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# Down-Regulation of the *kps* Region 1 Capsular Assembly Operon following Attachment of *Escherichia coli* Type 1 Fimbriae to D-Mannose Receptors

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**A differential-display PCR procedure identified the capsular assembly gene *kpsD* after *Escherichia coli* type 1 fimbrial binding to mannose-coated Sepharose beads. Limiting-dilution reverse-transcribed PCRs confirmed down-regulation of the *kpsD* gene, and Northern blot and *lacZ* fusion analyses showed down-regulation of the *kpsFEDUCS* region 1 operon. KpsD protein levels fell, and an agglutination test showed less K capsular antigen on the surface following the bacterial ligand-receptor interaction. These data show that binding of type 1 fimbriae (pili) to D-mannose receptors triggers a cross talk that leads to down-regulation of the capsule assembly region 1 operon in uropathogenic *E. coli*.**

Uropathogenic *Escherichia coli* (UPEC) strains are the primary causes of urinary tract infections in humans (14). A variety of virulence factors have been shown to be important for UPEC pathogenicity, including adhesins, hemolysins, capsules, and iron-acquiring proteins (35). Adherence to uroepithelial cells is a critical first step in the pathogenesis of UPEC strains. Both P and type 1 fimbriae (pili) are the most frequently observed pilus structures on *E. coli* cells isolated from the urinary tracts of patients (18, 25). Type 1 fimbriae bind to mannose-containing receptors found in the urinary tracts of mice and humans (12, 21). The ability to attach to these mannose receptors enables the bacteria to gain a foothold in the urinary tract that may lead to invasion into the bladder epithelium (20).

Several techniques have been used to try to elucidate what might be occurring within the bacterial cell following bacterial attachment or what may be important for in vivo survival of UPEC within a human host (3, 4, 34, 37). In this study, a differential-display PCR (DDPCR) was applied to determining what genes might be up- or down-regulated following binding of type 1 fimbriae to mannose receptors to obtain clues as to what might be occurring within the bacteria following entry and initiation of an infection within a human urinary tract.

The NU149 uropathogenic strain of *E. coli* (28) was grown in Luria broth as previously described (15) to allow for optimal expression of type 1 fimbriae, which was confirmed by an enzyme immunoassay with anti-149 pilus antiserum (30; data not shown). This culture was divided into two parts. One aliquot was reacted with Sepharose 4L beads (Sigma Chemical Co., St. Louis, Mo.), whereas the other aliquot was reacted with D-mannose-coated Sepharose beads (17). The interaction between strain NU149 cells and D-mannose-coated Sepharose beads resulted in 67% of the population binding to the beads, whereas NU149 cells mixed with plain Sepharose beads led to only 21% of the population either binding nonspecifically or being trapped by the beads. After 1.5 h, total RNAs were isolated from both populations by using a hot phenol extraction procedure (30) and treated twice with RNase-free DNase (Boehringer-Mannheim). A DDPCR that was previously described was performed, utilizing the PLCA2 primer to run the amplification (32). The DDPCR products were separated on 5% sequencing gels, and the numbers and intensities of bands were compared for the lane containing plain Sepharose versus that containing the D-mannose-coated Sepharose. Several bands were either missing in one lane compared to the other or had reduced intensity (data not shown). Each band was processed as previously described (32), using the PLCA2 primer to reamplify the DNAs. The resulting PCR products were ligated to pTZ18R plasmid DNA cut with SmaI (27). After verification that there was an insert (data not shown), each recombinant plasmid was sequenced, using M13 forward and reverse primers and the Sequenase 2.0 kit (USB, Cleveland, Ohio). One of the cloned DDPCR DNA products showed extensive homology with the *E. coli kpsD* gene (24). The DDPCR indicated that the *kpsD* transcript level was lower in the lane that represented binding to mannose-coated Sepharose beads (data not shown).

The *kpsD* gene is part of the *kpsFEDUCS* operon, also named capsule region 1, involved in assembly of capsular subunits that comprise the K antigen of *E. coli* (36). A single promoter drives transcription of the polycistronic *kpsFEDUCS* transcript. Strain NU149 appears to have a group II capsule gene locus structure organized into three regions. Regions 1 (*kpsFEDUCS*) and 3 (*kpsMT*) are very conserved at the genetic level and are involved in the assembly and transport of the capsular material. The final region, region 2 (*kfiABCD*), is

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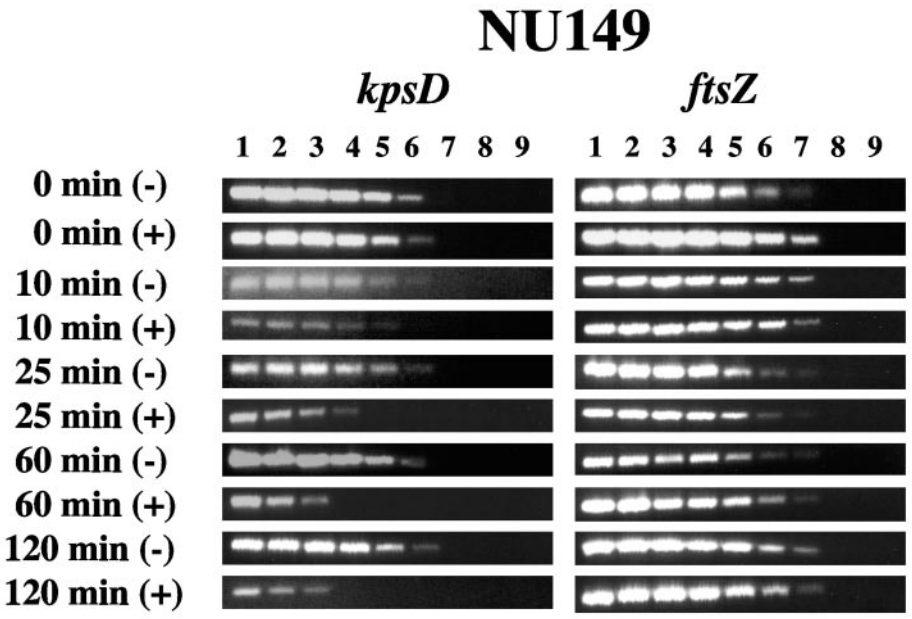


FIG. 1. Quantitative determination of *kpsD* and *ftsZ* transcript levels by LD-RT-PCR analysis. The cDNAs generated from strain NU149 cells mixed with plain Sepharose or D-mannose-coated Sepharose beads were examined at 0, 10, 25, 60, and 120 min. The KpsD1-KpsD2 and ECFtsZ1-ECFtsZ2 primer pairs were used to amplify serially twofold-diluted cDNAs targeting *kpsD* (367-bp product) and *ftsZ* (302-bp product) transcripts, respectively. The PCR amplifications were done a minimum of three times. All PCR products were electrophoresed on 1.5% agarose gels. The following dilutions of cDNAs were used: undiluted (lane 1), 1:2 (lane 2), 1:4 (lane 3), 1:8 (lane 4), 1:16 (lane 5), 1:32 (lane 6), 1:64 (lane 7), 1:128 (lane 8), and 1:256 (lane 9).

unique to each serotype and is directly involved in the biosynthesis of the capsular material (36).

To verify that there was a down-regulation of the *kpsD* gene following binding to the D-mannose-coated Sepharose beads, the NU149 strain was grown in Luria broth and divided into aliquots: one was reacted with the plain Sepharose beads, and the other was reacted with D-mannose-coated Sepharose beads. After 0, 10, 25, 60, and 120 min, total RNAs were extracted from both sets of cultures and converted into cDNAs as noted above. With these cDNAs, limiting-dilution reverse-transcribed PCRs (LD-RT-PCRs) were performed with the KpsD1 (5′-A ACGGACAGAAGTCGGATACG-3′) and KpsD2 (5′-TGTA ATAAGGAGGCGTAGACG-3′) primer pair, synthesized by Integrated DNA Technologies (Coralville, Iowa). The LD-RT-PCR conditions were as follows: an initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1 min, ending with a final elongation at 72°C for 7 min at the end of the last cycle. A 367-bp product was amplified. Each cDNA population was diluted twofold up through 1:256, and each dilution was PCR amplified. Amplification products were analyzed on 1.5% agarose gels, comparing the populations reacted with plain Sepharose versus those reacted with mannose-coated Sepharose beads. As a control, amplifications of each cDNA population were done using a primer pair specific for the *ftsZ* gene of *E. coli* that has been used previously (33). The results indicated that at time zero there was no difference between the *E. coli* cell populations. However, beginning at 10 min and proceeding through 120 min, there was a gradual decline in the level of *kpsD* transcripts in the mannose-coated Sepharose population compared to that in the plain Sepharose population that culminated in an eightfold decline after 120 min (Fig. 1). The level of *ftsZ* transcripts remained unchanged throughout the time course for both populations. This suggested that the ligand-receptor interaction between type 1 fimbriae and the mannose receptors led to the down-regulation of *kpsD* transcription.

To further substantiate that the binding of type 1 fimbriae to mannose receptors had an effect on capsule region 1 transcription in *E. coli* cells over time, the single promoter for the *kpsFEDUCS* gene cluster (region 1), which gives rise to an approximately 7.9-kb polycistronic transcript (36), was fused to *lacZ* by using plasmid pUJ9 (9). This was then moved to the single-copy-number plasmid pPP2-6 (33), resulting in plasmid pNLW4-46. *E. coli* AAEC189 cells (Δ*fim* Δ*lac* [5]) were cotransformed with pNLW4-46, containing the *kps-lacZ* reporter fusion, and pWRS1-17, which has the entire *fim* operon encoded on it (30). The recombinant AAEC189 cells were reacted with plain Sepharose beads, D-mannose-coated Sepharose beads, L-mannose-coated Sepharose beads, and plain Sepharose plus 50 mM free D-mannose. When tested for β-galactosidase levels at 0 and 1 h, all of the populations looked fairly similar, but at 8 h and then ultimately at 24 h there was more than a threefold difference in β-galactosidase units in the population that was mixed with D-mannose-coated Sepharose compared to that mixed with plain Sepharose (Fig. 2). The *kps* levels increased slightly in the population mixed with plain Sepharose and L-mannose-coated beads after a 24-h exposure. Although the kinetics of the down-regulation of the gene cluster and hence *kpsD* were different compared to the results of the LD-RT-PCR analysis, this can be explained by the need for transcription and translation of *lacZ* in a β-galactosidase assay

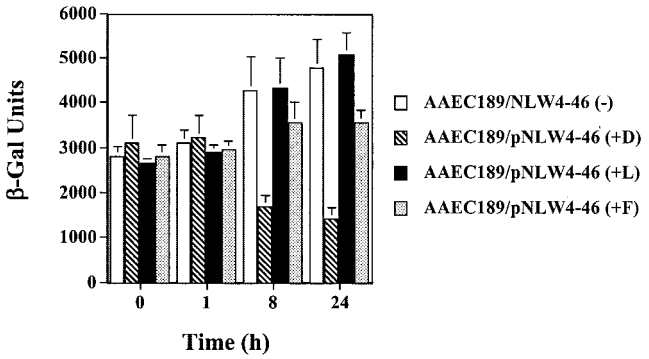


FIG. 2. Regulation of the *kpsFEDUCS* promoter following attachment to plain Sepharose (−), L-mannose-coated Sepharose (+L), D-mannose-coated Sepharose (+D), or plain Sepharose plus 50 mM free D-mannose (+F). The assays were done using a *kpsFEDUCS* promoter fused to *lacZYA* as a transcriptional fusion on a single-copy-number plasmid placed in strain AAEC189. The β-galactosidase (β-Gal) activity is expressed in Miller units; means ± standard deviations are indicated from three separate runs.

as opposed to merely transcription in the PCR-based assay. Moreover, mixing the bacterial cells with L-mannose-coated Sepharose did not affect the level of *kpsFEDUCS* operon (region 1) expression. On the other hand, the addition of 50 mM free D-mannose to the population mixed with plain Sepharose did affect region 1 expression, suggesting that merely having the interaction with D-mannose, regardless of whether it is bound to a bead or free, was affecting this expression.

To assess whether the attachment of the type 1 fimbrial adhesin to its receptor might be involved in the down-regulation of *kpsD*, wild-type strain NU14 cells (15) were compared to cells of strain NU14-1 (19), an *fimH* mutant strain missing the FimH adhesin, after mixing each bacterial population with D-mannose-coated Sepharose beads. Upon performing LD-RT-PCR analyses, the wild-type strain, NU14, had the same level of down-regulation of *kpsD* as strain NU149 had at the same time point (Fig. 3). However, the NU14-1 strain appeared to display the same level of *kpsD* expression at both the 0- and 120-min time points. Transcription of *kpsD* appears to

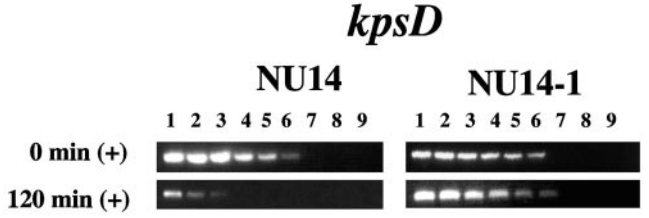


FIG. 3. Quantitative determination of *kpsD* transcript levels in strains NU14 (wild type) and NU14-1 (*fimH* mutant) by LD-RT-PCR analysis. The cDNAs generated from strain NU14 or NU14-1 cells mixed with D-mannose-coated Sepharose beads at 0 and 120 min were examined. The KpsD1-KpsD2 primer pair was used to amplify serially twofold-diluted cDNAs targeting *kpsD* (367-bp product) transcripts. The PCR amplifications were done a minimum of three times. All PCR products were electrophoresed on 1.5% agarose gels. The following dilutions of cDNAs were used: undiluted (lane 1), 1:2 (lane 2), 1:4 (lane 3), 1:8 (lane 4), 1:16 (lane 5), 1:32 (lane 6), 1:64 (lane 7), 1:128 (lane 8), and 1:256 (lane 9).

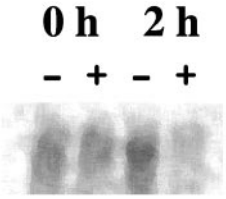


FIG. 4. Northern hybridization analysis of transcription of the *kpsFEDUCS* operon from total RNAs isolated from NU149 cells mixed with plain Sepharose (−) or D-mannose-coated Sepharose (+) beads after 0 and 2 h of exposure. Ten micrograms of total RNA from NU149 cells mixed with plain Sepharose beads or D-mannose-coated Sepharose beads was probed with a *kpsD* DNA fragment. After 6 days, the blot was developed with a phosphorimager and the amounts of *kpsFEDUCS* RNA (approximate size, 7.9 kb) were compared between lanes by using ImageQuant 5.2 software.

be negatively regulated by the ligand-receptor binding between type 1 fimbriae and D-mannose.

Northern blot hybridizations were then performed at two time points, 0 and 2 h, following the interaction of strain NU149 cells with either plain or D-mannose-coated beads. Total RNAs were isolated from the four samples as previously described. Ten micrograms of total RNA from each population was loaded on a 1% denaturing agarose gel, processed, and hybridized with the radiolabeled *kpsD* PCR product as described previously (30). Following a 6-day exposure on a phosphorimager screen (Amersham Biosciences, Piscataway, N.J.), the results with ImageQuant 5.2 software indicated a 6.9-fold difference in the *kpsFEDUCS* 7.9-kb transcript level after 2 h of mixing with D-mannose-coated beads compared to mixing with plain beads (Fig. 4). The level of *kpsFEDUCS* transcripts in the 2 h (−) lane increased by 1.8-fold, which was consistent with an increase observed in the β-galactosidase measurements (Fig. 2). Thus, the entire *kpsFEDUCS* operon is down-regulated following type 1 fimbrial binding to D-mannose residues, confirming the *lacZ* fusion results.

An examination of KpsD protein levels was then performed to determine if the transcriptional down-regulation carried over to the protein level. Again, two populations were examined: one reacted with D-mannose-coated beads and one reacted with plain Sepharose beads. The bacteria were lysed with lysozyme and sodium dodecyl sulfate, and total protein determinations were made for each group, using the Bio-Rad protein assay (Bio-Rad, Hercules, Calif.). Ninety-six-well microtiter plates were coated with 20 mg of total protein lysate in bicarbonate buffer as previously described (31). To measure KpsD protein levels, a rabbit anti-KpsD antibody (provided by Ian Roberts, University of Manchester) (2) was used at a concentration of 1:3,000 as a primary antibody in an enzyme-linked immunosorbent assay. Anti-rabbit immunoglobulin G labeled with alkaline phosphatase (Sigma) at a dilution of 1:3,000 was used as a secondary antibody, and the wells were developed with the substrate *p*-nitrophenylphosphate (Sigma) as previously described (31). Three time points were tested, 0, 8, and 24 h, comparing NU149 cells mixed with D-mannose-coated versus plain Sepharose at each time point. The results showed no difference in KpsD protein levels for both populations at time point zero but a twofold decline in KpsD after 8 h in the population reacted with D-mannose-coated beads and a

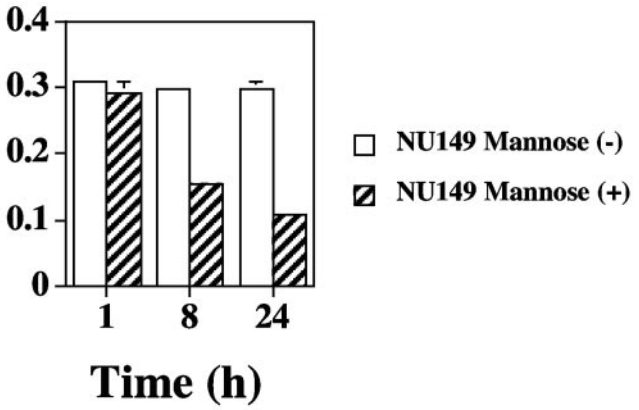


FIG. 5. Enzyme-linked immunosorbent assay analysis of the KpsD protein from strain NU149 cells mixed with plain or D-mannose-coated Sepharose beads for 0, 8, and 24 h. Optical densities at 405 nm ($O.D._{405}$) were determined, and means ± standard deviations are indicated from three runs. (−), plain Sepharose; (+), D-mannose-coated Sepharose.

further decline after 24 h in this population (Fig. 5). This confirmed not only that *kpsD* transcript levels dropped but that KpsD protein levels were also lower.

Although KpsD levels fell following adherence by type 1 fimbriated *E. coli* to mannose receptors, this alone did not confirm that capsular material was reduced on the surface of the bacterial cell. To address the potential role that this physiological change may have on the bacteria, the level of K capsular antigen was then assessed. Since the role of KpsD protein is to assemble capsule subunits onto the surface of the bacterial cell (36), a reduction in the assembly protein may affect the distribution of capsular antigen on the outer surface of the *E. coli* cells, which in turn could affect the association between the bacterial cell and the host cell that it is attached to in vivo. A past study that compared a *kpsD* mutant strain to the wild-type strain demonstrated a decline in capsular presentation on the exterior of the *E. coli* cells (6), so one would predict, since KpsD levels fell after type 1 fimbrial binding to mannose receptors, that less capsular antigen would reach the surface of the *E. coli* cells. *E. coli* strain NU14 has been typed as having the K1 antigen (16), but the serotype for strain NU149 is not known. A Directagen agglutination kit (Becton Dickinson, Sparks, Md.) was used to assay for K1 antigen on NU14 cells mixed with D-mannose-coated Sepharose or plain Sepharose at time zero and after 24 h. The time zero results demonstrated equal agglutination reactions when the population mixed with D-mannose-coated beads was compared to the population mixed with plain Sepharose (Fig. 6). However, the population mixed with D-mannose-coated beads displayed less agglutination (white clumps) after 24 h compared to the group mixed with plain Sepharose, demonstrating that less K1 antigen appeared to assemble on the surface of the *E. coli* cells because of the ligand-receptor interchange between the type 1 fimbriae and the mannose residues.

However, one could not rule out that the other biosynthesis genes involved in *E. coli* K-antigen production in region 2 (i.e., *kfiA, kfiB,* and *kfiD*) might be responsible rather than the effect from a reduction in KpsD levels. To address this point, an LD-RT-PCR procedure was then done using primers specific for the *kfiC* gene, found in region 2, involved in the biosynthesis of group II capsular antigen (36). The LD-RT-PCRs were performed with cDNAs from strain NU14 and the KfiC1 (5′-TCTTGCATATCATCGTTTGC-3′) and KfiC2 (5′-CAGCTC AGAATTCTGGCAA-3′) primer pair (Integrated DNA Technologies). The LD-RT-PCR amplification conditions were the same as those noted for amplification of *kpsD*. There was no difference in *kfiC* transcription between the cell population mixed with D-mannose-coated Sepharose compared to the population mixed with plain Sepharose beads at either 0 or 2 h (data not shown). Thus, the reduction in capsular antigen on the surface appears to be the result of lower KpsD levels and not a consequence of transcriptional regulation of at least one structural gene for capsules.

This study is the first to use a DDPCR technique to characterize changes in UPEC following the binding of the type 1 fimbrial adhesin FimH to its mannose receptor. DDPCR has been previously used to examine host cell responses following binding by bacteria (32) and to assess changes in the bacteria themselves (1). Our results show that a capsular assembly gene operon, region 1, containing the *kpsFEDUCS* gene cluster, is quickly down-regulated at the transcriptional level following bacterial binding, a reduction in the KpsD protein occurs, and less capsular polysaccharide is deposited on the surface of the *E. coli* cells. Although the data generated with the *fimH* mutant strain suggest that FimH contact with D-mannose receptors might be regulating *kps* gene cluster expression, we do not have enough information yet to prove a direct linkage. Certainly, we do not know the chain of events that ultimately leads to the down-regulation of the *kps* gene cluster following binding of type 1 fimbriae to D-mannose receptors.

UPEC strains adhere to bladder epithelial cells via the type 1 fimbrial adhesin FimH. The FimH adhesin is also responsible for the invasion of the bacteria into the bladder epithelium (20). The presence of a thick capsule surrounding the bacterial cells may hinder both tight adherence by the *E. coli* cells and the invasion process itself. Certainly, extracellular polysaccharide is important for the in vivo survival of the bacteria, exemplified by the recent signature-tagged mutagenesis study that indicated a pivotal role for the capsule in *E. coli* residing in the urinary tract (4). Although the presence of a capsule seems to

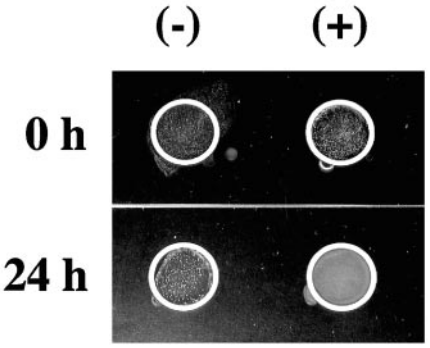


FIG. 6. Directagen analysis of K1 capsular expression in strain NU14. Bacterial cells were mixed with plain Sepharose (−) or D-mannose-coated Sepharose (+) beads and assayed after 0 and 24 h. White clumps of bacteria indicate a positive response.

be critical for long-term persistence within the urinary tract of man and mouse, it may also serve to hinder other key steps in the pathogenesis of UPEC within the urinary tract. A recent study suggests that the bacterial capsule may block intimate attachment mediated by protein antigen 43, found on the surface of UPEC strains (29). Previous studies with *Klebsiella pneumoniae* have indicated that the bacterial capsule impedes close adherence and invasion into epithelial cells (11, 26). Moreover, a bacterial capsule also negatively affects tight adherence and invasion by *Neisseria meningitidis* (10, 13).

Adherence of *E. coli* to a host cell through ligand-receptor binding does engender a cross talk between the bacterial cell and the host cell that is likely to result in greater fitness for the bacteria as a result of regulation of specific genes. Zhang and Normark (37) examined binding of P fimbriae to a receptor and identified a sensor-regulator gene essential for the bacterial iron starvation response. The gene was transcriptionally activated by the ligand-receptor interaction. Attached *E. coli* cells have significantly less OmpX outer membrane protein (23), which in turn can affect type 1 fimbriated *E. coli* attachment to abiotic surfaces (22). Certainly, other genes must be affected as well by ligand-receptor binding.

One can envision that *E. coli* cells entering the urinary tract from the outside require the capsule initially. Once the bacteria have bound loosely to bladder epithelial cells via type 1 fimbriae, the capsule becomes a steric hindrance for tight adherence by the bacteria and subsequent invasion. Down-regulation of the region 1 capsular operon, which includes *kpsD*, occurs quickly, which in turn leads to less capsular material distributed on the exterior of the bacterial cells. This might be advantageous to the bacteria because capsular subunits may accumulate in the cytoplasm as intermediates and be available for quick assembly if the conditions change. Mutations in region 1 capsule genes have previously resulted in such an accumulation of intermediate products (7, 8). The loss of capsular material would facilitate tight adherence and invasion by the bacteria to escape the immune system.

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