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A Novel Competence Gene, *comP*, Is Essential for Natural Transformation of *Acinetobacter* sp. Strain BD413

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***Acinetobacter* sp. strain BD413 (= ATCC 33305), a nutritionally versatile bacterium, has an extremely efficient natural transformation system. Here we describe the generation of eight transformation-affected mutants of *Acinetobacter* sp. strain BD413 by insertional mutagenesis. These mutants were found by Southern blot analysis and complementation studies to result from single *nptII* marker insertions at different chromosomal loci. DNA binding and uptake studies with one mutant, T205, revealed that the transformation deficiency of this mutant results from a complete lack of DNA binding and, therefore, uptake activity. A novel competence gene essential for natural transformation, named *comP*, was cloned by complementation of mutant T205. The nucleotide sequence of *comP* was determined, and its deduced 15-kDa polypeptide displays significant similarities to type IV pilins. Analysis of the ultrastructure of a transformation-deficient *comP* mutant and the transformation-competent wild-type strain revealed that both are covered with bundle-forming thin fimbriae (3 to 4 nm in diameter) and individual thick fimbriae (6 nm in diameter). These results provide evidence that the pilinlike ComP is unrelated to the piluslike structures of strain BD413. Taking all data into account, we propose that ComP functions as a major subunit of an organelle acting as a channel or pore mediating DNA binding and/or uptake in *Acinetobacter* sp. strain BD413.**

Uptake of exogenous DNA from the environment was the first mechanism of genetic exchange reported in bacteria (17). Today a large number of representatives of gram-negative and gram-positive bacteria are known to undergo natural transformation. Since transformation does not involve direct cell-to-cell interaction, it has been suggested that natural transformation most probably accounts for the exchange of DNA between distantly related microorganisms, providing a major mechanism for acquiring novel metabolic capabilities and adapting to changing environmental conditions (29, 30). A considerable amount of knowledge has been accumulated mainly with respect to the transformation systems in the gram-positive bacteria *Bacillus subtilis* and *Streptococcus pneumoniae* (11, 42). During the last 10 years an increasing amount of information has been collected concerning natural transformation in gram-negative bacteria, particularly with respect to determinants involved in natural transformation of pathogens, such as *Neisseria gonorrhoeae* (12) and *Haemophilus influenzae* (14). However, very limited information is available on the components of natural transformation systems of gram-negative bacteria inhabiting aquatic and terrestrial environments. To estimate the fate of released recombinant DNA and the influence of genetically engineered microorganisms on indigenous bacterial populations we need to understand the basic mechanisms of these DNA transfer processes in soil bacteria.

Representatives of the genus *Acinetobacter* capable of degrading a broad range of carbon sources are widely distributed in soil and water ecosystems. At least two *Acinetobacter* strains and their derivatives are naturally transformable (1, 26). One of these strains, formerly designated *Acinetobacter calcoaceticus* BD4 (26), has recently been renamed *Acinetobacter* sp. strain BD4. The high competence for natural transformation of strain BD4 and its miniencapsulated mutant, strain BD413, facilitated molecular studies of catabolic pathways (6, 10). The physiology of natural transformation in strain BD413 has been intensively studied. High competence is induced immediately after the transition from the lag phase to the exponential growth phase and gradually declines thereafter (33). DNA uptake is strongly dependent on divalent cations, such as Ca^{2+}, Mn^{2+}, or Mg^{2+} (28), and has been shown to require energy (32). Furthermore, *Acinetobacter* sp. strain BD413 does not discriminate between heterologous and homologous DNA and takes up DNA in the single-stranded form before it is integrated into the chromosome via a RecA-dependent recombination mechanism (32). To gain more insight into the mechanism of natural transformation of soil bacteria, we chose the highly transformable organism *Acinetobacter* sp. strain ADP239, a *pobA* (*p*-hydroxybenzoate hydroxylase) mutant of strain BD413, as a model bacterium to identify components of the DNA transformation system.

In this study we describe the generation of ADP239 mutants impaired in their ability to undergo natural transformation and report on the identification of a competence gene, designated *comP*. Although ComP displays significant amino acid similarity to type IV pilus subunits, it is not essential for pilus biogenesis. From our results we concluded that ComP functions in DNA binding and/or uptake. Due to its significant similarity to type IV pilins, we concluded that ComP is part of an oligomeric structure mediating DNA translocation through the outer membrane and periplasm in *Acinetobacter* sp. strain BD413.

MATERIALS AND METHODS

Strains and media. Strains and plasmids used in this study are shown in Table 1. Bacteria were grown in Luria-Bertani (LB) medium or in mineral medium (pH 6.8) containing (per liter of distilled water) 4.4 g of Na_2HPO_4, 3.4 g of KH_2PO_4, 1.0 g of NH_4Cl, 0.58 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of KNO_3, 0.067 g of $CaCl_2 \cdot 2H_2O$, 2 mg of $(NH_4)Mo_7O_{24} \cdot 4H_2O$, and 1 ml of trace element solution (45). Stock solutions of the carbon sources were sterilized and added to the media at final concentrations of 10 mM for succinate and 5 mM for benzoate or *p*-hydroxybenzoate. The following antibiotics were used: ampicillin (100 μg/ml), tetracycline (20 μg/ml), and kanamycin (20 μg/ml).

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant geno- and/or phenotype	Source or reference
Acinetobacter sp. strains		
BD413	Wild type	26
ADP239	Spontaneous *pobA* mutant of BD413	19
ADP197	*recA* mutant (*rec100*::Tn*5*) of BD413	15
Acinetobacter sp. strain ADP239 transformation-affected mutants		
T148	*pobA com*::*nptII*, Km^r	This study
T167	*pobA com*::*nptII*, Km^r	This study
T205	*pobA comP*::*nptII*, Km^r	This study
T206	*pobA comP*::*nptII*, Km^r	This study
T236	*pobA com*::*nptII*, Km^r	This study
T308	*pobA com*::*nptII*, Km^r	This study
T332	*pobA com*::*nptII*, Km^r	This study
T337	*pobA com*::*nptII*, Km^r	This study
T340	*pobA com*::*nptII*, Km^r	This study
E. coli strains		
DH5α	F^- *lacZ*Δ*M15 recA1 endA1 hsdR17 supE44* (*lacZYA argF*)	18
S17-1	*thi1 proA hsdR17* (r_k^-, m_k^+) *recA1*, tra^+ mob^+ Tp^r Sm^r	41
XL1-Blue	*recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1* (F *proA*$^+$ *B*$^+$ *lacZ*Δ*M15* Tn*10*)	8
Plasmids		
pBSK	Ap^r	Stratagene
pRK415	Tc^r, *lacp*/*o*	27
pUC4K	Ap^r Km^r	Pharmacia
pZR408	2.7-kb *Sst*I-*Acc*I *pobA* insert in pUC18	6
pRT14	14-kb *Xba*I fragment in pRK415, Tc^r	This study
pRT6	5-kb *Sst*I fragment of pRT14 in pRK415, Tc^r	This study
pRT2	2.7-kb *Hin*dIII-*Sst*I fragment of pRT6 in pRK415, Tc^r	This study
pRT1	1.6-kb *Pst*I fragment of pRT6 in pRK415, Tc^r	This study
pCP1	0.7-kb *Bgl*II-*Sca*I fragment of pRT6 in pRK415, Tc^r	This study

DNA isolation, manipulation, and sequencing. Standard genetic and molecular techniques were performed as described by Sambrook et al. (38). Restriction enzymes and T4DNA ligase were used as recommended by the manufacturers (Pharmacia Biotech Europe GmbH, Freiburg, Germany; Boehringer Mannheim GmbH, Mannheim, Germany). Plasmids were isolated by the alkaline lysis method, and chromosomal DNA was prepared by the method of Sambrook et al. (38). For cloning, competent cells of *Escherichia coli* were prepared and transformed by the method of Inoue et al. (23).

Probes for the hybridization experiments were labeled by incorporation of digoxigenin-11-dUTP with a random primed DNA labeling kit (Boehringer Mannheim GmbH). For Southern blots (43) DNA was transferred onto positively charged nylon membranes (GeneScreen Plus; DuPont, NEN Research Products, Dreieich, Germany) by vacuum blotting. Under low-stringency conditions the hybridizations were performed at 37°C as described by Anderson and Young (4). Hybridizations under high-stringency conditions were performed at 42°C in the presence of 40% formamide–5× SSC (1× SSC is 150 mM sodium chloride plus 15 mM sodium citrate, pH 7.0), and the filters were washed twice at 55°C in 0.1× SSC containing 0.1% sodium dodecyl sulfate. Subsequent detection of chemiluminescence was carried out by using a DIG luminescence detection kit (Boehringer Mannheim GmbH) according to the manufacturer's instructions. For autoradiography the membrane with the hybridized probe was exposed to Kodak X-Omat AR film.

DNA sequencing was carried out by the dideoxy chain termination method (39) by using an automated DNA sequencing system (ALF-Sequencer; Pharmacia Biotech Europe GmbH). The DNA was subcloned into pBluescriptII SK(+/−) (Stratagene, Heidelberg, Germany), and single-stranded DNA was isolated from *E. coli* XL1-Blue by using helper phage M13K07 (46). For primer walking, fluorescein-labeled oligonucleotides were purchased from NAPS GmbH, Göttingen, Germany. The sequences were analyzed by using a software package from the Genetics Computer Group of the University of Wisconsin (9).

Generation of mutants and screening for MMS sensitivity. Mutants defective in natural transformation were generated by insertional mutagenesis like that described by Palmen et al. (31). Genomic DNA of a *pobA* (*p*-hydroxybenzoate hydroxylase) mutant strain of BD413, designated ADP239 (Table 1), was digested with *Bam*HI, and the resulting fragments of genomic DNA were ligated to the kanamycin resistance marker (*nptII*) from pUC4K (Pharmacia Biotech Europe GmbH), which was isolated as a 1.3-kb *Bam*HI fragment from an agarose gel. Competent ADP239 recipient cells were prepared by adding 10 μl of succinate (1 M) to a 5-ml overnight culture. After incubation for 30 min at 30°C the ligation mixture containing 2 μg of donor DNA was added to 1 ml of cells spent in culture medium and was incubated for 45 min at 30°C. To allow expression of kanamycin resistance (Km^r) 2 ml of LB medium was added, and the suspension was incubated at 30°C for 4 h. Appropriate dilutions were then plated onto nutrient agar plates containing kanamycin, and Km^r transformants were selected. To identify transformation-deficient mutants, the Km^r transformants were replica plated onto mineral plates overlaid with *Hin*dIII-digested chromosomal DNA of BD413. Transformation-deficient *Acinetobacter* mutants were identified after overnight incubation at 30°C by their failure to grow on *p*-hydroxybenzoate as they could not acquire the *pobA* wild-type allele. The proficiency of homologous recombination in these mutants was tested as described by Gregg-Jolly and Ornston (16) by spreading dilutions of overnight cultures onto fresh LB medium plates containing 0.04 to 0.06% methylmethane sulfonate (MMS). After 24 h of incubation at 30°C, colony counts on MMS plates were compared with colony counts on plates that did not contain MMS. RecA mutant strain ADP197 was used as a control.

Transformation and conjugation procedures and construction of an *Acinetobacter* sp. strain BD413 genomic library. To determine the maximal natural transformation frequencies of transformation-impaired mutants, 1-ml overnight cultures were inoculated into 25 ml of mineral succinate medium. Samples (0.5 ml) were taken at different times during growth and incubated for 1 h at 30°C in growth medium with 1 μg of chromosomal DNA of BD413. After this incubation 50 μg of DNase was added, and appropriate dilutions of the cell suspensions were spread onto mineral agar containing *p*-hydroxybenzoate to select *pobA*$^+$ transformants.

A genomic library of strain BD413 was constructed by using pRK415 as the vector. *Xba*I-digested genomic DNA of *Acinetobacter* sp. strain BD413 was ligated into pRK415 and transformed into competent cells of *E. coli* S17-1. Spot matings of the transformation-deficient recipients with recombinant *E. coli* S17-1 donor cells carrying pRK415 recombinant plasmids were performed by mobilization of the plasmids and a filter mating technique. Filters with a mixture of donor and recipient strains at a 1:9 ratio were incubated overnight at 30°C on the surfaces of LB medium plates. Cells were then suspended in mineral medium, and appropriate dilutions were plated onto LB agar containing 20 μg of tetracycline per ml. The ability of transconjugants to take up DNA via natural transformation was determined by spotting transconjugants onto agar containing *p*-hydroxybenzoate as a carbon source and the selective antibiotic, as well as an overlay of 0.1 μg of chromosomal DNA of strain BD413. Cultures were incubated at 30°C overnight.

DNA uptake and binding studies. For DNA binding and uptake studies pZR408 template DNA (Table 1) was radiolabeled with α-^{35}S-dATP (10 μCi/μg of DNA) by using a nick translation system (Gibco-BRL GmbH, Eggenstein, Germany). A 10-ml portion of competent cells in mineral medium was incubated with 40 ng of α-^{35}S-dATP-radiolabeled DNA per ml at 30°C. At the times indicated below, samples (0.5 ml) were taken and immediately separated from

the medium by centrifugation (1 min, 14,000 × *g*) through silicon oil (0.2 ml; $\delta^{25°C} = 1.02$ g/ml). The top layer and the silicone oil were removed, and the tip of the cup containing the cell pellet was cut off and placed in a scintillation vial. Cells were lysed by addition of 0.5 ml of NaOH (3 M) and incubation at 20°C for 12 h. Then 5 ml of Optifluor scintillation fluid (Canberra-Packard, Frankfurt, Germany) was added, and the radioactivity was determined with a scintillation counter (Tri-Carb 1900TR; Canberra-Packard). The values were corrected for the 1,100 cpm detected at time zero. This value was taken as the value for rapid specific and nonspecific attachment of DNA since 2 min of sample preparation is sufficient to allow DNA binding to *Acinetobacter* cells (28).

To determine the amount of DNA bound to the cell surface, 2-ml cultures were grown in succinate mineral medium, collected in the mid-log phase (optical density at 600 nm, 1) by centrifugation, and transferred into 1 ml of mineral medium containing 10 mM EDTA to inhibit DNA uptake. The cell suspensions were incubated for 5 min 30°C with 1 μg of α-^{35}S-dATP-labeled DNA (40 μCi/μg of DNA). Subsequently, cells were collected by centrifugation, washed twice, resuspended in 1 ml of mineral medium containing EDTA, and split into two 0.5-ml fractions. To determine the amount of DNase-insensitive DNA bound to the cells, one of the fractions was mixed with DNase I (100 μg/ml) and subsequently incubated for 2 min at 20°C. After this treatment all cells were collected, washed once, and resuspended in 1 ml of mineral medium. Then 0.2 ml of the cell suspension was mixed with 5 ml of Optifluor scintillation fluid (Canberra-Packard), and the radioactivity was determined with a scintillation counter (Tri-Carb 1900TR; Canberra-Packard). The amount of radioactivity found in the cell pellet after DNase I treatment was assumed to represent the level of nonspecific binding and attachment to the cell surface and was subtracted from the other values.

Electron microscopy. Cells of an overnight culture of mutant T205 grown in mineral medium were carefully washed and then negatively stained with 2% (wt/vol) phosphotungstic acid. After drying on Formvar-coated copper grids, the cells were viewed with a Philips model EM301 transmission electron microscope at 80 kV.

Twitching motility. Twitching motility was analyzed by streaking an overnight culture on a freshly prepared plate of agar containing (per liter of distilled water) 5 g of tryptone, 2.5 g of yeast extract, 5 g of NaCl, and 20 g of agar. Since a humidified atmosphere facilitates twitching motility, the culture plates were incubated for 24 h at 30°C in a gas-tight jar which contained a petri dish filled with water. The cells were analyzed for twitching motility by the appearance of spreading zones along the central streak of growth.

Nucleotide sequence accession number. The complete pRT2 DNA sequence data (2,690 kb) have been deposited in the GenBank database under accession no. AF012550.

RESULTS

Generation and genetic characterization of noncompetent and transformation-deficient mutants of *Acinetobacter* sp. strain ADP239. To generate transformation mutants, chromosomal DNA of strain ADP239 was digested with *Bam*HI and the resulting fragments were ligated randomly to a 1.3-kb *Bam*HI fragment of pUC4K containing a *nptII* marker gene encoding Km^r. After transformation of the ligation mixture into competent cells of *pobA* mutant strain ADP239, Km^r transformants were selected. Out of 17,500 colonies obtained which contained random insertions of the Km^r marker gene (*nptII*), we identified eight transformation-deficient mutants on the basis of their reduced ability to acquire the *pobA* gene during transformation with wild-type DNA, which was indicated by a failure to grow in the presence of *p*-hydroxybenzoate. Five of these mutants, designated T167, T205, T308, T337, and T340, were found to be completely noncompetent. Three mutants, designated T148, T236, and T332, had 10×- to 23×-reduced maximal transformation frequencies of 2.0×10^{-5} to 4.6×10^{-5} transformant/viable cell compared to competent ADP239 cells, which exhibited a maximal transformation frequency of 4.6×10^{-4} transformant/viable cell in the early logarithmic growth phase. All of the defective mutants exhibited kanamycin resistance, a transformation-deficient or noncompetent phenotype, and the characteristic ADP239 phenotype of PobA dysfunction preventing growth with *p*-hydroxybenzoate even after growth for more than 100 generations under nonselective conditions. All eight mutants showed a high proficiency of DNA repair during growth in the presence of the DNA-alkylating agent MMS, excluding the possibility that the transformation-deficient phenotypes were a result of an impaired RecA function.

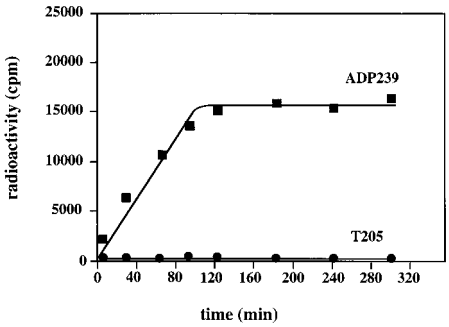

FIG. 1. DNA uptake by *Acinetobacter* sp. strain ADP239 and transformation-deficient mutant strain T205. Competent cells (10 ml; 1×10^9 cells ml^{-1}) of strain ADP239 and mutant strain T205 were incubated in mineral medium supplemented with 40 ng of α-^{35}S-dATP-radiolabeled DNA per ml at 30°C. At the times indicated, 0.5-ml samples were taken and the radioactivity of the cells was determined as described in Materials and Methods.

Analysis by Southern hybridization of the mutant DNA under high-stringency conditions revealed that the *nptII* gene hybridized with single chromosomal DNA fragments differing in size. These results provided evidence that the phenotypes of the eight mutants resulted from single marker gene insertions at different loci within the chromosome of strain ADP239. In the following experiments we focused on one of the mutants, T205.

DNA uptake and binding defect of mutant T205. As shown in Fig. 1, strain ADP239 took up DNA at a linear rate, which reached a maximum of 16,700 cpm after 120 min. In contrast, mutant T205 did not take up any DNA. This property of mutant T205 could have resulted from a defect either in the uptake or the binding of DNA. To determine the relative extents of DNA binding of strains T205 and ADP239, a binding assay under uptake-inhibitory conditions was performed (see Materials and Methods). Determination of the cell-bound radioactivity revealed a DNase-sensitive label of 1.25×10^4 dpm in the cell pellet of competent strain ADP239, whereas a DNase-sensitive label of only 500 dpm was found in the cell pellet of noncompetent strain T205 (data not shown). These results show that in comparison to the DNA binding to strain ADP239, the binding of DNA to mutant T205 was reduced by 96%. Since this value represents the lower limit of detection of specific binding, these experiments gave conclusive evidence that mutant T205 was defective in binding and, therefore, uptake of DNA.

Identification of a plasmid clone that complements the transformation deficiency of mutant T205. In order to identify the gene affected in mutant T205, a plasmid library of *Xba*I-digested chromosomal DNA of *Acinetobacter* sp. strain BD413 was constructed in pRK415 (Table 1). This plasmid library was mobilized into transformation-deficient mutant T205, and Tc^r transconjugants were screened in the presence of wild-type genomic DNA for their ability to undergo natural transformation to allow growth on *p*-hydroxybenzoate. This led to the identification of plasmid pRT14, which fully restored competence in strain T205 to wild-type levels (Fig. 2). Restriction analysis, subcloning, and complementation studies revealed that the ability to complement the transformation deficiency of T205 resided in a 2.7-kb *Hin*dIII-*Sst*I fragment, designated pRT2 (Fig. 2).

All of the strain T205 transconjugants tested exhibited the *nptII*-encoded Km^r phenotype, and plasmid pRT2 could be

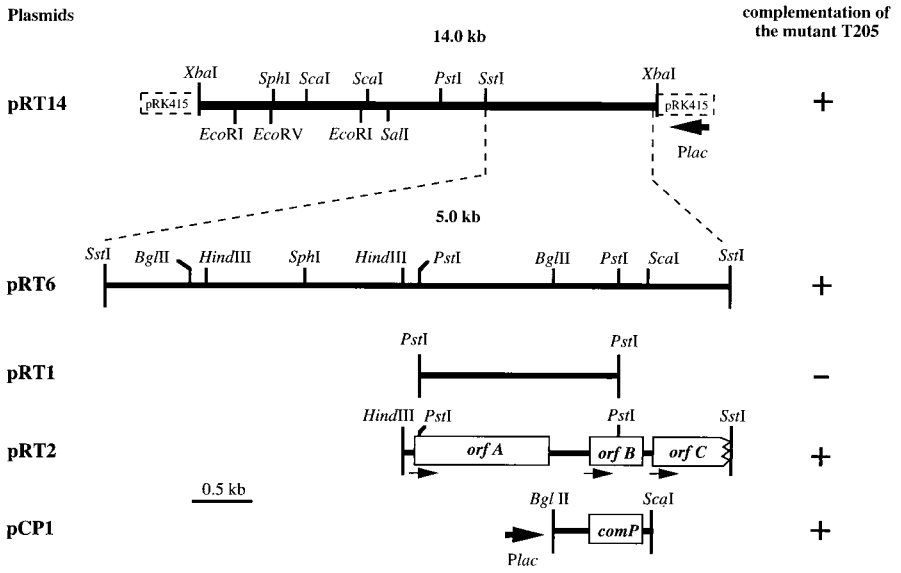

FIG. 2. Complementation analysis and physical map of plasmids used in this study. pRK415 was used as the cloning vector. The locations of important restriction sites, two complete open reading frames, designated *orfA* and *orfB* (= *comP*), and one incomplete open reading frame, designated *orfC*, are indicated. P*lac*, *lac* promoter of pRK415. The direction of transcription of *orfA*, *orfB*, and *orfC* determined by sequence analysis is indicated by the small arrows beneath pRT2.

recovered from all of the transconjugants. This suggested that mutant T205 was complemented by pRT2 in *trans*. The complementation of the transformation deficiency was found to be independent of the insert orientation with respect to the *lac* promoter (Fig. 2, complementation with pRT14, pRT2, and pCP1). This indicates that the wild-type allele complementing the transformation deficiency was expressed under the control of its native promoter.

Identification and sequence analysis of *comP*, a gene required for natural transformation in *Acinetobacter* sp. strain BD413. The complete nucleotide sequence of pRT2 (2,690 bp) was determined for both strands. Analysis of this DNA region revealed three open reading frames in the same orientation, designated *orfA*, *orfB*, and *orfC* (Fig. 2). *orfA* extended from nucleotide position 155 (ATG) to nucleotide position 1186 (TAA). The second open reading frame, *orfB*, had a size of 444 bp and extended from nucleotide position 1556 (ATG) to nucleotide position 1999 (TGA). *orfC* started at nucleotide position 2061 (ATG) and was found to be incomplete. In order to identify the gene responsible for complementation of the transformation deficiency of mutant T205, *orfB* was subcloned as a 0.7-kb *Bgl*II-*Sca*I fragment (pCP1) (Fig. 2) and subjected to complementation analysis. Transformation competence in mutant T205 was completely restored when *orfB* was provided in *trans*, which suggested that the transformation deficiency of mutant T205 resulted from a mutation in *orfB*. Due to this function *orfB* was designated *comP*.

To see whether *orfA* and *orfC* are also affected in mutant T205, a Southern analysis was performed. Chromosomal DNAs of ADP239 and mutant T205 were digested with *Hin*dIII and probed with *comP*. These studies revealed that the probe hybridized to a single 8-kb *Hin*dIII chromosomal DNA fragment from ADP239 but not to DNA from mutant T205 (data not shown). Since the *Hin*dIII-*Sst*I fragment of pRT2 did not contain a *Bam*HI site and this site was used for *nptII* marker insertion to generate mutant T205, this experiment indicated that a *Bam*HI fragment containing at least *orfA*, *orfB*, and *orfC* was deleted in mutant T205. Since pCP1 was able to restore transformation of mutant T205, a function of *orfA* and *orfC* in transformation was excluded. Additional hybridization studies performed under low-stringency conditions with *comP* as the probe and *Hin*dIII-digested genomic DNA of strain ADP239 always revealed a single hybridization signal with a single 8-kb DNA fragment (data not shown). These results suggest that *comP* is a single-copy gene.

The T205-complementing gene *comP* encodes a putative protein of 147 amino acids with a molecular weight of 15,000. *comP* is preceded by a ribosomal binding site and is terminated by three successive stop codons consisting of TGA and TAG (Fig. 3). Within a 370-bp spacer region between *orfA* and *comP* a conserved −24 (GG)–−12 (GC) site (5) for a presumptive σ^{54} (RpoN)-dependent promoter region was found. Downstream of *comP* an inverted repeat sequence could possibly function as a stem-loop structure for a transcriptional terminator.

Similarity of ComP to pilins of type IV pili. Database searches revealed significant amino acid similarity between ComP and pilins, the structural components of type IV pili in a number of bacteria (Fig. 4). The highest similarities were found to PilA of *Pseudomonas aeruginosa* (63% similarity, 48% identity), to TfpA of *Moraxella nonliquefaciens* (60% similarity, 43% identity), and to PilE of *N. gonorrhoeae* (52% similarity, 40% identity). Particularly within the first 40 amino acids of the N-terminal domains, the levels of identity were remarkably high, ranging from 76 to 93%. However, even the rest of the protein was 38 to 52% similar and 24 to 37% identical to pilins. Pilins have in common a short leader peptide, a cleavage motive for an endopeptidase, a hydrophobic N-terminal domain, a pair of cysteine residues near the carboxy terminus, and a characteristic protein size of 145 to 160 amino acids (22). These features were also found in ComP. Because of the high degree of similarity and the structural features we concluded that ComP is closely related to type IV pilins.

ComP is not essential for piliation and twitching motility. To address the question of whether ComP, analogous to the structural component of type IV pili (PilE) in *N. gonorrhoeae*, exhibits an essential function in both pilus biogenesis and natural transformation (37), we generated a defined mutant, des-

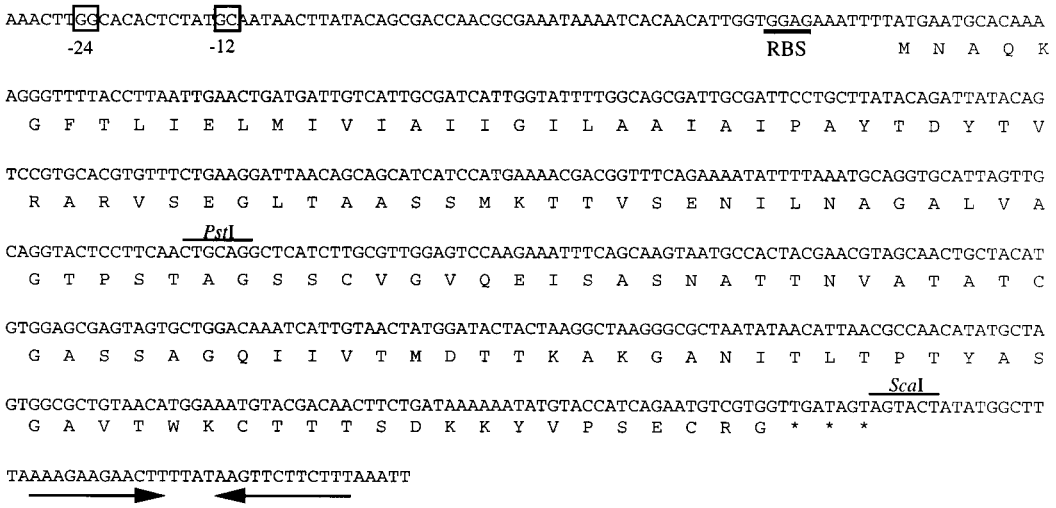

FIG. 3. Nucleotide sequence and deduced amino acid sequence of *comP*. The presumptive ribosomal binding site (RBS) and important restriction sites are indicated. The conserved −24/−12 regions for a putative σ^{54} binding site are enclosed in boxes. Downstream of *comP* an inverted repeat structure for a putative transcriptional terminator is indicated by arrows. The asterisks indicate stop codons.

ignated T206. This mutant was generated by deleting an internal 1.6-kb *Pst*I fragment from pRT2 and replacing this DNA fragment with a Km^r marker (*nptII*). This construct was then introduced into the chromosome of ADP239, resulting in noncompetent mutant strain T206. This mutant carried a defined deletion of *orfA* and the first 216 bp (49%) of *comP*.

Our electron microscopic investigation led to the identification of two different types of pili on the surface of transformation-deficient *comP* mutant T206, bundle-forming thin fimbriae with a diameter of 3 to 4 nm and thick fimbriae 6 nm in diameter (Fig. 5). Mutant T205 and transformation wild-type strain ADP239 had an identical piliation phenotype (data not shown). The production of these two types of pili by strains of *A. calcoaceticus* has been reported previously (20). Analysis of various *Acinetobacter* strains revealed that the thick pili mediate a special kind of surface translocation, termed twitching motility (21). Since the presence of thick pili does not exclude a defect in twitching, T205 and T206 were tested for their twitching ability, and we found that the mutants were not impaired in twitching (data not shown). These results provide substantial evidence that transformation factor ComP does not have an essential function in pilus biogenesis but is required for natural transformation.

DISCUSSION

Despite the importance of natural transformation among bacteria in microbial ecosystems, nearly nothing is known about the components required for the biogenesis of DNA transformation systems in soil microorganisms. In this paper we describe the generation of eight independent transformation-impaired mutants of the soil bacterium *Acinetobacter* sp. strain BD413. Using one of those mutants, we identified and characterized *comP*, a gene involved in transformation. From transport studies we concluded that ComP is essential for DNA binding and/or transport.

Clues to the structure and function of ComP can be derived from its similarity to pilins, the structural components of type IV pili. Type IV pili have been thoroughly investigated in several pathogenic bacteria (44); they are important virulence factors that function as bacterial adhesions and are also involved in twitching motility (21). A number of studies have shown that proteins with similarity to pilins are present in a wide range of gram-negative and gram-positive bacteria. Several of these pilinlike proteins, such as XcpT, XcpU, XcpV, and XcpW in *P. aeruginosa*, Pul's in *Klebsiella oxytoca*, OutG, OutH, OutI, and OutJ in *Erwinia* spp., ExeG, ExeH, ExeI, and ExeJ in *Aeromonas hydrophilia*, and XpsG, XpsH, XpsI, and XpsJ in *Xanthomonas campestris*, have been found to be involved in the secretion of various proteins (for a review see reference 22). Recently, even a pilin itself, PilE of *N. gonorrhoeae*, was found to be involved in natural DNA transformation (37).

The strong amino acid similarities suggest that transformation factor ComP, the pilinlike components of protein secretion systems, and the pilin itself are structurally similar. Pilus structure has been thoroughly studied in *N. gonorrhoeae* and *P. aeruginosa*, and the results have led to the proposal that the pilin subunits are arranged in a helical manner with five subunits per turn (34, 35). The pilus is inserted into the cytoplasmic membrane, spans the periplasmic space, and is extended into the medium. Pilinlike proteins involved in protein secretion systems are thought to function as portholes for import or export of macromolecules through the outer membrane, periplasm, and cytoplasmic membrane in gram-negative bacteria and through the cell wall and cytoplasmic membrane in gram-positive bacteria (22). However, it has to be noted that more than one protein is involved in building the structure. By analogy, we propose that ComP is assembled into a channel spanning at least the periplasmic space and the outer membrane of *Acinetobacter* sp. strain BD413. Since a loss of ComP was not reflected by a loss of a structure, as revealed by electron microscopy, it is evident that the ComP-derived channel does not extend into the medium. On the other hand, it is conceivable that the pilus consists of more than one component, one of which might be ComP. A loss of ComP would then not necessarily be reflected by a loss of the pilus. We expect that analysis of our mutant collection and Western blot and electron microscopic studies performed with ComP antibodies will shed more light on the structure of the DNA binding and uptake system and the function of ComP.

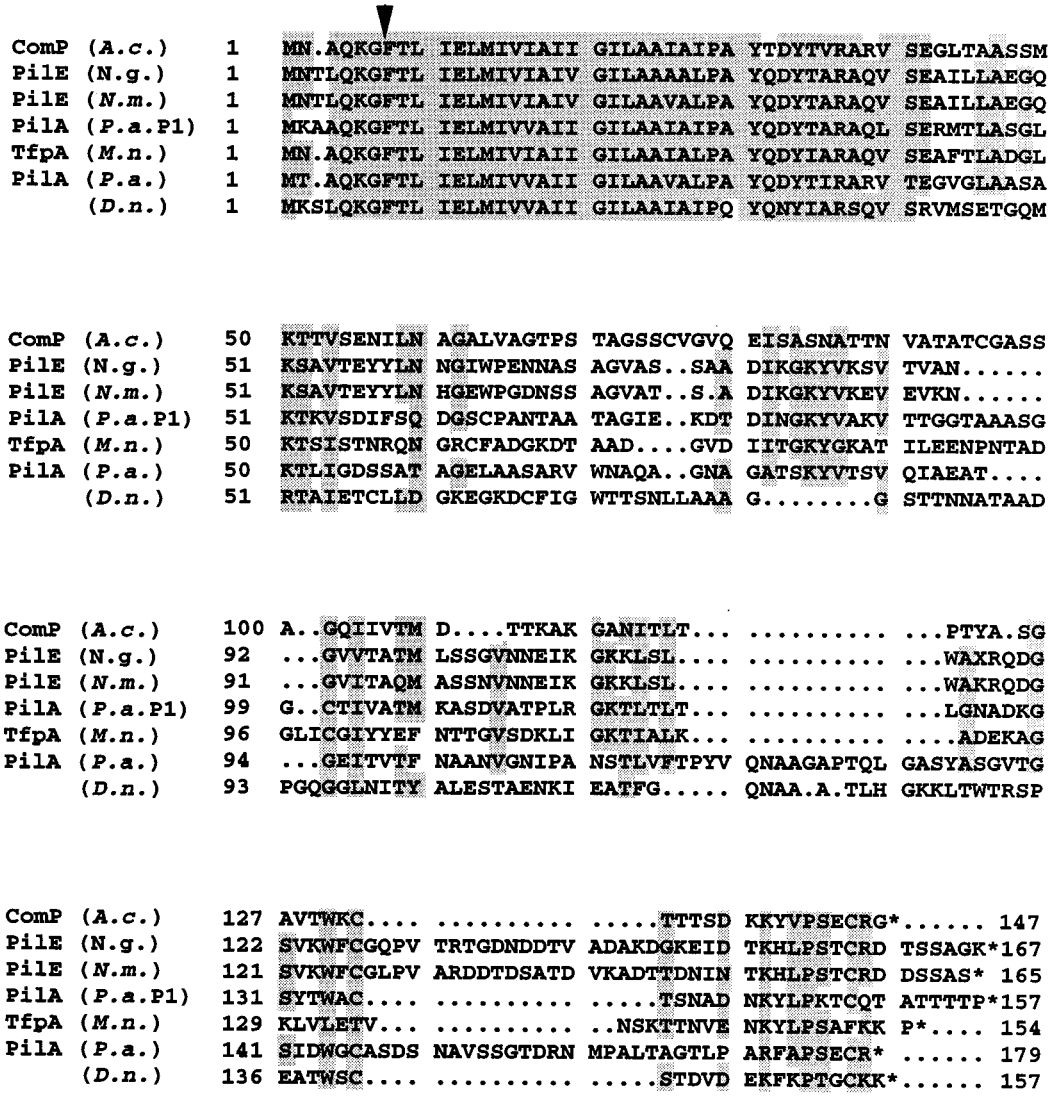

FIG. 4. Alignment of the ComP sequence with the sequences of PilE from *N. gonorrhoeae* (N.g.) (accession no. X66895) and *Neisseria meningitidis* (*N.m.*) (Z49820), PilA from *P. aeroginosa* (*P.a.*) (L37109) and *P. aeruginosa* P1 (*P.a.*P1) (M21651), TfpA from *M. nonliquefaciens* (*M.n.*) (M59707), and a prepilin from *Dichelobacter nodosus* (*D.n.*) (M13765). The arrowhead indicates the assumed cleavage site for a prepilin peptidase. Identical and homologous residues are shaded.

We do not interpret our results to mean that pili are involved in DNA uptake during natural transformation; rather, we believe that channellike structures spanning the outer membrane and periplasm, such as pili, protein secretion systems, and DNA uptake systems used in natural transformation, possess similar architectures, as has been suggested by Hobbs and Mattick (22) and Pugsley (36). However, the mechanism of transport and the structural features determining substrate specificity have to be resolved. Since leader sequences of pilins, pilinlike proteins involved in protein secretion, and ComP involved in natural transformation in *Acinetobacter* sp. strain BD413 are nearly identical, a common leader peptidase could be involved in their maturation. An example of this is the prepilin peptidase (PilD) of *P. aeruginosa*, which cleaves the prepilin PilA and the prepilinlike proteins XcpT, XcpU, XcpV, and XcpW of the protein secretion system (22). Experimental evidence for linkage of DNA transport and pilus formation has been presented for *N. gonorrhoeae*, in which a mutation in phase-variable pilus assembly and adherence factor PilC (24, 25) and the major subunit of the pilus, PilE (13, 40), revealed that the genes exhibit a dual function in pilus biogenesis and natural transformation (37). Although we cannot exclude the possibility that ComP has a function in pilus biogenesis, the presence of thin and thick pili on the cell surface independent of the *comP* deletion provides clear evidence that ComP is not essential for the biogenesis of these pili.

In competent *B. subtilis* cells three different competence factors, designated ComG-3, ComG-4, and ComG-5, were found to share similarities with pilins (2). It has been suggested that the ComG proteins are organized into a specific structure essential for DNA binding and DNA transport through the cell wall and the cytoplasmic membrane of *B. subtilis* (3, 7). Sequence alignments of ComP with the ComG proteins revealed no similarities apart from the typical type IV pilus leader sequence (data not shown), and therefore we concluded that assembly of ComP probably forms an oligomeric structure that resembles a rudimentary pilus but probably is quite different from the distinct ComG multimeric structure involved in DNA

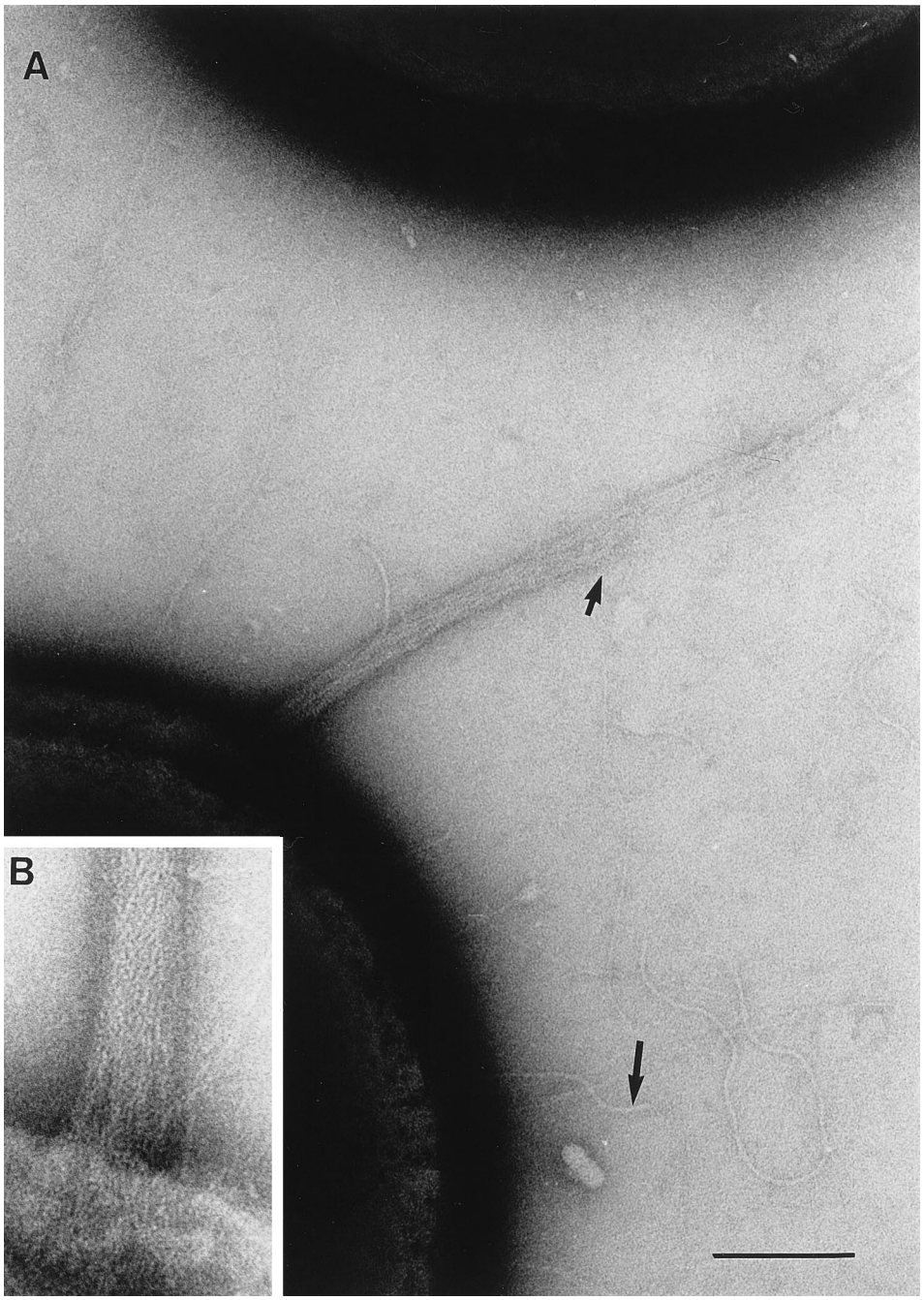

FIG. 5. Intact pili on the surface of *comP* mutant strain T206. Electron microscopic investigations were conducted with phosphotungstic acid-stained cells simultaneously grown to the logarithmic phase in mineral medium with succinate as the carbon source. (A) Representative sample showing thick pili (long arrow) and bundles of thin pili (short arrow) on the surface of mutant T206. Bar = 0.16 μm. (B) Membrane insertion site of a bundle of thin pili. Magnification, ×190,000.

translocation through the peptidoglycan layer and cytoplasmic membrane of *B. subtilis*. Although it seems not unreasonable that the ComG proteins in *B. subtilis* and the ComP protein in *Acinetobacter* sp. strain BD413 have a common evolutionary origin, they have diverged considerably to fulfill their functions in DNA transport processes in gram-negative or gram-positive bacterial strains.

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