JOURNAL OF BACTERIOLOGY, Sept. 2006, p. 6449–6459 Vol. 188, No. 18
0021-9193/06/$08.00+0 doi:10.1128/JB.00453-06
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# Characterization of Stg Fimbriae from an Avian Pathogenic *Escherichia coli* O78:K80 Strain and Assessment of Their Contribution to Colonization of the Chicken Respiratory Tract

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Received 1 April 2006/Accepted 7 July 2006

**In a previous study, *ecs-3*, a sequence from avian pathogenic *Escherichia coli* (APEC) O78:K80 strain χ7122, was found to be expressed in vivo in infected chicken tissues. The region encompassing *ecs-3* carries a fimbrial gene cluster that is a putative ortholog of the *stg* fimbrial gene cluster of *Salmonella enterica* serovar Typhi. This APEC fimbrial gene cluster, which we have termed *stg*, is a member of a distinct group of related fimbriae that are located in the *glmS-pstS* intergenic region of certain *E. coli* and *S. enterica* strains. Under the control of the pBAD promoter, the production of Stg fimbriae was demonstrated by Western blotting and immunogold electron microscopy with *E. coli* K-12. Transcriptional fusions suggest that *stg* expression is influenced by the carbohydrate source and decreased by the addition of iron and that Fur plays a role in the regulation of *stg* expression. *stg* sequences were associated with APEC O78 isolates, and *stg* was phylogenetically distributed among *E. coli* reference strains and clinical isolates from human urinary tract infections. Stg fimbriae contributed to the adherence of a nonfimbriated *E. coli* K-12 strain to avian lung sections and human epithelial cells in vitro. Coinfection experiments with APEC strain χ7122 and an isogenic Δ*stg* mutant demonstrated that compared to the wild-type parent, the Δ*stg* mutant was less able to colonize air sacs, equally able to colonize lungs, and able to more effectively colonize tracheas of infected chickens. Stg fimbriae, together with other adhesins, may therefore contribute to the colonization of avian respiratory tissues by certain APEC strains.**

Avian pathogenic *Escherichia coli* (APEC) is associated mainly with extraintestinal diseases in chickens, turkeys, and other avian species and causes severe economic losses to the poultry industry (12). The most common form of these diseases is avian colibacillosis, which starts as a respiratory infection (airsacculitis) and is frequently followed by generalized infections such as perihepatitis, pericarditis, and septicemia (12). APEC belongs to a limited number of serogroups, of which O1, O2, and O78 are the most common (12). Phylogenetic analyses have indicated that most APEC strains belong to a few distinct clonal groups (19, 59). Several virulence factors, such as adhesins, iron sequestering systems, capsular and lipopolysaccharide antigens, and toxins, have been reported for APEC (27).

Although many virulence factors are required for bacterial pathogenicity, adhesin-mediated colonization of different tissues is one of the earliest events occurring during an infection. Adhesins are either assembled into hair-like appendages (fimbriae or pili) or are directly associated with the bacterial cell surface (afimbrial adhesins) (55). Several adhesins have been described so far for APEC, including type 1 (F1A), P (F11), and AC/I (avian *E. coli* I) fimbriae, curli, and the temperature-sensitive hemagglutinin (Tsh) (12, 27). P fimbriae have been reported mostly for human uropathogenic *E. coli* and are present in only a minority of APEC isolates (12, 27). The expression of P fimbriae in the air sacs and other internal organs but not in the trachea of inoculated chickens supports the idea that they are important only in later stages of infection (47). Type 1 fimbriae are characterized by the ability to adhere to D-mannose residues of epithelial cells and mucosa (24). They were shown to be expressed by bacteria colonizing the respiratory tracts of experimentally infected chickens (14) and may contribute to the initial colonization of the avian respiratory tract (12, 47). AC/I fimbriae, which belong to the S fimbrial adhesin family, mediate adherence to avian epithelial tissues in vitro and in vivo and have been found on a limited number of O78 APEC isolates (3, 61). Curli are thin, coiled aggregative structures found on the surfaces of most *E. coli* isolates (27, 41). Curli promote binding to extracellular matrix proteins and major histocompatibility complex class I molecules (42, 53) and also agglutinate chicken erythrocytes (9). Curli cloned from an APEC O78 strain mediated the internalization of *E. coli* K-12 by eukaryotic cells (20). Tsh, a temperature-sensitive hemagglutinin identified from APEC strain χ7122 (O78:K80:H9) (48), was shown to be associated with pathogenic isolates of high lethality for day-old chicks (16).

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TABLE 1. Bacterial strains and plasmids used in this study

| Strain or plasmid | Characteristics[a] | Reference or source |
|---|---|---|
| *E. coli* K-12 strains | | |
| DH5α | F⁻ λ⁻ φ80 Δ(*lacZYA-argF*)*endA1 recA1 hsdR17 deoR thi-1 supE44 gyrA96 relA1* | Invitrogen |
| MG1655 | F⁻ λ⁻ *rph-1* | 8 |
| ORN103 | *thr-1 leu-6 thi-1* Δ(*argF-lac*)*U169 xyl-7 ara-13 mtl-2 gal-6 rspL tonA2 minA minB* Δ(*fimEACDFGH*) | 43 |
| ORN172 | *thr-1 leuB thi-1* Δ(*argF-lac*)*U169 xyl-7 ara-13 mtl-2 gal-6 rspL tonA2 supE44* Δ(*fimBEACDFGH*)::*kan pilG1* | 60 |
| QC2517 | MG1655 *recD1901*::Tn*10* Δ*fur*::*cat* | 17 |
| *E. coli* clinical strains and derivatives | | |
| CFT073 | Uropathogenic *E. coli* strain O6:K2:H1 | 58 |
| χ7122 | Wild-type APEC O78:K80:H9, *gyrA* Nal$^r$ | 49 |
| EDL933 | *E. coli* O157:H7, EHEC strain | 45 |
| QT51 | Strain χ7122 Δ*lacZYA* | This study |
| QT302 | χ7122 Δ*stgABCD*::*kan* | This study |
| QT826 | QT51 Δ*fur*::*cat* | This study |
| QT865 | QT826::pIJ79, P*stg-lacZ* single-copy integrant | This study |
| QT891 | QT51::pIJ79, P*stg-lacZ* single-copy integrant | This study |
| Plasmids | | |
| pBAD18-Cm | Arabinose-inducible vector, Cm$^r$ | 22 |
| pCP20 | FLP helper plasmid, temperature-sensitive replication, Cm$^r$ and Ap$^r$ | 11 |
| pCR-XL-TOPO | High-copy cloning vector, Km$^r$ | Invitrogen |
| pIJ2 | 6.0-kb PCR fragment of *stgABCD* cloned into pCR-XL-TOPO; Km$^r$ | This study |
| pIJ14 | XbaI and HindIII fragment of pIJ2 containing the *stg* operon cloned into pACYC184, Cm$^r$ | This study |
| pIJ32 | PCR fragment containing the *stgA* promoter cloned into pRS415, Ap$^r$ | This study |
| pIJ39 | PCR fragment of *stgABCD* cloned into pBAD18-Cm, Cm$^r$ | This study |
| pIJ79 | pST76-K::P*stg-lacZ* | This study |
| pKD3 | FRT-flanked kanamycin cassette template | 11 |
| pKD46 | Red recombinase expression plasmid, Ap$^r$ | 11 |
| pRS415 | Operon fusion vector, Ap$^r$ | 52 |
| pST76-K | Suicide vector pSC101, temperature-sensitive replicon, Km$^r$ | 46 |

[a] EHEC, enterohemorrhagic *E. coli*; Nal, naladixic acid.

Experimental infection studies showed that although Tsh contributes to the development of lesions in the air sacs, other virulence factors must contribute to the pathogenicity of strain χ7122 in the lower respiratory tract and extrarespiratory tissues (16).

A number of putative adhesin-encoding genes of strain χ7122 that were expressed in vivo in chicken tissues were previously identified by the selective capture of transcribed sequences (15). One of the pathogen-specific fragments identified (*ecs-3*) shared homology at the peptide level with putative fimbrial ushers present in *E. coli* O157:H7 and *Salmonella enterica* serovar Typhi. The related fimbrial gene clusters are located between the conserved genes *glmS* and *pstS*. These fimbriae also share some similarities with the long polar (LP) fimbriae of *S. enterica* serovar Typhimurium and *E. coli* strains EDL933 O157:H7 and 83/39 (6, 37, 56), each of which is encoded by operons located between the conserved genes *yhjX* and *yhjW*. This report describes the identification and characterization of the fimbrial gene cluster corresponding to fragment *ecs-3* of APEC strain χ7122. We have termed the genes encoding these fimbriae *stg*, as the predicted fimbrial system that they encode is most similar to Stg of serovar Typhi and is distinct from LP fimbriae encoded by gene clusters within the *yhjW-yhjX* regions in serovar Typhimurium and certain *E. coli* strains.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, media, and growth conditions.** The bacterial strains and plasmids used in this study are listed in Table 1. In addition, selected clinical or environmental isolates from various sources were used to screen for the presence of *stgC*. The 72 members of the *Escherichia coli* reference (ECOR) collection represent a diversity of *E. coli* strains that have been phylogenetically grouped by multilocus enzyme electrophoresis (MLEE) (39). The 298 APEC clinical isolates were previously described elsewhere (16). Thirty-two *E. coli* fecal isolates from healthy poultry were kindly provided by John M. Fairbrother (University of Montréal). APEC and avian *E. coli* environmental isolates were previously classified for virulence on the basis of lethality for 1-day-old chicks following subcutaneous inoculation, where lethality class 1 (LC1) corresponds to the high-lethality class, LC2 to the low-lethality class, and LC3 to the nonlethal class (16). Among the total number of APEC isolates tested, the serogroup of 187 isolates was determined. Human extraintestinal pathogenic *E. coli* (ExPEC) isolates included a diversity of strains from urosepsis and other extraintestinal infections in the United States. These strains were grouped phylogenetically by either MLEE or multiplex PCR (10). Bacteria were routinely grown in Luria-Bertani (LB) broth (Gibco) or on tryptic soy agar (TSA) (Gibco) at 37°C. Additional media used were MacConkey agar (Gibco) and M9 medium (35). When required, antibiotics were added at the following concentrations: 50 μg/ml for kanamycin (Km), 100 μg/ml for ampicillin (Ap), 50 μg/ml for chloramphenicol (Cm), and 15 μg/ml for nalidixic acid. For plasmid pBAD18-Cm and its derivative, chloramphenicol was used at a concentration of 10 μg/ml. The transformation of *E. coli* strains was routinely carried out by using calcium/manganese-based or electroshock methods as described previously (23).

**DNA and genetic manipulations.** Standard methods were used for the isolation of bacterial genomic DNA, DNA manipulation, and cloning (51). Restriction enzymes and DNA ligase used in this study were purchased from New England Biolabs (NEB), Invitrogen, or Amersham-Pharmacia and used according to the suppli-

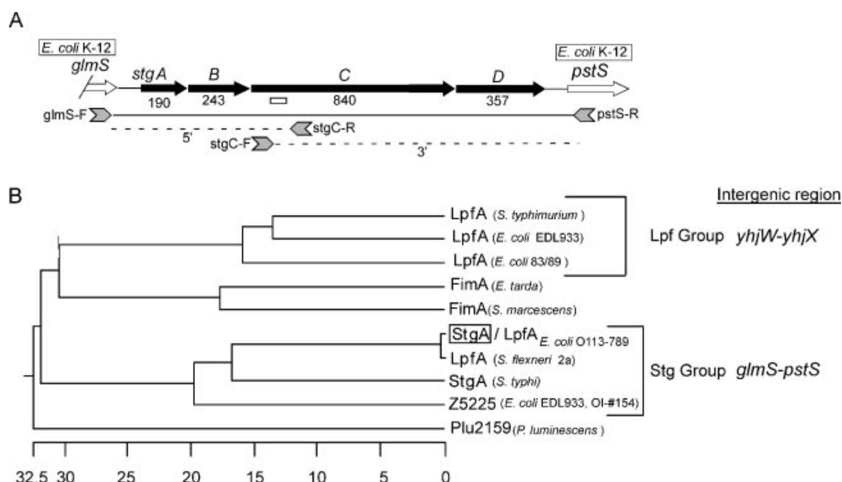


FIG. 1. (A) The *glmS-pstS* intergenic region of APEC strain χ7122. Black arrows indicate the four ORFs of the *stg* gene cluster. Numbers indicate the predicted number of amino acids. White arrows indicate corresponding genes present in *E. coli* K-12. Gray arrows indicate positions of primers used for cloning the *stg* encoding region (glmS-F and pstS-R) and for screening the presence of *stg* sequences among isolates (stgC-F and stgC-R). The small white rectangle corresponds to the region amplified for screening the presence of *stg* sequences among isolates. The dashed lines indicate the 5′ and 3′ products amplified to assess the presence of a full-length *stg* gene cluster among isolates. (B) Phylogram based on Clustal analysis of proteins sharing highest identities/similarities with the predicted *stgA* gene product. The APEC StgA protein is highlighted within a box. Fimbrial proteins from gene clusters inserted in the *yhjW-yhjX* region, which includes LpfA of serovar Typhimurium, cluster together and belong to the LP fimbrial group. Fimbrial proteins from gene clusters inserted in the *glmS-pstS* region, including StgA from *E. coli* and serovar Typhi, comprise a distinct cluster and belong to the Stg fimbrial group. Entries from top to bottom correspond to GenBank accession numbers P43660, C86029, AAO22843, AAO52822, P22595, AAL18161, AAN45247, CAD03135, AAG58930, and CAE14452. The scale indicates percent difference in similarity. *E. tarda*, *Edwardsiella tarda*; *P. luminescens*, *Photorhabus luminescens*; *S. flexneri*, *Shigella flexneri*; *S. marcescens*, *Serratia marcescens*.

ers' recommendations. Recombinant plasmids, PCR products, and restriction fragments were purified using plasmid mini-prep, PCR cleanup, and gel extraction kits (QIAGEN) as recommended by the supplier.

**Identification, cloning, and sequencing of the APEC *stg* gene cluster.** The *stg* gene cluster was amplified from genomic DNA of strain χ7122 using Elongase enzyme mix (Invitrogen Life Technologies) with the primers glmS-F (5′-GATC TTCTACACCGTTCCGC-3′) and pstS-R (5′-TTACGCCACCGGAAGAACC G-3′) (Fig. 1A). The 5.8-kb PCR product was purified and cloned into the vector pCR-XL-TOPO using the TOPO XL PCR cloning kit (Invitrogen), resulting in plasmid pIJ2. The cloned fragment was subcloned from pIJ2 into vector pACYC184 at the XbaI and HindIII restriction sites, resulting in vector pIJ14, which was then transformed into strain MG1655. Sequencing was achieved by the generation of transposon mutants by transduction using phage λ::Tn*5*seq1 (36), a derivative of Tn*5* that contains universal T7 and SP6 sequencing primers at its extreme ends. Total plasmid DNA of the transductants was isolated and transformed into calcium/manganese-based competent DH5α cells. The locations of the Tn*5*seq1 insertions were determined by PCR using the primer Tn*5*seq1-left (5′-AAGCTCGGATCTAATACGAC-3′) located within the transposon and primers glmS-F and pstS-R. Restriction enzyme digestions with NotI were also performed to determine the locations of Tn*5*seq1 insertions. Selected pIJ14::Tn*5*seq1 clones were sequenced by DNA LandMarks, Inc. (St. Jean sur Richelieu, Québec, Canada) using universal primers T7 and SP6.

**Preparation of StgA-specific antisera.** NDSAYTAIDAEGKAE, a purified peptide corresponding to the C-terminal portion of StgA, was coupled to keyhole limpet hemocyanin and used to immunize two New Zealand White rabbits. Peptide synthesis and anti-StgA (α-StgA) antiserum production were provided by New England Peptide, Inc. Antiserum was absorbed consecutively using whole cells of *E. coli* K-12 strain ORN172 grown at 37°C.

**Immunogold labeling and TEM.** The production of *stg*-encoded fimbriae in an *E. coli* clone was examined by transmission electron microscopy (TEM). The *stg* operon was placed under the control of the tightly regulated arabinose promoter of pBAD18-Cm. A 5,142-bp PCR product from plasmid pIJ2 was amplified using Elongase enzyme mix (Invitrogen Life Technologies) and the primers NheI-F (5′-ATGCTAGCAAGTGATGATTCATGGTAAAGG-3′) and HindIII-R (5′-TGTGAAGCTTAAAATCCCACATGTC-3′). The PCR purified product was then digested with HindIII and NheI and cloned into plasmid pBAD18-Cm. The resulting plasmid pIJ39 and the control plasmid were transformed into the nonfimbriated *E. coli* K-12 Δ*fim* mutant strain ORN172. Static bacterial cultures were grown overnight at 37°C on TSA plates with 10 μg/ml chloramphenicol and 0.05% arabinose. The bacteria were recovered by centrifugation and allowed to adhere to a carbon-Formvar-coated copper grid. For immunogold labeling, bacteria were mixed with α-StgA antiserum (1:1,000) and gold-labeled anti-rabbit immunoglobulin G (12-nm diameter) and then negatively stained with uranyl acetate. The grids were allowed to dry before being analyzed with a Philips EM300 electron microscope.

**Preparation of fimbrial extracts.** Bacteria were grown on large plates of tryptic soy agar medium supplemented with 10 μg/ml chloramphenicol and 0.05% arabinose for the induction of fimbrial synthesis. Following growth at 37°C for 16 to 18 h, the bacteria were harvested and resuspended in 5 ml of 75 mM NaCl–0.5 mM Tris-HCl (pH 7.0). They were then incubated at 60°C for 30 min and pelleted by centrifugation (3,000 × *g* for 15 min). The supernatant was then transferred to another tube, and an aliquot was taken for precipitation with concentrated trichloroacetic acid to obtain a final ratio of 1:10. The tube was incubated for 15 to 20 min on ice, followed by centrifugation at 20,000 × *g* for 15 min at 4°C. The protein pellet was washed twice with 0.5 M Tris-HCl–0.5 M EDTA (pH 12.0) and resuspended in 0.5 M Tris-EDTA at 1/10 of the supernatant initial volume.

**Western blotting.** Fimbrial extracts were separated by sodium dodecyl sulfate (SDS)-15% polyacrylamide gel electrophoresis minigels as previously described by Laemmli. Proteins were either stained with Coomassie brilliant blue or transferred to nitrocellulose membranes (Bio-Rad) using a Mini Trans-Blot electrophoretic cell (Bio-Rad) for 60 min at 100 V. The membrane was blocked with StartingBlock supplemented with 0.05% Tween 20 (Pierce). Incubations with primary (1:5,000) and secondary (1:25,000) antibodies were carried out for 1 h at room temperature. SuperSignal West Pico chemiluminescent substrate (Pierce) was used for detection.

**Construction of mutants of APEC strain χ7122.** The deletion/inactivation of *lacZYA*, *stgABCD*, or *fur* genes in strain χ7122 or derivatives was obtained using the lambda red recombinase system as described previously by Datsenko and Wanner (11). For the generation of the Δ*lacZYA* mutant of strain χ7122, the primers LacIKO-F (5′GCAGCGTATCAGGCAATTTTTATAATTTAAACTG ACGTGTAGGCTGGAGCTGCTTC-3′) and LacZKO-R (5′-GGTTTCCCGA CTGGAAAGCGGGCAGTGAGCGCAACGCCATATGAATATCCTCCTTA G-3′), containing regions homologous to terminal portions of *lacI* or *lacZ*, were used to amplify the *cat* gene flanked by FRT (FLP recognition target) sites from the template plasmid pKD3. Gene disruption was carried out by electroshock

transformation of strain χ7122 carrying plasmid pKD46 with the PCR fragment containing the chloramphenicol resistance cassette. Mutants were colony purified once at 37°C and then tested for the loss of pKD46 by selecting for ampicillin sensitivity. To eliminate the FRT-flanked *cat* gene encoding chloramphenicol resistance, strains were transformed with the helper plasmid pCP20 that encodes FLP recombinase and mediates excision of FRT-flanked sequences. Ampicillin-resistant transformants were selected at 30°C and then colony purified nonselectively at 43°C. The strains were then tested for a loss of resistance to both chloramphenicol (loss of the FRT-flanked *cat* gene) and ampicillin (encoded on plasmid pCP20), a loss of the pCP20 plasmid, and the presence of all four native plasmids of strain χ7122. The Δ*lacZYA* derivative of strain χ7122 was named QT51. The same technique was used for the construction of the Δ*stgABCD*::*kan* mutant of strain χ7122. The primers used were stgKO-F (5′-TATAAGTGATG ATTCATGGTAAAGGATATATTATATCAATGTGTAGGCTGAGCTGCT TC-3′) and stgKO-R (5′-TAGCGCACAATCTCAACAGTTATCGTCGCTGT TGCAGTAACATATGAATATCCTCCTTAG-3′). The resulting strain was named QT302. A *fur* mutant of strain QT51 was created as follows. Briefly, a Δ*fur*::*cat* allele derived from *E. coli* strain QC2517 (17) was amplified with primers CMD18 (5′-ATTCTAGACTGCTGCTGGGCATCCC-3′) and CMD19 (5′-ACTCTAGACACTCCGACATCCCAAGC-3′). The Δ*fur*::*cat*-containing amplicon was transferred to strain QT51 by homologous recombination using the lambda red recombinase method described above. A *fur* mutant of strain QT51 was designated QT826.

**Generation of single-copy *stg-lacZ* transcriptional fusions and β-galactosidase assay.** The *stgA* promoter region was amplified from plasmid pIJ2 by using Elongase enzyme mix (Invitrogen Life Technologies) and the primers CMD56-R (5′-TAGGATCCAGCATTAGAGATGCCAGAG-3′) and CMD57-F (5′-CGG AATTCAAAGGCACCGACGTTGAC-3′). The PCR product was digested with EcoRI and BamHI and cloned into pRS415 (52), resulting in plasmid pIJ32. A segment of pIJ32 containing the P*stg-lacZ* fusion was amplified by PCR using Elongase and primers CMD57-F (see above) and CMD173-R (5′-TAGCATGC GGAAGTAGGCTCCCATGAT-3′). The PCR product containing P*stg* fused to the *lacZ* gene was digested with EcoRI and SphI and cloned into suicide vector pST-76K (46), generating pIJ79. To generate single-copy P*stg-lacZ* fusions in the APEC strains QT51 and *fur* derivative QT826, pIJ79 was integrated into the genomes of these strains by homologous recombination as described previously (46). A strain carrying a single integrated copy of P*stg-lacZ* in QT826 was designated QT865. A strain carrying a single integrated copy of P*stg-lacZ* in QT51 was designated QT891.

The β-galactosidase assays were performed as previously described elsewhere (35). For the assays, *E. coli* strains were grown overnight (18 h) at 37°C on M9 agar plates supplemented with 0.2% (wt/vol) of the following carbohydrates: glycerol, mannitol, sorbitol, arabinose, ribose, xylose, glucose, mannose, and maltose or other carbon sources (pyruvate, acetate, and succinate) at 0.3% (wt/vol). To investigate the influence of iron on expression, cultures were grown on M9 plates supplemented with glucose and either 150 μM of the chelator dipyridyl (iron-limiting conditions) or 10 μM of $FeCl_3$ (iron-replete conditions). Bacterial suspensions were prepared by resuspending cultures in 2 ml of Z buffer (60 mM $NaHPO_4 \cdot 7H_2O$, $NaH_2PO_4 \cdot H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, 50 mM β-mercaptoethanol), and samples were adjusted with Z buffer to obtain an optical density at 600 nm of 0.6. A 100-μl volume of each sample was then transferred into 900 μl of Z buffer to which 40 μl of chloroform and 20 μl of 0.1% SDS solution were added, and tubes were then vortexed for 10 s. Following 5 min of incubation at 28°C, *O*-nitrophenyl-β-D-galactopyranoside was then added and samples were incubated for another 3 min. The reaction was stopped by the addition of 500 μl of a 1 M $Na_2CO_3$ solution (35).

**Presence of *stg* sequences among *E. coli* strains.** The presence of *stg* sequences was investigated among different *E. coli* strains by PCR amplification of a 208-bp segment of the *stgC* gene. Crude DNA extracts of strains were prepared by alkaline lysis. Primers specific to the putative usher encoding gene *stgC*, stgC-F (5′-TCTGGTTCACATACACTACG-3′) and stgC-R (5′-CCAATCATAATCT GGCTTCT-3′) (Fig. 1A), were used for screening, as the coding sequences of usher genes are typically conserved within a fimbrial type that may comprise a number of variant major subunit or adhesin alleles (24, 28). PCR conditions were as follows: 95°C for 1 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min and then an extension period of 72°C for 1 min. The reactions were carried out using *Taq* DNA polymerase (NEB). Specificity of the primers was confirmed by the amplification of the fragment in strain χ7122 and no amplification in strains known not to contain the *stg* operon on the basis of their genome sequences (MG1655, CFT073, and EDL933). For 25 of the *stgC*-positive strains, the *glmS-pstS* intergenomic regions were also analyzed by PCR amplification of the 5′ and 3′ regions by using the glmS-F/stgC-R and stgC-F/pstS-R primer pairs, respectively (Fig. 1A).

**Bacterial association with human epithelial cells.** The ability of strains ORN103 and ORN103(pIJ2) to adhere to epithelial cell monolayers was assessed using human bladder-derived (UM-UC-3; ATCC no. CRL-1749) and human intestine-derived (INT407; ATCC no. CCL-6) epithelial cell lines. For both quantitative and qualitative assays, epithelial cells were grown to semiconfluence (about $3.5 \times 10^5$ cells/ml) in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum and rinsed three times before the adherence assays. Bacteria were grown overnight on TSA plates containing kanamycin and resuspended in phosphate-buffered saline (PBS), pH 7.4, to an optical density at 600 nm of 0.6 ($\sim 3 \times 10^8$ CFU). Approximately $10^6$ CFU was added to each well. The 24-well plates were then centrifuged at 800 rpm for 5 min, incubated at 37°C in 5% $CO_2$ for 90 min, and rinsed three times. PBS-0.1% deoxycholic acid sodium salt was added to each well, and samples were diluted and spread on TSA plates containing kanamycin for enumeration by viable colony counts. Data are expressed as the percentage of the initial inoculum introduced. Assays were carried out in triplicate wells in two separate experiments.

**Bacterial adherence in vitro to chicken lung sections.** Adherence assays were performed by using a method adapted from Nowicki et al. (38). Lung tissues from two chickens were removed and frozen in Tissue-Tek OCT compound (Sakura) at −80°C. Tissues were cut in 5-μm sections on glass slides using a cryostat (Miles 4553) and fixed with methanol. For the adherence assay, bacteria were grown overnight on TSA plates with appropriate antibiotics and suspended in PBS, pH 7.4, containing 1% (wt/vol) bovine serum albumin. Fifty microliters of a $5 \times 10^9$ bacterial suspension was added to the tissue sections, which were incubated in a moist chamber at 4°C for 45 min. Slides were rinsed twice in PBS, pH 7.4, for 5 min with gentle agitation and then fixed and stained using a Diff-Quick stain set (Dade Behring, Inc.). Adherence was observed using a Nikon Eclipse E800 microscope equipped with a Nikon Coolpix 990 digital camera.

**Chicken infection model.** An *E. coli* intratracheal infection model using 2-week-old White Leghorn chickens preinfected with infectious bronchitis virus was used as described previously (2). Briefly, 14 axenic chickens (12 days old) were infected with a $10^4$ mean embryo infective dose of infectious bronchitis virus serotype Massachusetts strain M41 by the oculonasal route. Two days later, each bird was inoculated via the trachea with a 100-μl mixed suspension of $1.6 \times 10^8$ CFU containing APEC strain χ7122 (56%) and its isogenic Δ*stgABCD*::*kan* derivative, QT302 (44%), which were cultured overnight in LB broth at 37°C without agitation. Six days following coinfection with the *E. coli* strains, chickens were euthanized and necropsied. Bacterial counts were determined from the whole homogenized trachea and the left lung and from swabbings of the caudal thoracic air sacs as previously described (14). The numbers of wild-type and mutant bacteria were obtained by determining the numbers of colonies that were sensitive (wild-type strain χ7122) and resistant (Δ*stgABCD*::*kan* mutant strain QT302) to kanamycin.

**Statistical analyses.** Statistical analyses were performed using the Prism 4.0b software package (GraphPad Software). The chi-square test was used to compare frequency distribution of *stg* sequences among *E. coli* isolates. For coinfections, the Mann-Whitney test was used. The *t* test was used for a comparison of adherence to tissues and for β-galactosidase assays.

**Nucleotide sequence accession number.** The nucleotide sequence of the *stg* encoding region of strain χ7122 was assigned GenBank accession no. AY530785.

## RESULTS

**Identification of the *stgABCD* gene cluster and phylogenetic relationship with other fimbrial gene clusters.** Pathogen-specific transcripts of strain χ7122 were previously identified in a chicken infection model by selective capture of transcribed sequences (15). Transcript *ecs-3* shares sequence identity at the peptide level with the putative fimbrial ushers StgC of serovar Typhi strain CT18 (44) and Z5222 of *E. coli* O157:H7 strain EDL933 (45). In serovar Typhi strain CT18 and *E. coli* O157:H7 strain EDL933, *stgC* and gene Z5222, respectively, are parts of fimbrial operons located between the conserved genes *glmS* and *pstS*. By using primers corresponding to *glmS* and *pstS*, a 5,842-bp DNA fragment was amplified from strain χ7122 genomic DNA and cloned (Fig. 1A). In strain χ7122, a region spanning 5,423 bp and containing four open reading frames (ORFs) that comprise a fimbrial gene cluster is inserted

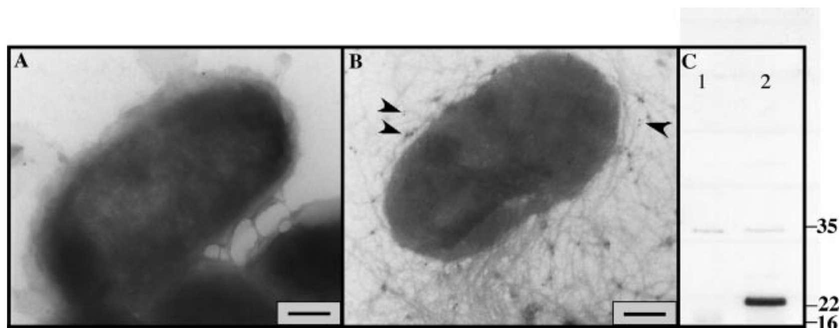


FIG. 2. (A and B) Immunodetection of Stg fimbriae and immunogold labeling with anti-StgA antiserum and observation by transmission electron microscopy. Bars = 200 nm. Strain ORN172(pIJ39) was grown overnight on TSA plates at 37°C without arabinose (A) or with 0.05% arabinose (B). Arrows indicate some of the StgA-labeled gold particles associated with fimbrial structures. (C) Immunoblot of fimbrial extracts obtained from ORN172(pIJ39) cells grown under the same conditions as those described above. Samples were migrated on an SDS-15% polyacrylamide gel electrophoresis gel and incubated with anti-StgA-specific antiserum. Lane 1, extract from cells grown without arabinose; lane 2, extract from cells grown with 0.05% arabinose. Molecular mass markers are indicated to the right. A band reacting with StgA-antiserum, at an apparent molecular mass of 23 kDa, was detected only in cells induced with arabinose.

1 nucleotide 3′ of the *glmS* stop codon, resulting in a replacement of a 205-bp span within the corresponding *glmS-pstS* intergenic region in *E. coli* K-12 strains. The *glmS-pstS* intergenic region from strain χ7122 exhibits a G+C content of 43%, which is considerably less than the average 50.8% G+C content of *E. coli* K-12 (8).

Thus far, a number of fimbrial gene clusters inserted into either the *glmS-pstS* region or the *yhjW-yhjX* intergenic region have been identified in different *E. coli* strains (13, 25, 37, 56) and all of the gene clusters encoding these fimbriae have been termed *lpf*, based largely on amino acid identity to LP fimbrial proteins of serovar Typhimurium. Sequencing of the *glmS-pstS* intergenic region from strain χ7122 led to the identification of a fimbrial gene cluster that shares high similarity to *lpf* gene clusters from *E. coli* O113:H21 (13) and *E. coli* 789 (25). Analyses of the current DNA database demonstrate that there are two main groups of putative or confirmed fimbrial systems present in either *E. coli* or *S. enterica* strains that demonstrate more similarity to either LP fimbriae of serovar Typhimurium or Stg fimbriae of serovar Typhi. This is exemplified by a phylogenetic tree based on Clustal analysis of predicted fimbrial gene products sharing the highest identity-similarity scores with APEC StgA (Fig. 1B). Phylograms for APEC StgB, StgC, and StgD and related gene products similarly resulted in the generation of the same two distinct groupings (data not shown). On the basis of a comparison of predicted gene products, the gene clusters located within the *glmS-pstS* intergenic region in *E. coli* strains are all more closely related to one another than to those inserted in the *yhjX-yhjW* intergenic region, which is the insertional location of the *lpf* gene cluster in serovar Typhimurium. On the basis of these results, we have termed the fimbrial gene cluster present in the *glmS-pstS* intergenic region of strain χ7122 *stg*, as the fimbrial gene products it encodes are orthologous to those of Stg fimbriae of serovar Typhi and are similarly encoded in the *glmS-pstS* intergenic region. The *stgABCD* gene cluster of strain χ7122 contains four ORFs (Fig. 1A). The predicted sizes of the APEC *stgABC* gene products are identical to those of the predicted products of the $lpf_{O113}$ and $lpf_{789}$ gene clusters, whereas the $lpfD_{O113}$ gene product is predicted to be 265 amino acids long (13) compared to a predicted 357-amino-acid precursor for the *stgD* and $lpfD_{789}$ gene products.

**Immunodetection of Stg fimbriae.** As the environmental conditions that are favorable for the expression of *stg* from its native promoter are unknown, we used the tightly regulated expression of *stg* genes from the arabinose-inducible promoter of plasmid pBAD18-Cm to induce the expression of Stg fimbriae. *stg* genes were cloned into plasmid pBAD18-Cm, generating plasmid pIJ39, and transformed into the fimbria-negative *E. coli* K-12 strain ORN172. Strain ORN172(pIJ39) was grown overnight at 37°C on TSA plates containing 0.05% arabinose, and cells were then analyzed by TEM following immunogold labeling with α-StgA antiserum. In the absence of arabinose, no fimbriae were visualized and very few gold particles were observed (Fig. 2A). When ORN172(pIJ39) was grown in the presence of arabinose, peritrichous filamentous structures were observed and immunogold labeling was localized primarily to these structures (Fig. 2B). Fimbrial extracts of strain ORN172(pIJ39), grown in the presence or absence of arabinose, were examined by Western blotting to confirm the specificity of StgA detection. Following Western blotting of the extracts, a specific band, with an apparent molecular mass of 23 kDa reacting with the α-StgA antiserum, was present in the extract of strain ORN172(pIJ39) grown in the presence of arabinose. Liquid chromatography-mass spectrophotometry analysis of the trypsin-treated product that reacted specifically with α-StgA antiserum confirmed that this band corresponded to the predicted StgA protein (data not shown). The StgA-specific band was absent when ORN172(pIJ39) was grown without arabinose (Fig. 2C). In addition, no fimbriae or StgA-specific bands were observed with either immunogold TEM or Western blotting of fimbrial extracts of the vector control strain ORN172(pBAD18-Cm) in either the absence or presence of arabinose (not shown).

**Transcription of the *stg* operon is regulated by carbon source and iron.** To investigate the regulation of *stg* gene expression in the native APEC strain, a *stg*::*lacZ* fusion was inserted into the chromosome of strain QT51, a Δ*lacZYA* derivative of strain χ7122, generating strain QT891. Strain QT891 was used to determine the influence of a number of

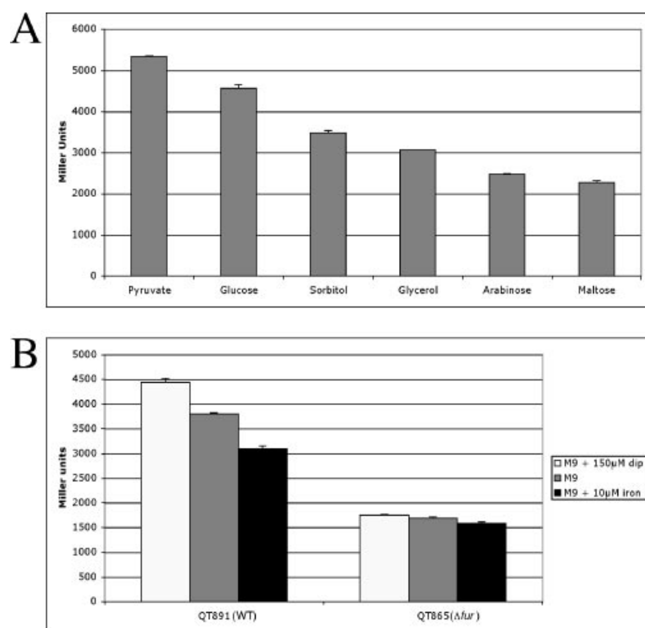


FIG. 3. (A) β-Galactosidase activity expressed from the P*stgA*::*lacZ* fusion in APEC strain QT891 grown overnight at 37°C on M9 minimal medium plates supplemented with different carbohydrates or other carbon sources. (B) Effect of iron on β-galactosidase activity expressed from the P*stgA*::*lacZ* fusion in APEC strain QT891 and isogenic Δ*fur* strain QT865. M9, M9-glucose. Error bars indicate standard deviations.

carbon sources on *stg* expression. The expression of the promoter fusion following overnight growth on M9 plates with pyruvate, glucose, sorbitol, glycerol, arabinose, or maltose as a carbon source is presented (Fig. 3A). β-Galactosidase was expressed at higher levels in M9 medium with either pyruvate or glucose as the carbon source (Fig. 3A). Among carbon sources tested, maltose and arabinose gave the lowest expression values. Promoter expression in strain QT891 was 1.17-fold higher in M9 medium with pyruvate, the carbohydrate with the highest promoter expression, than in M9 medium with glucose (M9-glucose). The lowest promoter expression was in M9 medium containing maltose, in which the mean expression was 0.43 times that observed when cells were grown in M9-pyruvate ($P < 0.0001$). In M9 medium containing sorbitol, glycerol, or arabinose, promoter expression levels were intermediate. Of the several other carbohydrates that were tested, ribose, xylose, mannose, acetate, succinate, and mannitol showed intermediate expression levels similar to that of sorbitol (results not shown).

In order to determine whether iron availability or Fur, the global iron-dependent regulator of *E. coli*, had a regulatory effect on the *stg* promoter in an APEC strain, a P*stg-lacZ* single-copy integrant of strain QT826 was constructed and termed QT865. The effects of various iron concentrations on β-galactosidase expression by strains QT891 and QT865 were then assessed. As shown in Fig. 3B, there was a significant decrease in β-galactosidase expression in strain QT891 with increasing amounts of iron ($P < 0.005$). Specifically, the expression decreased by 14% from M9-glucose containing 150 µM dipyridyl (M9-dipyridyl) to M9-glucose alone. A further decrease, 30% lower than that in M9-dipyridyl, was observed in M9 supplemented with 10 µM iron. Compared to that for strain QT891, *stg* promoter expression under all conditions was decreased for the isogenic Δ*fur* derivative QT865 ($P < 0.0001$) (Fig. 3B). For M9-dipyridyl, expression was 2.5-fold higher in QT891 than in QT865. Furthermore, in M9-glucose, promoter expression was 2.2-fold higher in QT891, and similarly, in M9 medium with 10 µM of added iron, expression was 1.96-fold higher. In addition, iron availability also had a less marked effect on β-galactosidase expression levels in the Δ*fur* strain QT865 ($P < 0.02$). These results indicate that increased iron concentrations decreased P*stgA* activity in the APEC wild-type strain background and that Fur appears to contribute to increased expression of *stg* regardless of iron availability in the medium.

**Distribution of *stgC* among the ECOR collection, *E. coli* isolates from urinary tract infections, and avian *E. coli*.** To investigate whether the *stg* gene cluster demonstrates a phylogenetic distribution among the ECOR collection, PCR analysis was performed using the stgC-F and stgC-R primers, which are specific for a 208-bp fragment of *stg*C (Fig. 1A). Among the 72 members of the ECOR collection, which were arranged into five phylogenetic groups (A, B1, B2, D, and E) on the basis of multilocus enzyme electrophoresis (39), the presence of *stg* sequences was significantly associated with phylogenetic groups B1 and D relative to the other groups ($P < 0.001$) (Table 2). *stg* sequences were present in all of the strains belonging to group B1 and 75% of the strains belonging to group D, whereas only 8% of group A strains and none of the B2 or E group strains contained *stg* sequences.

To complement the ECOR strains, 91 ExPEC isolates from human disease were also investigated for the presence of *stg* sequences. The ExPEC isolates tested belonged to phylogenetic group A, B1, B2, or D, as determined either by MLEE or by a triplex PCR method (10, 32). Analysis demonstrated that, as with the ECOR collection, *stg* sequences were highly associated with ExPEC isolates belonging to phylogenetic groups B1 and D relative to groups A and B2 ($P < 0.001$). No *stgC*-positive isolates belonging to phylogenetic group B2 were iden-

TABLE 2. Distribution of *stg*-positive strains within phylogenetic groups among the ECOR collection and ExPEC isolates

| Phylogenetic group | Value for *E. coli* from: | | | |
|---|---|---|---|---|
| | ECOR collection | | Human extraintestinal infections | |
| | Total no. of strains | No. of *stg*-positive strains[a] (%) | Total no. of isolates | No. of *stg*-positive isolates[a] (%) |
| A | 25 | 2 (8) | 15 | 7 (47) |
| B1 | 16 | 16 (100)[c] | 11 | 8 (72)[c] |
| B2 | 15 | 0 (0) | 24 | 0 (0) |
| D | 12 | 9 (75)[c] | 41 | 37 (90)[c] |
| E | 4 | 0 (0) | NA[b] | NA |
| Total | 72 | 27 (38) | 91 | 52 (57) |

[a] Strains positive by PCR amplification using the stgC-F and stgC-R primers.
[b] NA, not applicable.
[c] For both the ECOR collection and ExPEC isolates, *stg* sequences were significantly associated with phylogenetic groups B1 and D relative to groups A and B2 ($P < 0.001$).

TABLE 3. Distribution of *stg* among APEC and environmental isolates according to lethality class

| Lethality class[b] | Value for *E. coli* isolates from: | | | |
|---|---|---|---|---|
| | APEC | | Environment | |
| | Total no. of isolates | No. of *stg*-positive isolates[a] (%) | Total no. of isolates | No. of *stg*-positive isolates (%) |
| LC1[b] | 222 | 101 (46) | 1 | 1 (100) |
| LC2 | 38 | 18 (47) | 12 | 3 (25) |
| LC3 | 38 | 14 (37) | 19 | 4 (21) |
| Total | 298 | 133 (45)[c] | 32 | 8 (25) |

[a] Positive PCR amplification using the stgC-F and stgC-R primers.
[b] Lethality classes were defined as follows: LC1, 50% lethal dose of $<10^8$ CFU; LC2, 50% lethal dose of $\geq 10^8$ CFU; LC3, not lethal at $\geq 10^8$ CFU (16).
[c] *stg* sequences were significantly associated with APEC isolates relative to environmental isolates ($P < 0.05$).

tified in ExPEC (Table 2). As the *stg* fimbrial gene cluster was initially identified in the APEC O78 strain χ7122, the presence of *stg* sequences was also investigated in APEC isolates (Table 3). *stg* sequences were present in 133 of 298 (44.6%) of APEC isolates. *stg* was significantly associated ($P < 0.05$) with APEC isolates relative to those from the feces of healthy poultry, since only 8 of 32 (25%) of environmental isolates contained *stg* sequences (Table 3). There was no significant association with the presence of *stg* sequences and the lethality class of APEC or environmental isolates (Table 3). Among the most common serogroups associated with avian disease (O1, O2, and O78), *stg* was significantly associated with APEC isolates belonging to serogroup O78 (Table 4). That is, *stg* sequences were present in 90.7% of the APEC isolates belonging to serogroup O78, compared to 14.3% of serogroup O1 isolates, and none of strains belonging to serogroup O2 were *stgC* positive (Table 4). The determination of the phylogenetic grouping of APEC isolates by triplex PCR demonstrated that strains from serogroups O1 and O2 belonged to phylogenetic group B2, whereas O78 strains never belonged to this phylogenetic group. This finding correlates with the low prevalence of *stg* sequences observed in ECOR and ExPEC strains belonging to group B2 (Table 2).

For 25 of the *stgC*-positive strains, the conserved size and location of *stg* genes were assessed by PCR amplification of overlapping regions: the 5′ region using the glmS-F/stgC-R primers and the 3′ region using the stgC-F/pstS-R primers

TABLE 4. Association between the O serogroups of APEC strains and the presence of *stg* sequences

| Serogroup | No. of *stg*-positive strains[a] (%) | No. of strains tested |
|---|---|---|
| O1 | 4 (14.3) | 28 |
| O2 | 0 (0) | 46 |
| O78 | 78 (90.7)[c] | 86 |
| Other[b] | 12 (44.4)[c] | 27 |

[a] Positive PCR amplification using the stgC-F and stgC-R primers.
[b] Other serogroups included in this grouping are O6, O8, O11, O15, O18, O21, O22, O35, O45, O54, O55, O71, O83, O115, and O131.
[c] *stg* sequences were significantly associated with strains belonging to serogroup O78 or "other serogroups" relative to strains from serogroups O1 and O2 ($P < 0.001$).

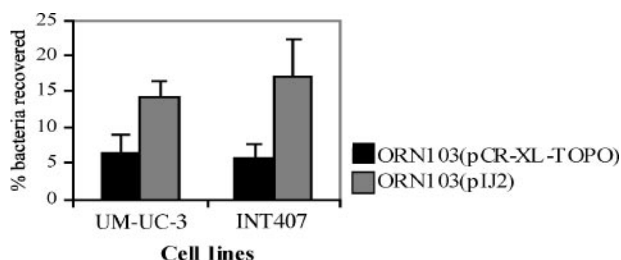


FIG. 4. Bacterial adherence assays using human UM-UC-3 kidney and INT407 intestinal epithelial cell lines. The percentage of the initial bacterial inoculum associated with epithelial cells after 90 min of incubation is indicated. Bacteria containing *stg* genes (pIJ2) were significantly more adherent ($P < 0.001$) to both cell lines than was the negative control strain ORN103(pCR-XL-TOPO). Error bars indicate standard deviations.

(Fig. 1A). Twenty-four of 25 *stgC*-positive isolates amplified DNA fragments of the same size as those from strain χ7122. These results suggest that the genomic location of *stg* at the *glmS-pstS* intergenic region is conserved and that most *E. coli stgC*-positive isolates are likely to contain complete full-length copies of *stg* gene clusters.

**Contribution of *stg* genes to adherence of *E. coli* to human epithelial cells and chicken respiratory tissues.** As *stg* genes were identified in 57% of the 91 ExPEC isolates tested from humans, we determined whether the *stg* gene cluster could confer adherence of *E. coli* to human bladder epithelial cells (UM-UC-3) and intestinal epithelial cells (INT407). Plasmid pIJ2 (*stgABCD*) was transformed into strain ORN103, and adherence assays were performed using ORN103(pCR-XL-TOPO) as a negative control. Strain ORN103 is an *E. coli* K-12 derivative that lacks type 1 fimbriae and does not produce any other known adhesins (43). ORN103(pIJ2) adhered to both epithelial cell lines to a significantly greater extent than did the negative control strain ORN103(pCR-XL-TOPO) (Fig. 4). Compared with control strain ORN103(pCR-XL-TOPO), ORN103(pIJ2) was at least twice as adherent to UM-UC-3 cells (14.2% of initial bacteria recovered versus 6.5% for ORN103; $P < 0.001$) and at least three times more adherent to INT407 cells (17.2% of initial bacteria recovered versus 5.6% for ORN103; $P < 0.001$). To examine the capacity of Stg fimbriae to mediate adherence to avian respiratory tissues, ORN103(pIJ2) and a control strain were tested for adherence to chicken lung sections in a qualitative assay. ORN103 cells containing *stg* demonstrated adherence in a clustered fashion to both lung and tracheal tissues, whereas the control strain adhered to a much lesser degree (Fig. 5). Although the adhered clusters of ORN103(pIJ2) cells were not uniformly distributed, the clustered adherence pattern was not observed for the control strain, with very few bacteria adhering to tissues. Adherence tests of wild-type strain χ7122 compared to that of Δ*stg* mutant strain QT302 to either human epithelial cells or chicken lung sections did not demonstrate any discernible differences in adherence, even when 0.5% mannose was added to inhibit adherence mediated by type 1 fimbriae (data not shown).

**Contribution of *stg* to colonization of chicken respiratory tissues in vivo.** To assess whether *stg* contributes to respiratory colonization of APEC strain χ7122, competitive coinfections

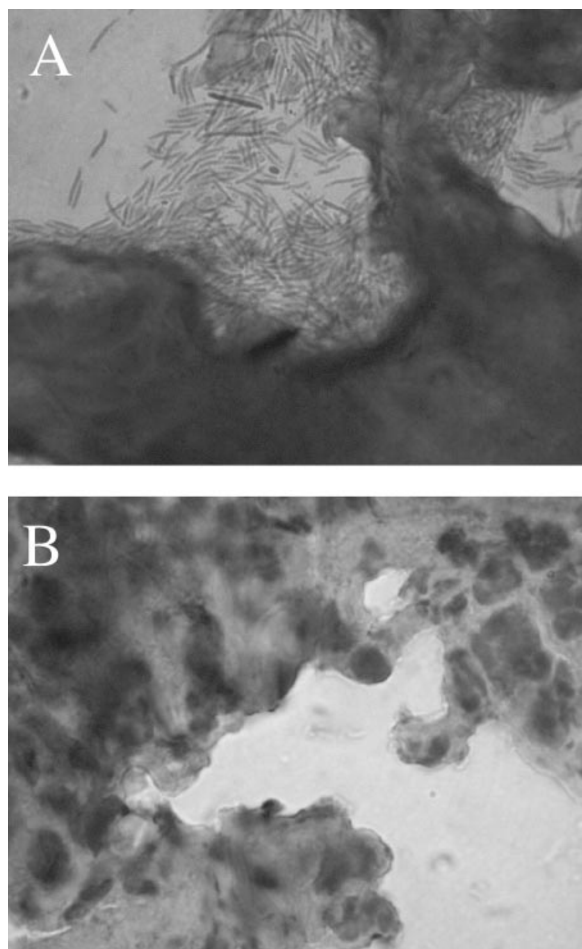


FIG. 5. Adherence of Stg-positive strain ORN103(pIJ2) (A) and Stg-negative control strain ORN103(pCR-XL-TOPO) (B) to chicken lung sections. Bacteria were grown on TSA, and 50 μl of a $5 \times 10^9$ bacterial suspension was added to the tissue sections. Slides were stained using a Diff-Quick stain set.

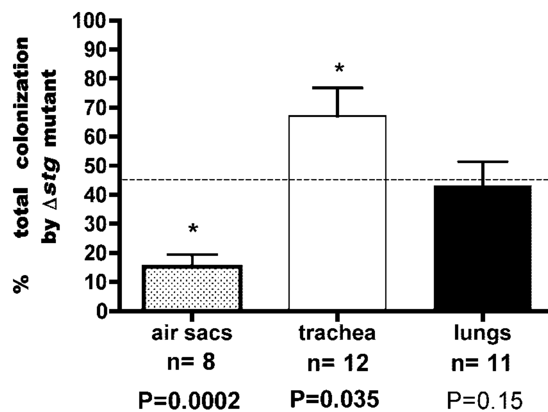


FIG. 6. Comparative colonization of respiratory tissues 6 days following coinfection of chickens with APEC strain χ7122 and isogenic Δ*stgABCD*::*kan* derivative QT302. Bars represent the mean proportion (as a percentage) of the Δ*stg* mutant compared to the total CFU of bacteria recovered. Error bars indicate standard errors of the means. Asterisks indicate significant differences in the proportions of Δ*stg* mutant and wild-type APEC χ7122 recovered from tissues compared to the input ratio. *P* values obtained using the Mann-Whitney test are indicated. The dashed line indicates the initial input ratio of the inoculum of the Δ*stg* mutant. *n* = number of tissue samples from which either strain could be recovered from 12 analyzed tissues.

with APEC strain χ7122 and its isogenic Δ*stgABCD* mutant derivative strain QT302 were tested in a chicken intratracheal infection model in 14 White Leghorn chickens (2 weeks old). Two of the 14 inoculated chicks died 4 days postinoculation and were not included in the experiment. Six days postinfection with *E. coli*, bacterial numbers in the trachea, air sacs, and lungs of chickens were determined. Figure 6 presents the mean proportion of the Δ*stgABCD* mutant relative to the total bacterial numbers recovered in each of the tissues of infected chickens. In the air sacs, the wild-type strain significantly outcompeted the Δ*stgABCD* mutant. By contrast, in the trachea, the Δ*stgABCD* mutant significantly outcompeted the wild-type parent. Levels of colonization in the lungs by the wild-type parent and the Δ*stgABCD* mutant were similar.

## DISCUSSION

Sequencing of the *glmS-pstS* intergenomic region of APEC strain χ7122 identified a four-gene operon that we have named the *E. coli* ortholog of *stg* from serovar Typhi (Fig. 1A). The *stg* gene cluster is most similar to other putative fimbrial operons in *E. coli* (O island 154) and serovar Typhi (*stg*) that are also located between *glmS* and *pstS* (Stg fimbrial group) (Fig. 1B). Database and alignment analyses demonstrate that APEC Stg fimbriae are more closely related to this distinct group of fimbriae than to other related fimbriae located in the *yhjW-yhjX* intergenic region, such as LP fimbriae of serovar Typhimurium (6) and LP fimbriae of *E. coli* (37, 56) (Fig. 1B).

The *stg* gene cluster from strain χ7122 is highly similar to fimbrial gene clusters that were named *lpf* in *E. coli* O113:H21, rabbit enteropathogenic *E. coli* O15:H7 (13), and *E. coli* 789 (25). The conserved locations of the *stg* and closely related gene clusters within a number of different *E. coli* strains and its absence from *E. coli* K-12 and pathogenic strains CFT073 and EDL933 prompted us to investigate whether this fimbrial gene cluster was phylogenetically distributed. Screening of the ECOR collection for *stg* sequences demonstrated that *stg* is associated with phylogenetic groups B1 and D and absent from phylogenetic group B2 (Table 2). The screening of a number of ExPEC clinical isolates similarly indicated the presence of *stgC* in most *E. coli* isolates from phylogenetic groups B1 and D, whereas it was absent from all B2 ExPEC isolates tested (Table 3). Further, the screening of APEC isolates demonstrated that *stg* is common among isolates of serogroup O78, which do not belong to phylogenetic group B2, but rare among those from serogroups O1 and O2, which commonly belong to group B2 (Table 4).

These results indicate that the *stg* gene cluster is phylogenetically distributed in *E. coli* and largely absent from *E. coli* strains belonging to phylogenetic group B2. Some molecular phylogeny studies suggest that the B2 group represents the most ancestral lineage of *E. coli* (18, 29). The *stg* gene cluster may have been acquired relatively recently by horizontal gene transfer since it exhibits an overall G+C content of 43%, which is considerably lower than the *E. coli* K-12 mean genomic DNA

G+C content of 50.8%. Further evidence supporting the probable horizontal acquisition of the *stg* gene cluster is its genomic location. The 3′ terminal end of the *glmS* gene and its terminator are a preferential insertion site for transposon Tn*7*, which has been termed *att*Tn*7* (21). Taken together, these results suggest that the acquisition of *stg* genes likely occurred after the branching of the B2 lineage and that *stg* genes may have been acquired by certain *E. coli* strains belonging to other lineages. In a similar way, the related *stg* and *lpf* operons may have been acquired or lost by certain clonal lineages or serovars of *Salmonella enterica* during its evolution (5, 57).

Several environmental factors, such as carbon source, aliphatic amino acids, iron, temperature, and electron acceptors other than oxygen, can regulate fimbrial expression (30). *lacZ* fusion assays using the *stgA* promoter indicate that this operon is transcribed in a wild-type strain background and that carbohydrate source can affect expression levels (Fig. 3A). The carbohydrate source is known to affect the expression of many bacterial genes, including those encoding fimbriae. Glucose may inhibit fimbrial expression by catabolite repression (30). However, following growth on M9 glucose medium, cells expressed the second highest level of *stg* promoter activity after pyruvate. Overall, expression results from the *stg-lacZ* fusions suggest that Stg fimbrial expression differed depending on the carbon source but was not greatly inhibited by any of the sources tested.

The *ecs-3* sequence corresponding to a fragment of the *stgC* gene was initially identified as an APEC-specific gene expressed in the pericardium of infected chickens (15). In systemic tissues such as the pericardium, it is likely that iron availability to the bacterial cells is limited due to iron sequestration by host ferroproteins. This is supported by the identification of aerobactin and salmochelin siderophore-encoding genes that were also expressed in the pericardial tissues of infected chickens (15). Siderophore-encoding genes are typically repressed in iron-replete conditions as their transcription is reduced by the Fur-$Fe^{+2}$ complex. The expression of the *stgA* promoter decreased as the amount of available iron in the medium increased, suggesting that *stg* expression is iron regulated (Fig. 3B). However, in the *fur* mutant strain, this decrease was much less striking, and overall promoter expression was about twofold lower in the *fur* mutant compared to that in the wild-type APEC strain. Fur acts mainly as a repressor of the *E. coli* iron acquisition genes under iron sufficiency, as the Fur-$Fe^{+2}$ complex actively binds to regulatory regions (known as Fur boxes) to inhibit the transcription of these genes. Analysis of the region 5′ of *stg* demonstrated a number of putative Fur-binding sites; however, matches to Fur consensus sequences were low. In addition, by using a Fur titration assay, the *stg* promoter encoded on a high-copy plasmid did not deregulate Fur-mediated repression of an iron-regulated *iucC*::*lacZ* fusion under iron-replete conditions, suggesting that Fur is unlikely to interact with the *stg* promoter (data not shown). Several genes are positively regulated by Fur and apparently do not possess a Fur box (1, 33). Using transcriptional profiling, McHugh et al. (34) recently identified 101 genes in *E. coli* K-12 that are regulated by the $Fe^{2+}$-Fur complex. Forty-eight of these genes were induced by Fur (34). Hence, in some cases and as we observed with *stg*, Fur may enhance the expression of certain genes. However, the involvement of Fur may be indirect and implicate other regulators (1).

The introduction of the *stg* genes into a nonfimbriated *E. coli* K-12 strain conferred the production of fimbrial structures. Stg fimbriae were peritrichously distributed, and anti-StgA antibodies were associated with fimbrial structures following immunogold labeling and electron microscopy. In addition, the antiserum specifically reacted with a band corresponding to the major fimbrial subunit following Western blotting (Fig. 2). Stg fimbriae and other fimbriae belonging to this group of adhesins mediate bacterial attachment to a number of cell/tissue types. The expression of Stg fimbriae increased the adherence of nonfimbriated *E. coli* K-12 cells to both human epithelial cell lines and avian respiratory tissues. Specifically, Stg fimbriae mediated increased adherence to human bladder (UM-UC-3) and intestinal (INT407) cell lines. Similarly, in nonfimbriated *E. coli* K-12 strains, $lpf_{O113}$ conferred increased adherence to CHO-K1 cells (13) and *lpf* of *E. coli* EDL933 conferred increased adherence to HeLa and MDBK cells (56). Moreover, LP fimbriae of *E. coli* EDL933 demonstrated a clustered adherence pattern to cell lines. A similar clustered adherence pattern to INT407 or UM-UC-3 cells as well as chicken lung sections was observed from *E. coli* K-12 cells expressing APEC Stg fimbriae. In seeming contrast, for in vitro adherence assays, we observed no difference in the adherence of the wild-type APEC strain compared to that of its isogenic Δ*stg* mutant to epithelial cells or chicken respiratory tissues. However, this actually is not surprising, as APEC strain χ7122 produces other known adhesins (e.g., type 1 fimbriae, Tsh, and curli) that may mediate adherence to cells. Similarly, Bäumler et al. (7) demonstrated that LP fimbriae of serovar Typhimurium mediated the adherence and invasion of Hep-2 cells but were not essential for adhesion to T-84, INT407, or HeLa cell lines. However, the *lpf* operon of serovar Typhimurium was also shown to be involved in the colonization of murine ileal Peyer's patches of mice in vivo (7). Recently, a direct role for $Lpf_{789}$ in the adherence of *E. coli* strain 789 to human kidney epithelial cells in vitro was demonstrated (25).

A number of adhesins that mediate adherence of APEC to avian tissues or cells have been identified. These include type 1 fimbriae (12, 27), P fimbriae (26, 47), AC/I fimbriae (3, 61), curli (9, 20), and Tsh (16, 48). Although several adherence factors were identified in different APEC strains, none were demonstrated to be essential for the initial stages of infection in chickens (2, 15, 27, 31). Other adhesins may therefore be involved in the initial stages of colonization of the chicken respiratory tract. Evidence that Stg fimbriae contribute to adherence in avian respiratory tissues was obtained by performing adhesion assays on chicken lung tissue sections using *E. coli* strain ORN103(pIJ2). The strain harboring the *stg* operon adhered to chicken lung tissues to a greater extent than did the control strain (Fig. 5). The observation that Stg fimbriae mediated adherence of nonfimbriated *E. coli* K-12 to avian frozen tissue sections suggested a possible role for this adhesin in the colonization of the avian respiratory tract by certain APEC strains. Coinfection studies with the APEC wild-type strain and a Δ*stgABCD* derivative, QT302, demonstrated that the loss of Stg fimbriae resulted in a decreased capacity to colonize the air sacs (Fig. 6). These results are the first obtained that demonstrate an individual role for an adhesin that promotes the

colonization of APEC to avian respiratory tissues. Further, since the air sacs are an initial port of entry for APEC and are commonly colonized by APEC during infection, Stg fimbriae, in concert with other adhesins, such as curli, type 1 fimbriae, or Tsh, may contribute to initial steps of APEC pathogenesis. By contrast, the loss of Stg fimbriae resulted in an improved capacity to colonize the trachea (Fig. 6), which is analogous to the enhanced tracheal colonization ability associated with the loss of the type 1 adhesin by APEC O2 strain MT78 (2). It is not immediately clear how the inactivation of a fimbrial adhesin can result in improved colonization of certain tissues. As has been noted for type 1 fimbriae, it is possible that the expression of a fimbrial adhesin in certain tissues may be a disadvantage by promoting adherence to mucus glycoproteins or specific receptors on phagocytic cells, which could result in increased bacterial clearance or killing (40, 47). In addition, it is possible that the inactivation of one fimbrial adhesin may result in greater expression of another adhesin, which can result in increased adherence to cells or tissues. The expression and phase variation of different adhesins involves cross talk between fimbrial systems, and recent evidence with ExPEC strains causing urinary tract infections demonstrated that the inactivation or constitutive expression of a fimbrial system can alter the expression of other adhesins (54). In human ExPEC strains, type 1 and P fimbriae have been shown to contribute to virulence in urinary tract infection models (4, 50). Since *stg* was associated with certain ExPEC and APEC strains and Stg fimbriae mediated adherence to both avian tissues and bladder epithelial cells, it will be of interest to investigate the contribution of Stg fimbriae in concert with other known adhesins to the pathogenesis of APEC as well as ExPEC associated with urinary tract infections.

## ACKNOWLEDGMENTS

Kind thanks to K. Hantke, P. Orndorff, B. Wanner, and J. M. Fairbrother for the gifts of strains or plasmids.

Funding for this project was provided by Natural Sciences and Engineering Research Council of Canada (NSERC) individual research grants to C.M.D. and F.D., the Office of Research and Development, Medical Research Service, Department of Veterans Affairs, and National Research Initiative (NRI) Competitive Grants Program/United States Department of Agriculture grant 00-35212-9408 (J.R.J.). M.H.L. was the recipient of a Fondation Armand-Frappier Scholarship, and S.L. received a summer studentship from NSERC.

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