INFECTION AND IMMUNITY, Aug. 2005, p. 4626–4633 Vol. 73, No. 8
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.8.4626–4633.2005

Capsule and Fimbria Interaction in *Klebsiella pneumoniae*

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Received 29 September 2004/Returned for modification 30 December 2004/Accepted 18 March 2005

The capsular polysaccharide and type 1 fimbriae are two of the major surface-located virulence properties associated with the pathogenesis of *Klebsiella pneumoniae*. The capsule is an elaborate polysaccharide matrix that encases the entire cell surface and provides resistance against many host defense mechanisms. In contrast, type 1 fimbriae are thin adhesive thread-like surface organelles that can extend beyond the capsular matrix and mediate D-mannose-sensitive adhesion to host epithelial cells. These fimbriae are archetypical and consist of a major building block protein (FimA) that comprises the bulk of the organelle and a tip-located adhesin (FimH). It is assumed that the extended major-subunit protein structure permits the FimH adhesin to function independently of the presence of a capsule. In this study, we have employed a defined set of *K. pneumoniae* capsulated and noncapsulated strains to show that the function of type 1 fimbriae is actually impeded by the concomitant expression of a polysaccharide capsule. Capsule expression had significant effects on two parameters commonly used to define FimH function, namely, yeast cell agglutination and biofilm formation. Our data suggest that this effect is not due to transcriptional/translational changes in fimbrial gene/protein expression but rather the result of direct physical interference. This was further demonstrated by the fact that we could restore fimbrial function by inhibiting capsule synthesis. It remains to be determined whether the expression of these very different surface components occurs simply via random events of phase variation or in a coordinated manner in response to specific environmental cues.

Klebsiella spp. account for up to 8% of all nosocomial infections in the Western world, placing them among the eight most infectious agents in hospitals (35). The most important species in the genus is *Klebsiella pneumoniae*, an opportunistic pathogen that is commonly associated with hospital-acquired urinary tract infections (UTI), pneumonia, septicemia, and wound infections. As far as nosocomial UTI is concerned, *K. pneumoniae* is second only to *Escherichia coli* as the major causative agent and accounts for 6 to 17% of all infections. These infections are often linked to the use of catheters, indicating that bacterial adhesion and biofilm formation are important for the establishment of the infection. Of particular concern is the recent rapid and extensive spread of antibiotic-resistant strains (particularly against β-lactam antibiotics), highlighting the need to gain a better understanding of the mechanisms by which this organism causes disease.

K. pneumoniae bacteria are commonly found in both clinical and nonclinical settings. Their nonclinical habitats encompass the gastrointestinal tracts of mammals, as well as environmental sources, such as soil, surface waters, and plants (4). Several recent studies have shown that environmental isolates are indistinguishable from human clinical isolates with respect to biochemical and virulence properties (32, 49). *K. pneumoniae* produces a number of virulence factors that contribute to pathogenesis, including fimbrial adhesins, siderophores, O antigens, and capsular antigens. The capsule is considered to be the dominant virulence property and consists of an elaborate layer of surface-associated polysaccharides, the composition of which is very much strain dependent. In *K. pneumoniae*, at least 77 distinct polysaccharides (designated the K antigens) have been reported (29, 35). Capsule polysaccharides contribute to pathogenesis by mediating resistance to phagocytosis and killing by serum (34, 55). Additional functions include protection against desiccation and attack from phages. Almost all members of the *Enterobacteriaceae* are able to produce a capsule, and this property has been strongly associated with extraintestinal infections, such as septicemia, meningitis, and UTI (20, 30, 33, 52). Strains causing UTI, meningitis, and sepsis are generally also resistant to the bactericidal action of human serum.

Another group of virulence factors produced by *K. pneumoniae* are fimbrial adhesins, protein structures that recognize a wide range of molecular motifs and provide targeting of the bacteria to specific tissue surfaces in the host (19). *K. pneumoniae* produces two major types of fimbriae, type 1 fimbriae and type 3 (Mrk) fimbriae (14). Type 1 fimbriae are thin, rigid, adhesive surface organelles found on virtually all members of the *Enterobacteriaceae* (19). On a fimbriated bacterium, several hundred of these organelles radiate peritrichously from the surface to a distance of about 1 μm. These fimbriae have been most extensively studied in *E. coli*; however, the corresponding structures of *K. pneumoniae* are highly similar with regard to genetic composition and regulation (14). Type 1 fimbriae exert their adhesive properties by virtue of the FimH adhesin, a minor organelle component located at the tips of the fimbriae (5). The FimH adhesin recognizes mannose-containing glyco-

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proteins that are present on many mammalian host tissues, such as the surface of the urinary tract. This enables the bacteria to target, attach to, and eventually colonize the uroepithelium (6, 25, 27). Type 1 fimbriation has been correlated with urovirulence (6). Expression of type 1 fimbriae is phase variable due to a flip-flop-type control system based on an invertible DNA switch located immediately upstream of the major-subunit gene. A promoter located in the switch drives the expression of the *fim* genes (28). On or off orientation of the switch results in a fimbriated or bald phenotype, respectively. Two tyrosine class recombinases, encoded by *fimB* and *fimE*, catalyze the inversion of the switch (18), with FimB having a preference for off-to-on switching and FimE having a preference for on-to-off switching (13). Type 3 fimbriae are 2- to 4-nm-wide and 0.5- to 2-μm-long structural organelles predominantly composed of a major-subunit protein (MrkA) in addition to an adhesin (MrkD) (2). They mediate attachment to the basolateral surfaces of tracheal epithelial cells and to components of basement membranes (51).

The capsule and fimbriae are prominent structural components of the *K. pneumoniae* cell surface and play important roles in its survival and pathogenicity. However, there are conflicting reports in the literature regarding their simultaneous expression by *K. pneumoniae*. In a primary investigation, Tarkkanen et al. reported that 29 of 32 encapsulated UTI *K. pneumoniae* strains were capable of expressing type 1 fimbriae (52). Conversely, Matatov et al. (26) identified an inverse relationship between capsule and type 1 fimbria expression. They screened a panel of strains derived from sepsis and UTI sources and showed that the encapsulated sepsis isolates did not produce type 1 fimbriae, while the majority of UTI isolates expressed type 1 fimbriae but were noncapsulated (26). In addition, data were presented which indicated that the encapsulated strains were unable to assemble functional fimbriae (26). A study by Sahly et al. (40) partially backed the latter findings, inasmuch as they reported that none of their capsulated stains produced noticeable type 1 fimbriae. Against this background, we have investigated possible interference between type 1 fimbriae and capsule.

MATERIALS AND METHODS

Bacterial strains and growth conditions. In this study, we primarily used *K. pneumoniae* strain C105 and its noncapsulated derivative C105NCV (26, 50). A second capsulated-noncapsulated strain pair [264(1) and 264(1)NCV] was also employed. *K. pneumoniae* strain 264(1) is of serotype O7:K67 (10). A noncapsulated version, 264(1)NCV, was isolated essentially as previously described (46) and does not react with anti-K67 serum. *E. coli* MS428 is a *fim* mutant of *E. coli* K-12 strain MG1655 (38). *E. coli* MC1061 (New England BioLabs) was used for all intermediate cloning work. Cells were grown at 37°C on solid or in liquid Luria-Bertani (LB) medium supplemented with the appropriate antibiotics unless otherwise stated.

DNA manipulations. Isolation of plasmid DNA was carried out using the QIAprep Spin Miniprep kit (QIAGEN). Restriction endonucleases were used according to the manufacturer's specifications (New England BioLabs). PCRs were done as previously described (48). Amplified products were sequenced to ensure fidelity of the PCR using the ABI PRISM BigDye Terminator cycle-sequencing ready-reaction kit (PE Applied Biosystems). Samples were run on a Perkin-Elmer ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems) as described in the manufacturer's specifications.

Plasmid constructions. The FimB expression plasmid pJKS60 was constructed as follows. The *fimB* gene was amplified by PCR from *E. coli* K-12 strain MG1655 using the primers ms17 (5′-GCGCGCCATGGAGAATAAGGCTGATAAC) and ms18 (5′-GGGCCAAGCTTCTATAAAACAGCGTGACG) containing NcoI and HindIII sites, respectively. The resulting PCR product was digested with NcoI and HindIII and inserted into the NcoI/HindIII site of the pBAD/myc-HisA plasmid (15) to produce plasmid pJKS60. In this construct, expression of the *fimB* gene is under the control of the arabinose-inducible *araBAD* promoter. The construct was sequenced to ensure fidelity of the PCR.

Blotting. Whole-cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride microporous membrane filters as described previously (48). Western blotting was performed using polyclonal antiserum against purified type 1 fimbriae of *E. coli* K-12. Receptor blots to detect FimH were carried out essentially as described previously (43) using α-D-mannose coupled to bovine serum albumin (BSA) as the primary reagent. Specific α-D-mannose binding was visualized by incubating the filter with anti-BSA rabbit serum and subsequently with horseradish peroxidase-conjugated anti-rabbit immunoglobulin serum. Tetramethylbenzidine was used as the substrate.

Agglutination of yeast cells. The capacity of bacteria to express a D-mannose-binding phenotype was assayed by their ability to agglutinate yeast (*Saccharomyces cerevisiae*) cells on glass slides. Bacterial cells were mixed with yeast cells (5%), and the time until agglutination occurred was measured.

Switch orientation assay. Samples were taken at regular intervals during the *fimB* induction assay and placed immediately on ice. After all samples were collected, they were centrifuged, and the cells were resuspended in 100 μl H_2O. The cells were lysed by boiling them for 5 min, and 1 μl of this suspension was used as template DNA to PCR amplify the *fim* switch. PCR was performed with primers 414 (5′-CGTTTCGCTGGCATCTGGGAAAG) and 415 (5′-AATGCACGGTCCCGCCGTTGACC) using the following program: 1 cycle of 94°C for 2 min; 25 cycles of 94°C for 15 s, 20°C for 30 s, 72°C for 30 s; 1 cycle of 72°C for 7 min. The products were purified, cut with BsaBI, and separated on a 2% Tris-borate-EDTA gel.

Biofilm assay. Biofilm formation on plastic surfaces was monitored in 96-well polystyrene microtiter plates essentially as previously described (41, 45). Briefly, cells were grown under hydrodynamic conditions for 24 h in LB medium (containing 0.2% arabinose where appropriate) at 37°C, washed to remove unbound cells, and stained with crystal violet. Quantification of bound cells was performed by addition of acetone-ethanol (20:80) and measurement of the dissolved crystal violet at an optical density of 600 nm.

TEM. Cells for transmission electron microscopy (TEM) were prepared from freshly grown colonies. Bacterial ultrastructure was examined by TEM as previously described with minor modifications (31). A Formvar-coated carbon-reinforced copper grid (400 mesh) was applied, film side down, on a droplet of a thick bacterial suspension on a paraffin strip. The grid was dried on filter paper and stained for 20 s on droplets of 1% phosphotungstic acid, and excess liquid was sucked off with filter paper. Electron microscopy was carried out using a Philips Morgagni D 268 electron microscope at 80 kV.

RESULTS

Expression of type 1 fimbriae is affected by the presence of a capsule in *K. pneumoniae*. The vast majority of *K. pneumoniae* cells express a pronounced polysaccharide capsule. However, noncapsulated variants (NCVs) arise spontaneously. An isogenic pair of *K. pneumoniae* strain C105, differing only in the ability to express K35 capsule antigen, was recently characterized (50). To examine whether fimbria expression is affected by the concomitant expression of a capsule, we transformed the C105/C105NCV pair with the high-copy-number plasmid pPKL4, which harbors the *fim* gene cluster from *E. coli* K-12. A classical assay for monitoring type 1 fimbria-mediated adhesion to eukaryotic cells is agglutination of yeast cells. When overnight cultures of the two strains were compared, we observed that the noncapsulated variant produced a rapid agglutination of yeast cells (5 s) while the capsulated strain agglutinated yeast cells in approximately double the time (10 to 12 s). In order to probe whether this phenomenon applied to other *K. pneumoniae* strains, we employed another isogenic *K. pneumoniae* wild-type/NCV pair, i.e., 264(1)/264(1)NCV, differing only in the ability to express K67 capsule. When strains 264(1) and 264(1)NCV were transformed with plasmid pPKL4

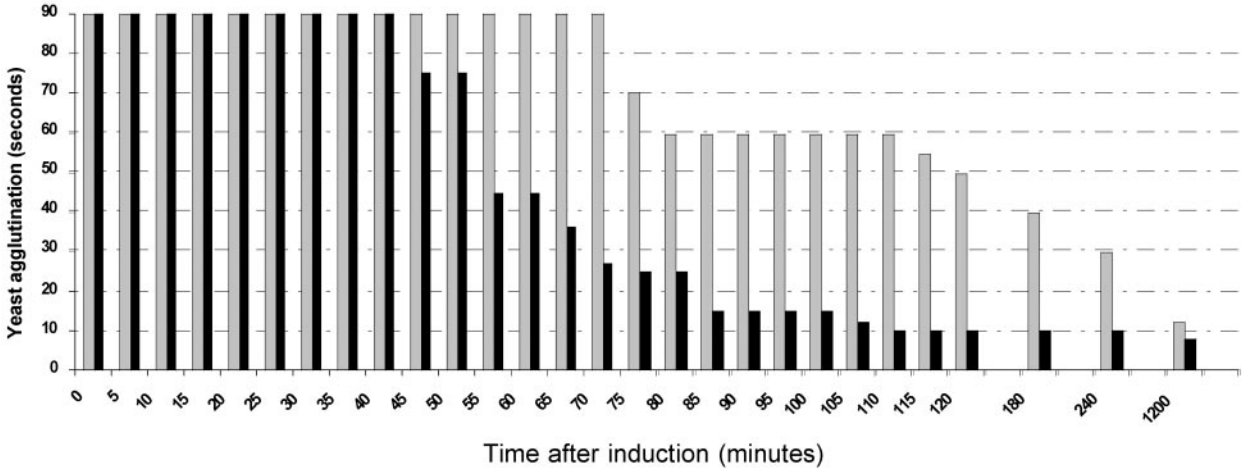

FIG. 1. Yeast cell agglutination profile of *K. pneumoniae* strains C105 (grey bars) and C105NCV (black bars) after induction of FimB expression. Bacterial cells were mixed with a 5% suspension of yeast cells on a glass slide, and the time until agglutination occurred was recorded. The assay is a direct measurement of functional FimH expression. The growth rates of the two strains during the course of the experiment were identical. Data from a single experiment are presented; the experiment was repeated three times, and the results were essentially the same.

and the resultant transformants were tested for yeast agglutination, the noncapsulated version agglutinated considerably faster than the parent. This result was seen in repeated analyses of overnight cultures and prompted us to examine the effect in more detail.

The presence of a capsule delays the ability to detect type 1 fimbria expression. In *E. coli*, type 1 fimbria expression is phase variable and controlled by two recombinases (18). The FimB recombinase efficiently promotes off-to-on switching, while the FimE recombinase primarily promotes on-to-off switching (13, 18). *K. pneumoniae* and *E. coli* contain virtually identical chromosomally encoded *fim* gene clusters with similar regulatory mechanisms (14, 18, 37). In order to devise a mechanism by which we could control expression of the *K. pneumoniae* type 1 fimbria genes, we cloned the *E. coli fimB* gene behind the arabinose-inducible P_{ara} promoter (plasmid pJKS60). This plasmid was then transformed into the *K. pneumoniae* C105/C105NCV isogenic strains. The strains were grown to mid-exponential phase, induced with arabinose, and tested for type 1 fimbria expression by yeast agglutination (Fig. 1). Fimbria expression was first detected in the noncapsulated variant 45 min after arabinose induction, and within 110 min, rapid agglutination (10 s) was observed. In contrast, the capsulated C105 strain did not produce detectable agglutination until 75 min after induction and maintained a significantly slower agglutination profile throughout the course of the experiment.

Capsule expression does not affect switching of the *fim* promoter. One explanation for the above-mentioned results is that capsule expression affects fimbria expression at the transcriptional level, i.e., switching of the *fim* promoter (either directly or indirectly). To explore this possibility, we examined the orientation of the chromosomally encoded *fim* switch at selected time points during the arabinose induction experiment described above. In this assay, the *fim* switch region is PCR amplified and then digested with BsaBI restriction endonuclease, which cuts asymmetrically within the switch. The off-specific products are 204 bp and 446 bp in size, while the on-specific products are 322 bp and 328 bp. We observed that in both strains the first detectable "on" fragments appeared 15 min after induction with arabinose (Fig. 2). No significant difference in "on" and "off" fragments was observed within the course of the experiment when relative time points were compared. The apparent diminution of the off products in accordance with *fimB* induction in C105(pJKS60) may be due to subtle variations in the PCR. However, when Fig. 1 and Fig. 2 are compared, there appears to be a 30-min lag phase between the start of transcription and functional presentation of the fimbrial organelle at the cell surface (noncapsulated strain), with a further lag phase of 30 min required for penetration of the capsule (capsulated strain). Taken together, the results suggest that capsule expression per se does not affect switching of the *fim* promoter. Also, although *K. pneumoniae* has a switch sequence that is virtually identical to that of *E. coli*, it has not been demonstrated previously to be invertible. We have now shown this to be the case.

Capsule expression does not affect the amount of fimbriae produced. Another explanation for the effect of capsule expression on fimbrial function could be that the amount of fimbriae present at the cell surface is reduced in capsulated strains. To examine this possibility, we employed a combination of Western and receptor blotting and electron microscopy. The C105/C105NCV strains (containing pPKL4 for *fim* expression) were assessed for production of the type 1 fimbrial adhesin (FimH) by Western blotting employing type 1 fimbrial antisera aginst whole-cell lysates (Fig. 3A). The FimH protein was found to be produced in similar amounts in both strains. The same result was obtained using a receptor blot employing α-D-mannosylated BSA (Fig. 3B). We also examined the strains for the production of type 1 fimbriae by transmission electron microscopy (Fig. 4). No difference was observed in the amounts of fimbriae observed at the surfaces of the capsulated and noncapsulated cells, indicating that capsule expression does not affect the synthesis or assembly of type 1 fimbriae. Taken together, the results indicate that the most likely mech-

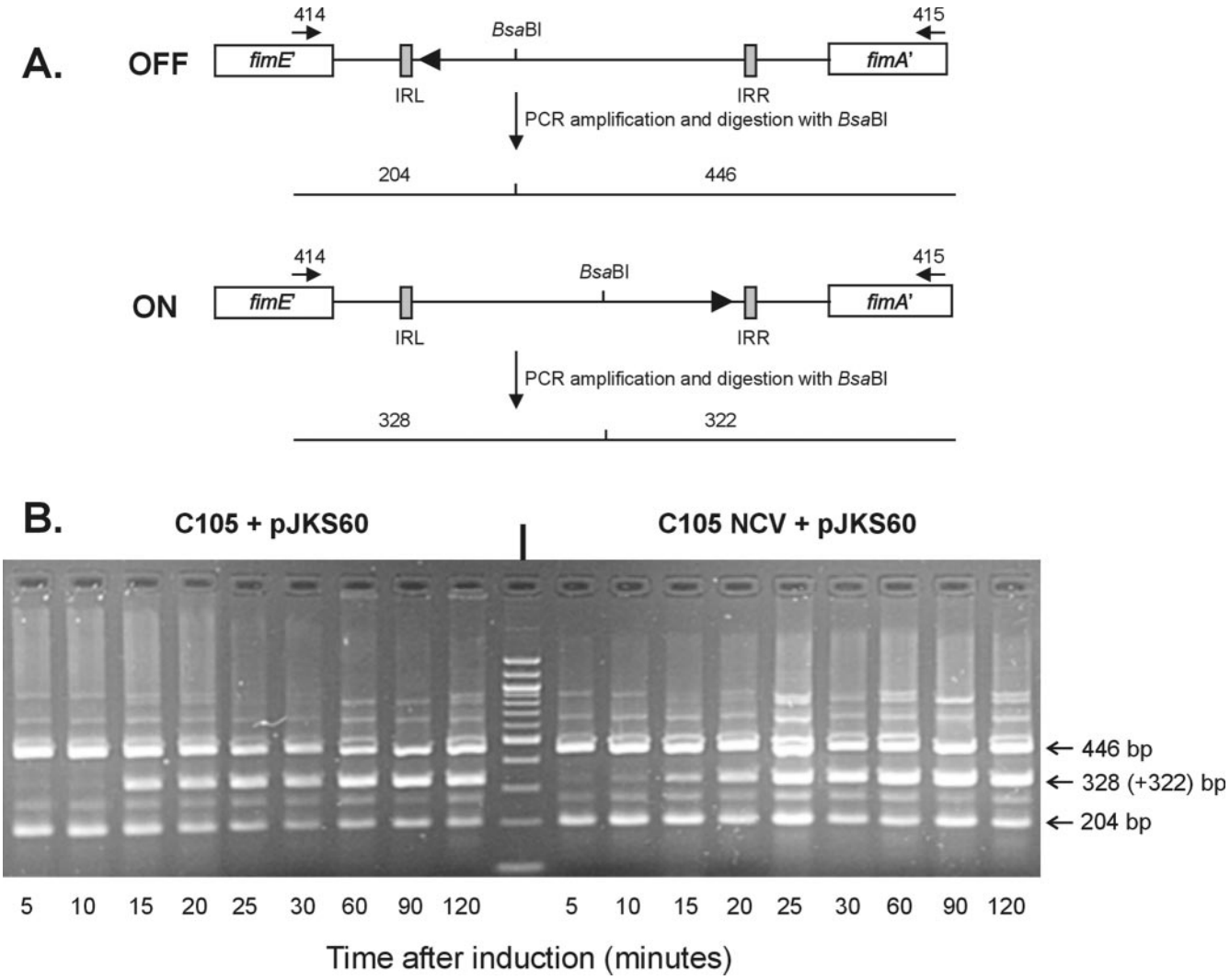

FIG. 2. In vitro recombination assay for monitoring of the influence of the capsule on FimB-mediated switching. (A) Map of the phase switch region in both on and off orientations. Indicated are the positions of the primers used in the PCR amplification and the sizes of the DNA fragments resulting from BsaBI digestion. IRL, inverted repeat left; IRR, inverted repeat right. (B) Acrylamide gel electrophoresis showing the amounts of on and off DNA fragments obtained after BsaBI digestion. Samples were taken at regular time intervals after induction of FimB expression, and the switch orientation was monitored. On fragments (204 and 446) and off fragments (322 and 328) are indicated. A DNA size marker (100 bp to 1,200 bp, increasing by regular 100-bp increments) is shown in the middle of the gel.

anism by which capsule expression effects type 1 fimbrial function is physical interference.

Inhibition of capsule synthesis enhances type 1 fimbrial function. Bacterial capsule expression can be inhibited by several agents, including salicylate and bismuth (8). In a recent study, bismuth-2,3-dimercaptopropanol (BisBAL) was shown to reduce *K. pneumoniae* O1:K2 capsule expression by approximately 90% in defined medium (9). To directly demonstrate that the presence of a capsule on the bacterial surface interferes with fimbrial function, we grew the capsulated *K. pneumoniae* C105 strain containing the inducible FimB recombinase in LB medium with and without BisBAL. Consistent with our previous data, the inhibition of capsule synthesis by the addition of subinhibitory concentrations of BisBAL resulted in a twofold increase in the speed of yeast cell agglutination (data not shown).

Capsule expression hinders fimbria-mediated biofilm formation. We and others have previously demonstrated that type 1 fimbriae confer excellent biofilm-forming properties upon *E. coli* (36, 41, 44, 45). Although it seems reasonable to assume that *K. pneumoniae* type 1 fimbriae possess similar functional properties, such studies have not been reported previously. Furthermore, our encapsulated-nonencapsulated strain set provides a unique background in which to test for capsule-mediated interference of this property. To perform these experiments, the C105/C105NCV strains were transformed with our *fimB* expression plasmid (pJKS60) or a vector control (pBADMyc-HisA) and investigated for the ability to form biofilms on an abiotic surface (in this case, polystyrene microtiter plates) (Fig. 5). Our results show that (i) type 1 fimbriae from *K. pneumoniae* can promote biofilm formation on abiotic surfaces, (ii) this property is blocked by the concomitant expres-

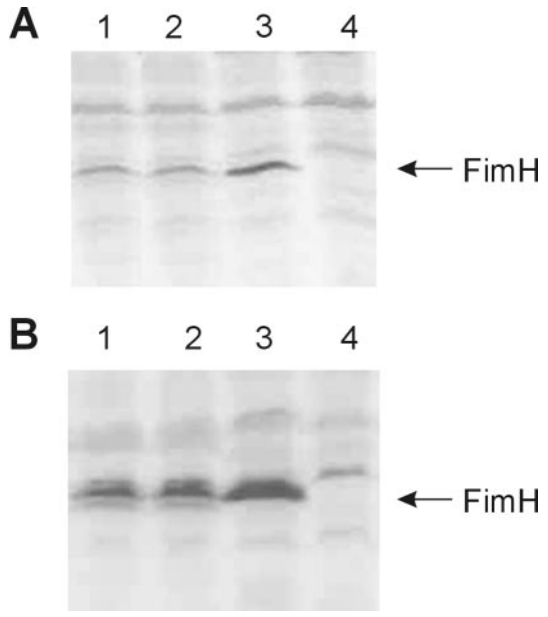

FIG. 3. Western blot (A) and receptor blot (B) of total whole-cell lysates of recombinant cells reacted with either type 1 fimbrial antisera or α-D-mannosylated BSA, respectively. Lanes 1, C105 containing pPKL4; lanes 2, C105NCV containing pPKL4; lanes 3, MS428 containing pPKL4 (positive control); lanes 4, MS428 containing pBR322 (negative control). The FimH protein is indicated. Equal amounts of FimH were detected in both capsulated and noncapsulated *K. pneumoniae* cells (lanes 1 and 2).

sion of a capsule, (iii) the capsule actually reduces bacterial adhesion to such surfaces, and (iv) the expression of type 1 fimbriae may interfere with other adhesion mechanisms of *K. pneumoniae*. We attribute the last observation to fimbrial shielding of shorter adhesins as previously observed in *E. coli* (16). The physical shielding of short nonfimbrial adhesins by the capsule has also been demonstrated (42). Taken together, the results provide further evidence that capsule expression physically interferes with the function of type 1 fimbriae.

DISCUSSION

Bacteria express a number of surface structures that enable them to interact with and survive in changing environments, e.g., flagella in order to swim and adhesins in order to attach. These surface components have highly diverse structures, and it must be implicit that they physically interfere with each other in such a way that the activity of one may sometimes be obstructed by the presence of another. As an example, we have previously demonstrated that both fimbriae and the capsule physically block the action of the much shorter Ag43 adhesin in bacteria (16, 42). Such surface structure interference must have important consequences for the interplay of bacteria with the environment. Here, we have studied the interference between the capsule and type 1 fimbriae. The capsule is a thick polysaccharide matrix that surrounds the entire cell, provides resistance against host predation, and is associated with the later developmental stages of complex biofilm structures that display enhanced resistance to antibiotics. Fimbriae are also required for the formation of biofilms, but predominantly during the initial adhesion phase. Intriguingly, although the long polymorphic structure of fimbriae allows them to penetrate the capsule, their adhesive properties are actually impeded by its presence, suggesting that the expression of these two major surface components may be controlled in a coordinated manner.

The capsule and type 1 fimbriae are two of the most prominent virulence factors of *K. pneumoniae* (11, 24, 33, 54). Capsular polysaccharides are produced by the majority of uropathogenic *Klebsiella* strains and play a significant role in the murine UTI model. A recent study reported that isogenic capsulated and unencapsulated *Klebsiella* strains display significant differences in binding to human cell lines, suggesting that the capsule might mask the activities of other surface-located adhesins (50). The capsule shielding effect has also been demonstrated in adherence studies with other organisms, including *E. coli* (39, 42), *Neisseria meningitides* (53), and *Haemophilus influenzae* (47). In this study, we have demonstrated by several means that the function of type 1 fimbriae is also affected by the presence of a capsule on the bacterial cell surface. First, the capacity of type 1 fimbriae to mediate yeast cell agglutination (a measure of function) was significantly different in the isogenic C105 and C105 NCV strains. Second, induction of functional fimbrial expression via overexpression of the FimB recombinase was slower in the capsulated strain. Finally, fimbrial function could be enhanced by the addition of capsule-inhibitory substances.

In a previous study of selected *K. pneumoniae* UTI isolates, it was reported that capsule formation prevents the assembly of functional type 1 fimbriae on the bacterial surface (26). The authors suggested that capsule expression most likely interferes at the level of fimbrial assembly in the outer membrane. Our data show that capsule expression does not effect inversion of the switch-located *fim* promoter. However unlike the previous study, we did not observe that the presence of a capsule prevents the formation of type 1 fimbrial organelles at the cell surface. Indeed, fimbrial expression was observed in both capsulated and noncapsulated strain backgrounds. Furthermore, the number of fimbriae visualized on the capsulated strain was similar to that of the noncapsulated strain. We therefore hypothesize that the capsule-mediated effect on fimbrial function is most likely due to physical interference between these two very different surface structures. In light of the fact that fimbriae on the surface of a cell can vary considerably in length (0.1 to 2.0 μm) and our electron micrographs show that the capsule extends 0.3 to 0.5 μm out from the cell surface, there seems to be ample room for physical interference. There are a number of mechanisms by which capsule could interfere with fimbrial function: (i) the fimbriae (specifically FimH) may be masked by capsular material, (ii) FimH adhesin molecules that penetrate the capsule may be coated by the polysaccharide and thus rendered nonfunctional, (iii) the presence of a capsule may result in structural weak points within the fimbrial organelle that induce breakage, and (iv) the presence of a capsule may retard the flexibility of fimbriae that is required to achieve optimal adhesion to multiple receptors on the same target cell. An additional mechanism of functional interference relates to the precise location of FimH in the fimbrial structure. FimH is thought to be located on the tip of the fimbrial organelle as an integral part of a short fibril structure (17). A

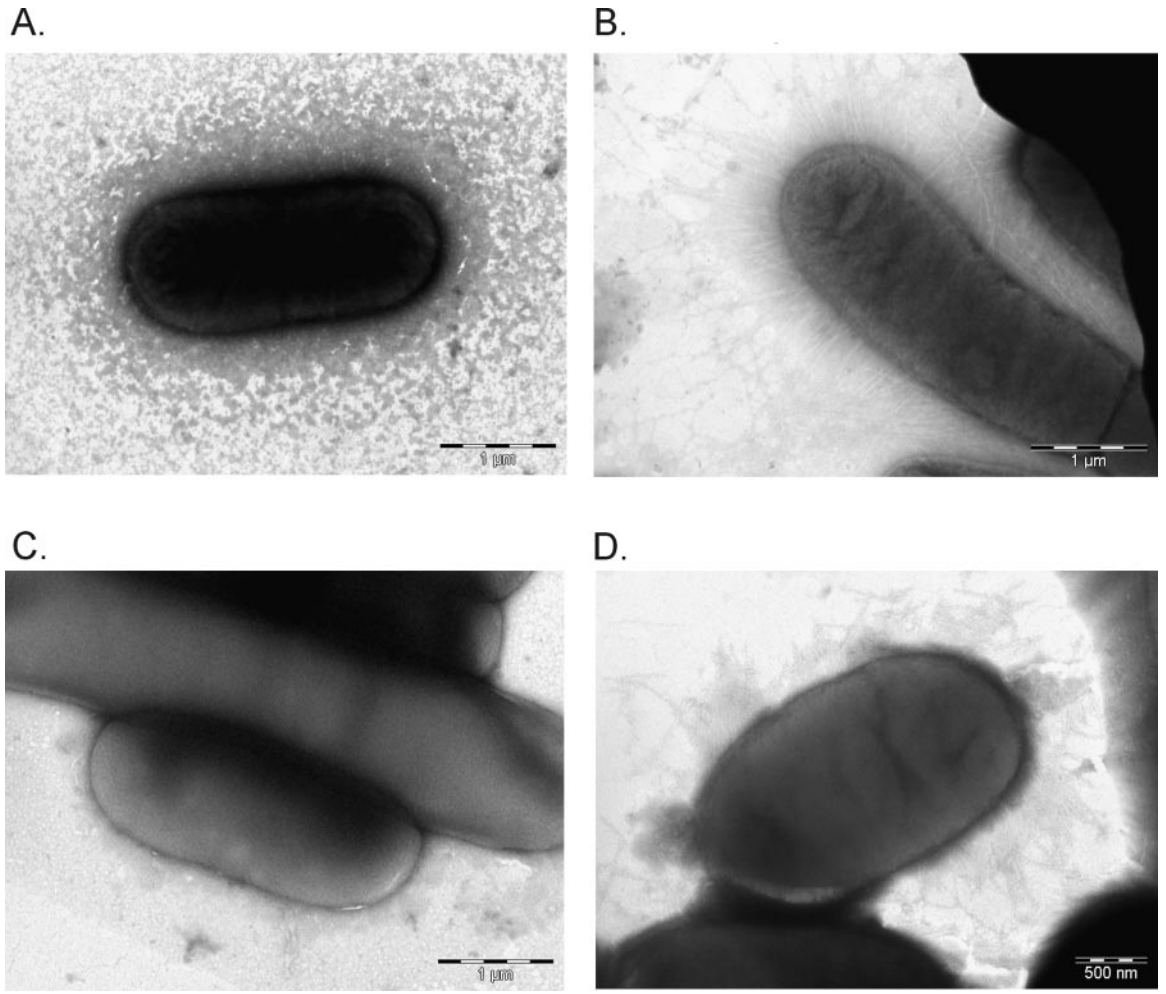

FIG. 4. Transmission electron micrographs of *K. pneumoniae* strains expressing different combinations of capsule and type 1 fimbriae. (A) C105 (capsule$^+$); (B) C105 plus pPKL4 (capsule$^+$ fimbriae$^+$); (C) C105NCV (capsule$^-$); (D) C105NCV plus pPKL4 (capsule$^-$ fimbriae$^+$). Scanning of multiple cells by TEM did not reveal any significant differences in the numbers of fimbriae between C105 and C105NCV (A versus C) or between C105(pPKL4) and C105NCV(pPKL4) (B versus D).

model of fimbrial biosynthesis supports this view (5). However, several independent studies have additionally suggested that FimH is interspersed along the fimbrial shaft (1, 22). Incorporation of FimH at such points in the organelle structure would also be consistent with the reduced level of yeast agglutination observed in the capsulated strain, since they would be masked by the extended polysaccharide matrix.

The regulation of capsule gene expression is complex, with overlapping regulatory circuits. Capsule expression has been reported to be phase variable in several organisms, including *Bacteroides fragilis* (21), *Neisseria meningitidis* (7), and *Campylobacter* (3). In *K. pneumoniae*, capsule production is influenced by environmental conditions (12). Arguably, differential expression of capsules would intermittently result in a noncapsulated state in which fimbriae can function at optimal efficiency. At present, it is hard to say whether bacteria actually coordinate the production of capsules and fimbriae. A recent report suggests that the binding of type 1 fimbriae to D-mannose receptors triggers the down-regulation of the capsule assembly region 1 operon in uropathogenic *E. coli* (46). Alter-

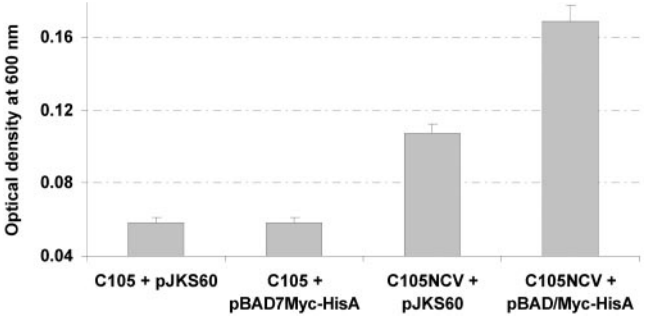

FIG. 5. Biofilm formation by the capsule-producing *K. pneumoniae* strain C105 containing either pJKS60 (FimB$^+$) or pBAD/Myc-HisA (control) and the capsule-negative strain C105NCV containing either pJKS60 (FimB$^+$) or pBAD/Myc-HisA (control). FimB-promoted type 1 fimbria expression promoted an enhanced biofilm formation phenotype only in the absence of any capsular material. The strains were grown under hydrodynamic conditions in LB media containing 0.2% arabinose on polystyrene microtiter plates. Adhered cells were stained with 0.1% crystal violet, and the absorbance was measured at 600 nm. Shown are the averages of readings from three experiments (plus standard deviations).

natively, since both fimbriae and capsule expression are phase variable, random switching between the two surface structures may be sufficient to ensure that a percentage of the population has an appropriate phenotype. It is also possible that bacteria actually compromise the function of fimbriae for the advantage conferred by the presence of a capsule in some environmental scenarios.

The controlled induction of the FimB recombinase enabled us to monitor the kinetics of fimbrial appearance in the capsulated and noncapsulated strains. The two strain types could be directly compared, since there was no difference in FimB-mediated conversion of the switch-located promoter from the "off" to the "on" state. In the C105NCV noncapsulated strain, a lag period of approximately 30 min was observed between the change to "on" orientation of the switch and the detection of functional surface-located fimbriae. This time period is consistent with previous reports (23, 56). Of particular relevance to this study, however, was the fact that an additional 30-min period elapsed before the synthesized fimbriae could penetrate the capsule in sufficient quantities to be detected in a functional form. Furthermore, this functional difference was maintained (albeit to a lesser extent) after extended growth of the induced strains.

An important aspect of this study was the finding that capsulation interfered with but did not abolish the function of type 1 fimbriae. These organelles have been comprehensively studied and are representative of an entire class of structurally similar adhesins, all of which protrude approximately 1 μm from the bacterial surface. Examples include the P, S, and F1C fimbriae of uropathogenic *Escherichia coli* (19). The implications of these findings are far ranging, because they mean that the functions of these other fimbrial types may also be affected by the presence of a capsule. Most strains of *K. pneumoniae* and *E. coli* that cause UTI produce a capsule. In line with our data, several other studies have actually reported that capsulated bacteria adhere poorly to epithelial cells and exhibit severe reduction in the ability to invade compared to their noncapsulated counterparts (26, 40). The FimH adhesin has also been shown to mediate invasion of human bladder epithelial cells (25). Based on the results of this study, one might suspect that this phenotype could be hampered by the concomitant expression of a capsule.

Type 1 fimbriae promote bacterial adhesion, invasion, and biofilm formation, all of which are traits closely associated with bacterial virulence. Capsulation protects bacteria that live in close association with a mammalian host against several defense mechanisms. Together, the two surface components play a significant role in biofilm formation, with most models predicting that they are expressed at different phases, fimbriae for initial adhesion and capsular polysaccharides during the development of mature biofilm structures. The future challenge is to decipher how bacteria orchestrate the expression of these two important types of surface structures in order to make use of their full survival and virulence capabilities.

ACKNOWLEDGMENTS

We thank Eva M. Nielsen, Statens Serum Institut, for providing *K. pneumoniae* strains 264(1) and 264(1)NCV. Also, we thank Birthe Jul Jorgensen for expert technical assistance.

This work was supported by grants from the Australian National Health and Medical Research Council (301163) and the Danish Medical Research Council (22-03-0462 and 2052-03-0013).

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Editor: V. J. DiRita