. Shown are the sequences of ComP of *Acinetobacter* sp. strain BD413 (A.c.) (34), PilE from *P. aeruginosa* (P.a.) (40), PilE from *N. gonorrhoeae* (N.g.) (3), PilE from *N. meningitidis* (N.m.) (45), PilA from *P. aeruginosa* T2A (6), PilE from *L. pneumophila* (L.p.) (44), prepilin from *Dichelobacter nodosus* (D.n.) (4), PulG from *K. pneumoniae* (K.p.) (37), OutG from *Erwinia carotovora* (Er.car.) (36), XcpT from *Pseudomonas alcaligenes* (P.a.) (13), XcpT from *Pseudomonas putida* (P.p.) (accession no. AF062532), and ComG3 and ComG4 from *B. subtilis* (B.s.) (1).

Unexpectedly, induction of expression led to growth inhibition of the host. These findings strongly suggest that the fusion products of MalE and complete ComE or ComF proteins are toxic to *E. coli* cells; this might be due to the pilin-like structural features, indicating a pilin-analogous processing and export of these proteins. This suggestion is supported by the finding that *malE-comE* or *malE-comF* fusions devoid of the first 34 or 53 codons (encoding the hydrophobic N termini) are expressed in *E. coli*. Expression was followed by purification of the fusion proteins by affinity chromatography. Coomassie staining of the purified, SDS-PAGE-separated MalE-ComE and MalE-ComF fusions led to the detection of chimeric proteins of the expected sizes (Fig. 3). Factor Xa treatment of the preparations revealed polypeptides with molecular masses of 15 and 10 kDa, which match exactly the deduced masses of the N-terminally deleted versions of ComE and ComF.



**ComE and ComF are involved in natural transformation.** The similarity of ComE and ComF to pilin-like polypeptides such as the competence factor ComP of *Acinetobacter* sp. strain BD413, together with the close physical association of *comE* and *comF* with *comC*, led us to speculate that ComE and ComF might play a role in natural transformation of *Acinetobacter* sp. strain BD413. To address this question, we generated insertion mutants and analyzed the mutants with respect to their transformation phenotypes.

Plasmid pRK13 (Fig. 1) was subjected to transposon mutagenesis, using the suicide vector pLOF/Km carrying a mini-Tn*10* element (18). Restriction analysis of one resulting plasmid, pRK13-8, revealed a mini-Tn*10* insertion in *comF*, 0.6 kb downstream of *comC* (Fig. 1). The plasmid pRK13-8 was digested with *Xba*I, generating a 6.1-kb *Xba*I fragment carrying the kanamycin resistance gene plus 0.4 kb upstream of *comF* and 3.6 kb downstream of *comF* (Fig. 1). This DNA fragment was used to transform *Acinetobacter* sp. strain ADP239, and transformants were selected on kanamycin agar. Thereby, the wild-type copy of *comF* was successfully replaced by a mutated version, as verified by Southern hybridization. Most interestingly, the *comF* mutant T501 was found to have a 1,000-fold-reduced transformation frequency ($3.2 \cdot 10^{-7}$ transformants/viable cell in the early logarithmic growth phase) compared to the transformation wild-type strain ADP239 ($1.6 \cdot 10^{-4}$ transformants/viable cell). To clearly elucidate a role for *comF* in natural transformation and to exclude any polar effect of the transposon insertion, a 475-bp fragment carrying *comF* was amplified from plasmid pSE13 via PCR and cloned into the broad-host-range plasmid pRK415 in the correct orientation with respect to the *lac* promoter. The resulting plasmid, pCR5 (Table 1), was able to restore the wild-type transformation phenotype. These results give clear evidence that *comF* is involved in DNA translocation of *Acinetobacter* sp. strain BD413.

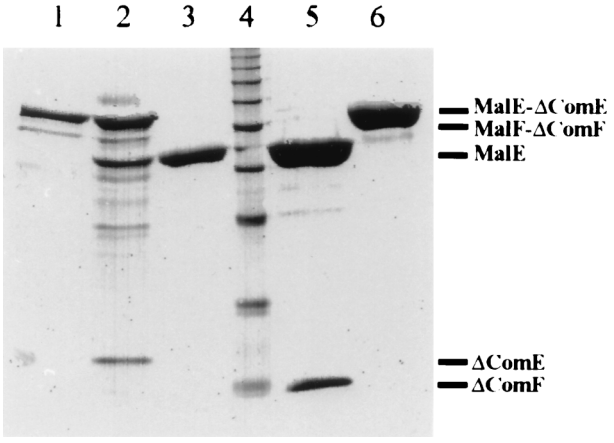



FIG. 3. SDS-PAGE of heterologously expressed ComE and ComF. 5′-deleted *comE* (102 bp deleted) and *comF* (159 bp deleted) were amplified via PCR and fused to *malE* by using the vector pMalc2. The fusion proteins were isolated by affinity chromatography and separated on an SDS–15% polyacrylamide gel. Lane 1, untreated MalE-ComE; lane 2, MalE-ComE treated with factor Xa; lane 3, MalE; lane 4, molecular mass standards; lane 5, MalE-ComF treated with factor Xa; lane 6, untreated MalE-ComF.

To determine whether *comE* is also involved in natural transformation, the chromosomal *comE* gene was replaced by a mutated version (see Materials and Methods). The resulting mutant, T503, was found to have 10-fold-reduced transformation frequency ($1.6 \cdot 10^{-4}$ transformants/viable cell), but the wild-type transformation phenotype was restored by pRK13-8 (Fig. 1). No complementation was found with pCR5, encoding ComF. These results demonstrate that ComE is also involved in natural transformation of strain BD413.

**Effect of *comE* and *comF* mutations on piliation, pilus-mediated twitching motility, adhesion, lipase secretion, and degradation of hexadecane.** The significant similarities of ComE and ComF to type IV pilins raised the question of whether ComE and ComF are involved not only in DNA translocation but also in other functions requiring pili or pilus-like structures, such as protein secretion and twitching motility. First, there was no difference in piliation of mutants T501 and T503 and the wild-type transformation strain ADP239, as revealed by electron microscopy. All strains contained on their surfaces the two characteristic types of pili; bundle-forming thin fimbriae with a diameter of 3 to 4 nm and thick fimbriae of 6 nm in diameter were identified. The thin pili have been shown to be important for adherence to hydrocarbons and for agglutination of the cells (38), whereas the thick pili mediate a special kind of surface translocation termed twitching motility (17). However, mutants T501 and T503 were not impaired in twitching or adherence to hexadecane. These results strongly suggest that ComE and ComF are not part of the type IV pili of *Acinetobacter* sp. strain BD413.

Recently, a mutant of *Acinetobacter* sp. strain BD413 unable to grow on hexadecane and defective in secretion of lipase was described (31). Complementation studies led to the identification of a gene, *xcpR*, encoding a pilin-like protein similar to components of GSPs in gram-negative bacteria, indicating a pilus-like structure involved in growth on hexadecane and in lipase secretion. However, mutants T503 and T501 were not impaired in hexadecane degradation or lipase secretion, suggesting that the DNA uptake machinery is unrelated to the proposed GSP in *Acinetobacter* sp. strain BD413.

## DISCUSSION

In this study, we have identified two competence genes, *comE* and *comF*, of *Acinetobacter* sp. strain BD413. ComE, ComF, and the previously described competence factor ComP (34) are similar to pilins and pilin-like components involved in transport of biomolecules across the cytoplasmic membrane, periplasmic space, and outer membrane. Interestingly, the competence factors from *Acinetobacter* are more closely related to pilins than to pilin-like components involved in protein secretion. ComE and ComF group with PilE from *P. aeruginosa* and *Legionella pneumophila*, whereas ComP is more closely related to PilE from *N. gonorrhoeae* and *Neisseria meningitidis*. ComP, ComE, and ComF are assumed to be the structural subunits of a DNA-translocating pilus-like structure. The presence of multiple pilin-like genes which are all involved in natural transformation has so far only been observed in *Acinetobacter* sp. strain BD413, *B. subtilis*, and *S. pneumoniae*. From the effect of the mutation on the transformation frequency, it is tempting to speculate that ComE is indirectly implicated in natural transformation (*comE* mutants exhibit a 10-fold-reduced transformation frequency) whereas ComF and ComP are probably more directly involved in natural transformation (*comF* mutants exhibit a 1,000-fold-reduced transformation frequency, while *comP* mutants are completely transformation negative). Apparently, the absence of ComE or ComF can be tolerated (although to different degrees), or ComE or ComF can be replaced by ComP to some extent. On the other hand, ComE or ComF cannot substitute for ComP.

The similarities between the *Acinetobacter* competence factors and the prepilins raised the central question of whether ComE and ComF are involved in pilus biogenesis and whether the *Acinetobacter* sp. strain BD413 pili are directly involved in DNA binding and uptake. A dual function of pilins had been reported for the structural subunit of gonococcal pili, PilE, which was found to be essential for pilus biogenesis and DNA translocation. Nevertheless, the question of whether pili are directly involved in DNA binding and uptake has not yet been settled. Experimental data suggest that the gonococcal pilus fibers themselves are neither essential nor sufficient for DNA uptake (39). On the other hand, the observation that gonococcal PilT mutants were defective in DNA uptake and pilus-mediated twitching motility but expressed morphologically intact pili indicated that the gonococcal pilus itself is essential for DNA translocation (48). The finding of morphologically intact pili on the surface of transformation-affected *comE* and *comF* mutants, together with the finding of intact pili functions such as twitching motility and adhesion, suggests that *comE* and *comF* are not essential in the biogenesis and function of the two types of pili found in *Acinetobacter* sp. strain BD413. The same is true for ComP (34). Together with the finding that ComE, ComF, and ComP are very important for DNA transformation, this led us to the proposal that the pilus structures are not involved in DNA transformation. It remains to be seen whether pili and the DNA transformation system in *Acinetobacter* sp. strain BD413 are related or unrelated systems.

Pilin-like components have already been identified as playing a major role in GSPs, also referred to as type II secretion pathways. GSPs are involved in translocation of extracellular proteins across the outer membrane in a wide range of gram-negative bacteria (24, 35, 46). The observation that competence factors of *B. subtilis* and *N. gonorrhoeae* and components of GSPs are similar to proteins involved in the biogenesis of type IV pili (19) has brought forth the hypothesis that evolutionarily related general systems of surface-associated protein complexes transport biomacromolecules across the cell enve-

lope. Based on these findings, the question of whether the pilin-like competence factors ComE and ComF exhibit a dual function in the DNA translocation system and GSP of *Acinetobacter* sp. strain BD413 becomes obvious. The GSP system in *P. aeruginosa* has been intensively investigated (10). These studies have clearly shown that the formation of adhesive type IV pili and the secretion of exoenzymes are related but nevertheless independently operating processes. The components of these two systems in *P. aeruginosa* include distinct sets of proteins, but the different systems share at least PilA, the major subunit of type IV pili, and PilD, the prepilin peptidase (25, 26).

Recently the first subunit of the GSP in *Acinetobacter* sp. strain BD413 was identified (31). This subunit, XcpR, is similar to PilB, a type IV pilus assembly factor of *P. aeruginosa*. From the finding that an XcpR mutant (WH399) was not impaired in natural transformation (31), together with the unaffected lipase secretion phenotype of the transformation-affected *comE* and *comF* mutants, we conclude that the GSP and the DNA transformation machinery in *Acinetobacter* sp. strain BD413 are functionally distinct systems.

Secondary-structure analysis suggests that ComE and ComF have α-helical, hydrophobic N termini, and N-terminal hydrophobic α-helices are highly conserved among pilins. There exists experimental evidence that the N termini of pilins are of importance for polymerization of the pilus to an intact homomeric helical pilus fiber, and the hydrophobic packing and flexibility of the N-terminal α-helices of pilins within a pilus fiber are discussed as being important features for pilus bending and twisting (11, 32, 33). Molecular and biochemical studies of type IV pili have led to a model of pilus architecture. According to this model, type IV pili are inserted into the cytoplasmic membrane and span the periplasmic space and the outer membrane via a helical arrangement of the structural subunits (32). The N-terminus similarities among ComE, ComF, ComP, and pilins strongly suggest that ComE and ComF as well as ComP are anchored in the cytoplasmic membrane and polymerized into a heteromeric helical structure, a rudimentary pilus structure which mediates DNA import. Experiments to define this structure are presently under way in our laboratory.

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