Revisiting the Importance of Virulence Determinant magA and Its Surrounding Genes in *Klebsiella pneumoniae* Causing Pyogenic Liver Abscesses: Exact Role in Serotype K1 Capsule Formation

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(See the editorial commentary by Fang et al, on pages 1268–1269.)

**Background.** Mucoviscosity-associated gene A (magA) is proposed to play a decisive role in the pathogenesis of liver abscesses due to *Klebsiella pneumoniae*. Although some investigators consider MagA to be a putative O-antigen ligase, it is also reportedly associated with the K1 antigen.

**Methods.** Using magA-positive serotype K1 *K. pneumoniae* STL43 isolated from a patient with liver abscess, we constructed 3 bacterial mutants by targeting genes within the same transcription unit, including magA, wcaG, and rfbP. The virulence of these mutants was determined by neutrophil phagocytosis and inoculation of mice. Transmission electron microscopy and Western blot analysis were used to define their surface polysaccharides.

**Results.** STL43 was resistant, and all 3 mutants were highly susceptible, to phagocytosis. None of the mutant strains caused death in mice at the lethal dose of STL43. In contrast to previous reports, transmission electron microscopy revealed that all 3 mutants were nonencapsulated. Analysis of surface polysaccharides revealed that all 3 mutants retained their O antigen but lost their K antigen/capsule. Furthermore, amino acid analysis showed that MagA shared a conserved domain of Wzy, the serotype-specific capsular polysaccharide polymerase.

**Conclusions.** In accordance with the bacterial polysaccharide gene nomenclature (BPGN) scheme, MagA should be renamed WzyKpK1, the capsular polymerase specific to *K. pneumoniae* serotype K1.

*Klebsiella pneumoniae*, with its mucoid phenotype, is one of the most important known nosocomial pathogens, causing urinary tract infections, pneumonia, wound infections, and septicemia in immunocompromised patients [1]. Liver abscess due to community-acquired *K. pneumoniae* was first documented in Taiwan. Although it has since become a global disease, it is still most frequently reported in Asia [2]. More than one-half of infected patients also have diabetes mellitus, whereas the rest are otherwise healthy individuals, and underlying diabetes mellitus predisposes individuals to various complications, including metastatic septic meningitis or endophthalmitis [3].

Previous searches for the pathogenic mechanisms of *K. pneumoniae* infection have identified several bacterial virulence factors, such as lipopolysaccharide (LPS), which contains the O antigen, and capsular polysaccharide (CPS), which contains the K antigen [1]. Lost or decreased expression of either the O or K antigen renders *K. pneumoniae* less mucoid and less virulent. Strong associations exist between the 77 K serotypes and 9 O serotypes that are currently recognized; for instance, K1 and K2 almost always coexist with O1, and K3 almost always coexists with O2 [4]. Some serotypes
have related virulence to the capsular serotypes, with K1 and K2 being the most virulent [5]. Although it is rare in Western countries, the K1 serotype is common in Taiwan [6], and it is recognized as a major factor contributing to the pathogenesis of liver abscesses due to *K. pneumoniae* [7].

To identify other unique factors vital to the virulence process of *K. pneumoniae* liver abscesses, mutant strains with mini-Tn5 mutagenesis were generated in a previous study [8]. Of the 2500 mutants generated, 20 strains that had lost their wild-type mucoviscosities were chosen for further investigation. One novel gene was found to be more prevalent in strains causing liver abscess than in strains causing septicemia; this gene was designated *magA* (mucoviscosity-associated gene A; GenBank accession no. AB085741 and GI no. 31044072). The *magA* mutant that remains encapsulated was susceptible to phagocytosis and was not lethal to mice. *MagA* was proposed to be a putative O-antigen ligase *WaaL from a Protein Basic Local Alignment Search Tool (BLAST-P) search, whereas its upstream gene product Wzx is an O-antigen flippase [9].

Although *magA*, which is known to be specific to the K1 serotype [10], has been used in various *K. pneumoniae* virulence studies [11], the exact functions of *magA* and its associated genes are still unclear. In the present study, we constructed mutants of *magA* and 2 other genes within the same transcription unit, to investigate their roles in *K. pneumoniae* virulence.

### MATERIALS AND METHODS

**Bacterial strains, plasmids, and culture conditions.** A *K. pneumoniae* strain, STL43, that caused liver abscess in a 33-year-old Taiwanese was *magA* positive, of serotype K1, and hypermucoid. The bacterial strains and plasmids used in the present study are presented in Table 1. For general use, bacteria were routinely cultured in Luria-Bertani broth or agar at 37°C. When required, 50 μg/mL kanamycin or 34 μg/mL chloramphenicol was added to the media. BIND (brilliant green agar containing inositol-nitrate-deoxycholate), a selective medium of *K. pneumoniae* [13], was used for conjugation.

**Construction of mutant strains.** The coding regions for transposase, ampicillin resistance, and kanamycin resistance were removed from pUTmini-Tn5 Km vector (Biomedal). The remaining regions, which consisted of an R6K origin of replication and an *mob*P4 origin of transfer, were ligated with a kanamycin resistance cassette to construct a new plasmid for insertional mutagenesis. The final constructed plasmid, designated “pUT-kmy,” was used for further insertional mutagenesis by means of an established method [9].

Primer pairs designed for mutagenesis and genotype confirmation are shown in Table 2. With the wild-type chromosome used as a template, the polymerase chain reaction (PCR)–amplified fragments of target gene were excised by *Eco*I and *Not*I and then were ligated into the *Not*I-*Eco*I site of pUT-kmy. The resultant plasmid constructs were electroporated into *Escherichia coli* S17-1λpir, followed by conjugation with the wild-type strain. Transconjugations were selected using the *Klebsiella*-selective medium BIND supplemented with 50 μg/mL kanamycin. The colony grown in BIND with kanamycin was the *K. pneumoniae* strain that had the kanamycin-resistant pUT-kmy inserted into the target gene.

The mutant genotype was confirmed by PCR performed with one primer pair (known as “PUT-F3A” and “PUT-R1”) de-

### Table 1. Bacterial Strains and Plasmids Used in the Present Study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristic</th>
<th>Reference or manufacturer</th>
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<tbody>
<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
<td></td>
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</tr>
<tr>
<td>STL41</td>
<td>Wild type (O1:K2)</td>
<td>Present study</td>
</tr>
<tr>
<td>STL43</td>
<td>Wild type (O1:K1)</td>
<td>Present study</td>
</tr>
<tr>
<td>STL43ΔmagA</td>
<td>Nonpolar <em>magA</em> mutant</td>
<td>Present study</td>
</tr>
<tr>
<td>STL43ΔwcaG</td>
<td>Nonpolar <em>wcaG</em> mutant</td>
<td>Present study</td>
</tr>
<tr>
<td>STL43ΔrfbP</td>
<td>Nonpolar <em>rfbP</em> mutant</td>
<td>Present study</td>
</tr>
<tr>
<td>STL43ΔwbbO</td>
<td>Nonpolar <em>wbbO</em> mutant</td>
<td>Present study</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pUTmini-Tn5 Km</td>
<td>Parent vector of pUT-kmy</td>
<td>Biomedal</td>
</tr>
<tr>
<td>pUT4K</td>
<td>Kanamycin resistance cassette [12]</td>
<td></td>
</tr>
<tr>
<td>pUTkmy</td>
<td>Suicide vector for insertional mutagenesis</td>
<td>Present study</td>
</tr>
<tr>
<td>pHSG398</td>
<td>For complementation</td>
<td>Takara</td>
</tr>
<tr>
<td>pHSG398-magA</td>
<td>pHSG398 containing intact <em>magA</em> gene</td>
<td>Present study</td>
</tr>
<tr>
<td>pHSG398-wcaG</td>
<td>pHSG398 containing intact <em>wcaG</em> gene</td>
<td>Present study</td>
</tr>
<tr>
<td>pHSG398-rfbP</td>
<td>pHSG398 containing intact <em>rfbP</em> gene</td>
<td>Present study</td>
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</table>

signed outside the NotI-EcoRI restriction sites of pUT-kmy and one pair (known as “ORF-OF1” and “ORF-OR1”) designed outside the target gene. The PCR results achieved with the use of primer pairs PUT-F3A and ORF-OF1 or PUT-R1 and ORF-OR1 were positive for the target gene mutant but negative for the wild-type strain.

**Trans-complementation.** Fragments of the intact genes were amplified by PCR performed using the primers described above. After digestion with EcoRI and BamHI, the gene fragments were cloned into a chloramphenicol-resistant pHSG398 plasmid (Takara). These constructed plasmids were transformed into their corresponding kanamycin-resistant isogenic strain, Luria-Bertani agar plates were supplemented with kanamycin and chloramphenicol.

**Phagocytosis assay.** Isolation of neutrophils from healthy volunteers and labeling of bacteria with fluorescein isothiocyanate (FITC) were performed as described elsewhere [14]. A mixture of labeled bacteria, neutrophil suspensions, and phosphate-buffered saline (PBS [pH 7.4]) was incubated for 0 or 10 min in a shaking water bath at 37°C. Ethidium bromide was added before measurement to suppress the extracellular fluorescence. The FITC fluorescence was detected using a FACScan (Becton Dickinson Immunocytometry Systems). The mean percentage of neutrophils that carried FITC-stained bacteria after 10 min of incubation in 6 successive results was used as the phagocytosis rate.

**Inoculation of mice with wild-type or mutant strains.** Six-week-old male BALB/c mice were used for inoculation. A standard inoculum of $2 \times 10^6$–$6 \times 10^6$ cfu of *K. pneumoniae* in the midlogarithmic phase of growth was diluted in 100 µL of PBS and injected intraperitoneally. Six mice were used to test the effects of each strain and were observed for 1 week after inoculation.

**Transmission electron microscopy.** Transmission electron microscopy (TEM) was used to visualize the capsular structures of thin sections of bacteria by means of an established method [15]. Colonies were scraped from Luria-Bertani plates, and the cells were resuspended in PBS at an optical density value (measured at 600 nm) of ∼0.5. The cells were incubated in 0.5 mL of 20% cationized ferritin solution (EMS) in PBS. Thin sections of bacteria were prepared and examined using an electron microscope (JEM-1230; JEOL), operating at 80 kV, at the National Defense Medical Center in Taipei, Taiwan, Republic of China.

**Agglutination test.** The serotyping of *K. pneumoniae* strains was performed using *Klebsiella* antisera Seiken (Denka Seiken), which were prepared by immunizing rabbits with bacterial strains of serotype K1–K6 administered separately. A drop of type-specific antiserum or 0.9% saline was put onto a glass slide, to which a drop of a dense suspension of test

### Table 2. Primers Used in the Present Study

<table>
<thead>
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<th>Primer</th>
<th>Sequence (5′→3′)</th>
<th>Purpose</th>
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</thead>
<tbody>
<tr>
<td>kan-F2</td>
<td>AACTGCGAAGACAATCTTCGATGACAAAGGCTGAC</td>
<td>Amplification of kanamycin-resistant cassette</td>
</tr>
<tr>
<td>kan-R2</td>
<td>AACTGCGAAGACAATCTTCGATGACAAAGGCTGAC</td>
<td>Confirmation of genotype after insertion mutagenesis</td>
</tr>
<tr>
<td>PUT-F3A</td>
<td>CAGGATATGCAGATGAATAGAC</td>
<td>Confirmation of STL43a magA genotype</td>
</tr>
<tr>
<td>PUT-R1</td>
<td>ATAGGTATACGGGTGTTGGAC</td>
<td>Confirmation of genotype after insertion mutagenesis</td>
</tr>
<tr>
<td>magA-OF2</td>
<td>GGAATTCCTCTGCTTTGATTCCTTCT</td>
<td>Confirmation of magA mutant, STL43a magA</td>
</tr>
<tr>
<td>magA-IR2</td>
<td>AGTTAGCTGGTGTGCATAG</td>
<td>Construction of magA mutant, STL43a magA</td>
</tr>
<tr>
<td>wcaG-OF2</td>
<td>TATTTTTGCTGAGATAGTGA</td>
<td>Construction of wcaG mutant, STL43a wcaG</td>
</tr>
<tr>
<td>wcaG-IR2</td>
<td>GTAAGGTGGAATGAGATAGTGA</td>
<td>Construction of wcaG mutant, STL43a wcaG</td>
</tr>
<tr>
<td>kan-F2</td>
<td>AACTGCGAAGACAATCTTCGATGAC</td>
<td>Construction of magA mutant, STL43a magA</td>
</tr>
<tr>
<td>kan-R2</td>
<td>AACTGCGAAGACAATCTTCGATGAC</td>
<td>Construction of magA mutant, STL43a magA</td>
</tr>
<tr>
<td>PUT-F3A</td>
<td>CAGGATATGCAGATGAATAGAC</td>
<td>Construction of magA mutant, STL43a magA</td>
</tr>
<tr>
<td>PUT-R1</td>
<td>ATAGGTATACGGGTGTTGGAC</td>
<td>Construction of magA mutant, STL43a magA</td>
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<td>magA-OF2</td>
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<td>Construction of magA mutant, STL43a magA</td>
</tr>
<tr>
<td>magA-IR2</td>
<td>AGTTAGCTGGTGTGCATAG</td>
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<td>wcaG-OF2</td>
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<td>wcaG-IR2</td>
<td>GTAAGGTGGAATGAGATAGTGA</td>
<td>Construction of wcaG mutant, STL43a wcaG</td>
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strain was also added. Agglutination was then observed under light microscopy.

**Western blot analysis of cell-surface polysaccharides.** Cell-surface polysaccharides, including CPS and LPS, were extracted using a modified hot water/phenol extraction method [16]. Samples were analyzed using 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After the gel was transferred to polyvinylidene difluoride membrane with the use of Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad), Western immunoblotting was performed using 1:500 *Klebsiella* antiserum Seiken (Denka Seiken) and 1:1000 anti-rabbit immunoglobulin (Ig) G (whole molecule)–peroxidase (Sigma). The membrane was stained with SigmaFast 3-3′-diaminobenzidine with a Metal Enhancer Tablet Set (Sigma).

**RESULTS**

*Phenotype of the mutant strains.* To study whether *magA* was responsible for a unique function or whether it had a role different from that of its associated genes in the same transcription unit, we performed functional analyses of 3 genes—*magA*, *wcaG*, and *rfbP*—that were previously described in a cluster of *magA* [9]. PCR analysis revealed that all 3 genes were present in our mucoid study strain STL43. Three mutants, STL43Δ*magA*, STL43Δ*wcaG*, and STL43Δ*rfbP*, were obtained by the homologous recombination of the pUT-kmy suicide vector and were verified by interruption of target genes with specific PCR primer sets.

Morphologically, STL43Δ*magA* was nonmucoid and appeared to be indistinguishable from the other 2 mutants. All 3 mutants displayed similar/comparable mucoviscosities.

**Susceptibility to phagocytosis and lethality to mice.** Because the 2 genes had the same effect as that of *magA* on mucoviscosity, we evaluated their contribution to virulence by inoculating 6 mice and employing a human neutrophil phagocytosis assay with flow cytometry. Complementation studies of the 3 mutants were performed using the chloramphenicol-resistant plasmid pHSG398, which carries the entire open-reading frame of the interrupted gene.

The phagocytosis rate was considered to be the percentage of neutrophil-harboring FITC-stained bacteria after 10 min of incubation. Wild-type STL43 had a mean phagocytosis rate (± standard deviation [SD]) of 8.2% ± 2.0%, implying that the wild-type strain was highly resistant to phagocytosis. Each of 3 mutants was markedly susceptible to phagocytosis, with phagocytosis rates ranging from 88.4% to 88.9%, whereas their complementation strains significantly restored phagocytosis resistance partially (Figure 1). In a mouse inoculation test using wild-type STL43, all mice died within 3 days of injection, whereas the 3 mutants were nonlethal to mice at a similar dosage.

**TEM.** The wild-type and mutant bacteria were labeled with cationized ferritin, which is useful for microscopic examination of the bacterial capsule, and capsule morphological findings were examined using TEM (original magnification, ×60,000). The morphology of the wild-type STL43 capsule (Figure 2) was similar to that of *E. coli* B44 [15]. Capsule deficiency was observed in all 3 mutants, because cationized ferritin did not bind to their surfaces. Therefore, *magA*, *wcaG*, and *rfbP* are all responsible for capsule synthesis in *K. pneumoniae*.

**Agglutination of mutants by reactions with K1 and K2 antiserum.** We performed an agglutination test to probe for the interaction between the *K. pneumoniae* strains and the antiserum that was originally used for capsule serotyping.
type, mutant, and complementation strains were each mixed separately with saline or K1 or K2 antiserum. No spontaneous agglutination was observed when any test strain was mixed with saline, and the wild-type serotype K1 strain STL43 agglutinated in the K1, but not the K2, antiserum (Figure 3). Despite capsule loss, all 3 mutant strains agglutinated with the K1 antiserum and, unexpectedly, also with the K2 antiserum (Figure 3). Furthermore, all the complementation strains restored the wild-type phenotype and allowed agglutination with the K1, but not the K2, antiserum (Figure 3).

**Western blot analysis.** To assess whether other surface antigens, such as O antigen, were responsible for agglutinations in both K1 and K2 antiserum, we extracted CPS and LPS from the bacteria, separated them by means of SDS-PAGE, and analyzed the results using Western blot analysis and silver staining, the latter of which detects LPS but not CPS. Silver staining revealed that the O-antigen mutant STL43ΔwbbO had lost its LPS, whereas the 3 mutant strains had retained their O antigens, similar to wild-type STL43, serotype K2 wild-type STL41, and the magA complementation strain (Figure 4A).

In Western blot analysis performed with Seiken K2 antiserum, both CPS and LPS were detected in K2 strain STL41 but were absent in the LPS-deficient strain STL43ΔwbbO (Figure 4B). Wild-type K2 *K. pneumoniae* possesses the O1 antigen, and the aforementioned results indicate that the Seiken K2 antiserum consists of anti-K2 and anti-O1 antibodies. The O1 antigens of wild-type STL43, the 4 other mutants, and the complementation strain of STL43ΔmagA were also detected by this method, but CPS was not detected in any of these strains in this blotting (Figure 4B).

In Western blot analysis performed using Seiken K1 antiserum, O1 LPS was detected only in serotype K2 STL41, whereas K1 CPS was detected only in the O-antigen mutant STL43ΔwbbO. Both CPS and LPS were detected in wild-type STL43 and the complementation strain STL43ΔmagA. All 3 mutant strains had lost their CPS but retained their LPS (Figure 4C).

Taken together, our results indicate that magA, wcaG, and rfbP contribute to capsule synthesis but do not affect LPS. In all 3 mutants, positive agglutination with K1 and K2 antisera was the result of an interaction between the anti-O1 antibodies in antiserum and the exposed O1 antigens in mutant strains.

**Analysis of MagA from the conserved domain.** We also analyzed the function of MagA, using the experimental results presented here in addition to those obtained by a search of conserved domains using Pfam [17]. Although amino acids 284–339 of MagA were comparable to the Wzy conserved domain (Wzy_C) of the O-antigen polymerase, a protein involved in the synthesis of O antigen [18] (Figure 5B), this putative function for MagA was contradicted by our experimental results showing that the O antigen is present in the ΔmagA mutant. Our results further indicated that MagA is associated only with CPS (K antigen) synthesis. Thus, MagA is a Wzy responsible for K- but not O-antigen polymerization. Several other proteins also contained Wzy_C as MagA, including ORF10 of the *cps* cluster of *K. pneumoniae* strain Chedid (O1:K2) (Figure 5C) [19], WaaL of *K. pneumoniae* 52145 (O1:K2) (Figure 5D) [20], Wzy of the *cps* cluster of *E. coli* (O9a:K30) (Figure 5E) [21], WaaL of *E. coli* K-12 (Figure 5F) [22], and WaaL of *Vibrio cholerae* (Figure 5G) [23].

**DISCUSSION**

Although MagA had been proposed to be a putative O-antigen ligase [9], our results showed that it contributed to capsular K-antigen synthesis and had no effect on O-antigen synthesis. In the present study, 2 mutants with gene disruption adjacent to MagA had shown an identical phenotypic and physiological characteristic on the basis of capsule morphology and virulence. MagA was further confirmed to play a role as the capsular polysaccharide polymerase Wzy.

In this study, the STL43ΔmagA mutant shared many of the characteristics of the *magA* mutant observed by Fang et al [8], even though each was constructed by different methods. Both mutants had lost their mucoviscosity, had become susceptible to neutrophil phagocytosis, and were not lethal to mice. Complementation of *magA* reversed resistance to phagocytosis to some extent. In contrast to the report by Fang et al [8], which...
Figure 3. Agglutination tests of 7 Klebsiella pneumoniae strains. Lane 1, 0.9% saline; lane 2, K1 antiserum; and lane 3, K2 antiserum. Images were photographed using a light microscope (original magnification, ×1000). STL43, wild type; STL43ΔmagA, magA mutant; STL43ΔmagA+ pHS398-magA, magA complementation strain; STL43ΔrfbP, rfbP mutant; STL43ΔrfbP+pHS398-rfbP, rfbP complementation strain; STL43ΔwcaG, wcaG mutant; STL43ΔwcaG+pHS398-wcaG, wcaG complementation strain.

indicated that the magA mutant remains encapsulated under light microscopy, our results showed complete capsule loss, compared with the wild-type parent strain (Figures 2 and 4). Because light microscopy with periodic acid–Schiff staining is a relatively less sensitive method of capsule identification than is TEM, interpretation will be influenced by other factors and requires an experienced microbiologist for interpretation of the results. However, capsule loss was further confirmed by Western blot analysis in the present study. In addition, capsule loss was also observed in strains with insertional mutagenesis of genes that are transcribed with magA. Compatible with the findings of our previous study [24], it is therefore evident that magA
Figure 4. Western blotting and silver staining of surface polysaccharides extracted from 7 *Klebsiella pneumoniae* strains. A, Silver staining. Western blot analysis performed using K1 (B) or K2 (C) antiserum. CPS, capsular polysaccharide; LPS, lipopolysaccharide; STL41, serotype O1:K2 wild type; STL43, serotype O1:K1 wild type; STL43ΔmagA, magA mutant; STL43ΔmagA ΔpHSG398--magA, magA complementation strain; STL43ΔrfbP, rfbP mutant; STL43ΔwbbO, O-antigen--deficient wbbO mutant; STL43ΔwcaG, wcaG mutant.

and its adjacent genes are responsible for capsule polysaccharide synthesis.

Chuang et al [9] reported a cross-reaction between serotype K1 and K2 conventional antisera, a phenomenon that we observed in the capsule-deficient mutants but not in the wild-type strain (Figure 3). Our results indicated that the agglutination of capsule-deficient mutants in both K1 and K2 antisera is actually the result of exposure of the O1 antigen on the cell surface after the loss of capsule polysaccharide K antigen.

Although a previous comparison of MagA and WaaL of *V. cholerae* ascribed MagA as a putative O-antigen ligase [9] with a 20% amino acid similarity, our analysis showed that they shared the Wzy conserved domain, similar to some Wzys and WaaLs (Figure 5). If MagA ligates O antigen to lipid A core, then O antigen should not be detected during SDS-PAGE of MagA mutants [25]. However, our study showed that magA mutant did not lose its O antigen. With reference to previous studies of serotype O1 *K. pneumoniae*, the real *waaL* is located between *wabK* and *wabM* within the *waa* gene cluster, the genes of which are involved in core lipopolysaccharide biosynthesis [25, 26]. Thus, to predict the function of a novel gene, similarity of the amino acid sequence alone may not be sufficient to identify the exact gene function.

With the remarkable versatility of surface polysaccharides among different bacteria, *E. coli* is the best model system for capsule study, and it has been classified into 4 groups with respect to its genetics, polymerization mechanisms, and regulation [27]. Group 1 *E. coli* synthesizes K antigen with the *wzx/wzy* system, whereas group 2 uses the same system to make O antigen. The flippase Wzx transfers the preformed oligosaccharide subunit across the plasma membrane, and then the
polymerase Wzy links each subunit to form the whole polysaccharide antigen. Because most studies have focused on group 2 *E. coli*, Wzy is generically depicted as an O-antigen polymerase and Wzx as an O-antigen flippase. Nevertheless, group 1 *E. coli* uses Wzy as a K-antigen polymerase and Wzx as a K-antigen flippase.

In *K. pneumoniae*, the genes responsible for capsule biosynthesis were first identified in the K2 serotype Chedid strain [18]. Capsule synthesis in *K. pneumoniae* resembles that in group 1 *E. coli*, using the wxwz/wzy system to synthesize its K-antigen capsule [27]. In the present study, we have shown that magA and its cotranscribed genes are necessary for capsule synthesis but not for O-antigen synthesis, leading us to suggest that MagA is not WaaL but, rather, the K-antigen polymerase Wzy and that Wzx is not the O-antigen flippase but, rather, the K-antigen flippase.

One of the major characteristics of *magA* is its specificity to serotype K1 *K. pneumoniae* [10, 28]. Such serotype specificity of wxwz and wywz has been extensively observed in group 2 *E. coli* and used for molecular O serotyping [29]. Thus, despite their similar functions, wxwz and wywz have diverse sequences and vary from serotype to serotype and among species. These genes are distinguished using the bacterial polysaccharide gene nomenclature (BPGN) scheme, which designates the origin of the polysaccharide gene by a subscript, such as *wzy*Eco for O-antigen polymerase for *E. coli* serotype O1 [30]. Because it was previously proposed that mature intracellular form–associated gene A should be designated as *magA* [31], and on the basis of the results in this study, we suggest that mucoviscosity-associated gene A should be designated as *wzycK*.

In addition to wywz (*magA*), Chuang et al [9] also identified several genes with unique specificity to serotype K1 *K. pneumoniae*, according to a survey of 36 *K. pneumoniae* serotype K1 strains and 38 non-K1 strains. Yu et al [32] also suggested that orf9 within the cps cluster of *K. pneumoniae* Chedid strain (01: K2) corresponds to *magA* in the K1 cps cluster. In contrast, our analysis showed that it is *orf10* that contains Wzy_C (Figure 4C), and BLAST-P results suggested that orf9 is actually related to mannosyltransferase. Similarly, with a limited number of 20 non-K1/K2 strains, the claim that orf9 is unique for *K. pneumoniae* serotype K2 may not be sufficiently conclusive. In our opinion, it is hard to justify that some genes other than wxwz and wywz are specific to some serotypes without fully surveying all 77 K serotypes of *K. pneumoniae* and the “nontypeable” strains.

In conclusion, the gene cluster that contains magA contributes to the capsule synthesis of serotype K1 *K. pneumoniae*, and Wzx is a K-antigen flippase, rather than an O-antigen flippase. With the confirmation that MagA is a K1-antigen–specific polymerase, and in accordance with the BPGN scheme, MagA should be renamed WzyKpK1, the capsular polymerase specific to *K. pneumoniae* serotype K1.

Acknowledgment

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References