INFECTION AND IMMUNITY, Dec. 1996, p. 5098–5105 Vol. 64, No. 12
0019-9567/96/$04.00+0

Characterization of the *fim2* and *fim3* Fimbrial Subunit Genes of *Bordetella bronchiseptica*: Roles of Fim2 and Fim3 Fimbriae and Flagella in Adhesion

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Received 21 March 1996/Returned for modification 11 June 1996/Accepted 5 September 1996

With DNA probes derived from the fimbrial subunit genes *fim2* and *fim3* of *Bordetella pertussis*, two homologous subunit genes of *Bordetella bronchiseptica* were identified and cloned. The nucleotide sequences of these genes were determined. Comparison of these nucleotide sequences with the *B. pertussis* fimbrial *fim2* and *fim3* subunit genes showed a pronounced homology. Therefore, the *B. bronchiseptica* genes were also designated *fim2* and *fim3*. Expression of the two *B. bronchiseptica* genes was demonstrated by Western blotting (immunoblotting) with polyclonal antiserum directed against the Fim2 and Fim3 fimbrial subunit proteins of *B. pertussis* and by enzyme-linked immunosorbent assay with monoclonal antibodies. After growth of *B. bronchiseptica* in the presence of $MgSO_4$, no expression of both fimbrial subunit genes was observed. While no fimbriae were expressed, expression of flagella was observed under these circumstances. A longer C-stretch (up to 19 cytosine residues) than the one in front of the *fim2* and *fim3* genes of *B. pertussis* is present in front of the *B. bronchiseptica* fimbrial genes. In adherence experiments, fimbriated (Bvg^+) as well as flagellated (Bvg^-) *B. bronchiseptica* bacteria were able to adhere to HeLa cells, whereas nonflagellated *B. pertussis* did not. This suggests that fimbriae as well as other factors (possibly flagella) contribute to adherence of *B. bronchiseptica* to eukaryotic cells.

All *Bordetella* species are able to colonize the respiratory tract. A number of virulence factors play a role in this colonization. In *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica*, expression of these virulence factors is coordinately regulated by the *bvg* locus (bordetella virulence genes) (25, 42). The proteins encoded by the two genes of the *bvg* locus (*bvgS* and *bvgA*), a two-component sensory transduction system similar to those occurring in many other bacteria (15, 41, 43), are responsible for reversible regulation of the production of virulence factors. Modulating environmental signals like nicotinic acid, a temperature of 25°C, and sulfate are sensed and lead to a reduced expression of genes encoding virulence factors, the so-called *vag* genes (virulence activated genes). This regulation is known as antigenic (phenotypic) modulation (17, 45). The *bvg* locus is also involved in the regulation of several virulence repressed genes (*vrg*) (5). The *vrg* genes are expressed in the Bvg^- state, i.e., in the presence of modulating signals. Five yet-unidentified *vrg* products in *B. pertussis* have been described (5). One of these products (Vrg-6) seems to play a role in colonization and lymphocytosis, as observed in a respiratory infection model in mice (5). The flagella of *B. bronchiseptica* may be regulated by other *vrg* genes (1, 2).

Besides antigenic modulation, the expression of virulence genes is also regulated by a genetic event known as phase variation. Phase variation has been described as the result of a frameshift mutation in the bvg locus that is inherited (41). Although in *B. pertussis* fimbrial expression is under the control of the *bvg* locus, transcription of the fimbrial subunit genes is independently regulated by the length of a stretch of cytosine residues (the C-stretch), in front of these subunit genes (47).

B. bronchiseptica is the causative agent of kennel cough in dogs and probably plays an opportunistic role in atrophic rhinitis in pigs (13, 34). Virulence factors that are expressed in the Bvg^+ state include adenylate cyclase (toxin with hemolytic activity) (20, 29, 31, 33, 46), filamentous hemagglutinin (6, 8, 46), and fimbriae (9, 19, 21, 26, 27, 32). Adherence of *B. bronchiseptica* to the epithelium of the respiratory tract is a prerequisite for infection. Both filamentous hemagglutinin and fimbriae play a role in the adherence of the bacterium (10, 25, 27, 46). In *B. pertussis*, at least three different fimbrial subunit genes, *fim2*, *fim3*, and *fimX* (9), are present; *fimX* is a silent gene or expressed at a very low level (35, 47).

To characterize the Fim2 and Fim3 fimbriae in *B. bronchiseptica*, the genes of both subunits were cloned. The nucleotide sequences of the genes including the promoter sequences were compared with the corresponding *B. pertussis* sequences. Expression and regulation of expression of the *fim2* and *fim3* genes of *B. bronchiseptica* were studied. Furthermore, the capacity for adhesion to HeLa cells of fimbriated (Bvg^+) and nonfimbriated but flagellated (Bvg^-) *B. bronchiseptica* was determined.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The wild-type *B. bronchiseptica* strain 685, isolated from dogs suffering from kennel cough, was obtained from J. M. Musser. This strain was grown in tryptose phosphate broth or on tryptose phosphate broth-agar (Difco Laboratories, Detroit, Mich.). The *B. pertussis* strain Wellcome 28 (36) was grown on Bordet-Gengou agar (Difco Labo-

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ratories). To repress the production of virulence factors in *B. bronchiseptica*, the growth media were supplemented with 20 mM $MgSO_4$. *E. coli* K-12 strain PC2495 was used as the host in the cloning experiments. The strain was grown on Luria-Bertani agar or in Luria-Bertani broth supplemented with 100 μg of ampicillin per ml, 50 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per ml, and 20 μg of isopropyl-β-D-thiogalactopyranoside (IPTG) per ml was added for identification of strains containing recombinant plasmids with a cloned DNA fragment. All bacterial cultures were grown at 37°C for 16 to 48 h.

DNA isolation and nucleotide sequence determination. Isolation of chromosomal DNA was carried out according to the method of Maniatis et al. (23). Plasmid isolation was carried out by the method of Birnboim and Doly (7). Chromosomal DNA fragments were isolated from TAE (40 mM Tris-acetate, 2 mM EDTA) (23) agarose gels with the Geneclean kit (Bio 101, Inc., La Jolla, Calif.). DNA fragments were ligated in pBluescript KS $M13^+$ and $M13^-$ (Stratagene, La Jolla, Calif.) for all cloning purposes. Nucleotide sequence determination was carried out with the T7 polymerase sequencing kit (Amersham) by the dideoxy chain termination method of Sanger et al. (37). PC/Gene computing software (release 6.5; Genofit, Geneva, Switzerland) was used for analyzing DNA sequence data. Restriction enzymes and T4 DNA ligase were purchased from Pharmacia and were used according to the instructions of the manufacturer. Plasmids were transformed to *E. coli* PC2495 by the $CaCl_2$ method of Dagert and Ehrlich (10).

Southern blotting. Southern blotting was carried out according to the method of Maniatis et al. (23).

SDS-PAGE and immunological techniques. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot (immunoblot) analysis were carried out with 15% gels as described by Laemmli (18) and Van Embden et al. (44), respectively. Equal amounts of bacterial cells were analyzed. Bacteria were harvested in phosphate-buffered saline (PBS) and diluted until an A_{600} of 1.0 was achieved. To 50 μl of this suspension, 20 μl of the final sample buffer (3× concentrated) was added. Polyclonal antibodies, raised against SDS-denatured serotype 2 or 3 fimbrial subunit proteins from *B. pertussis* (27), were used for detection of *B. bronchiseptica* fimbrial subunit proteins.

Fim2- and Fim3-specific monoclonal antibodies, raised against *B. pertussis*, kindly provided by J. Poolman, RIVM (National Institute of Public Health and Environmental Protection), Bilthoven, The Netherlands, were used in an enzyme-linked immunosorbent assay (ELISA) on flat-bottom microtiter plates coated with whole bacteria (100 μl of a 1:10 diluted suspension with an A_{600} of 0.1 in 15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6). The amount of bound goat anti-mouse immunoglobulin G-peroxidase conjugate (Nordic) was determined with ABTS (2,2′-azino-bis-3-ethylbenzthialzolin-6-sulfonic acid; Sigma) as the substrate. A_{405} was measured.

Electron microscopy. For electron microscopy, a *B. bronchiseptica* culture was diluted in PBS. Pilioform-coated copper grids were placed on 50 μl of bacterial suspension for 5 min. The grids were washed two times in H_2O. Staining was performed during 2 min in 1% tungstophosphoric acid (Merck). The grids were examined in a Philips EM 201 electron microscope.

Purification of flagella. *B. bronchiseptica* cells were grown in 500 ml of tryptose phosphate broth supplemented with 20 mM $MgSO_4$, harvested in the logarithmic growth phase, and washed in PBS. The bacterial pellet was suspended in 10 ml of 5 M NaCl, incubated for 30 min at 65°C, and slowly cooled to 20°C. The suspension was spun down at 15,000 × *g* at 4°C during 30 min, and the supernatant was centrifuged at 200,000 × *g* for 16 h at 4°C. The pellet was suspended in 200 μl of H_2O supplemented with 100 μg of phenylmethylsulfonyl-fluoride per ml and stored at −20°C. Purified flagella were analyzed by electron microscopy and PAGE.

Polyclonal antisera. Polyclonal antibodies were raised against SDS-denatured purified flagella in rats. Approximately 300 μg of flagellar protein was separated by SDS-PAGE, blotted onto nitrocellulose, and visualized by staining with 0.6% Ponceau Red in 3% trichloroacetic acid. The area on the filter containing the desired protein was cut out, shock frozen in liquid nitrogen, and homogenized. The homogenized material was suspended in 1 ml of distilled H_2O and stored at −20°C. For immunization, 0.5 ml of this material was mixed with an equal volume of Freund's complete adjuvant and inoculated intramuscularly in two Wistar rats on day 0. The rats were given a booster of 0.5 ml of homogenized flagellar proteins in an equal volume of Freund's incomplete adjuvant on day 28. Sera were collected at days −1, 20, 34, 41, and 48.

Binding of purified *B. bronchiseptica* flagella to HeLa cells. Human epithelial cells, HeLa 229 (American Type Culture Collection, Rockville, Md.), were seeded in 96-well flat-bottom ELISA plates at two concentrations, approximately 10×10^4 and 5×10^4 cells per ml, respectively, in 100 μl of Dulbecco's modified Eagle medium per well supplemented with 10% fetal calf serum (Gibco), 100 IU of penicillin per ml, and 100 μg of streptomycin per ml at 5% CO_2 and 37°C. After 2 days, the culture medium was removed, the cells were fixed with ethanol (−70°C) containing 5% (vol/vol) acetic acid and stored with fixative at −70°C until use. Before use, the plates were air dried, washed with PBS containing 0.05% Tween 80, pH 7.2 (PBS-T), and incubated for 1 h at 37°C with 200 μl of blocking buffer (PBS-T supplemented with 0.6% nonfat dry milk and 1% FCS) per well. The plates were washed three times with PBS-T and incubated overnight at 20°C with 100 μl (50 μl/ml) of purified *B. bronchiseptica* flagella per well. Subsequently, the plates were washed three times with PBS-T, and 100 μl of serial twofold dilutions of a rat polyclonal anti-flagellum antiserum were added. The plates were then incubated for 90 min at 37°C and washed three times with PBS-T, and 100 μl of horseradish peroxidase-conjugated rabbit anti-rat conjugate diluted 1:4,000 was added for 2 h at 37°C. After washing three times with PBS-T, 100 μl of the substrate tetramethylbenzidine (0.01% tetramethylbenzidine, 1.67% DMSO, 10 μl of H_2O_2 in 0.11 M sodium acetate buffer, pH 5.5) was added, and the reaction was stopped after 10 min by adding 100 μl of 2.0 M H_2SO_4. The optical densities were measured spectrophotometrically at 450 nm in a Titertek Multiscan.

Adherence of *B. bronchiseptica* to HeLa cells. Experiments investigating adherence of *Bordetella* spp. to HeLa cells were carried out as described by Garcia et al. (11). The number of bacteria per HeLa cell were counted to determine differences in adherence between the bacteria.

Nucleotide sequence accession numbers. The nucleotide sequences of the *B. bronchiseptica fim2* and *fim3* genes were submitted to the EMBL Data Library under accession numbers X74119 and X74120.

RESULTS

Nucleotide sequences of the *fim2* and *fim3* fimbrial subunit genes. The *B. bronchiseptica fim2* and *fim3* subunit genes could be identified on the basis of their homology with the *B. pertussis fim2* and *fim3* genes, respectively. An *Acc*I-*Pst*I DNA fragment of 1,000 bp was used as a probe for the *fim2* gene (21). A *Sal*I DNA fragment of 900 bp was used as a probe for the *fim3* gene (26). Hybridization of chromosomal DNA from *B. bronchiseptica* with the *fim2* or *fim3* probe revealed more than one band, due to homology between the *fim* genes. DNA was isolated from two regions of the agarose gel which contained *Pst*I fragments giving the strongest signal with either the *fim2* or the *fim3* probe. These fragments of about 2.3 and 2.8 kb, respectively, were cloned into the Bluescript vector. After transformation and colony lifting, two clones (designated pIVB3-402 and pIVB3-430) were identified by hybridization with either of the *fim* probes. The nucleotide sequences of both clones were determined. It appeared that pIVB3-402 contained a *fim* gene with a pronounced homology to *fim2*, while pIVB3-430 contained a *fim* gene with a pronounced homology to *fim2*, while pIVB3-430 contained a gene homologous to *fim3*. Therefore, these *B. bronchiseptica fim* genes were also designated *fim2* and *fim3*. Comparison of the nucleotide sequences of the *fim2* and *fim3* genes of *B. bronchiseptica* and *B. pertussis* revealed some differences (Fig. 1 and 2). The identity between the nucleotide sequences of the *fim2* genes is 74%, whereas the identity between the nucleotide sequences of the *fim3* genes is 94%. The *fim2* gene of *B. bronchiseptica* is one codon longer than its *B. pertussis* counterpart (Fig. 1). The *fim3* gene of *B. bronchiseptica* is three codons longer than the *fim2* gene and the *fim3* gene of *B. pertussis* (Fig. 1 and 2). The nucleotide sequences upstream of the *fim2* and *fim3* fimbrial subunit genes in *B. bronchiseptica* are almost identical to the corresponding sequences in front of the homologous *B. pertussis* subunit genes. The most pronounced difference is found in the C-stretch. The *fim2* C-stretch contains 18 cytosine residues (15 in *B. pertussis*), whereas the *fim3* C-stretch contains 19 cytosine residues (13 in *B. pertussis*). The regions described as the putative −10 region and the putative activator binding region in *B. pertussis* (47) are identical to the corresponding regions of the *fim2* and *fim3* promoter regions in *B. bronchiseptica* (Fig. 3).

Expression of fimbrial subunit genes in *B. bronchiseptica*. It has been proposed that the distance between a putative activator binding site and the −10 sequence influences the expression of the fimbrial subunit genes (47). This distance can be varied by the length of the C-stretch. A short C-stretch (7 cytosine residues), as found in the *fimX* promoter of *B. pertussis*, strongly reduces expression of the *fimX* gene. Since the C-stretch in front of the *fim2* and *fim3* genes of *B. bronchiseptica* is longer (two and six residues, respectively) than the C-stretch of the homologous genes in *B. pertussis* (Fig. 3), this

FIG. 1. Comparison of the nucleotide and amino acid sequences of the *fim2* fimbrial subunit genes of *B. bronchiseptica* (upper sequence) and *B. pertussis* (lower sequence) (21). Dots indicate identical nucleotides or amino acids. Dashes indicate gaps. The signal sequence is underlined.

FIG. 2. Comparison of the nucleotide and amino acid sequences of the *fim3* fimbrial subunit genes of *B. bronchiseptica* (upper sequence) and *B. pertussis* (lower sequence) (26). Dots indicate identical nucleotides or amino acids. Dashes indicate gaps. The signal sequence is underlined.

may also affect expression. Expression of the *fim2* and *fim3* genes in *B. bronchiseptica* was examined by Western blotting with polyclonal antiserum raised against denatured *B. pertussis* fimbriae. This shows that fimbrial subunit genes which do react with the polyclonal antiserum in *B. bronchiseptica* are expressed (Fig. 4). Furthermore, it was evident that these subunit genes are also under the control of the *bvg* locus, because no fimbrial subunit proteins were detected when *B. bronchiseptica* was grown in the presence of $MgSO_4$ (Fig. 4). From these data, it is impossible to tell which genes (*fim2*, *fim3*, or *fimX*) are expressed, due to the cross-reactivity of the polyclonal antisera used. This was solved by using monoclonal antibodies recognizing native Fim2 or Fim3 fimbriae of *B. pertussis*. An ELISA on whole *B. bronchiseptica* bacteria, using these monoclonal antibodies, confirmed that Fim2 and Fim3 fimbriae are produced in *B. bronchiseptica* and that they are subject to antigenic modulation (Fig. 5).

The *bvg* locus regulates flagellum production. As described by Akerly et al. (1, 2) we observed that flagella were produced by *B. bronchiseptica* when grown in the presence of 20 mM $MgSO_4$ (Fig. 6A). In the absence of $MgSO_4$, a mixture of fimbriated and flagellated bacteria is observed when the bacteria are grown until a stationary phase. In the logarithmic growth phase, only fimbriated bacteria were observed (Fig. 6B). Besides the expression of flagella or fimbriae, a difference in the shape of the bacteria was recognized. Flagellated bacteria were drawn out, whereas fimbriated bacteria were round. The fact that unfavorable conditions like the presence of sulfate or stationary culture conditions lead to flagellated bacteria may be regulated by the *bvg* locus.

Adhesion of bacteria and purified flagella to HeLa cells. To determine whether flagella play a role in adhesion to eukaryotic cells, both *B. bronchiseptica* and *B. pertussis* bacteria were grown in the presence (Bvg^- state) and absence (Bvg^+ state) of $MgSO_4$. In contrast to *B. bronchiseptica*, *B. pertussis* is not able to produce any flagella in the Bvg^- state. Adhesion of both bacteria to HeLa cells was determined. From these experiments, it was clear that *B. bronchiseptica* is able to adhere to HeLa cells in both states (Fig. 7A and B). Adherence experiments with *B. pertussis* showed that *B. pertussis* is able to adhere to HeLa cells only in its Bvg^+ state (Fig. 7C and D). This observation was confirmed by counting the number of

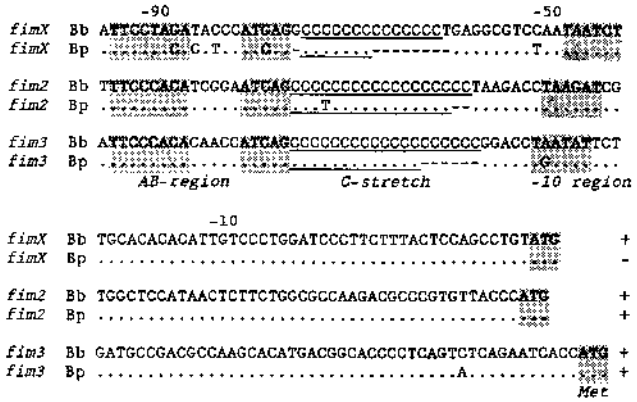

FIG. 3. Comparison of the promoter sequences of all known fimbrial subunit sequences of *B. bronchiseptica* (Bb) and *B. pertussis* (Bp) (47). The regions described as a putative activator binding (AB) region, −10 region, and start codon (*Met*) are marked. C-stretches are underlined. Dashes represent gaps, and dots indicate identical nucleotides. +, subunit genes are expressed; −, subunit genes are not expressed.

bacteria on at least 25 eukaryotic cells. The average number of attached bacteria per 25 HeLa cells was 1,060 for *B. bronchiseptica* and 580 for *B. pertussis*, grown in the presence of 20 mM $MgSO_4$. Without $MgSO_4$, 1,025 *B. bronchiseptica* and 37 *B. pertussis* bacteria were counted. Herewith a significant difference ($P = 0.0048$) was demonstrated between the flagellated *B. bronchiseptica* (Bvg$^-$) and nonflagellated *B. pertussis* (Bvg$^-$). To determine whether adherence in the Bvg$^-$ state might be caused by flagella, the flagellar proteins were purified and their adherence to HeLa 229 cells was examined. Serial twofold dilutions of purified flagella showed corresponding adherence to two different concentrations of HeLa 229 cells, demonstrated with polyclonal antiserum raised against purified flagella (Fig. 8). These results indicate that flagella apparently may play a role in adherence of *B. bronchiseptica.*

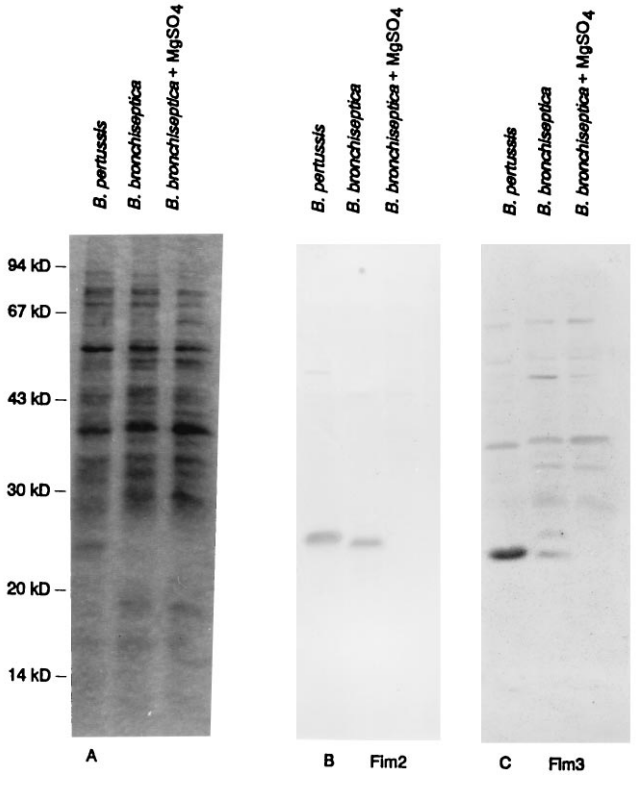

FIG. 4. Production of fimbrial subunits in *B. bronchiseptica*. Total cell lysates of *B. pertussis* (strain Wellcome 28) (36) and *B. bronchiseptica* (strain 685) grown on Bordet-Gengou agar and Bordet-Gengou agar supplemented with 20 mM $MgSO_4$, were analyzed on a Coomassie blue-stained gel (A). Corresponding Western blots were incubated with polyclonal antisera directed against denatured Fim2 (B) and Fim3 (C) fimbriae of *B. pertussis*.

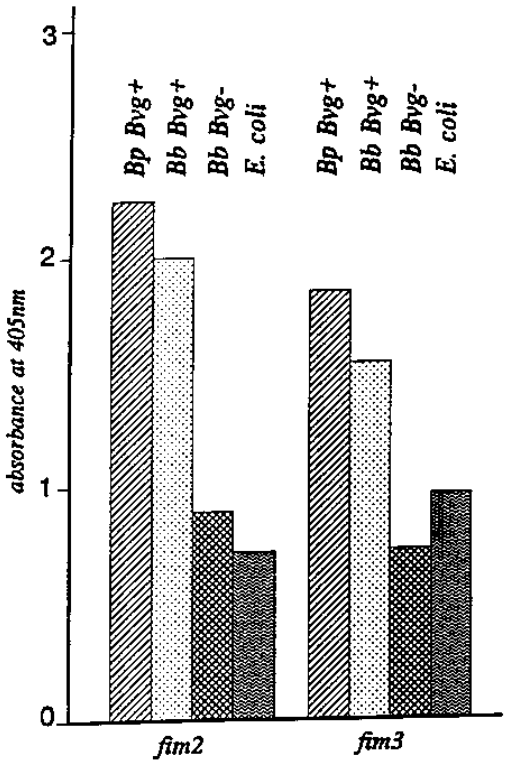

FIG. 5. Production of Fim2 and Fim3 fimbriae as determined by a whole-cell ELISA on *B. pertussis* (strain Wellcome 28) (Bp Bvg$^+$), *B. bronchiseptica* (strain 685), and *E. coli* PC2495 bacteria. Monoclonal antibodies against Fim2 and Fim3 fimbriae of *B. pertussis* were used. *B. bronchiseptica* was grown in the absence (Bb Bvg$^+$) or presence (Bb Bvg$^-$) of 20 mM $MgSO_4$.

DISCUSSION

B. pertussis, *B. parapertussis*, and *B. bronchiseptica* are closely related species within the genus *Bordetella* (28). Research on the genus *Bordetella* has mainly been focused on *B. pertussis*. In *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*, expression of virulence factors is controlled by a homologous *bvg* locus (4, 24). The fourth species, *Bordetella avium*, is slightly different, but there are indications for a *bvg*-like locus responding to similar environmental conditions (12). Most virulence factors are present in all four species (14), and all four species show phase variation superimposed on the regulation by the *bvg* locus (4, 12).

The *fim2* and *fim3* fimbrial subunit genes present in *B. bronchiseptica* are highly similar to the *fim2* and *fim3* genes of *B. pertussis*. By Western blotting and ELISA it was shown that the expression of the fimbrial subunit genes of *B. bronchiseptica* is regulated by the *bvg* locus and that the *fim2* and *fim3* genes in *B. bronchiseptica* are expressed. The C-stretches observed in *B. bronchiseptica* in front of the *fim2* (18 residues) and *fim3* (19

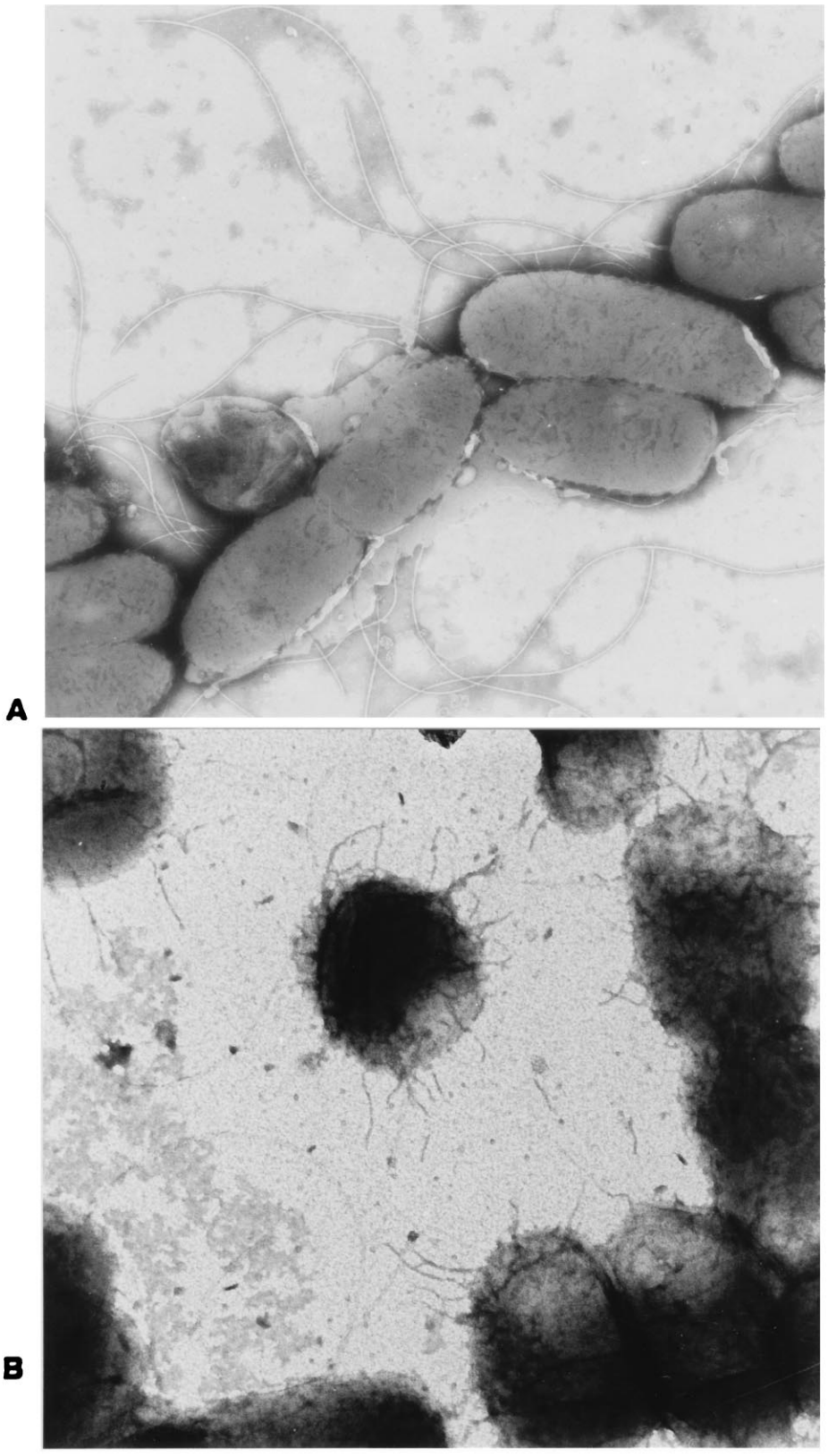

FIG. 6. Electron micrographs of wild-type *B. bronchiseptica* (strain 685) grown on tryptose phosphate agar in the presence (A) or in the absence (B) of 20 mM $MgSO_4$.

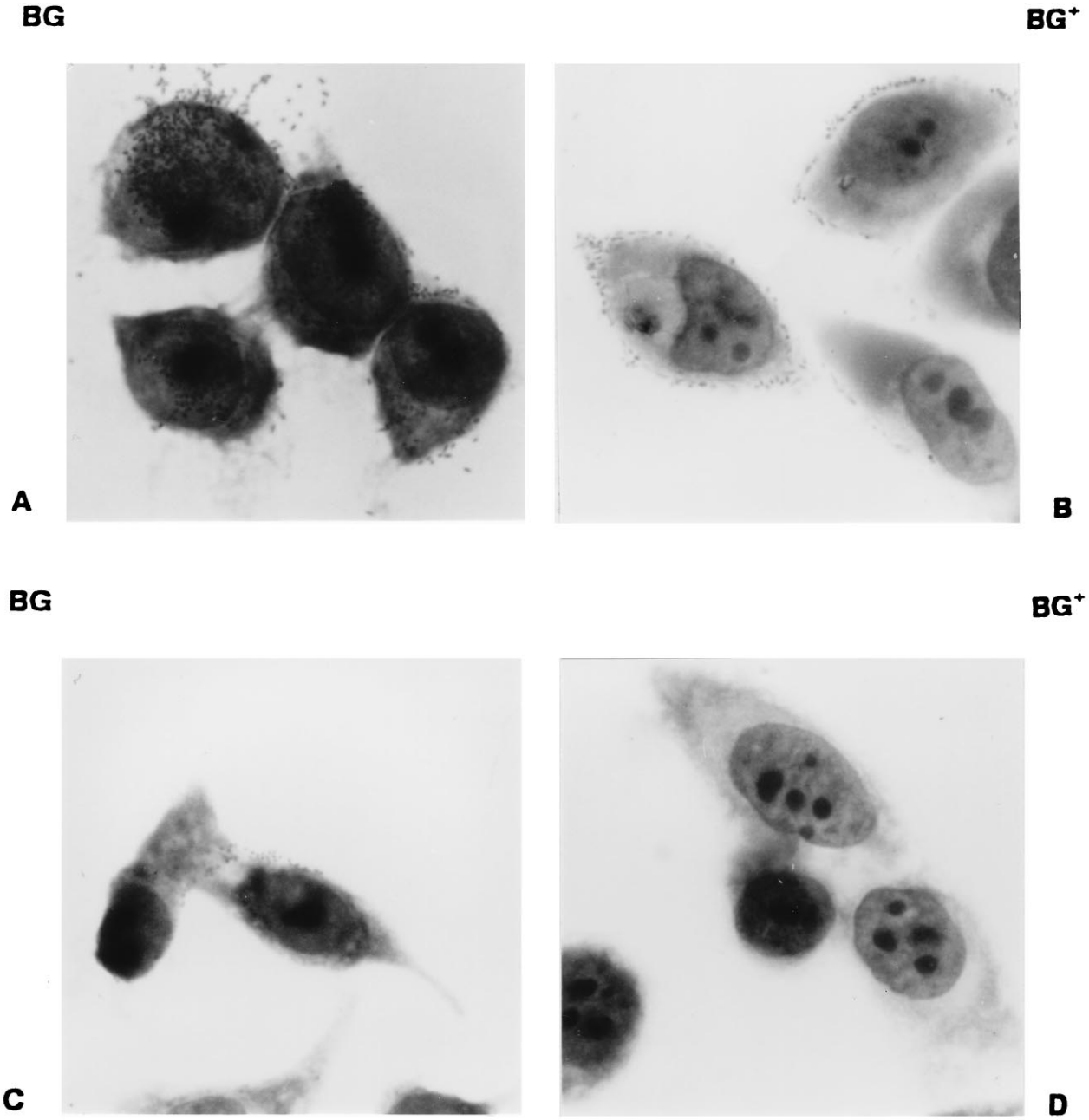

FIG. 7. Adherence of *B. bronchiseptica* 685 (A and B) and *B. pertussis* W28 (C and D) to HeLa 229 cells. Both strains were grown on Bordet-Gengou agar with (BG^+) and without (BG) 20 mM $MgSO_4$.

residues) genes are longer than those in *B. pertussis*. Since the genes are expressed, this probably means that only a minimum number of cytosines residues is required for their expression and that expression is not influenced by the length of the C-stretch once a minimal distance of about 22 bases (25 to 27 bases in *B. bronchiseptica*) is present between two regions described as the putative activator binding region and the putative −10 region by Willems et al. (47) (Fig. 3).

One of the differences between *B. bronchiseptica* and *B. pertussis* is the production of flagella by *B. bronchiseptica*, when the bacteria are grown in the presence of 20 mM $MgSO_4$. It is suggested for *Bartonella bacilliformis* and *Vibrio anguillarum* that flagella are an important component for invasion of human erythrocytes and fish epithelium, respectively (30, 39). For *B. bronchiseptica*, invasiveness in HeLa 229 cells of both the fimbriated as well as the flagellated phase is described (38). Since adhesion is a prerequisite for invasion, flagella may be a factor in adhesion. Experiments investigating adhesion to HeLa cells show that both in the Bvg^+ state as well as in the Bvg^- state, *B. bronchiseptica* is able to adhere to HeLa cells. Furthermore, purified flagella are able to adhere to HeLa 229 cells, which makes an adhesive role for another extracellular protein less likely. Therefore, the flagella of *B. bronchiseptica* may play a role in adherence to eukaryotic cells, as previously

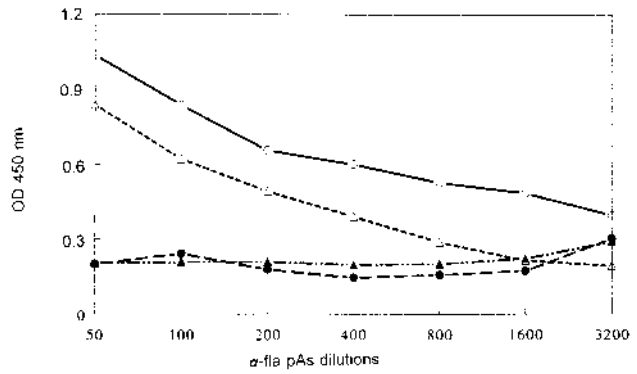

FIG. 8. Binding of purified flagella of *B. bronchiseptica* to HeLa 229 cells as determined in an ELISA. Two concentrations of HeLa cells (10×10^4 cells per ml [H10] and 5×10^4 cells per ml [H5]) were incubated with 50 μg of purified flagella per ml (+fl) and without flagella (−fl). Serial twofold dilutions of polyclonal antiflagellum antiserum (α-fla pAs) were used to detect the presence of flagella on the cells. OD, optical density. —○—, +fl and H10; —●—, −fl and H10; —-△—-, +fl and H5; —▲—, −fl and H5.

reported for *Salmonella typhimurium* (22). The exact role of flagella as a specific adhesin and virulence factor of *B. bronchiseptica* is still unknown.

In the genus *Bordetella*, several genes, although present, are not expressed by all species. Such a gene is the *fimX* subunit gene, which is silent in *B. pertussis* (32, 47). On the other hand, *B. bronchiseptica* and *B. parapertussis* contain a silent toxin gene (3). A similar situation might exist for flagellum production in *B. pertussis*. Hybridization experiments with an oligonucleotide probe, derived from the N-terminal amino acid sequence of the flagellin gene of *B. bronchiseptica*, give positive hybridization signals in *B. pertussis* DNA (1, 2), suggesting that the flagellin gene is present. For *E. coli*, it is estimated that at least 13 operons together, containing more than 60 genes, are required for synthesis and rotation of the flagellar apparatus (22). Akerly et al. have demonstrated that the expression of the flagella in *B. bronchiseptica* is regulated by the *bvg* locus, and the FlaA flagellin is functionally related to the FliC flagellin of *E. coli* (1, 2). Knapp and Mekalanos (16) and Beattie et al. (5) have shown that in *B. pertussis* several proteins are induced during growth in the presence of antigenic modulators (*vrg*). The activated genes might be involved in the production of flagella. The fact that the FlaA is not expressed (1) will result in the absence of flagella in *B. pertussis* in the presence of antigenic modulators.

In many other bacteria, flagella have been shown to play a role in virulence (30, 40). Thus, in view of the capacity of the flagellated phase to adhere to HeLa cells, flagella may also play a similar role for *B. bronchiseptica.*

ACKNOWLEDGMENTS

We thank C. J. A. H. V. van Vorstenbosch for performing the electron microscopy.

This work was supported by Solvay-Duphar B.V., Weesp, The Netherlands.

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Editor: B. I. Eisenstein