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# The *rcsA* Gene of *Klebsiella pneumoniae* O1:K20 Is Involved in Expression of the Serotype-Specific K (Capsular) Antigen

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**In *Klebsiella pneumoniae*, the ability to synthesize large amounts of capsular polysaccharide is an important correlate of virulence. We report the cloning of *rcsA* from *K. pneumoniae* serotype O1:K20 and demonstrate that *rcsA* is involved in the expression of the K antigen capsule. We have determined the nucleotide sequence for the *rcsA* gene from *K. pneumoniae* K20 and shown it to be identical to the sequence reported previously for *rcsA* from strain K21 (Allen et al., J. Gen. Microbiol. 133:331–340, 1987). Southern hybridization results indicate that this gene is widely distributed among different *Klebsiella* K serotypes. When cloned into *Escherichia coli* K-12, the *K. pneumoniae rcsA* gene caused a mucoid phenotype, resulting from the activation of colanic acid synthesis. Activation of colanic acid synthesis was not dependent on growth at low temperatures (≤30°C). The *K. pneumoniae rcsA* gene complemented *E. coli* K-12 *rcsA* mutations but could not complement defects in *rcsB*, suggesting that RcsA may be functionally homologous in these bacteria. The cloned *rcsA* gene also complemented a defect in nonmucoid strain K20 derivatives that normally produced only trace amounts of K20 antigen and were unable to assemble a wild-type capsular structure. Mutants that were K20-deficient were not complemented. The K antigen capsule of *K. pneumoniae* therefore joins a growing list of polysaccharide-synthetic systems in which "RcsA-like" proteins are involved.**

Members of the genus *Klebsiella* are opportunistic pathogens which cause pneumonia, bacteremia, and urinary tract and other infections. These organisms are a particular threat to compromised hosts (28). *Klebsiella* spp. characteristically produce large mucoid colonies due to the synthesis of large amounts of capsular polysaccharide (CPS; K antigen). There are 82 serologically distinct K antigen types in *Klebsiella* spp., although not all serotypes are associated with pathogenesis (27, 33). The expression of K antigen is an important virulence determinant in *Klebsiella* spp. since it plays a role in resistance to phagocytosis (36, 46, 47). Several workers have demonstrated that the size of the capsule and the rate of its synthesis are important in virulence in pulmonary (9, 10), intraperitoneal (12, 39), and burn (6) infection models. One consequence of higher levels of K antigen synthesis is the release of larger amounts of polysaccharide from the cell surface (9, 11), providing cell-free K antigen which could neutralize circulating anticapsular antibody (34). In addition, purified K antigens have been shown to exert a number of effects which would have a significant influence on pathogenicity. These effects include induction of immune tolerance (2, 29, 32) and impairment of the maturation and function of macrophages (49, 50). The nature of the mechanisms which regulate K antigen expression therefore represents an important and unresolved question in the pathogenesis of *Klebsiella* spp.

Although normally nonmucoid, many enteric bacteria (particularly *Escherichia coli* K-12 and *Salmonella* spp.) are capable of synthesizing a slime polysaccharide called colanic acid (or M antigen) (14, 16). The regulation of this polysaccharide has been the subject of detailed study. Three regulatory *rcs* (regulator of capsule synthesis) genes have been identified in *E. coli* K-12 (3, 15). The *rcsA* gene product is a positive regulator whose level is controlled by the activity of the Lon protease (40). RcsB and RcsC are positive and negative regulators, respectively, and have been proposed to function as the effector and sensor, respectively, of a two-component regulatory system (37). Although the regulatory system controlling *Klebsiella* K antigens has not been defined, there have been independent reports of *Klebsiella* genes which conferred a mucoid phenotype on *E. coli* K-12 hosts. The mucoid polysaccharide was identified as colanic acid, and activation of its synthesis was likely due to the *Klebsiella* genes interfering with the regulatory systems which normally maintain *E. coli* K-12 in a nonmucoid state. In 1987, Allen et al. (1) described two genes (termed *rcsA* and *rcsB*) from *K. pneumoniae* K21. The genes from *Klebsiella* spp. will be designated $rcsA_K$ and $rcsB_K$, to distinguish them from the *E. coli* K-12 $rcsA_E$ and $rcsB_E$ genes. $rcsA_K$ caused the activation of colanic acid synthesis in *E. coli* K-12 at 30°C; mucoidy was not evident at 37°C unless $rcsB_K$ was also present.

Extensive homology exists between the RcsA proteins of *Klebsiella* spp. and *E. coli* K-12 (41). The 220-bp $rcsB_K$ open reading frame (ORF) does not direct synthesis of any polypeptide in minicells (1), whereas $rcsB_E$ comprises 651 bases and is known to code for a protein with a predicted molecular weight of 23,656 (37). No role was established for $rcsA_K$ and $rcsB_K$ in *Klebsiella* K antigen expression. More recently, Nassif et al. (30, 31) described two further genes (designated *rmpA* and *rmpB*) in *K. pneumoniae* serotype K2, which also activated colanic acid synthesis in *E. coli* K-12. The *rmpA* and *rmpB* genes are located on a virulence plasmid and function in the expression of a mucoid polysaccharide which is apparently unrelated to the K2 antigen (30).

The present study was initiated in an attempt to resolve the mechanisms involved in K antigen expression in *Klebsiella* spp. We report the isolation of $rcsA_K$ from *K. pneumoniae* serotype K20 and establish that $rcsA_K$ is involved in the expression of K antigen in *K. pneumoniae*.

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TABLE 1. Bacteria and plasmids

| Strain or plasmid | Description | Source or reference |
|---|---|---|
| *K. pneumoniae* | | |
| 889/50 | Serotype O1:K20 | I. Ørskov |
| KD1 | O1:K20 $Str^r$ | 26 |
| KD2 | O1:$K^-$ $Str^r$ | 26 |
| KD8 | O1:K20 nonmucoid, $Str^r$ | This study |
| *E. coli* | | |
| LE392 | $F^-$ *hsdR514* ($r_K^-$ $m_K^+$) *supE44 supF58 lacY1* or Δ(*lacIZY*)*6 galK2 galT22 metB1 trpR55* $\lambda^-$ | 24 |
| JM109 | *endA1 recA1 gyrA96 thi hsdR17* ($r_K^-$ $m_K^+$) *relA1 supE44* $\lambda^-$ Δ(*lac-proAB*) (F′ *traD36 proAB lacI*$^q$ *lacZ*ΔM15) | 48 |
| VS20186 | MC4100 derivative, *lon-100 rcsA51*::Δ*kan* | V. Stout |
| VS20187 | MC4100 derivative, *lon-100 rcsB62*::Δ*kan* | V. Stout |
| Plasmids | | |
| pVK102 | Cosmid vector, $Tc^r$ $Km^r$ | 22 |
| pRK404 | RK2 derivative, $Tc^r$ $Mob^+$ $Tra^-$ | 8 |
| pRK2013 | RK2 derivative, $Km^r$ $Mob^+$ $Tra^+$ ColE1 | 7, 13 |
| pGEM-5Zf(+) | $Ap^r$ | Promega Biotech |
| pWQ100 | *Hin*dIII cosmid clone in pVK102, $RcsA_K^+$ $Tc^r$ | This study |
| pWQ200 | pGEM-5Zf(+) derivative containing 3 kb of pWQ100, $RcsA_K^+$ $Ap^r$ | This study |
| pWQ400 | pRK404 derivative containing 3 kb of pWQ100, $RcsA_K^+$ $Tc^r$ | This study |
| pWQ1000 | $His^+$ $O1^+$ $K20^+$ R-prime plasmid derived from pULB113 | 23 |

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1. Strain 889/50 is a representative of *K. pneumoniae* subsp. *pneumoniae*, and KD1 is a streptomycin-resistant derivative (26). Strains KD2 and KD8 are visibly nonmucoid mutants of strain KD1 and were obtained following *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine mutagenesis. Plasmids pVK102 and pRK404 were mobilized in triparental matings with a helper plasmid in *E. coli* HB101(pRK2013). All strains were grown on Luria broth (LB), supplemented where appropriate with streptomycin (100 μg/ml), ampicillin (100 μg/ml), kanamycin (50 μg/ml), and tetracycline (15 μg/ml).

**Recombinant DNA procedures.** Genomic DNA was isolated by the method of Hull et al. (20). Plasmid DNA was purified by an alkaline lysis method, and transformations were performed with $CaCl_2$-treated competent cells (24). Cosmid cloning in pVK102 was carried out with size-fractionated (approximately 20 kb) *Hin*dIII fragments from partial digests of chromosomal DNA. Ligation mixtures were packaged into lambda particles with a commercial in vitro packaging lysate (Promega Biotech Inc., Madison, Wisc.), and the recombinant lambda phages were transduced into *E. coli* LE392.

DNA-DNA (Southern) hybridizations were performed essentially as described elsewhere (24). DNA fragments isolated from low-melting-point agarose gels were radiolabeled with [α-$^{32}$P]ATP (New England Nuclear) by using a nick translation kit (Boehringer Mannheim Canada). Hybridization was carried out for 16 h at 42°C in a solution containing 7 ml of formamide, 2.1 ml of 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.027 ml of 10-mg/ml sheared salmon sperm DNA, 0.28 ml of 50× Denhardt's solution, 0.63 ml of 10% sodium dodecyl sulfate (SDS), and 0.28 ml of 0.5 M EDTA, in a total volume of 18 ml. Blots were washed twice with 2× SSC at room temperature, twice at 60°C in 0.1% SDS–0.1× SSC, and finally twice at room temperature in 0.1% SDS.

DNA sequencing was performed on DNA fragments cloned into the vector pGEM-5Zf(+) (Promega Biotech). Deletions were made from each end of the insert by exonuclease III digestion and the Erase-a-base system (Promega Biotech). Both strands were sequenced by the dideoxy chain termination method (35) and [α-$^{32}$P]ATP labeling.

Restriction enzymes, T4 DNA ligase, and alkaline phosphatase were purchased from either Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) or Boehringer Mannheim Canada (Laval, Québec, Canada) and used according to the manufacturer's instructions.

**Rocket immunoelectrophoresis.** Samples for immunoelectrophoresis were prepared from cells grown overnight at 37°C on LB plates. The growth from six plates was scraped into phosphate-buffered saline and resuspended by vigorous mixing. The cells were sedimented by centrifugation (15 min, 7,000 × *g*), yielding a supernatant containing cell-free polysaccharides. The cell pellet was extracted with 45% aqueous phenol at 65°C (44), and the aqueous phase, containing cell-associated polysaccharides, was collected and dialyzed against distilled water. Both cell-free and cell-associated samples were adjusted to 10 mM Tris-HCl (pH 7.2)–10 mM $MgCl_2$ and treated with RNase and DNase (final concentration, 5 μg/ml) for 2 h at 37°C. Proteinase K (5 μg/ml) was added, and the incubation was continued at 60°C for 2 h. The polysaccharides were then precipitated with 2 volumes of cold acetone, reconstituted in distilled water, dialyzed, and lyophilized. For immunoelectrophoresis, 10-μg samples were examined. The antibody was MAb4-15A, a monoclonal antibody raised against the K30 capsular antigen of *E. coli* (19); this antigen is identical in structure to the K20 antigen of *Klebsiella* spp. (21). Conditions for electrophoresis were reported previously (19).

**Electron microscopy.** Examination of the morphology of *K. pneumoniae* derivatives was performed as described previously (45). Briefly, the cell pellet from 2 ml of an exponential-phase culture was resuspended in 0.1 ml of phosphate-buffered saline, and capsule stabilization was achieved by addition of 0.02 ml of MAb4-15A. Samples were stained with ruthenium red and embedded. Sections were examined with a Philips EM300 transmission electron microscope operating at 60 kV.

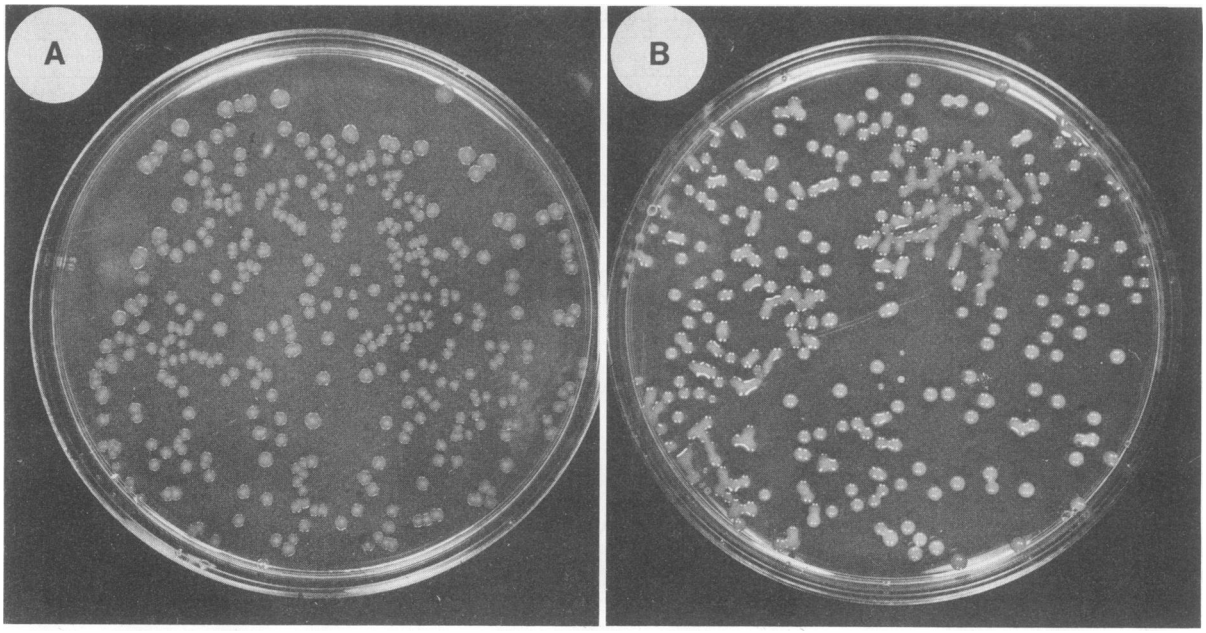


FIG. 1. Mucoid phenotype of *E. coli* LE392(pWQ100). (A) *E. coli* LE392; (B) *E. coli* LE392(pWQ100). Plates were incubated for 16 h at 37°C.

## RESULTS

**Cloning and characterization of the $rcsA_K$ gene from *K. pneumoniae* serotype K20.** Approximately 2,400 recombinants were isolated, each containing an average of 20 kb of *K. pneumoniae* DNA cloned in pVK102. Of these recombinants, 14 demonstrated a distinct mucoid phenotype (Fig. 1B). Phenol-extracted polysaccharides from the 14 mucoid recombinants were tested for reactivity with MAb4-15A, which recognizes the K20 antigen, and no reactivity was detected (Fig. 2A.) The polysaccharide from two mucoid recombinants was acid hydrolyzed and analyzed by gas-liquid chromatography. Both samples were found to contain fucose, glucose, and galactose in a molar ratio of approximately 2:1:2 (results not shown). This neutral sugar composition is that expected for colanic acid (14).

Plasmid DNA was isolated from each of the 14 mucoid recombinants and digested with several restriction endonucleases (results not shown). Eight plasmids contained a single 21.8-kb *Hin*dIII fragment; the other six plasmids contained additional smaller *Hin*dIII fragments. All 14 plasmids contained common *Pst*I and *Xho*I fragments. One plasmid, designated pWQ100, was selected for further study, and the physical map of the insert DNA is shown in Fig. 3.

Information about the location of the putative *rcs* gene(s) was obtained by cloning pWQ100 DNA from total and partial *Pst*I digests into pBR325. All mucoid recombinants which were recovered contained plasmids with two adjacent 1.5-kb *Pst*I fragments in common. These two fragments were copurified from agarose gels and ligated to pGEM-5Zf(+). Only plasmids containing both *Pst*I fragments (plasmid pWQ200) produced a mucoid phenotype in *E. coli* JM109. Deletions were constructed from both ends of the insert in pWQ200, and the putative *rcs* gene(s) was localized to approximately 950 bp spanning the junction of the two *Pst*I fragments (Fig. 3).

The mucoid phenotype conferred by pWQ200 in *E. coli* K-12 was not restricted to strain JM109; similar phenotypes occurred following introduction of the plasmid into *E. coli* strains HB101, LE392, DH1, and DH5α. The mucoid phenotype showed no absolute requirement for lower incubation temperatures, since strains grown at 37°C were clearly mucoid (Fig. 1). However, growth at 30°C and below appeared to stimulate slightly higher levels of colanic acid production. These observations, together with the relatively small amount of *K. pneumoniae* serotype K20 DNA involved, were not consistent with the phenotypes reported previously for either $rcsA_K$ $rcsB_K$ (1) or *rmpA rmpB* (30, 31).

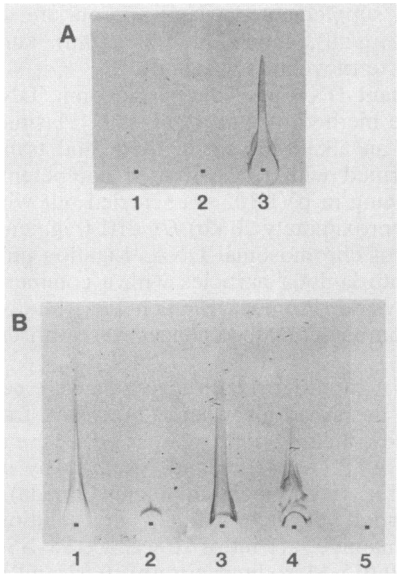


FIG. 2. Rocket electrophoresis of partially purified polysaccharides. Samples were analyzed with MAb4-15A, which recognizes the K20 antigen. (A) Cell-associated polysaccharides synthesized in *E. coli* backgrounds. Lanes: 1, *E. coli* LE392; 2, LE392(pWQ100); 3, control K antigen from a *Klebsiella* serotype O1:K20 strain. (B) Polysaccharides synthesized by *Klebsiella* derivatives. Lanes: 1, KD8, cell associated; 2, KD8, cell free; 3, KD8(pWQ400), cell associated; 4, KD8(pWQ400), cell free; 5, KD2(pWQ400), cell associated.

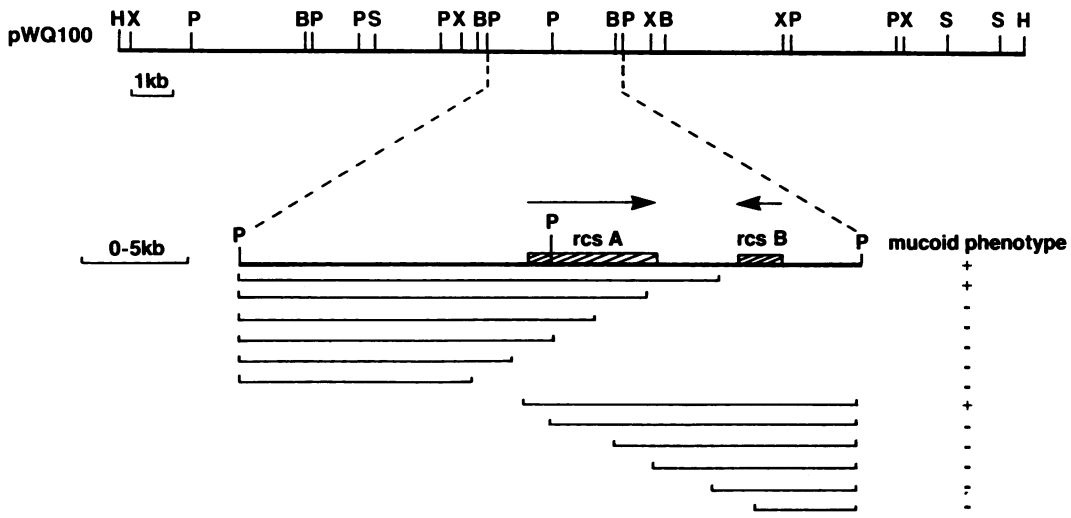


FIG. 3. Physical map of the *Klebsiella* $rcsA_K$ gene and adjacent sequences. pWQ100 contains 21.8 kb of *Klebsiella* DNA cloned in the cosmid vector pVK102. The 3-kb region of pWQ100 responsible for the mucoid phenotypes in *E. coli* K-12 strains is expanded, and the mucoid phenotypes of truncated derivatives of this region are indicated. Deletions were made in pWQ200, which contains the 3-kb region cloned in pGEM-5Zf(+). The positions and directions of transcription of $rcsA_K$ and $rcsB_K$ were determined by physical mapping and sequencing. The enzymes used were *Bam*HI (B), *Hin*dIII (H), *Pst*I (P), *Sal*I (S), and *Xho*I (X).

To resolve this inconsistency, the putative *rcs* gene(s) was sequenced. A single open reading frame of 624 nucleotides contained a sequence identical to that previously reported by Allen et al. (1) for $rcsA_K$ from *Klebsiella* serotype K21, and the position of the $rcsA_K$ gene is indicated in Fig. 3. The 3-kb insert in pWQ200 has not been sequenced in its entirety, but the untranslated open reading frame designated $rcsB_K$ (1) was identified approximately 360 bp from $rcsA_K$. However, based on the analysis of deletions (Fig. 3), this region of the insert is apparently not required for a mucoid phenotype at 37°C in *E. coli* K-12 under the conditions used here. Furthermore, the presence of $rcsB_K$ did not enhance the $rcsA_K$ mediated mucoid phenotype in *E. coli* K-12 at a growth temperature of 30 or 42°C.

**Complementation of *E. coli* K-12 $rcsA_E$ defects with cloned $rcsA_K$.** The mucoid phenotype observed in *E. coli* K-12(pWQ200) could be explained in two ways. First, $RcsA_K$ may function in the same way as $RcsA_E$ when introduced into *E. coli* K-12, with the mucoid phenotype resulting from the additional copies of $rcsA_K$. Alternatively, $RcsA_K$ may interact in a different way, coincidentally interfering with the normal regulation of colanic acid in *E. coli* K-12 and resulting in a mucoid phenotype. To resolve this question, pWQ200 was introduced into *E. coli* strains VS20186 (*lon-100 rcsA51*::Δ*kan*) *and VS20187 (lon-100 rcsB62*::Δ*kan). These strains contain a deletion in lon* and mini-*kan* insertions in *rcsA* and *rcsB*, respectively. pWQ200 complemented the $rcsA_E$ mutation in VS20186 and produced mucoid colonies; the $rcsB_E$ defect was not complemented. Deletion derivatives of pWQ200 lacking the $rcsB_K$ ORF gave identical results.

**Complementation of *K. pneumoniae* serotype K20 capsule synthetic defects with cloned $rcsA_K$.** To determine whether $rcsA_K$ functions in K antigen synthesis in *K. pneumoniae* serotype K20, pWQ400 was constructed. This plasmid contains the 3-kb $rcsA_K$ containing region from pWQ100 (Fig. 3) in the broad-host-range mobilizable plasmid pRK404. In *E. coli* K-12 hosts, pWQ400 confers mucoidy. pWQ400 was then transferred to a series of 20 nonmucoid mutants of *K. pneumoniae* serotype K20. Eighteen mutants were unaffected by introduction of pWQ400, but two strains, KD8 (pWQ400) and KD10(pWQ400), produced mucoid colonies which were indistinguishable from the wild-type *K. pneumoniae* serotype K20 strain (Fig. 4). Mutants KD8 and KD10 were subsequently found to have similar properties, and only KD8 is discussed further.

To determine the nature of the polysaccharide synthesized by KD8(pWQ400), extracts were examined by immunoelectrophoresis. K20 antigen was detected in both the cell-associated and cell-free extracts of KD8(pWQ400) (Fig. 2B). Surprisingly, KD8 does synthesize cell-associated K20 antigen, despite a nonmucoid colony morphology. Polysaccharide was also precipitated from culture supernatants of KD8, but it did not react with MAb4-15A (Fig. 2B). Subsequent analysis with O1-specific monoclonal antibodies (26) showed that this material was lipopolysaccharide (LPS) (results not shown). All *Klebsiella* serotype O1 strains examined appear to slough significant quantities of LPS during growth (44a). A mutant designated KD2 was also analyzed. KD2 has been shown previously to synthesize no K20 antigen (26), and introduction of pWQ400 or the original cosmid derivative pWQ100 did not alter this K20-deficient phenotype (Fig. 2B). Chemical analysis of the neutral sugars in the polysaccharide synthesized by KD8(pWQ400) indicated the composition expected of the K20 antigen and the absence of fucose, a marker of colanic acid. Based on estimation of total CPS by rocket immunoelectrophoresis, there was no apparent difference in the amount of K20 antigen synthesized by *K. pneumoniae* KD1 and KD8(pWQ400) (results not shown).

The morphology of KD8(pWQ400) was also examined by electron microscopy to determine whether the complementation of the defect in KD8 with cloned $rcsA_K$ gave complete restoration of the wild-type capsule. The capsule was stabilized by using MAb4-15A, confirming that the material visualized was in fact K20 antigen. The wild-type strain produced an extensive capsule, although it was evident that many of the cells were not completely enclosed within the capsule (Fig. 5A). We have previously reported a similar morphology for *K. pneumoniae* serotype K20 with a different method used to label the capsule (23). Points at which

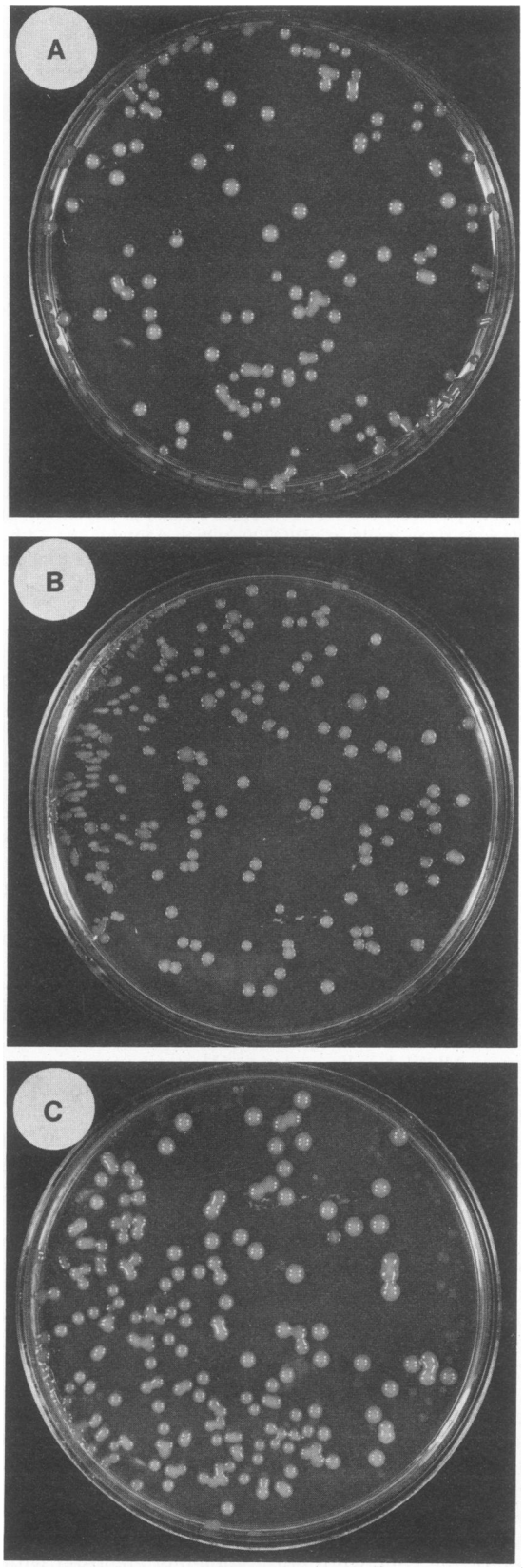


FIG. 4. Restoration of mucoidy in strain KD8 following transformation with pWQ400. (A) Serotype O1:K20 (strain KD1); (B) strain KD8; (C) KD8(pWQ400). Plates were incubated for 16 h at 37°C.

the capsule appears to be associated with the cell surface were evident in the wild-type strain (Fig. 5A and 6A). Strain KD8 produced no capsule. However, antibody-treated KD8 cells exhibited a distinct electron-dense layer on the outer leaflet of the outer membrane (Fig. 6B). Based on the agglutination of intact KD8 cells in MAb4-15A, the K20 antigen was determined to be surface exposed. The electron-dense layer may reflect the binding of antibody at the cell surface. Strain KD8(pWQ400) produced extensive capsular polysaccharide, although the degree of cell association between the capsule and the cell surface was markedly reduced in comparison to the wild type (Fig. 5B and 6C). The reasons for these differences remain unclear. A simple gene dosage effect does not appear to be responsible, since the morphology of KD8(pWQ400) could not be duplicated by introduction of pWQ400 into the wild-type strain. There was no detectable increase in the amount of K antigen synthesized in either *Klebsiella* strain KD1(pWQ400) or KD1(pWQ100).

**Distribution of $rcsA_K$ sequences among different *Klebsiella* K antigen serotypes.** Allen et al. (1) demonstrated the hybridization of $rcsA_K$ sequences to chromosomal DNA from serotypes K2, K12, K36, and K43. The Southern blot shown in Fig. 7 confirms and extends these observations. The $rcsA_K$ probe (consisting of the two *Pst*I fragments from pWQ200) hybridized to a 1.5-kb region in DNA from serotypes K1, K2, K7, K10, K16, and K20. Although only *Pst*I digests are shown, the probe also hybridized to a similar 13.4-kb fragment present in *Sal*I-digested DNA from each serotype. This fragment was predicted from the physical map of pWQ100 in Fig. 3. The *Klebsiella* $rcsA_K$ sequences showed no hybridization to *E. coli* LE392 DNA.

**Localization of the $rcsA_K$ gene on the chromosome of *K. pneumoniae* KD1.** Previously, we have demonstrated that transfer of the *his* region from an O1:K20 strain to *E. coli* K-12 recipients resulted in transconjugants which expressed both the O1 and K20 antigens (23). Transfer was achieved by using RP4::mini-Mu (pULB113). Southern hybridization was used to determine whether pWQ1000, a $His^+$ $K20^+$ R-prime plasmid, also carried $rcsA_K$. The $rcsA_K$ probe hybridized to *Pst*I-digested pWQ1000, giving the single 1.5-kb band expected (Fig. 7, lane 9). This result indicates that in *Klebsiella* spp., the $rcsA_K$ gene maps in the vicinity of the *his* locus. Due to the large size and instability of these R-prime plasmids, plus their tendency to give partial restriction enzyme digests, any determination of size is inaccurate, but pWQ1000 may contain at least 150 kb of *Klebsiella* DNA (23a).

## DISCUSSION

The *Klebsiella* $rcsA_K$ gene was first described for serotype K21 by Allen et al. (1). The gene consisted of 624 nucleotides and coded for a protein of 23.4 kDa. Cloned $rcsA_K$ caused induction of colanic acid synthesis in *E. coli* K-12 hosts at 30°C, but the presence of a second and apparently untranslated ORF, termed $rcsB_K$, was necessary to confer mucoidy at 37°C. In this study, we have identified $rcsA_K$ in a *K. pneumoniae* serotype K20 strain and confirmed that the genes from both serotypes have identical nucleotide sequences. The genes from both sources also give protein products of similar molecular weights (26a). However, in the study reported here, introduction of $rcsA_K$ into *E. coli* K-12 was sufficient to confer mucoidy at 37°C, regardless of the presence of the $rcsB_K$ ORF described by Allen et al. (1). The reasons for the differences in requirements are not clear. The genetic background of the *E. coli* K-12 strain is not respon-

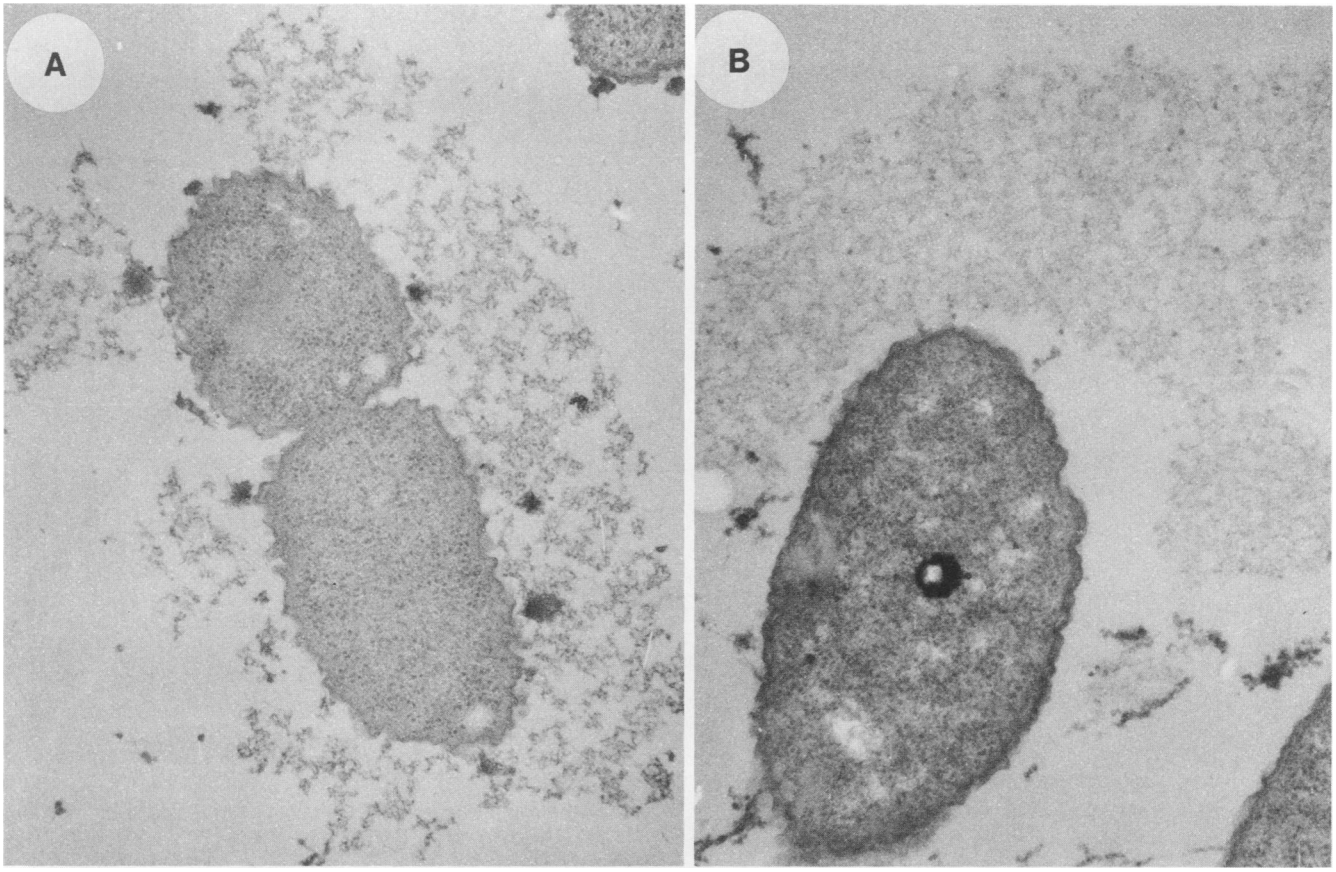


FIG. 5. Morphological examination of capsule expression in strain KD8(pWQ400). Late-exponential-phase cells were treated with MAb4-15A to stabilize the capsule and then examined by electron microscopy. Extensive capsule was synthesized by the wild-type *Klebsiella* serotype O1:K20 strain (A) and by strain KD8(pWQ400) (B). Magnification, ×50,000.

sible, since deletions lacking $rcsB_K$ give a mucoid phenotype in *E. coli* DH1, the strain used by Allen et al. (1). However, it is apparent that subtle differences in the growth medium used can markedly influence the amount of colanic acid synthesized (25).

Colanic acid is synthesized by a range of enteric bacteria, particularly in *E. coli* and *Salmonella* spp. It has also been detected by a chemical approach in *Aerobacter cloacae* (14, 16) and by serological methods in *Aerobacter aerogenes* (17). Despite extensive detailed analysis of the structures of *K. pneumoniae* K antigens (reviewed in reference 21), colanic acid has not to our knowledge been reported in this species. However, there are similarities between the structures of several *Klebsiella* K antigens and colanic acid (21), and these structural similarities often lead to serological cross-reactions (18, 33).

Complementation studies demonstrated for the first time that $rcsA_K$ is involved in the expression of the K antigen of *K. pneumoniae* serotype K20. Although the precise function of $RcsA_K$ in this system remains to be established, there are striking parallels with the *E. coli* K-12 system, in which $RcsA_E$ acts as a transcriptional activator (40). First, the cloned $rcsA_K$ gene from *Klebsiella* spp. complements *E. coli* K-12 $rcsA_E$ (but not $rcsB_E$) mutants. This is consistent with the observations of Coleman et al. (5), who recently showed that the *Klebsiella* serotype K21 $rcsA_K$ elevated transcription of *cps* genes in an *E. coli* K-12 *rcsA cps*::*lac* strain; the effect of this gene in an $rcsB_E$ background was not reported. These results indicate that RcsA proteins from these organisms are functionally homologous and that, as in wild-type *E. coli* K-12, $RcsA_K$ cannot activate colanic acid synthesis in the absence of functional $RcsB_E$. Second, there is extensive homology between the deduced amino acid sequences of the RcsA proteins for *Klebsiella* spp. and *E. coli* K-12 (41). Third, the *E. coli* K-12 $rcsA_E$ gene maps at 43 min, near *his* (44 min) (15), and we have demonstrated that the *K. pneumoniae* $rcsA_K$ gene also maps near *his*. Interestingly, the K antigen-synthetic genes are found near *his* in *K. pneumoniae* (23), and their counterparts (*cps* genes) are located at 45 min in *E. coli* K-12 (42). Finally, the *K. pneumoniae* K antigen mutants complemented by $rcsA_K$ are ones in which the amounts of CPS synthesized, rather than the synthetic ability, are affected. Introduction of multicopy $rcsA_K$ into *K. pneumoniae* serotype O1:K20 did not stimulate detectably higher levels of capsule synthesis. It is possible that in the wild-type strain, which already synthesizes large amounts of capsule, components other than $RcsA_K$ limit the levels of production.

Introduction of $rcsA_K$ into *K. pneumoniae* KD8 did not restore a completely wild-type phenotype. The reasons for the apparent differences in cell association of CPS in the wild-type strain versus KD8(pWQ400) are presently unclear. In most *Klebsiella* strains, the K antigen is firmly associated with the cell surface; in some strains, as much as 80% of the CPS sediments with the cells on centrifugation (43). However, it has not been determined whether the CPS is cova-

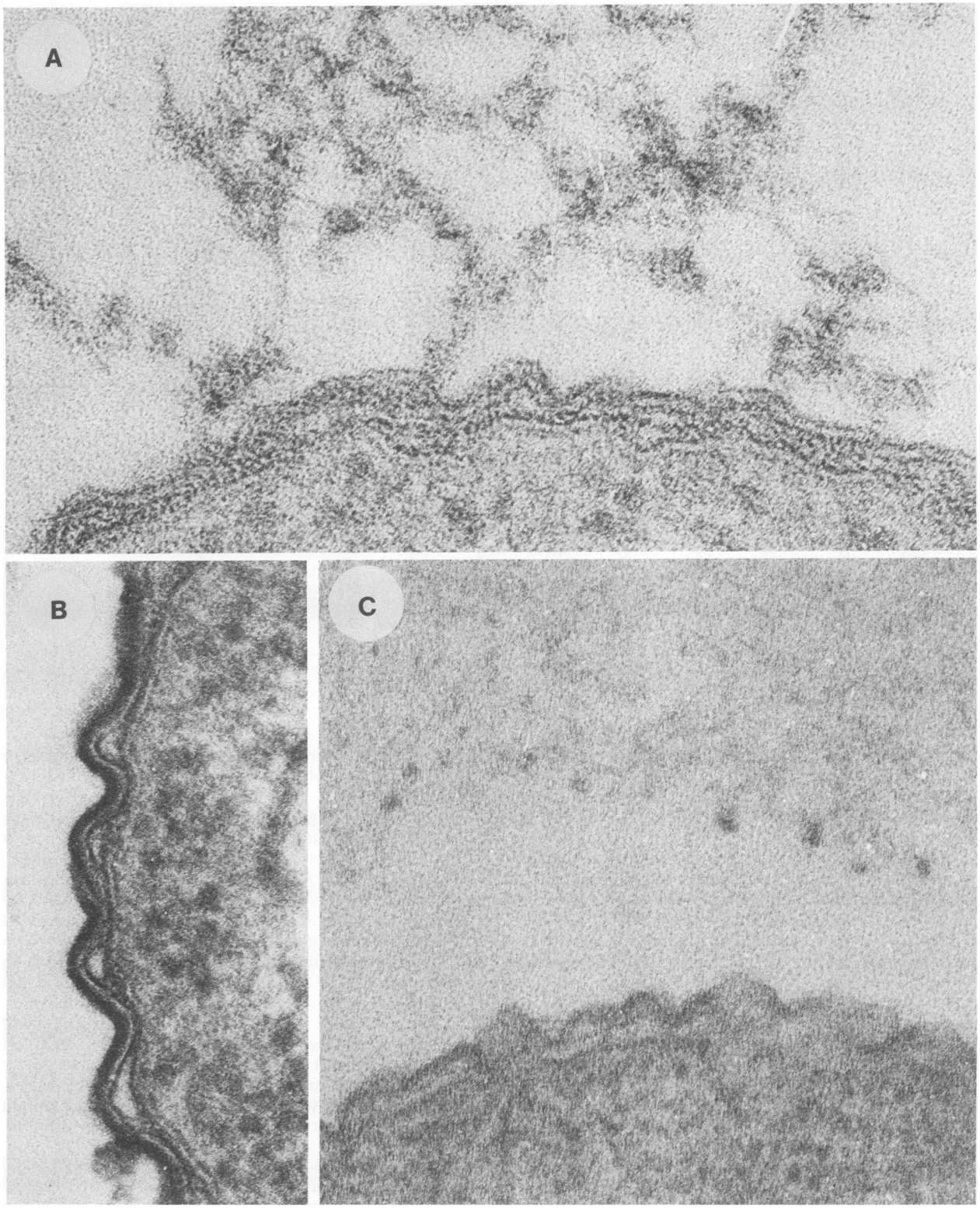


FIG. 6. Cell association of the K20 antigen. Note the frequent association of the capsular material with the cell surface in a *Klebsiella* serotype O1:K20 strain (A). The same degree of association was not evident in strain KD8(pWQ400) (C). No capsular structure was produced by strain KD8 (B). Magnification, ×250,000.

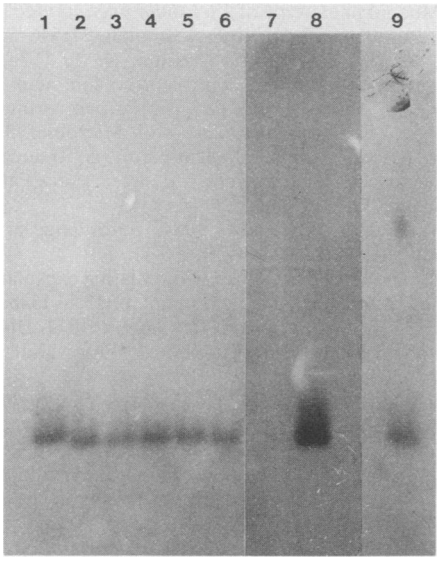


FIG. 7. Distribution of $rcsA_K$ sequences in different *Klebsiella* K serotypes and the identification of $rcsA_K$ on $His^+$ R-prime plasmids. The probe used in Southern blotting was a mixture of the two 1.5-kb *Pst*I fragments from pWQ200, containing $rcsA_K$ and adjacent sequences, as shown in Fig. 3. All DNA samples were digested with *Pst*I. Lanes 1 to 7 show chromosomal DNA from serotypes K1, K2, K7, K10, K16, and K20 and from *E. coli* LE392, respectively. Lane 8 contains pWQ200, the source of the probe. Lane 9 contains DNA from a $His^+$ R-prime plasmid designated pWQ1000.

lently bound to the surface via a linker molecule. Early reports of a role for LPS in the linkage of *Klebsiella* K antigens have not been confirmed and most likely reflect the contamination of isolated CPS preparations with LPS (38). Subsequent direct attempts to identify a covalently bound linker molecule at the reducing terminus of K antigens in *K. pneumoniae* serotype K20 and other Klebsiella strains have not been successful (38, 43, 44a), and it is possible that cell association of CPS does not require a linker molecule per se. While it is possible that strain KD8 contains an additional defect which directly affects an undefined linkage process, it is also conceivable that the restored biosynthetic system in strain KD8(pWQ400) is unbalanced in a way which indirectly affects cell association.

Based on the results presented here and those of other studies, it appears that there may be two distinct regulatory systems operating in the synthesis of extracellular polysaccharides in *Klebsiella* spp. The first involves the type-specific K antigen and $RcsA_K$. The other system would be the expression of mucoid polysaccharide and its control by RmpA and RmpB, as described by Nassif et al. (30, 31). Presumably this particular system must be confined to strains which carry the large virulence plasmid, from which *rmpA* and *rmpB* are derived (30); serotype K20 contains no large plasmids (25a). Interestingly, serotypes such as K1 and K2 which harbor the virulence plasmid have been shown to be particularly virulent in a mouse model (27). Indeed, it has been demonstrated that the *rmp*-mediated mucoid phenotype is correlated with virulence (30). The work presented here, together with earlier studies (1), has demonstrated that isolates of serotypes K1 and K2 also contain $rcsA_K$ sequences. Until the nature of the *rmp*-controlled mucoid polysaccharide is unambiguously established, assessment of possible interrelationships between $rcsA_K$ and *rmpA*/*rmpB* remains difficult.

In addition to *E. coli* K-12 and *Klebsiella* spp., an RcsA protein also functions in the synthesis of the extracellular polysaccharide of *Erwinia stewartii* (41) and *Erwinia amylovora* (4, 5). At least in *E. coli* K-12 and *Erwinia* spp., RcsA functions in a similar fashion, by acting either directly or indirectly as a transcriptional activator (5, 41). Both *Erwinia* RcsA proteins complement $rcsA_E$ defects in *E. coli* K-12 (4, 5, 41). There is extensive homology between the *Klebsiella* and *E. amylovora* RcsA proteins (5), as appears to be the case with *Klebsiella* and *E. coli* K-12 RcsA (41). It will be interesting to see if common aspects in these regulatory systems extend to other components, particularly RcsB and the putative environmental sensor, RcsC (37).

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