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Requirement for Genes with Homology to ABC Transport Systems for Attachment and Virulence of *Agrobacterium tumefaciens*

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Transposon mutants of *Agrobacterium tumefaciens* which were avirulent and unable to attach to plant cells were isolated and described previously. A clone from a library of *Agrobacterium tumefaciens* DNA which was able to complement these chromosomal *att* mutants was identified. Tn*3*HoHo1 insertions in this clone were made and used to replace the wild-type genes in the bacterial chromosome by marker exchange. The resulting mutants were avirulent and showed either no or very much reduced attachment to carrot suspension culture cells. We sequenced a 10-kb region of this clone and found a putative operon containing nine open reading frames (ORFs) (*attA1A2BCDEFGH*). The second and third ORFs (*attA2* and *attB*) showed homology to genes encoding the membrane-spanning proteins (*potB* and *potH*; *potC* and *potI*) of periplasmic binding protein-dependent (ABC) transport systems from gram-negative bacteria. The homology was strongest to proteins involved in the transport of spermidine and putrescine. The first and fifth ORFs (*attA1* and *attE*) showed homology to the genes encoding ATP-binding proteins of these systems including *potA*, *potG*, and *cysT* from *Escherichia coli*; *occP* from *A. tumefaciens*; *cysA* from *Synechococcus* spp.; and ORF-C from an operon involved in the attachment of *Campylobacter jejuni*. The ability of mutants in these *att* genes to bind to host cells was restored by addition of conditioned medium during incubation of the bacteria with host cells.

Infections of wound sites in dicotyledonous plants by *Agrobacterium tumefaciens* result in the formation of crown gall tumors. The mechanism of pathogenesis involves the transfer of a DNA fragment (T-DNA) from the bacterial Ti plasmid to the plant host cell (4). An early step in tumor formation is attachment of bacteria to the plant cell surface (15). This attachment is required for pathogenesis since all known nonattaching mutants are avirulent (3, 5, 16, 25). We have previously isolated three Tn*5* nonattaching mutants (16). These mutants all mapped to the same region of the bacterial chromosome (close to *met6*) and contained Tn*5* insertions in a large *Eco*RI fragment (22). In order to characterize the process of bacterial attachment and to identify the genes involved, we examined this region of chromosomal DNA in greater detail.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Bacterial strains and plasmids used in this study were as previously described (14, 16, 19). Bacteria were grown in Luria broth, nutrient broth, or minimal medium, and viable cell counts were determined as previously described (14). *A. tumefaciens* was grown at 25°C. *Escherichia coli* was grown at 37°C. Antibiotics were used at the following concentrations: carbenicillin, 50 mg/liter; tetracycline, 10 mg/liter; chloramphenicol, 100 mg/liter; neomycin, 20 mg/liter for liquid media and 60 mg/liter for agar media; and gentamycin, 50 mg/liter. A library of *A. tumefaciens* NT1 DNA cloned as *Sau3*a partial digestion products into the *Bam*HI site of pCP13 was obtained from S. Farrand. A library of *A. tumefaciens* NT1 DNA cloned as *Sal*I partial digestion products into the *Sal*I site of pVK102 was obtained from E. W. Nester (3).

In order to obtain transposon insertions in cloned DNA in *E. coli*, the Tn*3*HoHo1 system described by Stachel et al. (24) was used. Tn*3*HoHo1 is an artificial transposon which carries the β-lactamase gene but lacks a transposase. Plasmids containing transposon insertions were introduced into *A. tumefaciens* by conjugation with pRK2013 as a mobilizing plasmid for mating as previously described (14). *A. tumefaciens* mutants in which the wild-type DNA was replaced by DNA containing the transposon insertion were obtained by marker exchange. Bacterial mutants were characterized with respect to their ability to attach to carrot suspension culture cells, their virulence on *Bryophyllum daigremontiana*, motility, and cellulose synthesis as previously described (14, 16).

The effect of conditioned medium on bacterial attachment and virulence. Conditioned medium was prepared and tested for its effect on bacterial attachment as previously described (17). Briefly, the procedure was as follows. Wild-type *A. tumefaciens* cells were incubated for 6 to 8 h with freshly transferred carrot suspension culture cells in Murashige and Skoog (MS) medium. The carrot cells were then removed from the medium by filtration through Miracloth, and the conditioned medium was filter sterilized by using a 0.2-μM-pore-size filter. Sterile conditioned medium was stored at 4°C. Butanol extracts of MS medium and of conditioned medium were prepared by mixing equal volumes of *n*-butanol and medium, vortexing the mixture for 2 min, and separating the butanol and water phases by centrifugation at 500 × *g* for 5 min. The butanol and water phases were then dried under vacuum and redisolved in the original volume of sterile water. They were tested for their effect on bacterial attachment to carrot cells with a 1:10 dilution in MS medium. Their effect on bacterial virulence was assayed by spreading 0.1 ml on the surface of a freshly cut asceptic carrot root disc. The disc was incubated for 15 min. Then, 0.1 ml of a stationary-phase culture of bacteria grown in Luria broth was added to the surface of the carrot disc. Carrot discs were incubated for 6 weeks at 25°C in petri dishes containing 1% water agar. The discs were scored for tumor formation once a week.

Expression of transposon insertions. The transposon Tn*3*HoHo1 can be used as a promoter probe in *A. tumefaciens* since it contains a promoterless β-galactosidase gene at one end. *A. tumefaciens* does not have a β-galactosidase gene. β-galactosidase was measured in *A. tumefaciens* strains as described by Stachel et al. (24).

DNA sequencing and analysis of sequence data. DNA fragments to be sequenced were subcloned using the protocols described by Maniatis et al. (13) into pBluescript KS$^-$ (Stratagene) or into pBC KS$^-$ (Stratagene) which had been modified in our laboratory by the deletion of 4 bp so that the *Sac*I site was removed. The DNA sequence of the modified vector in this region was determined to be CCGCGGTGGCCAGCTTT. Plasmid DNA was purified as previously described (14), and double-stranded sequencing was carried out at the University of North Carolina Nucleic Acid Sequencing Facility with a model 373A DNA Sequencer (Applied Biosystems) with the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing was carried out by walking from the ends of the clones, using as primers artificial oligonucleotides identical to a sequence near the end of the region previously sequenced. These

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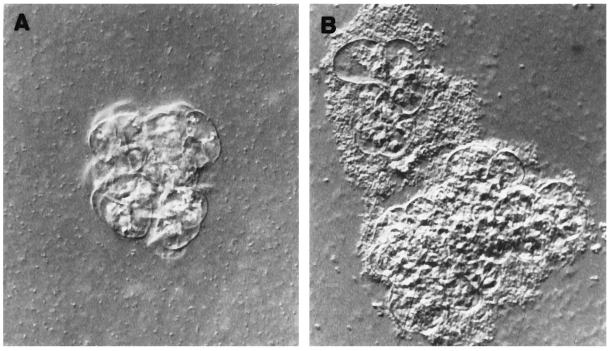

FIG. 1. Attachment of *A. tumefaciens* to carrot suspension culture cells as seen in the light microscope with living cells and Nomarski optics. (A) Nonattaching mutant Att-C43; (B) strain Att-C43 carrying plasmid pCP13.108. Note the absence of attachment in panel A and the presence of attachment in panel B. All cultures contained 10^8 bacteria per ml and 10^5 carrot cells per ml. The bacteria were incubated with the carrot cells for 20 h.

primers were synthesized by the University of North Carolina Oligonucleotide Synthesis Facility. Unless otherwise indicated, both strands of the DNA were sequenced. DNA sequences were aligned and analyzed by using the Genetics Computer Group (GCG) computer analysis programs. BlastX (1, 7) and Blocks (8) were used to search databases for protein amino acid sequences similar to those obtained by translating the DNA sequence. Similar sequences were aligned by using the Pileup program contained in GCG. Percent identity and homology were calculated with the GCG program Bestfit, using a gap weight of 3.0 and a gap length weight of 0.10. Probabilities that regions of the predicted protein sequence would be found in the membrane were determined by using the ALOM program for detecting membrane spanning proteins (11).

GenBank accession number. The GenBank accession number for the sequence reported here is L63540.

RESULTS

Identification of a chromosomal region containing genes required for attachment. Our laboratory has previously identified and mapped three closely-linked chromosomal Tn*5* mutants of *A. tumefaciens* which were avirulent and unable to bind to plant cells (16, 22). Random clones from a library of *A. tumefaciens* DNA were transferred to one of these mutants, Att-C43. Transconjugants were selected for the ability to grow on minimal medium containing tetracycline; this medium selected *A. tumefaciens* which contained a library clone. A heterogeneous population of *A. tumefaciens* transconjugants was then mixed with carrot suspension culture cells and incubated for 1 h at room temperature. The carrot cells were collected by filtration through Miracloth (Calbiochem), washed, and homogenized in a Waring blender to release any bound bacteria. The released bacteria were grown on minimal medium containing tetracycline, and individual colony isolates were tested for their ability to bind to carrot cells in the presence of tetracycline. The ability of the introduced plasmid to restore virulence was not tested because the plasmid was found not to be stably maintained in the absence of antibiotic selection (18).

Several overlapping cosmid clones that were able to restore to the mutant bacteria the ability to bind to carrot cells were identified. One such clone, pCP13.108, was kept and studied further (Fig. 1 and 2). This clone contained most of a large *Eco*RI fragment (more than 10 kb) which extended beyond the end of the insert. The original Tn*5* insertions which resulted in nonattaching mutants were in an *Eco*RI fragment of about 12 kb (16). The cloned DNA containing the Tn*5* insertion from Att-C43 hybridized to the large *Eco*RI fragment of pCP13.108 (data not shown). An additional larger cosmid clone, pG644, from the library of Cangelosi et al. (3), which overlapped pCP13.108 and contained all of the 12-kb *Eco*RI fragment, was identified by hybridization to the 1.5-kb *Pst*I fragment from the left end of pCP13.108 (Fig. 2).

Characterization of Tn*3*HoHo1 insertions. Transposon insertions were made at random in pCP13.108 and in the 4-kb *Eco*RI-to-*Bam*HI fragment from the left end of pG644 cloned into pRK415 (10). The position of insertions in the left end of the cosmid clones is shown in Fig. 2. The insertions were introduced into the *A. tumefaciens* chromosome by marker exchange. The position of the insertions was confirmed by Southern hybridization of the cloned 4-kb *Eco*RI-to-*Bam*HI fragment to *Eco*RI digested chromosomal DNA. Bacteria which carried the B215 insertion in the chromosome as a result of marker exchange grew very poorly in Luria broth or on Luria agar. They grew slowly in nutrient broth and in minimal medium. Bacteria which carried any of the other insertions exhibited normal growth rates. When marker exchanged into the bacterial chromosome, all of the transposon insertions in the left 10 kb of pCP13.108 caused the bacteria to become avirulent on leaves of *Bryophyllum daigremontiana*.

The ability of mutant bacteria carrying these transposon insertions to attach to carrot suspension culture cells was examined with the light microscope. No attachment was seen with insertions N004, B215, N016, N033, N013, B123, B124, A216, and B213 (Fig. 3C). Some attachment was observed with mutant bacteria carrying the B218, B112, and B117 insertions, but the attachment was much reduced and appeared abnormal (Fig. 3B). The lack of attachment of the C58::B215 mutant could be complemented in part by the introduction of pCP13.108 into the mutant (Fig. 3D). The lack of complete complementation was probably due to the fact that pCP13.108 was not stably maintained in these bacteria.

Since Tn*3*HoHo1 contains a promoterless β-galactosidase gene at one end, the expression of β-galactosidase in these Tn*3*HoHo1 mutants can be used to determine the direction of transcription of open reading frames (ORFs). All of the insertions in this region which were oriented to read from left to right gave β-galactosidase expression. Bacteria containing transposons that were oriented in the opposite direction showed no detectable β-galactosidase activity.

The effect of conditioned medium on attachment and virulence of mutants in *attA1A2BCDEFGH*. The presence of conditioned medium was previously shown to increase the ability of *A. tumefaciens* NT1 to bind to carrot cells (17). The effect of conditioned medium on the binding of *att* mutants was examined. Conditioned medium was able to restore the ability of all of the mutants carrying insertions in *attA1A2BCDEFGH* except C58::B215 to bind to carrot cells (Table 1 and Fig. 4). The failure of C58::B215 to be complemented by conditioned medium may be due to its poor growth in the MS medium in which the attachment assays were done. Mutants in *att* genes located outside this region were not complemented by conditioned medium (Table 1 and Fig. 4) (18).

MS medium by itself causes the growth of callus tissue which is difficult to distinguish from tumor tissue. Therefore, in order to examine the effect of conditioned medium on virulence of *att* mutants, it was necessary to fractionate the medium. Conditioned medium was extracted with butanol. The ability of the dried and redissolved water and butanol phases to complement the attachment of C58::B105 to carrot cells was tested. The water phase failed to complement the mutant while the butanol phase restored the ability of the mutant bacteria to bind to carrot cells. Attachment was measured after 60 min of incubation of the bacteria with carrot cells. The percent bacterial inoculum attached (mean ± standard deviation of a minimum of three measurements) for each addition to MS medium was

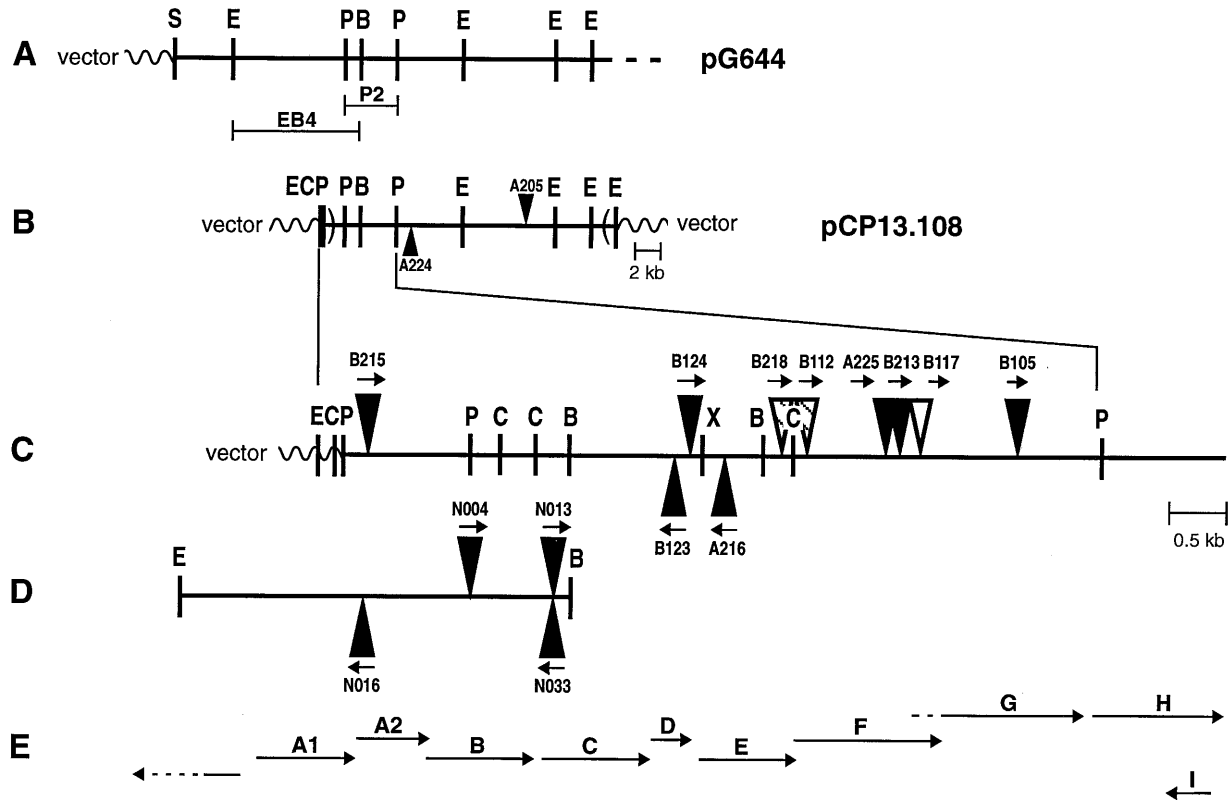

FIG. 2. Map of the cosmid clones pCP13.108 and pG644 carrying *att* genes. Panels A and B show the entire two cosmid clones. The right end of pG644 has not been determined. The locations of the *Pst*I fragment from pCP13.108 used to identify pG644 and of the *Eco*RI-to-*Bam*HI fragment subcloned into pRK415 to make Tn*3*HoHo1 insertions are shown in panel A. Panels C, D, and E are enlargements of the region containing the genes *attA1-attI*. Panel E shows the location and direction of ORFs. The location and orientation of Tn*3*HoHo1 insertions are indicated by triangles. All the transposon insertions resulted in an avirulent phenotype when used to replace the wild-type DNA in the parent bacterium. The insertions colored black also resulted in a nonattaching phenotype. The two striped insertions resulted in mutant bacteria which were able to bind to carrot cells, but the binding was distinguishable from that of the parent bacteria. The insertion indicated by an open triangle resulted in bacteria which were able to bind to carrot cells. Arrows indicate the direction of β-galactosidase transcription. Restriction sites are indicated as follows: E, *Eco*RI; C, *Cla*I; P, *Pst*I; B, *Bam*HI; X, *Xba*I; S, *Sal*I. The Tn*3*HoHo1 insertions shown in panel C were made in pCP13.108. The insertions shown in panel D were made in the *Eco*RI-to-*Bam*HI fragment subcloned into pRK415.

as follows: no addition, 10 ± 9; 1/10 volume of conditioned medium, 24 ± 6; 1/10 volume of the water phase from the butanol extractions of conditioned medium, 8 ± 8; 1/10 volume of the butanol phase from the butanol extractions of conditioned medium, 39 ± 11.

The effect of the butanol phase from the extraction of MS medium and of conditioned medium on the virulence of C58::B123 on carrot discs was examined. No tumors were formed when the disc was covered with the butanol extract of MS medium prior to the addition of bacteria, but when the disc was covered with the butanol extract of conditioned medium, mutant bacteria did cause the formation of tumors (Fig. 5).

DNA sequences and homologies of the *attA1A2BCDEFGH* genes. A region 10 kb long beginning at the *Eco*RI site at the left end of pG644 was sequenced. Nine ORFs were identified reading from left to right. On the 5′ side of the first ORF there is a space of 200 bp with no ORFs. To the left of this space is an ORF reading in the opposite direction from the ORFs described here (Fig. 2).

The first ORF, *attA1*, encodes a putative hydrophilic protein of 328 amino acids. This protein contains an ATP-GTP binding site motif A (P loop) and sequence blocks indicative of an ATP-binding protein involved in active transport. It has homology to many other proteins including the gene products of the *E. coli potG* (42% identity, 62% similarity), *E. coli potA* (38% identity, 62% similarity), and *Synechococcus* spp. *cysA* (41% identity, 64% similarity) genes (6, 12, 21). The *attA1* gene product also has significant homology to the putative protein encoded by the *attE* gene (36% identity, 61% similarity) downstream of the *attA1* gene (Fig. 6).

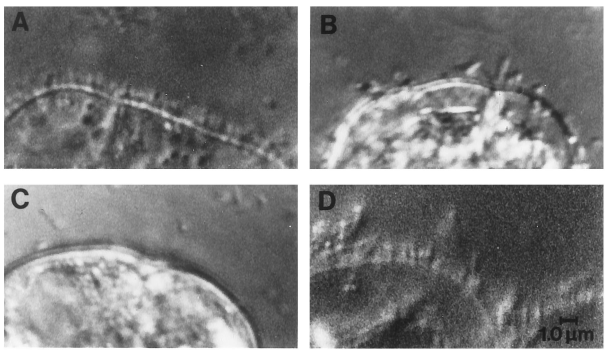

FIG. 3. Attachment of *A. tumefaciens* to carrot suspension cells as seen in the light microscope with living cells and Nomarski optics. (A) Wild-type parent strain C58. Note the numerous bacteria attached end on to the plant cell surface. (B) Mutant C58::B217. Note the sparse attachment and the random angle of the bacteria with respect to the plant cell surface. (C) Mutant C58::B215. No attached bacteria were seen. (D) Mutant C58::215 carrying the plasmid pCP13.108. Tetracycline was added to the medium. Note the partial restoration of attachment.

TABLE 1. Effect of conditioned medium on the attachment of various bacterial mutants

Bacterial strain	ORF containing Tn insertion[a]	% Inoculum[b] attached	
		MS medium[c]	CM medium[d]
C58	None (wild type)	35 ± 6	30 ± 10
C58::N016	*attA2*	4 ± 6	20 ± 8
C58::B215	*attA2*	2 ± 2	2 ± 2
C58::N004	*attB*	2 ± 6	15 ± 8
C58::N013	*attC*	0 ± 6	27 ± 13
C58::N033	*attC*	8 ± 8	25 ± 7
C58::B123	*attD*	1 ± 1	14 ± 3
C58::B124	*attE*	2 ± 3	23 ± 7
C58::B218	*attE*	3 ± 3	15 ± 1
C58::A225	*attF*	2 ± 2	14 ± 1
C58::B105	*attG*	10 ± 9	24 ± 6
C58::B213	*attG*	8 ± 7	19 ± 2
C58::A224	*attJ*	9 ± 8	1 ± 3

[a] ORF containing the Tn*3*HoHo1 insertion which resulted in the mutation.
[b] Mean ± standard deviation of a minimum of three measurements.
[c] Attachment was measured after 60 min of incubation of bacteria with carrot cells.
[d] Conditioned medium prepared by the incubation of C58 with carrot cells. Attachment was measured after 60 min of incubation of bacteria with carrot cells.

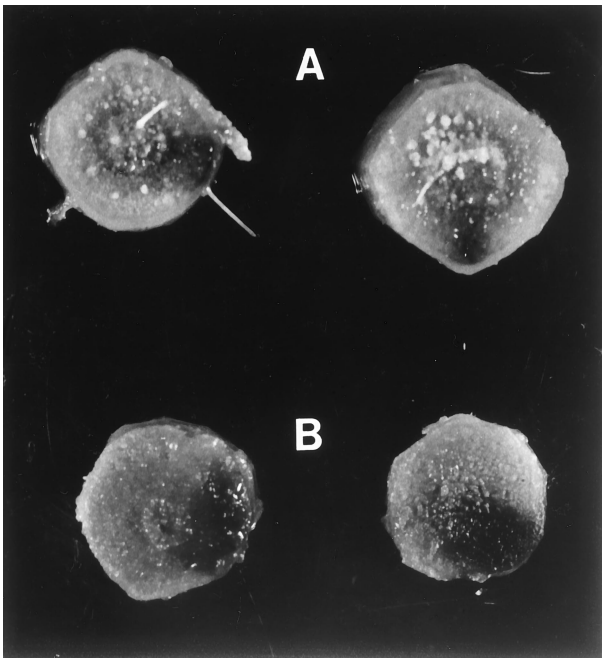

FIG. 5. The effect of conditioned medium on virulence of C58::B123 on carrot discs. (A) Carrot discs incubated with a butanol extract of conditioned medium for 15 min prior to the inoculation of C58::B123. (B) Carrot discs incubated with a butanol extract of MS for 15 min prior to the inoculation of C58::B123. Note the tumors present on the discs in panel A and the absence of tumors on the discs in panel B. The butanol extracts were dried and redissolved in water before use.

The second ORF, *attA2*, encodes a putative protein 300 amino acids long. The stop codon of the previous ORF (TGA) overlaps the start codon (ATG) of this ORF. A hydrophobicity profile of this protein shows six possible membrane-spanning domains (Fig. 7). This protein has homology to the *potB* (35% identity, 64% similarity) and *potH* (33% identity, 59% similarity) genes of *E. coli* (Fig. 8) (6, 21). The ORF ends with TGAACAGTG. The GTG is the first codon in the third ORF.

The third ORF, *attB*, encodes a putative protein of 285 amino acids. A hydrophobicity profile of this protein shows that it also has six possible membrane-spanning domains (Fig. 7). The protein has homology to *E. coli potC* (29% identity, 56% similarity) and *potI* genes (29% identity, 55% similarity) (Fig. 9) (6, 21). The ORF ends with a TAA codon 15 bp upstream from a possible Shine-Dalgarno sequence for the next ORF, *attC*.

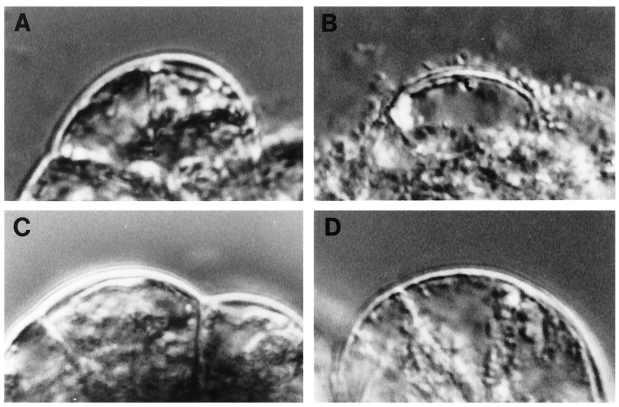

FIG. 4. Photomicrographs taken with Nomarski optics of live specimens showing the results of incubating bacteria with carrot cells for 24 h. (A) Bacterial mutant C58::B123 incubated with carrot cells in MS medium. (B) Bacteria mutant C58::B123 incubated with carrot cells in conditioned medium. (C) Bacterial mutant C58::A224 incubated with carrot cells in MS medium. (D) Bacterial mutant C58::A224 incubated with carrot cells in conditioned medium. Attachment of the first mutant to carrot cells could be promoted by conditioned medium. Attachment of the second mutant was unaffected by conditioned medium.

The putative protein encoded by *attC* is 339 amino acids long and has one possible membrane-spanning domain near the amino-terminal end (Fig. 4). This domain may represent a signal sequence. No significant homology of the predicted *attC* protein product to any protein in the databases was found. The ORF ends TGAATG with the ATG serving as a possible start for a short ORF (*attD*) encoding a hydrophilic protein of 96 amino acids. There is a space of 120 bp before the ATG at the start of the next ORF.

The next ORF (*attE*) encodes a hydrophilic protein of 264 amino acids. This protein contains an ATP-GTP binding site motif A (P-loop) and sequence blocks indicative of an ATP-binding protein involved in active transport. It has homology to many other proteins including octopine permease *occP* from *A. tumefaciens* (38% identity, 58% similarity), *E. coli* sulfate permease (39% identity, 60% similarity), *E. coli potA* (34% identity 57% homology), and ORF C (36% identity, 56% similarity) from an operon of *Campylobacter jejuni* known to be involved in attachment of this bacterium to intestinal cells (Fig. 6) (6, 20, 21, 26). The two putative ATP-binding proteins encoded in this region (the *attA1* and *attE* gene products) show homology to each other (36% identity, 61% similarity).

The stop codon of *attE* overlaps the start codon of *attF*, ATGA. This ORF encodes a putative protein of 420 amino acids which has three possible membrane-spanning domains (Fig. 7). No significant homology to this protein was found in the databases. The stop codon of *attF* overlaps a possible start codon of *attG*, ATGA. However, there is also another possible start for *attG* at an ATG located 168 bp upstream. If the upstream start is used, then this region of the DNA would be read in two different frames, one for *attF* and one for *attG*. The

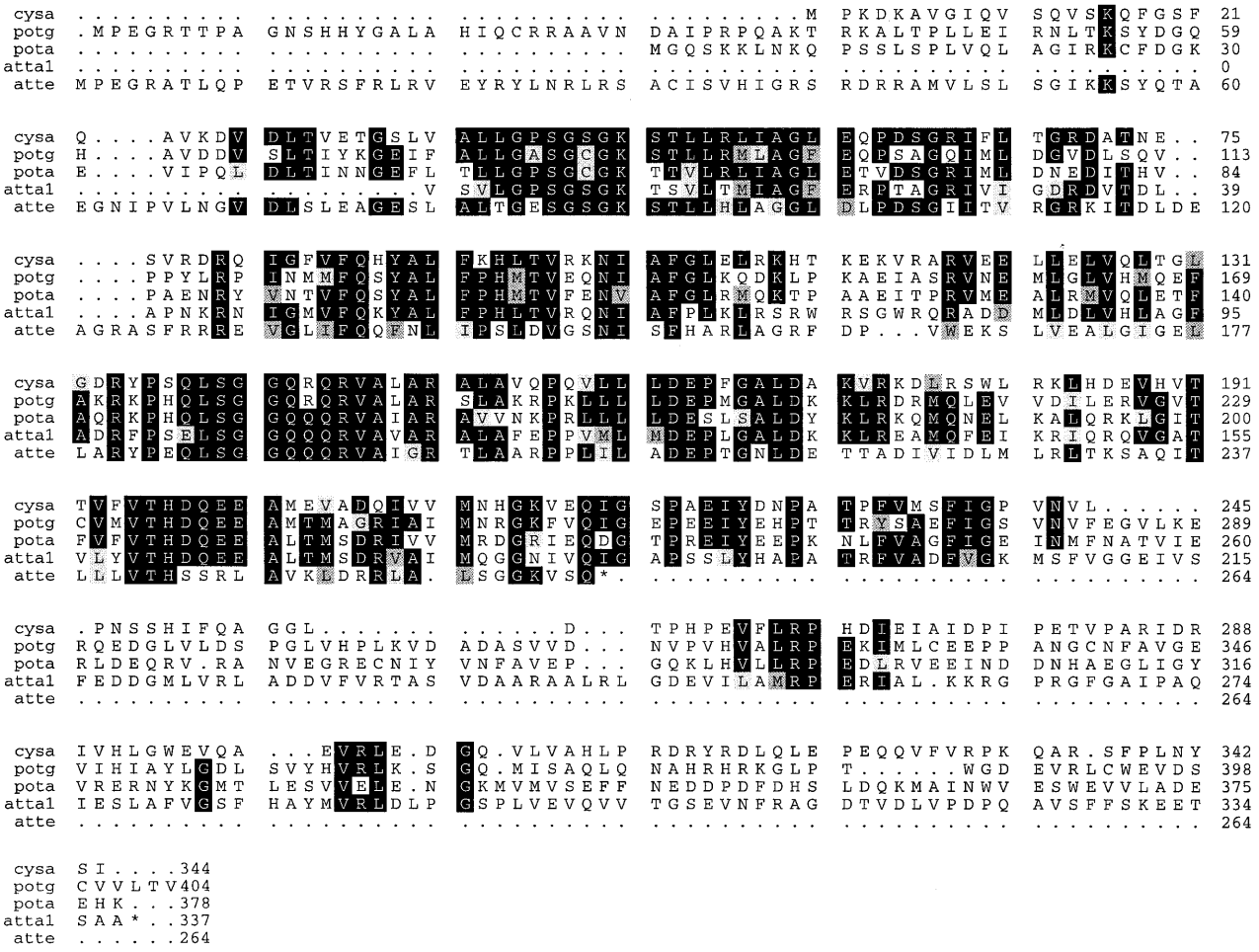

FIG. 6. Homology between *attA1*, *attE*, *potA*, *potG*, and *cysA*. Dark boxes indicate identical amino acids. Grey boxes indicate similar amino acids.

shorter of the two possible ORFs for *attG* encodes a putative protein of 382 amino acids with four possible membrane-spanning domains (Fig. 7). No sequences with significant homology to *attG* were found in the databases. There is a space of 60 bp between the end of *attG* and the start of *attH*, which encodes a putative protein of 355 amino acids. The protein contains one possible membrane-spanning region located at amino acids 5 to 22 which may represent a signal sequence. No sequences with significant homology to *attH* were found in the databases.

An ORF reading in the opposite direction to *attH* is found a few base pairs downstream of the end of *attH*, suggesting that *attH* is the last gene in this group of genes. The direction of transcription of all of these ORFs (*attA1A2BCDEFGH*) agrees with the direction of transcription determined from the Tn*3*HoHo1 insertions.

DISCUSSION

A region of the bacterial chromosome which contains genes required for bacterial attachment and virulence has been cloned. We have analyzed the left 10 kb of this region. It contains nine possible ORFs, one of which is short (*attD*, 96 amino acids predicted protein length). The arrangement of the genes suggests that all of them are transcribed as one operon although it is possible that a second promoter exists in the space between *attD* and *attE*. The other genes are so closely spaced that it is likely that they are cotranscribed. Four of these sequences have homology to genes known to be involved in ABC transport systems (also called periplasmic binding protein-dependent transport systems) (2, 9). The homology is closest to genes of the *pot* operons of *E. coli* which are involved in the transport of putrescine and spermidine into the cell and appear to play a role in the response of the bacteria to high osmotic strength. Homology was observed to the two membrane-spanning proteins of the *pot* operons; *attA2* showed homology to *potH* and *potB* and *attB* showed homology to *potC* and *potI* as well as *cysT*, which appears to play a similar role in the transport of sulfate (6, 21, 23).

attA1 and *attE* showed homology to the ATP-binding proteins of these transport systems. The homology was not significantly better for any particular one of the ATP-binding proteins, which included *occP*, *potA*, *potG*, *cysA*, and many other members of this family (12, 21, 23). Interestingly, among this group of proteins is ORF-C from *C. jejuni*, which is in an operon known to be required for attachment. However, no homology was found between any of the *A. tumefaciens att* gene products and PEB-1, which is thought to be the major cell-binding factor of *C. jejuni* (20). The predicted gene products of the other five *att* genes found in this region (*attC*, *attD*,

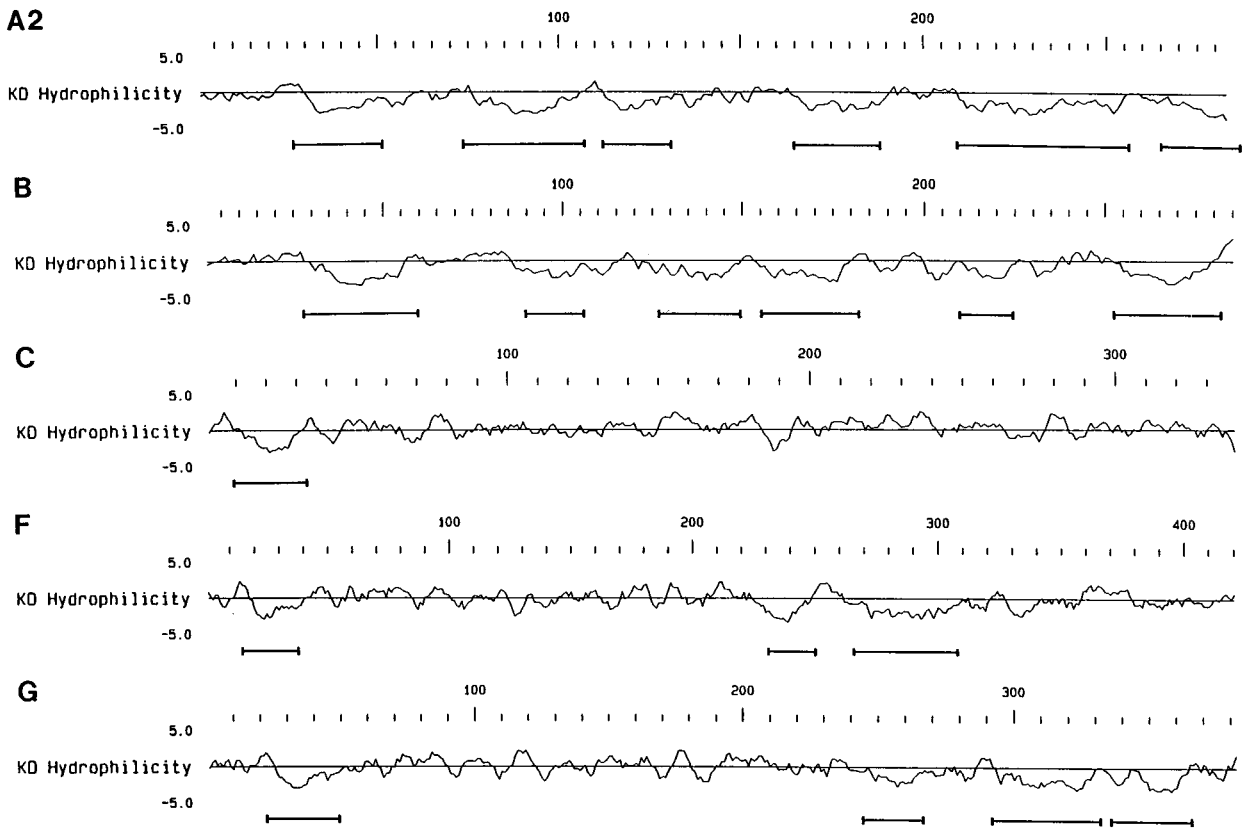

FIG. 7. Hydropathy plot of the predicted protein products of the *attA2*, *attB*, *attF*, and *attG* genes. The domains predicted by ALOM (12) to be localized in the membrane are underlined.

attF, *attG*, and *attH*) showed no significant homology to any protein in the databases.

The significance of the homology of *att* genes to genes encoding ABC transport systems is presently unclear. Many ABC transport systems function in uptake into bacterial cells. Others are involved in secretion. Thus homology to an ABC transport system does not indicate whether *att* gene products are involved in transport into or out of the cell. The ability of conditioned medium to restore binding of the mutants to carrot cells suggests that these *att* mutants fail to accumulate some substance in the medium which is required for bacterial attachment. The *att*-encoded ABC transporter could be involved in the secretion of this substance. Alternatively it could be involved in the transport into the bacteria of some signal from the plant which then results, indirectly, in the accumulation of the active substance in the medium.

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potb   . . . . . .  . . . . . .  . . . . . .  . . . M I V T I V G  W L V L F V F L . P  N L M I I G T S F .  25
poth   . . M S T L E P A A  Q S K P P G G F K L  W L S Q L Q M K H G  R K L V I A L P Y I  W L I L L F L L . P  F L I V F K I S L A  57
atta2  M S A S S L A S R A  I A N L Q S V M F S  T P S A K T Y S H A  G A L L L L L P A A  L M L L T G F V Y P  L A R L V A L S M P  60

potb   . L T R D D A S F V  K M V . . . . . .  . . . F T L D N Y T  R L L . D P L Y F E  V L L H S L N M A L  I A T L A C L V L G  73
poth   E M A R A I P P Y T  E L M E W A D G Q L  S I T L N L G N F L  Q L T D D P L Y F D  A Y L Q S L Q V A A  I S T F C C L L I G  117
atta2  D M S . . . . . .  . . . . . .  . . . . .  L E L Y I  R I F R D P L Y L N  V L F S T T V V A L  A V M C C C L L L G  98

potb   Y P F A W F L A K L  P H K V R P L L L F  L L I V P F W T N S  L I R I Y G L K I F  L S T K G Y L N E F  L L W L G V I D T P  133
poth   Y P L A W A V A H S  K P S T R N I L L L  L V I L P S W T S F  L I R V Y A W M G I  L K N N G V L N N F  L L W L G V I D Q P  177
atta2  F P V A N A M V R L  K H P W T L I L T G  C V L I P L W T S V  L V R S Y A W I V L  L Q R T G I I N Q A  L L S T K L I D E P  158

potb   I R I M F T P S A V  I I G L V Y I L L P  F M V M P L Y S S I  E K L D K P L L E A  A R D L G A S K L Q  T F I R I I I P L T  193
poth   L T I L H T N L A V  Y I G I V Y A Y V P  F M V L P I Y T A L  I R I D Y S L V E A  A L D L G A R P L K  T F F T V I V P L T  237
atta2  L R M I Y T Q G A V  I V A M T H V L L P  F M I M P I F A A L  R A I P P E Y V Q A  A M N L G S T R L H  A F R A I I V P L S  218

potb   M P G I I A G C L L  V M L P A M G L F Y  V S D L M G G A K N  L L I G N V I K V Q  F L N I R D W P F G  A A T S I T L T I V  253
poth   K G G I I A G S M L  V F I P A V G E F V  I P E L L G G P D S  I M I G R V L W Q E  F F N N R D W P V A  S A V A I . . I M  294
atta2  L P G I F A G C V I  S F V L A L G F Y V  T P A L V G G A G S  L M V A T L I G Q Q  T T V L L D W P F A  A A L S T S L L V V  278

potb   M G L M L L V Y W R  . . . A S R L L N K  K V E L E . 275
poth   L L L L I V P I M W  . . . F H K H Q Q K  S V G E H G 317
atta2  T L A V V I I F R R  T L S L S K G L N S  G I * . . . 300
```

FIG. 8. Homology between *attA*, *potB*, and *potH*. Dark boxes indicate identical amino acids. Grey boxes indicate similar amino acids.

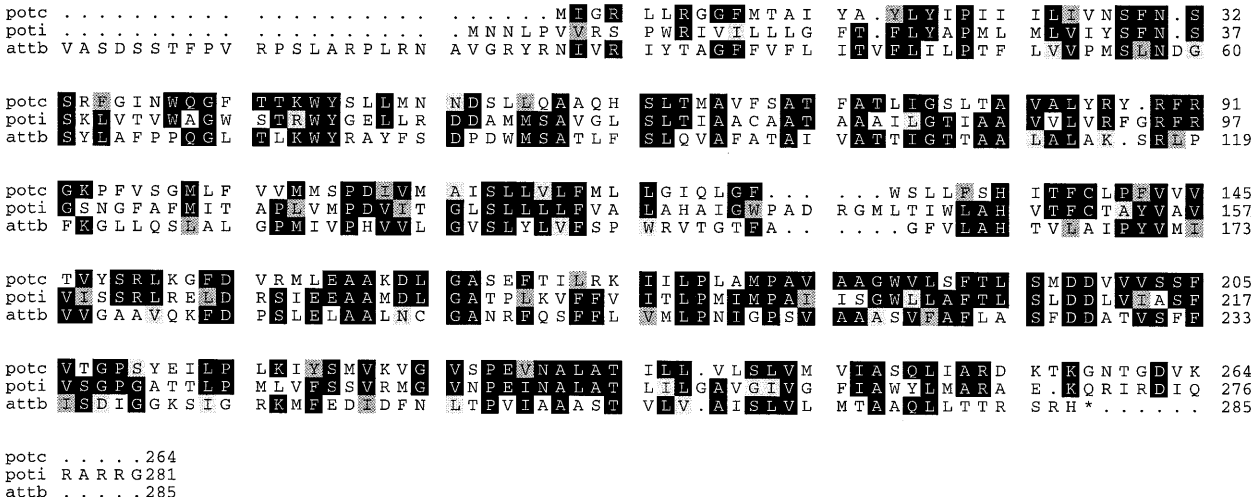

FIG. 9. Homology between *attB*, *potC*, and *potI*. Dark boxes indicate identical amino acids. Grey boxes indicate similar amino acids.

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