Consequences of Reduction of *Klebsiella pneumoniae* Capsule Expression on Interactions of This Bacterium with Epithelial Cells

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Most *Klebsiella pneumoniae* clinical isolates are fully encapsulated and adhere in vitro to intestinal cell lines with an aggregative pattern. In this study, the influence of the capsule on interactions with epithelial cells was investigated by creating an isogenic mutant defective in the synthesis of the capsule. Determination of the uronic acid content of bacterial extracts confirmed that the mutant did not produce capsular polysaccharides whereas, with the wild-type strain, the level of encapsulation was growth phase dependent and reached a maximum during the lag and early log phases. Assays performed with different epithelial cell lines, Int-407, A-549, and HEP-2, showed that the capsule-defective mutant demonstrated greater adhesion than did the wild-type strain and that the aggregative pattern was maintained, indicating that the capsule was not related to the adhesion phenotype. In contrast, when the mucus-producing HT-29-MTX cells were used, the encapsulated wild-type strain adhered more strongly than did the capsule-defective mutant. No invasion properties were observed with any of the capsular phenotypes or cell lines used. The *K. pneumoniae* adhesin CF29K was detected by Western blot analysis and enzyme-linked immunosorbent assay on the surface of transconjugants obtained after transfer of a conjugative plasmid harboring the CF29K-encoding genes into both the wild-type and the capsule-defective strains. The amounts of adhesin detected were greater in the capsule-defective background strain than in the wild-type encapsulated strain and were associated with an increase in the level of adhesion to Caco-2 cells. Moreover, RNA slot blot experiments showed that transcription of the adhesin-encoding gene was markedly increased in the capsule-defective mutant compared to the wild-type encapsulated background. These results suggest (i) that the capsule plays an active role during the initial steps of the pathogenesis by interacting with mucus-producing cells but is subsequently not required for the adhesin-related interaction with the epithelial cell surface and (ii) that the expression of the adhesin is modulated by the presence of a capsule at a transcriptional level.

*Klebsiella pneumoniae* is a paradigm of opportunistic bacteria among gram-negative bacilli responsible for nosocomial infections. Extended-spectrum β-lactamase production, an event probably linked to a growing use of cephalosporins as therapeutic agents, was first described in this species. However, antibiotic selection pressure is not sufficient to explain the persistence and spread of opportunistic strains. Previous epidemiological studies showed that nosocomial infections due to *K. pneumoniae* are preceded by colonization of the gastrointestinal tract (7, 21). Contamination of different sites of the body from this reservoir is likely to occur via exogenous or endogenous processes.

The colonization of mucous membranes by bacteria is linked to an adhesion process involving specific adhesins on the bacterial surface (16). Several pili involved in either adhesion to bladder epithelial and tracheal ciliated cells (type 1 pili) or adhesion to kidney epithelium through the type V collagen (type 3 pili) have been described in *K. pneumoniae* isolates (12, 13, 33). To reproduce the interactions between *K. pneumoniae* and the intestinal mucus, Caco-2 and Intestine-407 cell lines have been used in vitro adhesion assays (6, 9). Three adhesion phenotypes have been described to date: a diffuse pattern of adhesion where bacteria spread all over the cell surface, an aggregative phenotype in which bacteria clump onto the cells, and a localized pattern associated with microcolonies (15, 20). A nonfimbrial adhesin named CF29K, as well as a fimbrial one, KPF28, have been shown to be responsible for the diffuse adhesion phenotype (6, 9). No such adhesins have been characterized in *K. pneumoniae* strains showing either aggregative or localized phenotypes. Electron microscopic observations revealed that bacteria expressing aggregative adhesion are surrounded by a capsule material which is probably involved in bacterium-eucaryotic cell and bacterium-bacterium interactions (15).

Little is known about the involvement of capsular polysaccharides in bacterial interactions with mucosal surfaces. The presence of a capsule was shown to reduce the adhesion of enterotoxigenic *Escherichia coli* to pig intestinal epithelial cells (27) and of Neisseria meningitidis to both mucosal and Chang cells (29, 35, 36). Similar results were obtained when comparing a wild-type *Haemophilus influenzae* type b isolate with its capsule-deficient mutant in adhesion assays with Chang epithelial cells (31). It was recently demonstrated that the capsule of *H. influenzae* type b inhibits adhesion by masking the bacterial fibrils and therefore impairing the bacterium-eucaryotic cell receptor recognition (30). Thus, in at least some cases, capsules may reduce adherence rather than mediating it.

Since most clinical and environmental isolates of *K. pneumoniae* are fully encapsulated, it is likely that the first contacts with human mucosal surfaces occur via bacterial capsular polysaccharides. The following experiments were undertaken to...
TABLE 1. Strains and plasmids used in this work

<table>
<thead>
<tr>
<th>Strains or plasmid</th>
<th>Relevant characteristics</th>
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<tr>
<td>K. pneumoniae</td>
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<tr>
<td>LM21</td>
<td>Wild type; K35</td>
<td>This study</td>
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<td>LM21 Rif&lt;sup&gt;+&lt;/sup&gt;</td>
<td>K. pneumoniae LM21, Rif&lt;sup&gt;+&lt;/sup&gt;, capsule-defective mutant</td>
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<td>LM21(cps)</td>
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<td>CF504</td>
<td>Clinical isolate carrying cf29K</td>
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<td>CH067</td>
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<td>Kn&lt;sup&gt;r&lt;/sup&gt;; source of Kn&lt;sup&gt;r&lt;/sup&gt; cassette</td>
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<td>pLD55</td>
<td>Suicide vector, derivative of pBluescript II (SK&lt;sup&gt;+&lt;/sup&gt;), Tet&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This study</td>
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<td>pS1-3</td>
<td>pUC18 plus 2500 bp of cps region</td>
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<td>pS1-3ΔKn</td>
<td>pS1-3 with 500-bp deletion</td>
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<td>pSFB1-3ΔKn</td>
<td>pLD55 containing orf1–3ΔKn</td>
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demonstrate whether the capsule was involved in the interactions of K. pneumoniae with epithelial cells. We created a capsule-defective mutant of a K. pneumoniae aggregative isolate advantage of the recent description by Arakawa et al. of the genomic organisation of the 29-kb region responsible for the K. pneumoniae capsular polysaccharide synthesis (2). The nucleotide sequence analysis revealed 19 open reading frames (ORF), some of which were highly conserved among the different capsular phenotypes. In this paper we describe the mutant construction by allelic exchange techniques and present evidence of the role of capsule in the interactions with several epithelial cell lines. We also discuss CF29K adhesive expression at the cell surface according to the level of bacterial encapsulation.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Bacterial strains were stored at −20°C in Luria-Bertani (LB) broth (Difco, Paris, France) containing 15% glycerol and were cultivated on LB agar supplemented with the appropriate antibiotics at the following concentrations: ampicillin, 50 μg/ml; tetracycline, 20 μg/ml. Bacterial growth curves were obtained with an overnight culture of the K. pneumoniae strain and was incubated at 37°C in a shaker for 8 h. Bacterial growth was monitored by measuring the optical density at 620 nm, and the numbers of CFU were quantified by plating serial dilutions of the suspension on LB agar plates. For qualitative adhesion experiments, assays were performed as described above, except that eucaryotic cells were seeded onto glass coverslips. After being washed, the cells were fixed with methanol, stained with 20% Giemsa, and examined under a light microscope.

Invasion assays. Confluent monolayers of the different cell lines were rinsed twice with 1 ml of PBS. Bacteria (10<sup>9</sup>) were suspended in the appropriate medium containing 2% d-mannose and added to the cells. After 1 h of incubation at 37°C, the cell monolayers were rinsed four times with 1 ml of PBS, and gentamicin was added to each well at 100 μg/ml. After 3 h of incubation, four washes were performed with PBS and intracellular bacteria were released by addition of 1 ml of 0.5% Triton X-100 (Sigma) and quantified by plating appropriate dilutions on LB agar plates. Adhesion was expressed as the number of CFU adherent to eucaryotic cells per monolayer. For qualitative adhesion experiments, assays were performed as described above, except that eucaryotic cells were seeded onto glass coverslips. After being washed, the cells were fixed with methanol, stained with 20% Giemsa, and examined under a light microscope.

Extraction and quantitation of capsular polysaccharides. Capsular polysaccharides were extracted according to the method previously described by Domencio et al. (10). Samples (500 μl) of bacterial cultures were removed at various times and mixed with 100 μl of 1% Zwittergent 3-14 detergent (Calbiochem, Meudon, France) in 100 mM citric acid (pH 2.0). This mixture was incubated at 50°C for 20 min. After it was centrifuged for 5 min at 14,000 rpm in a no. 5415C Eppendorf centrifuge, 300 μl of the supernatant was transferred to a new tube and absolute ethanol was added to a final concentration of 80%. The mixture was placed at −20°C for 20 min. After centrifugation (14,000 rpm), the supernatant was decanted and the pellet was dissolved in 200 μl of distilled water.

Polysaccharides were then quantitated by measuring the amount of uronic acid (4). A 1,200-μl volume of 0.025 M tetraborate in concentrated H<sub>2</sub>SO<sub>4</sub> was added to 200 μl of the sample to be tested. The mixture was vigorously vortexed and heated in a boiling-water bath for 5 min. The mixture was cooled, and 20 μl of 0.15% 3-hydroxydiphenol (Sigma-Aldrich Chimie, L’Île d’Abeau, France) in 0.5% NaOH was added. The tubes were shaken, and absorbance measurements...
were made at 520 nm. The uronic acid concentration in each sample was determined from a standard curve of D-mannuronic acid (Sigma-Aldrich Chimie). In parallel, serial dilutions of the bacterial culture were plated to determine the number of CFU/mL. Bacterial cell content was expressed as CFU/mL.

**Construction of a capsule-defective mutant.**

The genomic organization of a serotype K2 *K. pneumoniae* isolate cps region responsible for capsular polysaccharide synthesis has been determined by Arakawa et al. (2). Only some parts of this 29-kb region are highly conserved among the different K serotypes as demonstrated by hybridization assays and PCR amplifications (2, 14). To create a capsule-defective mutant of *K. pneumoniae* LM21 (serotype K35), a 2,560-bp fragment was amplified from a highly conserved region including a putative promoter-polycistronic operon by using the following primers designed from the published *K. pneumoniae* Chedid (serotype K2) isolate sequence: kcps1, 5'-GCCGATGCCGGAAAGAGCTTATATCA-3' and kcps3, 5'-GCCGATGGTTATATGGCGCA-3'. Chromosomal DNA isolated from the wild-type *K. pneumoniae* LM21 was subjected to 30 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 4 min of extension at 68°C. The resulting 2,560-bp PCR product was cloned into the BamHI site of pUC18 (Boehringer Mannheim, Meylan, France), resulting in plasmid pS1-3. This plasmid was then deleted from 509 bp internal to the cloned fragment and comprising the upstream promoter region located just upstream of the first ORF (ORF3) of a polycistronic structure by digesting with BclI and self-ligating to form pS1-3A. An *aph*3″ kanamycin-resistance-encoding cassette (Pharmacia Biotech, Saclay, France), including a translation termination, digested by BamHI was inserted into the BclI site of plasmid pS1-3A. The resulting plasmid, pS1-3Kn, was then digested with BamHI. The cloned fragment was subcloned into the BamHI site of the *K. pneumoniae* plasmid pLD55, a lambda pir vector (24). Plasmid pSFB1-3Kn was then introduced into *K. pneumoniae* LM21 by mating experiments carried out by patch mutagenesis. The resulting colonies were streaked out onto counterselection agar plates containing tetracycline (20 μg/ml) and kanamycin (20 μg/ml). The transconjugants resulting from a double recombination event were screened for the loss of tetracycline resistance.

**Construction of a capsule-defective mutant.**

Chromosomal DNA was prepared from wild-type *K. pneumoniae* LM21 and from mutant LM21 cps (pSFB1-3Kn) as described by Sambrook et al. (28). The chromosomal DNA was digested with appropriate restriction endonucleases and separated by agarose gel electrophoresis (0.7% agarose). The probes used contained either the kanamycin cassette or an internal restriction endonuclease fragment from the capsule-encoding region of *K. pneumoniae* LM21. For labeling, genomic DNA from the mutant and DNA probes specific for the kanamycin cassette and the 2,050 bp of the DNA fragment from the fragment of the wild-type *K. pneumoniae* CRM904 to *K. pneumoniae* LM21 Rf and to *K. pneumoniae* LM21 (pSFB1-3Kn). Transconjugants were selected on Mueller-Hinton agar (Sanofi-Pasteur, Marne la Coquette, France) containing 20 μg/ml rifampicin (rif) and ceftazidime (10 μg/ml) and were used for hybridization experiments.

**Bacterial surface protein analysis.**

Bacterial surface proteins were extracted as previously described (6) by heating at 60°C for 20 min with gentle agitation. After centrifugation at 10,000 × g for 20 min, the supernatant was brought to pH 4.0. The resulting proteins were collected by precipitation and resuspended in PBS. Extracted proteins (15 μg for each sample) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 12% acrylamide for each sample) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 12% (wt/vol) acrylamide for the separation gel (18). Western immunoblotting conditions for the adsorbed anti-CF29K antisera were reported previously (6).

**Quantitative analysis of the CF29K adhesin by ELISA.**

The amount of CF29K adhesin expressed from the same plasmid-encoded gene in the different strain backgrounds was estimated by an enzyme-linked immunosorbent assay (ELISA) with optimal concentrations of the anti-CF29K polyclonal antibody (6) and anti-CS31A biotinylated rabbit immunoglobulin G recognizing the CF29K adhesin and provided by J. P. Girardeau (Laboratoire de Microbiologie, Institut National de la Recherche Agronomique, Theix, France). ELISA microtiter plates (ICN Biomedicals, Orsay, France) were coated overnight with anti-CF29K antibodies. After nonspecific binding sites were blocked by addition of 1% bovine serum albumin in PBS, whole bacteria were added to the wells and incubated for 2 h at 37°C. After several washes with 0.1% Tween 20 in PBS, biotin-labeled anti-CS31A antibodies were added in excess and a streptavidin-alkaline phosphatase complex (Bio-Rad, Ivry Sur Seine, France) was added. For signal development, the wells were incubated with p-nitrophenylphosphate (Sigma-Aldrich) and the optical density was determined at a wavelength of 405 nm.

**Characterization of capsular polysaccharide production by the mutant.**

As shown in Fig. 3, the colonies obtained after overnight culture of the *K. pneumoniae* mutant on agar plates were quite different from the ones obtained with wild-type *K. pneumoniae* LM21; they were much smaller and did not exhibit the smooth phenotype characteristic of surface polysaccharide-producing colonies. Since capsule synthesis is known to be dependent on the physiological state (23), specific quantitation of capsular polysaccharides was performed with both the wild-type and mutant strains at different times of the growth curve. With the wild-type *K. pneumoniae* strain (Fig. 4A), the amount...
of uronic acid reached a maximum during the lag and the early log phases (180.2 ng of uronic acid/10^6 CFU after 40 min of incubation) and then constantly declined. After 300 min, the level of capsule remained minimal (5.5 ng of uronic acid/10^6 CFU). Similar experiments performed with the mutant strain showed that the level of uronic acid remained low (below 30 ng/10^6 CFU) at all points of the growth curve (Fig. 4B), indicating that this mutant was in fact defective in the synthesis of capsular polysaccharides.

Influence of the capsule on the interactions of *K. pneumoniae* with epithelial cells. Previous adhesion experiments performed with samples of the wild-type *K. pneumoniae* LM21 removed at various time of the growth curve revealed that the level of adhesion to Int-407 cells was inversely proportional to the level of capsule detected; i.e., adhesion was minimal during the lag and early log phases and maximal with bacterial samples taken during the late exponential and stationary phases (data not shown). For these reasons and because we wished to compare the behavior of the wild-type capsule-producing strain with its capsule-defective mutant, we performed adhesion assays with bacteria collected during the early log phase (2 h postinoculation) and incubated for 1 h only with eucaryotic cells. The results obtained with the different cell lines, namely, Int-407, A-549, and HEp-2, cells are presented in Fig. 5. For all cell lines tested, the capsule-defective mutant adhered more than the wild-type strain; the greatest difference was observed with the type II pneumocyte-like cell line, A-549 (the level of adhesion being eight times higher for the mutant strain than for the wild-type strain). Microscopic observations after Giemsa staining showed bacteria from the mutant strain adhering to the epithelial cells via an aggregative phenotype, identical to the one observed with the wild-type strain (data not shown).

Interactions with mucus-producing cells were investigated with HT-29-Rev MTX 10^5 differentiated cells. After 2 h of culture, bacteria were added to the confluent monolayer containing an average of 5 × 10^5 cells. Determination of the number of CFU associated with the cells indicated that the encapsulated wild-type strain adhered to these mucus-producing cells to a higher level (three times higher) than did the capsule-deficient strain, in contrast to the results obtained with the previous cell lines tested (Fig. 5).

To determine if the loss of capsule production would favor an invasion phenotype, gentamicin resistance assays were performed with both the capsule-defective mutant and the wild-type strain, using Int-407, HEp-2, and A-549 cells. Intracellular bacteria were detected with neither strain after 3 h of incubation in a gentamicin-containing medium.

Influence of the capsule on the expression of *K. pneumoniae* adhesin at the bacterial cell surface. To determine the influence of encapsulation on the adhesin-mediated interaction with epithelial cells, we introduced the self-transmissible pCFF504 plasmid harboring the genes coding for a known

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![Diagram](https://example.com/diagram.png)

**FIG. 1.** Construction of the capsule-defective mutant *K. pneumoniae* LM21(cps). (A) A 2,560-bp DNA fragment was amplified from *K. pneumoniae* LM21 chromosomal DNA by PCR and ligated into the BamHI site of pUC18 to obtain pS1-3. An internal BclI 509-bp fragment was deleted and replaced by the Knr cassette, and the construct was cloned into the BamHI site of pLD55. (B) Allelic exchange mutagenesis. After introducing pSFB1-3Kn into *K. pneumoniae* LM21, a single recombination event leads to the formation of a cointegrate (KnTetr) and a double recombination event leads to the mutated copy (Kn') after loss of the suicide vector (Tet').
adhesin from \textit{K. pneumoniae}, the CF29K adhesin (6), into both the \textit{K. pneumoniae} LM21 wild-type strain and its capsule-defective mutant. Adhesion to Caco-2 cells was at least three times higher with transconjugants obtained with the capsule-defective mutant, CH009, as recipient than with the mutant itself (4.70 $\pm$ 10$^3$ and 1.35 $\pm$ 10$^3$, respectively), whereas no difference was observed between the wild-type strain, LM21, and its transconjugant, CH067, (2.24 $\pm$ 10$^2$ and 2.80 $\pm$ 10$^2$, respectively). Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of similar amounts of total bacterial surface protein extracts of the two types of transconjugants revealed that only transconjugants obtained with the mutant strain as recipient produced large amounts of the CF29K adhesin as detected by immunoblotting with specific anti-CF29K antibodies (Fig. 6B, lane 4). Plasmid pCFF504 had been previously introduced into an \textit{E. coli} K-12 recipient strain (6), known to produce low levels of extracellular polysaccharides. The surface extract analysis revealed similar results, i.e., a higher quantity of CF29K protein detected with this transconjugant than with the \textit{K. pneumoniae} CF504 donor wild-type strain (Fig. 6, lanes 1 and 2).

To quantify the differences observed in the previous immunoblot experiments, an ELISA was performed with antibodies raised against the CF29K protein and whole bacteria. Regardless of the number of bacteria used in the assay, the binding curves showed that the amounts of CF29K adhesin detected were greater with both the capsule-defective mutant and the \textit{E. coli} K-12 backgrounds compared to the corresponding encapsulated wild-type \textit{K. pneumoniae} strains, respectively LM21 and CF504 (Fig. 7). Finally, \textit{cf29A} transcription was examined in both the wild-type and the capsule-defective transconjugants by probing RNA extracts with a \textit{cf29A}-specific probe. As shown in Fig. 8, \textit{cf29A} expression was strongly increased in the capsule-defective mutant compared to the fully encapsulated wild type.

**DISCUSSION**

A mutant defective in the synthesis of capsule was created by allelic exchange by using a lambda pir-dependent vector in a \textit{K. pneumoniae} strain. Based on the genomic sequence encoding the capsule of a K2 serotype \textit{K. pneumoniae} isolate (2), a DNA fragment was amplified from the \textit{K. pneumoniae} LM21 wild-type strain serotype K35 in a conserved capsule-encoding region. This fragment was deleted from an internal sequence encompassing the putative promoter sequence of a polycistronic operon involved in both capsule polysaccharide assembly and transport to the cell surface. An \textit{aph3}$^\text{’}$ cassette was inserted in this deleted fragment, and a double-recombination event was screened after introducing this construction in a \textit{l}-pir-dependent vector. To our knowledge, this is the first report of the use of a \textit{l}-pir system in bacteria belonging to the genus \textit{Klebsiella}. The resulting modification in capsular polysaccharide expression at the cell surface was appreciable by streaking out the mutant on an agar plate. Because \textit{K. pneumoniae} serotype K35 capsular polysaccharides include acidic saccharides such as uronic acids (11), the level of encapsulation was estimated by quantifying uronic acids. We demonstrated that the mutant strain had at least sevenfold less capsular material than the wild type and that the capsule levels observed in the mutant strain were similar to background levels observed with other nonencapsulated \textit{K. pneumoniae} strains (14, 25).
We noted that the level of encapsulation of the K. pneumoniae wild-type strain was affected by the growth phase. This level was maximal 40 min after the beginning of growth and decreased during the exponential and stationary phases. These results are in agreement with a previous study showing that a K1 K. pneumoniae strain produced maximum levels of capsular polysaccharides at low growth rate, i.e., when bacteria are dividing slowly (23). Moreover, Whitfield et al. (38) demonstrated that the synthesis of E. coli K1 capsule occurred 10 min after a temperature upshift and no further increase occurred after 45 min. Since the biosynthesis of capsule in K. pneumoniae is controlled by a two-component regulatory system (1, 22), it is likely that encapsulation is modulated by the environmental milieu and can shift very quickly.

In a previous study, we showed that the adhesion of a K. pneumoniae aggregative isolate to Int-407 cells was maximal at the end of the exponential phase and during the stationary phase (15). Similar results were obtained with the wild-type K. pneumoniae LM21 in the present study. Thus, the expression of the adhesion phenotype is inversely correlated with the level of encapsulation, suggesting that during infection, the loss of capsule favors the process of adhesion to epithelial cells.

We demonstrated by performing adhesion assays with the isogenic capsule-defective mutant and intestinal cell lines, laryngeal cells, and epithelial cells derived from the lungs that this strain adhered more efficiently than the encapsulated K. pneumoniae strain. The greatest adhesion was obtained with the A-549 cells derived from a human lung carcinoma. Since K. pneumoniae is a pathogen frequently involved in pneumonia, this type of cell could be the preferential target of this organism’s adhesins. The adhesion of the capsule-defective mutant was higher in all cell lines used, and the aggregative adhesion pattern was similar in all cases. This result demonstrated that the capsule itself was not responsible for the aggregative phenotype but in fact impaired adhesion to these cell lines. Several other examples of polysaccharide capsules disrupting bacterial interactions with epithelial cells have been reported: adhesion experiments with enterotoxigenic E. coli demonstrated that capsule inhibits the recognition of pig intestinal cells by K99 pili (27) and the presence of the group B streptococcus capsule attenuated the adherence of this bacteria to A-549 cells (32). Likewise, adhesion of Neisseria meningitidis to human epithelial cells was enhanced by the loss of the polysialic acid capsule.

**FIG. 4.** Plots of capsule quantities related to the growth phase of wild-type K. pneumoniae LM21 (A) and mutant K. pneumoniae LM21(cps) (B). Growth curves were determined by measuring the number of CFU (solid triangles). Capsule was quantified by measuring the quantity of uronic acid per 10⁶ CFU (open squares). Values for uronic acid contents are the mean of measurements made in triplicate.

**FIG. 5.** Adherence assays performed with Int-407, A-549, HEP-2 and HT-29-MTX 10⁻⁶ cell lines with the wild-type K. pneumoniae LM21 (solid bars) and the capsule-defective mutant LM21(cps) (hatched bars). The results are expressed in CFU per monolayer. The data are the mean of measurements made in triplicate.

**FIG. 6.** SDS-PAGE (A) and Western blot (B) of bacterial surface proteins from K. pneumoniae CE504 (lane 1), E. coli transconjugant CI604 (lane 2), K. pneumoniae CH007 (lane 3), and K. pneumoniae CH009 (lane 4). Molecular weight marker positions are shown for carbonic anhydrase (30,000) and ovalbumin (43,000).
When the adhesion abilities of *Haemophilus influenzae* type b strain and its isogenic capsule-negative mutant were compared, it was found that the mutant strain demonstrated a greater adhesion to Chang epithelial cells (31).

Unlike the results obtained with the epithelial cell lines Int-407, A-549, and HEp-2, adhesion experiments performed with the mucus-producing HT-29-Rev MTX 10^6 cells revealed that the capsule-defective mutant adheres less than the strongly the wild-type encapsulated strain. Although the difference was not very great, it was reproducible and would indicate that the presence of mucus components on the cell surface favors the establishment of interaction with polysaccharide-surrounded bacteria.

Introduction of an adhesin-encoding gene by conjugation into both the wild-type encapsulated strain and its capsule-defective mutant induced higher levels of adhesion to Caco-2 cells, but only with the latter transconjugant. Two independent possible explanations concerning the higher adhesion level observed with bacteria expressing little capsular polysaccharide material can be proposed. Because of the absence of capsule, the adhesin protein would be much more accessible at the cell surface. The masking of adherence factors by capsular polysaccharide-surrounded bacteria is strain related.

FIG. 7. Anti-CF29K antibody-binding analysis of the whole bacteria: *K. pneumoniae* CH067 and CH009 (A) and *K. pneumoniae* CF504 with its transconjugant *E. coli* CF604 (B). The abilities of the bacteria from different backgrounds to bind the anti-CF29K antibodies were measured by ELISA. Microtiter plates precoated with anti-CF29K antibodies were incubated with different quantities of the whole *K. pneumoniae* CH067 (open squares), *K. pneumoniae* CH009 (solid squares), *K. pneumoniae* CF504 (solid triangles), or *E. coli* CF604 (open triangles). To detect bound bacteria, wells were incubated with biotinylated anti-CS31A antiserum; streptavidin-alkaline phosphatase and an appropriate substrate were added, and the optical density at 405 nm was monitored. The optical density measured at 405 nm from the negative control (no bacteria added) was equal to 0.130.

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FIG. 8. Regulation of cf29A transcription by the presence of capsule. For the RNA slot-blot analysis: 1 or 5 μg of RNA isolated from *K. pneumoniae* CH067 (encapsulated) and CH009 (nonencapsulated) was hybridized with a cf29A specific probe (left). The same blot was probed with an rnbB7 probe as a standard for the total amount loaded onto the membrane (right).
role by interacting with the mucus layer whereas its presence is a disadvantage when bacteria come in contact with the underlying epithelial cells. The expression of the two bacterial surface factors would be closely synchronized and influenced by external factors. It has been recently demonstrated with Salmonella typhi that the cell surface-associated polysaccharide (antigen Vi) prevents the secretion of both invasion proteins and flagellin and that this control occurs at both transcriptional and posttranscriptional levels and is environment dependent (3). Further studies are required to determine the genetic organization involved in the K. pneumoniae coordination of adhesin and capsule expression.

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