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A *selC*-Associated Genomic Island of the Extraintestinal Avian Pathogenic *Escherichia coli* Strain BEN2908 Is Involved in Carbohydrate Uptake and Virulence

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The complete nucleotide sequence and genetic organization of a new genomic island (AGI-3) isolated from the extraintestinal avian pathogenic *Escherichia coli* strain BEN2908 is reported. This 49,600-bp island is inserted at the *selC* locus and contains putative mobile genetic elements such as a phage-related integrase gene, transposase genes, and direct repeats. AGI-3 shows a mosaic structure of five modules. Some of these modules are present in other *E. coli* strains and in other pathogenic bacterial species. The gene cluster *aec-35* to *aec-37* of module 1 encodes proteins associated with carbohydrates assimilation such as a major facilitator superfamily transporter (Aec-36), a glycosidase (Aec-37), and a putative transcriptional regulator of the LacI family (Aec-35). The *aec-35* to *aec-37* cluster was found in 11.6% of the tested pathogenic and nonpathogenic *E. coli* strains. When present, the *aec-35* to *aec-37* cluster is strongly associated with the *selC* locus (97%). Deletion of the *aec-35–aec-37* region affects the assimilation of seven carbohydrates, decreases the growth rate of the strain in minimal medium containing galacturonate or trehalose, and attenuates the virulence of *E. coli* BEN2908 for chickens.

Escherichia coli, a commensal inhabitant of the gastrointestinal tract of mammals and birds, is also the causative agent of several diseases in animals and human worldwide. Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection they are causing. ExPEC strains are responsible for a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (35, 54).

Avian pathogenic *Escherichia coli* (APEC) strains belong to the ExPEC group. They are mainly responsible for a respiratory disease in poultry usually followed by a systemic infection and a fatal septicemia. Characteristic fibrinopurulent lesions are aerosacculitis, pericarditis, and perihepatitis. APEC strains can also be involved in localized infections such as omphalitis, salpingitis, swollen head syndrome, and cellulitis (2, 17). APEC isolates commonly belong to serogroups O1, O2, O5, O8, O18, O35, and O78 (4, 17). Various virulence factors of APEC strains, such as adhesins (F1 and P fimbriae and curli), anti-host defense factors (OmpA, Iss, lipopolysaccharide, and K1), iron acquisition systems (aerobactin, Iro proteins, yersiniabactin, and the Sit iron acquisition locus), autotransporters (Tsh and Vat), and the IbeA protein have been identified (20, 21, 25, 36–38, 42, 46, 50). Using various genomic approaches, several putative virulence factors of APEC strains have been identified (9, 20, 38, 58). However, the above components cannot explain all the disease process, suggesting the existence of other, unidentified components.

It is well described that pathogenicity factors can be encoded by mobile genetic elements (transposons, phages, plasmids, integrons, and genomic islands) which are capable of horizontal gene transfer. Genomic islands (GEIs) are clusters of chromosomal genes that are often associated with tRNA genes (28). GEIs often possess genes or cryptic pseudogenes coding for mobility-related elements such as phage genes, insertion sequence elements, transposases, and origins of replication (28). They have a modular organization and carry gene clusters that encode a wide range of functions that contribute to the adaptation of bacteria to the environment and/or to the development of symbiosis, antibiotic resistance, or virulence. In the last case, GEIs are designated pathogenicity islands (PAIs).

GEIs can be unstable due to homologous recombination between their flanking direct repeats. These direct repeats are often homologous to phage attachment sites. The *selC* tRNA locus has been shown to be a hot spot for integration of foreign DNA, and particularly of PAIs: PAI-1 in the uropathogenic *E. coli* strain 536, the locus of enterocyte effacement (LEE) in enteropathogenic and enterohemorrhagic *E. coli* strains, the toxigenic invasion locus A in the enterotoxigenic *E. coli* strain H10407, the locus of proteolysis activity in the Shiga toxin-producing *E. coli* strain 4797/97, SPI-3 in *Salmonella enterica*, and SHI-2 in *Shigella flexneri* (5, 7, 23, 24, 44, 57). The *selC* locus is also the attachment site for the *E. coli* retronphage ΦR73 (61).

As *selC* is a hot spot for insertion of PAIs, we searched for the presence of foreign DNA inserted at the *selC* locus of the avian ExPEC strain BEN2908. We thus identified and characterized a new genomic island containing genes involved in carbohydrate uptake and virulence.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The main bacterial strains and plasmids used in this study are described in Table 1. *E. coli* strain BEN2908, O2:K1:H5 (*fim*$^+$ *iut*$^+$ *ibeA*$^+$), is a nalidixic acid-resistant derivative of strain MT78 that was isolated from the trachea of a chicken with a respiratory infection

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TABLE 1. Relevant strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
E. coli strains		
BEN2908	Pathogenic avian isolate O2:K1:H5 *fim*$^+$ *iut*$^+$ *ibeA*$^+$ Nal^r	16
BEN2269	Nonpathogenic avian isolate, O2:K1$^-$:H$^-$, formerly EC79	16
BEN2929	BEN2908 Δ(*aec-35-36-37*)::Kan^r	This study
TOP10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*Δ*M15* Δ*lacX74 recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL* (Str^r) *endA1 nupG*	Invitrogen
Plasmids		
pKD4	Plasmid carrying a kanamycin resistance cassette and oriRγ origin	14
pKD46	Red recombinase expression plasmid, temperature-conditional replicon, Amp^r	14
pCR2.1	TA cloning vector, Kan^r Amp^r, high copy number	Invitrogen
pBEN182	pCR2.1 containing the *aec-35* to *aec-37* genes of AGI-3	This study

(16, 25). *E. coli* strain BEN2269 was isolated from the intestine of a healthy chicken and is nonpathogenic (16). For prevalence analysis a total of 285 *E. coli* strains were also used, including 240 avian isolates (205 pathogenic and 35 nonpathogenic), 37 strains isolated from humans with extraintestinal diseases, 7 isolates from animals (other than poultry) with extraintestinal syndrome, and the nonpathogenic K-12 MG1655 strain as a negative control.

Strains were grown in Luria-Bertani broth (LB) at 37°C. When necessary, ampicillin at 100 μg ml^{-1}, kanamycin at 50 μg ml^{-1} or nalidixic acid at 30 μg ml^{-1} were added. For growth rate experiments, overnight LB cultures were centrifuged, washed twice, and resuspended in the same volume in a minimal medium (100 mM NaCl, 30 mM triethanolamine HCP [pH 7.5], 5.0 mM NH_4Cl, 2.0 mM NaH_2PO_4, 0.25 mM Na_2SO_4, 0.05 mM $MgCl_2$, 1.0 mM KCl) (66). The strains were then cultured in triplicate at 37°C in 100-well, sterile, covered microplates (Labsystems, Helsinki, Finland). Each well contained 300 μl of the above minimal medium supplemented with 5 mM Na-D-galacturonate or 5 mM trehalose and 3 μl of the bacterial suspension. The plates were incubated in a Microbiology Reader Bioscreen C apparatus (Labsystems, Helsinki, Finland) and the optical density was measured at 450 nm every 15 min, after shaking. The generation time was calculated as described by Miller (43).

DNA preparation. Plasmids and cosmids were purified using Nucleobond AX100 or AX500 columns (Macherey-Nagel, Düren, Germany). Genomic DNA was prepared using a NucleoSpin tissue kit from Macherey-Nagel (Düren, Germany) or by the boiling method (56).

PCR and primers. The primers used in this study are listed in Table 2. PCRs were performed in 25 μl containing 500 nM of the forward and reverse primers, 200 μM of each deoxynucleoside triphosphate (Finzyme, Ozyme France), 1 U of *Taq* DNA polymerase (New England Biolabs Inc.), and 2 mM $MgCl_2$ in a PCR buffer containing 1 mM KCl, 1 mM $(NH_4)_2SO_4$, 200 μM $MgSO_4$, 0.1% Triton X-100, 2 mM Tris-HCl, pH 8.8 (New England Biolabs Inc.). The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min/kb. A final extension step at 72°C for 7 min was included. Reactions were performed in a Perking Elmer thermocycler (GeneAmp 9700, Applied Biosystems).

DNA sequencing and sequence analysis. Cosmid clones were sequenced by primer walking. Nucleotide sequencing was performed by Genome Express (Meylan, France). Sequences were assembled and analyzed using the VectorNTI package (InforMax, Inc., Bethesda, Md.). Coding sequences were predicted using GeneMark (39). Homology searches were performed by using the BLAST server from the National Center for Biotechnology Information.

Construction of the mutant strain BEN2929 (BEN2908Δ*aec-35-37*::Kan^r) and plasmid pBEN182. The *aec-35* to *aec-37* chromosomal DNA region was deleted by recombination with the PCR product as described by Datsenko and Wanner (14). Briefly, the method of Tung et al. was used to electroporate *E. coli* BEN2908 cells with pKD46, a temperature-sensitive plasmid carrying the Red recombinase system from the λ bacteriophage under the control of the arabinose-inducible P_{BAD} promoter (14, 62). The Red recombinase system allowed the replacement of the *aec-35* to *aec-37* DNA region by a kanamycin resistance cassette obtained by amplifying pKD4 template plasmid using primers cat75 and cat76 (Table 2). The 5′ ends of primers cat75 and cat76 are homologous to the 5′ and 3′ ends of the *aec-35–aec-37* region, respectively. The amplified product was then inserted into *E. coli* BEN 2908 carrying pKD46 by electroporation and a kanamycin-resistant and ampicillin-sensitive clone was isolated. The replacement of the *aec-35–aec-37* gene cluster was confirmed by PCR using primers cat47 and cat82 (flanking the deleted region) (Table 2) and by Southern blot with a kanamycin probe generated by PCR amplification of pKD4 using primers cat51 and cat52 (Table 2). The mutant was named BEN2929 and stored at −70°C.

Cloning of the *aec-35* to *aec-37* DNA region was carried out after amplification of this region from BEN2908 chromosomal DNA using primers cat75′ and cat76′ (Table 2). The amplified region was cloned into pCR2.1-TOPO (Invitrogen), leading to plasmid pBEN182. The construction was verified by sequencing the inserted region.

Hybridization. For Southern blot hybridization, DNA restriction fragments generated by BlnI digestion were submitted to electrophoresis and transferred to a Hybond-N+ membrane (Amersham Pharmacia Biotech). The 1.5-kb fragment carrying the kanamycin resistance gene obtained by PCR amplification from plasmid pKD4 using primers cat51 and cat52 (Table 2) was labeled by using enhanced chemiluminescence with the ECL RPN3000 kit (Amersham Pharma-

TABLE 2. Oligonucleotide primers used for PCR

Primer	Sequence[a]	Reference
K260	GAGCGAATATTCCGATATCTGGTT	41
K261	CCTGCAAATAAACACGGCGCAT	41
K255	GGTTGAGTCGATTGATCTCTGG	41
CA8R	CCACCTTCACCGGCATAAC	This study
CA8F	CATCGGTGATACCTGTCGCC	This study
cat75	ACATTACGCTTATTTTCCCTTGACCGG AAGAATCAGAGGCTGTGGTTTCAgtgt aggctggagctgcttc	This study
cat76	ACTCTTACAGTGCCTTTATTTGGCCAT GATTTACCCTCTACAATAAATTAcata tgaatatcctccttag	This study
cat75′	ACATTACGCTTATTTTCCCTTGACCGG AAGAATCAGAGGCTGTGGTTTCA	This study
cat76′	ACTCTTACAGTGCCTTTATTTGGCCAT GATTTACCCTCTACAATAAATTA	This study
cat47	CTTTGAAGTGCACGATAACC	This study
cat82	GGTGGAGCTGCATGACAAGG	This study
cat51	GTGTAGGCTGGAGCTGCTTC	This study
cat52	CATATGAATATCCTCCTTAG	This study
IC79	AATCGGAGTTGGCTCTTTCC	This study
IC36	CGTGCCCGCGAGAAAGTATTGG	This study
IC39	CATCCGGTGGGGTGATTATGAGTCA	This study
IC64	AAGCCTATTTTGTCAGCAGTAACGCCA	This study
IC45	GGATAATGCTGGTCAGTGGCAGGAA	This study
IC50	GCCCTGCTTCCACGACACTTGC	This study
IC37	TGCGATAATGCCTTGCTGATGCTTT	This study
IC60	TGTCGCAGAAGCCTCAGCACCTC	This study
IC43	GGTTCAGCCGCATGGATTGCC	This study
IC44	CAAGTTCCTGCCACTGACCAGCATT	This study
IC80	CAAGTGGGGACTTCATTGCT	This study
SelC1	GCGTGTATTAGGCGGAAAAAAC	This study

[a] For gene inactivation, sequences complementary to the kanamycin resistance cassette are in lowercase letters.

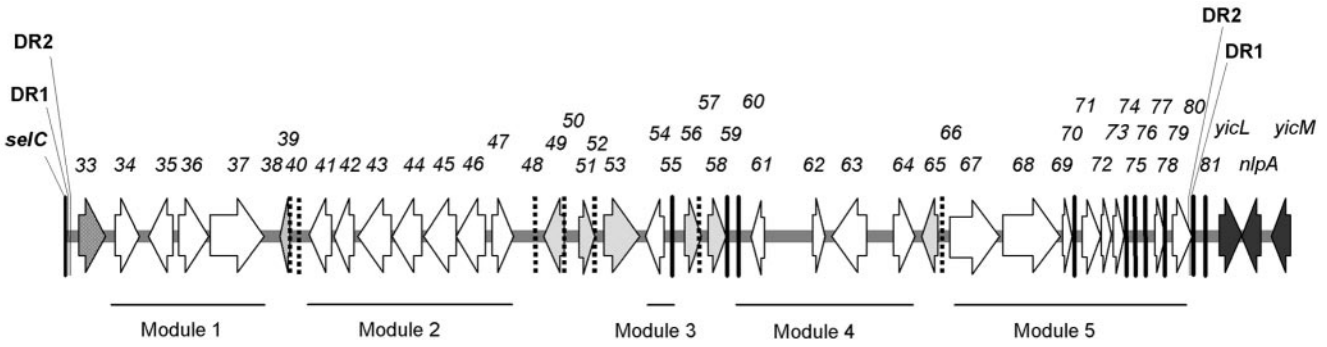

FIG. 1. Genetic organization of the AGI-3 genomic island. Open reading frames larger than 360 bp are indicated as dark grey arrows for core genome genes, as a grey arrow for the phage-like integrase gene *aec-33*, as light grey arrows for mobility genes, and as white arrows for genomic island-associated genes. ORFs smaller than 360 bp (120 amino acids) are indicated in bold black lines or in dotted black lines (mobility genes). ORFs are labeled consecutively from 33 to 81 and correspond to *aec-33* to *aec-81*. DR, direct repeat.

cia Biotech) according to the manufacturer's protocol. Hybridization, washes, and detection were performed as instructed by the kit.

Experimental colibacillosis. An in vivo virulence assay was conducted as described previously with some modifications (25). Briefly, 25-day-old White Leghorn specific-pathogen-free chickens (line PA12) from the Institut National de la Recherche Agronomique experimental farm were inoculated in the right thoracic air sac with a 0.1-ml suspension containing 5×10^6 CFU. The inocula were prepared from bacteria cultivated in LB at 37°C with agitation to an optical density at 600 nm of 0.6.

Groups of 20 and 19 animals were inoculated with strain BEN2908 and mutant strain BEN2929, respectively. One control group of three animals was inoculated with the avian nonpathogenic strain BEN2269. Blood samples were collected 24 and 48 h postinoculation and serial dilutions were plated onto Drigalski agar plates for bacterial quantification. Animals were euthanized 48 h postinoculation by injection of Nesdonal (Rhône-Mérieux, Lyon, France), and necropsied. A piece of liver was collected and after homogenization in saline, serial dilutions were plated onto Drigalski agar plates supplemented when needed with nalidixic acid (30 μg ml^{-1}) or kanamycin (50 μg ml^{-1}). Macroscopic fibrinous lesions were observed and scored (air sacs, 0 to 4; heart, 0 to 2; and liver, 0 to 2) as previously described (42).

Bacterial growth assay in 90% normal chicken serum. Blood from 2-week-old specific-pathogen-free chickens was collected and allowed to clot for 1 h at room temperature. Sera were then pooled and stored at 4°C until used. Fifty milliliters of LB was inoculated with 100 μl of bacteria that had been grown for 24 h in LB and then incubated at 37°C with agitation to an optical density at 600 nm of 0.3. After centrifugation, the bacterial pellet was washed twice and resuspended in 1 ml of sterile Tris buffer (50 mM Tris-HCl, 2 mM $MgCl_2$, 0.4 mM $CaCl_2$, 40 mM NaCl, pH 8.4); 100 μl of this bacterial inoculum was incubated at 37°C in 900 μl of fresh serum. Bacterial counts at 0, 1, 2, and 3 h were obtained by plating 10-fold serial dilutions onto LB agar plates. Slide agglutination did not reveal the presence of antibodies specifically directed against O2 *E. coli* in the serum used.

Phenotype microarray tests. The effect of the *aec-35–aec-37* gene cluster on the carbon metabolism of strain BEN2908 was tested in Phenotype microarray plates (Biolog Inc., Hayward, California) (8). These are 96-well microtiter plates containing different carbohydrates dried on the bottom of each well. When a carbohydrate is assimilated, it allows the respiration of the bacteria and the electrons produced are transferred to an indicator dye (tetrazolium violet), resulting in a purple color. Cells were streaked onto LB agar plates supplemented with the appropriate antibiotic (nalidixic acid, kanamycin, or ampicillin) and grown overnight at 37°C. Individual colonies were then picked up from the surface of the plates using a cotton swab and resuspended (at an optical density at 450 nm of 0.08) in the minimal medium containing the indicator dye provided by the supplier (IF0 inoculating fluid). The suspensions were then distributed (100 μl per well) into PM1 and PM2 microplates containing a panel of 190 different carbon sources. The plates were incubated aerobically at 37°C for 24 h and the reduction of the tetrazolium dye was then quantified by measuring the optical density at 590 nm (enzyme-linked immunosorbent assay reader Multiskan Ascent).

Statistical analysis. Statistical analysis of the data from the in vivo virulence assay was done by applying a Mann-Whitney test. Values were arranged in increasing numbers, dead animals being given the highest rank. Exact *P* values were calculated with the StatXact software (version 5.0; Cytel Software, Cambridge, MA) and $P < 0.05$ was considered significant. The prevalence data were analyzed by using the chi-square test. Exact *P* values were calculated with the software StatXact and $P < 0.05$ was considered significant. The phenotype microarray data were analyzed online (http://home.clara.net/sisa/*t* test.htm) by using Student's *t* test for statistical significance.

Nucleotide sequence accession number. The AGI-3 nucleotide sequence has been assigned GenBank accession no. AY857617.

RESULTS

Identification and sequencing of a genomic island inserted at the *selC* locus of the APEC strain BEN2908. To search for the presence of foreign DNA inserted at the *selC* locus of the avian ExPEC strain BEN2908, we first checked the integrity of the locus. For that aim we tried to amplify the *selC* locus (527 bp in strain MG1655) with primers K260 and K261 (Table 2) located upstream and downstream of *selC*, respectively, in *E. coli* strain MG1655 (41). No PCR product was obtained with strain BEN2908, suggesting the insertion of foreign DNA at the *selC* locus.

As the regions flanking PAIs inserted at *selC* are relatively well conserved, we then used the K255 primer (matching the left end of the locus of enterocyte effacement) and the K260 primer (located upstream of the *selC* locus) to screen by PCR a previously made genomic cosmid library of the strain (Table 2) (40). One of the cosmids (CA8), which yielded a PCR product of 418 bp, was sequenced by primer walking. As the insert in CA8 did not cover the entire DNA region inserted at *selC*, the cosmid library was screened again by PCR with primers CA8R and CA8F matching the 216-bp end of the insert of CA8 (Table 2). Cosmid DG6, which yielded the predicted PCR product of 216 bp, was identified. After sequencing of the DG6 insert by primer walking, the right end of the DNA region inserted at the *selC* locus was identified.

The DNA region inserted at *selC* was named AGI-3 for *A*PEC *G*enomic *I*sland 3. AGI-3 is inserted at the 3′ end of *selC* (Fig. 1). By comparison with the genome of the nonpathogenic *E. coli* strain K-12 MG1655, the insertion leads to a deletion of 1,903 bp comprising the *yicK* gene (6). AGI-3 is 49,600 bp long and contains two direct repeats: DR1 of 16 bp, TTCGACTCCTGTGATC, and DR2 of 21 bp,TTTGGGGGT (A/T)CTT(T/A)(A/T)GGGGGT. One DR1 is located at one

TABLE 3. Genetic features of the identified AGI-3 open reading frames

ORF	G+C content (%)	Size (aa)	Position[a] (bp)	Similarities with representative sequence in database (Blastp E value)[b]	Accession no.	Conserved domain	Predicated function
aec-33	45	394	598–1782	Putative prophage integrase, *E. coli* CFT073 (0.0)	NP_756353.1	cd00801	Integrase
aec-34	46	347	2194–3237	ShiA homolog, *E. coli* CFT073 (0.0)	NP_756354.1	None	Reduction of inflammation
aec-35	44	356	C 3635–4705	Putative transcriptional regulator, *E. coli* CFT073 (0.0)	NP_756356.1	smart00354, pfam00532	Transcriptional regulator LacI family
aec-36	42	452	4910–6268	Hexuronate transporter, *E. coli* CFT073 (0.0)	NP_756357.1	pfam00083	Sugar (and other) transport
aec-37	43	795	6277–8664	Putative glucosidase, *E. coli* CFT073 (0.0)	NP_756359.1	pfam01055	α-Glycosidase
aec-38	55	151	C 9306–9761	IS*1* protein InsB, *E. coli* EDL933 (3e-61)	AAG55747.1	pfam03400	Transposase
aec-39	53	91	C 9680–9955	IS*1* ORFB, *Shigella flexneri* 2a str. 2457T (7e-42)	NP_836645.1	pfam03811	Transposase
aec-40	51	70	C 9772–9984	Putative iso-IS*1* ORF, *Shigella flexneri* (5e-25)	AAK18551.1	None	Unknown
aec-41	44	320	C 10588–11550	Putative fructokinase, *E. coli* EDL933 (9e-61)	NP_311269.1	cd01167	Fructokinase
aec-42	50	302	C 11664–2572	No significant similarities			
aec-43	49	497	C 12691–14184	Glycosylhydrolase, *Bacteroides thetaiotaomicron* VPI-5482 (9e-37)	AAO76867.1	smart00640	Glycosylhydrolase
aec-44	46	420	C 14199–15461	No significant similarities			
aec-45	56	477	C 15558–16991	Sucrose hydrolase, *E. coli* (e-152)	AAP79503.1	smart00640	Glycosylhydrolase
aec-46	49	412	C 17011–18249	Sugar transporter FruP, *Bacillus megaterium* (6e-98)	AAM19070.1	pfam01306	Sugar transporter
aec-47	52	311	18551–19486	Putative sucrose-specific transcriptional regulator, *Yersinia pestis* KIM (2e-86)	NP_669120.1	smart00354, pfam00532	Transcriptional regulator
aec-48	49	127	C 20425–20808	(ORF truncated) Possible transposase remnant, *Yersinia pseudotuberculosis* IP 32953 (1e-60)	CAF25366.1	pfam00665	Transposase
aec-49	56	301	C 20793–21698	Hypothetical ORF in IS*2*, *Klebsiella pneumoniae* (e-162)	AAR07888.1	pfam00665	Transposase
aec-50	51	136	C 21656–22066	IS*2* ORFA, *Shigella flexneri* 2a str. 2457T (3e-60)	NP_838126.1	pfam01527	Transposase
aec-51	53	225	22338–23015	Unknown protein encoded by ISEc8, *E. coli* CFT073 (e-115)	NP_755534.1	pfam01527	Transposase
aec-52	55	115	23015–23362	Unknown protein encoded by ISEc8, *E. coli* CFT073 (4e-60)	NP_755535.1	pfam05717	Transposase
aec-53	57	523	23382–24953	IS*66*-like transposase, *E. coli* (0.0)	CAD33772.1	pfam03050	Transposase
aec-54	55	268	C 25214–26020	(ORF truncated) Putative arabinose exporter (MFS superfamily), *Acinetobacter* sp. ADP1 (3e-29)	YP_047119.1	COG2814	Carbohydrate transport and metabolism
aec-55	54	129	C 26375–26764	(ORF truncated) Oxidoreductase, aldo/ketoreductase family, *Silicibacter pomeroyi* DSS-3 (3e-31)	YP_165172.1	pfam00248	Oxidoreductase
aec-56	52	247	26921–27664	(Truncated ORF) IS*100* transposase, *Yersinia pestis* (e-122)	YP_094025.1	COG4584, partial cd00093	Transposase
aec-57	55	132	27544–27942	(Truncated ORF) Transposase for insertion sequence IS*100*, *Yersinia pestis* biovar Medievalis str. 91001 (e-71)	AAS60948.1	Partial COG4584	Transposase
aec-58	50	260	27939–28721	Insertion sequence IS*100*, ATP-binding protein, *Yersinia pestis* biovar Medievalis str. 91001 (e-132)	NP_993067.1	smart00382	Transposase
aec-59	53	141	C 28827–29252	Hypothetical protein SF2980, *Shigella flexneri* 2a str. 301 (1e-69)	NP_708754.1	None	Unknown
aec-60	55	129	C 29249–29638	Hypothetical protein SF2981, *Shigella flexneri* 2a str. 301 (1e-59)	NP_708755.1	None	Unknown
aec-61	59	189	C 29799–30368	ORF31, *E. coli* RW1374 (3e-91)	CAI43834.1	None	Unknown
aec-62	51	169	32482–32991	ORF33, *E. coli* RW1374 (2e-96)	CAI43836.1	None	Unknown
aec-63	45	500	C 33313–34815	Hypothetical protein YfjI, *E. coli* CFT073 (0.0)	NP_755527.1	None	Unknown
aec-64	58	303	35989–36900	YeeP protein, *E. coli* (e-162)	CAE85196.1	COG3596	GTP binding protein
aec-65	52	225	C 37213–37890	Putative transposase, *E. coli* RW1374 (e-129)	CAI43898.1	pfam00665	Transposase
aec-66	50	115	C 38079–38426	Unknown protein encoded by IS*911* within prophage CP-933L, *E. coli* EDL933 (2e-46)	AAG58804.1	pfam01527	Transposase
aec-67	58	727	38411–40594	(Truncated ORF) Adhesin AIDA-I precursor, *E. coli* (0.0)	BAA15832.1	cd01344, pfam03797	Autotransporter
aec-68	52	840	40709–43231	ORF36, *E. coli* RW1374 (0.0)	CAI43839.1	None	Unknown
aec-69	48	151	43307–43762	Hypothetical protein c3665, *E. coli* CFT073 (2e-83)	NP_755540.1	None	Unknown
aec-70	55	77	43841–44074	ORF39, *E. coli* RW1374 (8e-37)	CAI43842.1	None	Unknown
aec-71	57	272	44174–44992	ORF40, *E. coli* RW1374 (e-146)	CAI43843.1	pfam06067	Unknown
aec-72	54	161	45047–45532	ORF41, *E. coli* RW1374 (4e-84)	CAI43844.1	pfam03230	Antirestriction
aec-73	58	163	45533–46024	YeeS protein, *E. coli* 536 (1e-74)	CAE85201.1	pfam04002	DNA repair protein
aec-74	53	73	46094–46315	ORF44 (YeeT), *E. coli* RW1374 (5e-34)	CAI43847.1	None	Unknown
aec-75	57	122	46478–46846	Putative structural protein, *E. coli* EDL933 (4e-64)	AAG55773.1	None	Unknown
aec-76	53	124	46936–47310	ORF45, *E. coli* RW1374 (4e-66)	CAI43848.1	None	Unknown
aec-77	51	162	47307–47795	YeeW, *E. coli* RW1374 (5e-87)	CAI43849.1	None	Unknown
aec-78	50	80	47762–48004	ORF104, *E. coli* RW1374 (1e-29)	CAI43905.1	None	Unknown
aec-79	48	281	48089–48934	Z1226 protein, *E. coli* 536 (e-151)	CAD33792.1	None	Unknown
aec-80	39	135	48980–49387	(Truncated ORF) Hypothetical protein c4580, *E. coli* CFT073 (5e-34)	NP_756440.1	Partial COG3943	Unknown
aec-81	34	122	49530–49898	Hypothetical protein c4581, *E. coli* CFT073 (3e-36)	NP_756441.1	None	Unknown

[a] C indicates ORFs transcribed on the complementary strand.
[b] str., strain; MFS, major facilitator superfamily.

TABLE 4. Orthologs of Aec-35, Aec-36, and Aec-37 found in the genomes of sequenced pathogenic bacteria

Species and strain	Homolog (% amino acid similarity)		
	Aec-35	Aec-36	Aec-37
Escherichia coli CFT073	C4494 (99)	C4494 (100)	C4496 and C4495 (99)
Shigella sonnei Ss046	SSO_0802 (87)	SSO_0801 (94)	SSO_0800 (91)
Erwinia carotovora subsp. *atroseptica* SCRI1043	ECA1966 (75)	ECA1967 (89)	ECA1968 (84)
Yersinia pseudotuberculosis IP 32953	YPTB3091 (74)	YPTB3092 (85)	YPTB3093 (83)
Yersinia pestis biovar Orientalis CO92	YP00846 (74)	YP00847 (85)	YP00848 (83)
Yersinia pestis biovar Mediaevalis KIM	Y3231 (74)	Y3232 (85)	Y3233 (83)
Yersinia pestis biovar Mediaevalis 9100	YP3543 (74)	YP3544 (85)	YP3545 (83)
Burkholderia cepacia R18194	Not present	Bcepa03004976 (62)	Bcepa03004977 (59)
Ralstonia solanacearum GMI1000	Not present	RSc1080 (62)	RSc1081 (61)

end of AGI-3 (3′ extremity of *selC*) and the second DR1 is located inside *aec-80*. DR1 of AGI-3 was found to also flank PAI-I of *E. coli* 536 and the toxigenic invasion locus A PAI of *E. coli* H10407, and corresponds to half of the *attP* site of phage ϕR73 (18, 24, 32). One DR2 is located between DR1 and *aec-33*, and the second DR2 is located inside *aec-80*. AGI-3 was predicted to contain 49 open reading frames (ORFs) numbered sequentially from *aec-33* to *aec-81* (*aec* for *a*vian *E. c*oli) (Table 3, Fig. 1). The overall G+C content of AGI-3 is 49.25%, which is close to the average of the *E. coli* genome (51%). Nevertheless, the G+C content shows variability across the AGI-3 genomic island (Table 3).

Putative mobile genetic elements present on AGI-3. AGI-3 contains genes and putative mobile elements homologous to bacteriophage and insertion sequence (IS) elements that could promote horizontal gene transfer. The *aec-33* gene potentially encodes a polypeptide of 394 amino acids that is 86% similar to the integrase of the retronphage ϕR73, belonging to the tyrosine recombinase family (61). Aec-33 is identical to putative phage integrases identified in pathogenicity islands located at the *selC* locus of both *E. coli* strains CFT073 (ORF c4491) and 4797/97 (ORF L01), and almost identical (more than 90% of similar amino acids) to those found in PAIs of *Shigella flexneri* 2a strain 301 (ORF SF3698), *E. coli* EDL933 (ORF Z5087), *Yersinia pseudotuberculosis* IP 32953 (ORF YPTB3886), and *Photorhabdus luminescens* subsp. *laumondii* TTO1 (ORF plu0125) (11, 22, 33, 51, 57, 65). Fourteen ORFs of AGI-3 are homologous to IS elements or transposons of the IS*1*, IS*1222*, IS*2*, IS*Ec8*, IS*66*, IS*100*, or IS*911* type (Fig. 1 and Table 3). Only the IS*1* element (*aec-38* to *aec-40*) seems to be intact and to possess its left and right terminal inverted repeats (47). All the other elements are disrupted either by frameshift or by integration of another IS element. These data strongly suggest that AGI-3 has evolved by repeated recombination events.

Genetic features of AGI-3. Among the 49 ORFs identified, 35 are not related to mobility. These 35 ORFs can be divided into five modules bound by either repeats, insertion elements, or bacteriophage sequences (Fig. 1, Table 3).

Module 1, which is composed of gene *aec-34* and of the *aec-35–aec-37* gene cluster, was also found to be present at the same location and with the same organization in the uropathogenic *E. coli* strain CFT073 (99% identical nucleotide) (Table 4) (65). The G+C content (average: 43%) of the genes in module 1 is lower than the average for the *E. coli* genome (51%) (Table 3). The *aec-34* gene encodes a protein highly homologous to ShiA of *Shigella flexneri*, involved in the attenuation of inflammation (31). The *shiA* gene is located downstream of the integrase gene of the SHI-2 pathogenicity island present at the *selC* locus (44).

The *aec-35* to *aec-37* gene cluster is potentially involved in carbohydrate transport and metabolism. The *aec-35* product is homologous to transcriptional regulators of the LacI family. Aec-35 has a helix-turn-helix DNA-binding motif (position 5 to 26) and a sugar binding domain of the LacI family (position 73 to 313). The *aec-36* product shares similarity (62% similar amino acids) with ExuT of *E. coli* K-12 MG1655, a major facilitator superfamily transporter of the anion:cation symporter family 14 (48). ExuT is involved in galacturonate uptake in *E. coli* and in other bacteria such as *Erwinia chrysanthemi* and *Ralstonia solanacearum* (26, 45, 63). Aec-36, like ExuT, is predicted to be an integral membrane protein.

The *aec-37* gene encodes a putative glycosylhydrolase of family 31. This family is very diverse and contains alpha-glucosidases, glucoamylases, sucrase-isomaltases, alpha-xylosidases, alpha-glucan lyases, and isomaltosyltransferases (13). The *aec-35–aec-37* region is present in the sequenced genome of other pathogenic bacterial species (Table 4). The cluster is present in *Shigella sonnei*, *Yersinia pestis* biovar Orientalis CO92, *Yersinia pseudotuberculosis* IP32953, *Yersinia pestis* biovar Medievalis KIM and 91001, and *Erwinia carotovora* subsp. *atroseptica* SCRI1043 (3, 11, 15, 49, 60). In these species, the organization of the *aec-35–aec-37* gene cluster is the same as in *E. coli* BEN2908 and CFT073 but the cluster is not inserted at the same genomic location. The genes *aec-36* and *aec-37* but not *aec-35* are also present in *Burkholderia cepacia* and in *Ralstonia solanacearum* GMI1000 (55). In these two species, the *aec-36* and *aec-37* orthologs are linked to a transcriptional regulator that is divergently transcribed but that does not show any similarity with Aec-35.

Module 2 is composed of the *aec-41* to *aec-47* genes. The gene products show homologies with proteins putatively involved in carbohydrate transport and metabolism (putative fructokinase, glycosylhydrolases, and sugar transporter of the major facilitator oligosaccharide:H^+ symporter family 5). Such an arrangement of genes has not been described before.

Module 3 includes ORFs *aec-54* and *aec-55*. Due to the insertion of the IS elements *aec-53* and *aec-56*, the predicted products of *aec-54* and *aec-55* are truncated compared to their homologs (a putative arabinose exporter of *Acinetobacter* sp. strain ADP1 and an oxidoreductase of the aldo/ketoreductase family of *Silicibacter pomeroyi* strain DSS-3) (Table 3). Thus, module 3 is probably nonfunctional.

Module 4, comprising ORFs *aec-59* to *aec-64*, is very similar to the region containing *ORF29* to *ORF34* in the enterohemorrhagic *E. coli* strain RW1374, located in a 111-kb pathogenicity island inserted at the *pheV* locus (34). The products of the ORFs of module 4 are of unknown function.

Module 5 is composed of *aec-67* to *aec-81*. A nearly identical region is present in the 111-kb pathogenicity island of *E. coli* strain RW1374 (*ORF35*, *ORF36*, and *ORF39* to *ORF47*) (34). This module is also present in the *she* pathogenicity island of *Shigella flexneri* 2a strain 301 inserted at the *pheV* locus, in genomic island I of *E. coli* strain Nissle 1917 inserted at the *serX* locus, and in two different genomic islands of *E. coli* strain CFT073, inserted at the *serX* and *pheV* loci (1, 27, 65). Various ORFs of module 5 show homology with the CP4-44 prophage of *E. coli* K-12 MG1655 (6). Gene *aec-67* product is highly related to the AIDA-I precursor encoded by the *flu* gene. However, *aec-67* is truncated at its 5′ end by the insertion of the IS*911* transposase gene, leading to a deletion of 380 amino acids compared to the native AIDA-I precursor of *E. coli* MG1655. AIDA belongs to the autotransporter protein family, which comprises many virulence factors (29).

Cluster *aec-35 to aec-37* is implicated in carbohydrate metabolism and virulence. As the products of *aec-35* to *aec-37* are homologous to proteins implicated in carbohydrate transport and metabolism, we tested the role of this gene cluster in the metabolism of strain BEN2908. For that aim, the *aec-35–aec-37* region (5,124 bp, Fig. 1) was replaced by a kanamycin resistance cassette by the method of Datsenko and Wanner, leading to mutant strain BEN2929 (BEN2908Δ*aec-35-37*::Kan^r) (14).

The capacity of wild-type strain BEN2908 and mutant BEN2929 to metabolize 190 different carbon sources was then tested using PM1 and PM2 phenotype microarrays from Biolog. A statistically significant reduction in the metabolism of D-mannitol ($P = 0.0073$), α-D-glucose ($P = 0.0081$), α-D-lactose ($P = 0.0074$), D-xylose ($P = 0.0309$), D,L-malic acid ($P = 0.0035$), D-galacturonic acid ($P = 0.0085$), and D-threhalose ($P = 0.0084$) was observed for the mutant strain BEN2929 (Fig. 2). The wild phenotype was restored by complementation of the deleted mutant BEN2929 with the entire *aec-35–aec-37* region previously cloned into the high-copy-number plasmid pCR2.1-Topo (strain BEN2929/pBEN182). When tested on PM1 and PM2 phenotype microarray plates, the complemented strain metabolized the above-listed carbohydrates as well as the wild-type strain, confirming that at least one of the *aec-35–aec-37* genes is involved in the metabolism of these carbohydrates (Fig. 2). Furthermore, the generation times of the wild-type and the mutant strains were measured in minimum medium containing Na-D-galacturonate or trehalose. In both conditions, the generation time of the mutant strain was twofold less than those of the wild-type strain (1,500 min versus 750 min and 750 min versus 375 min in the presence of Na-D-galacturonate or trehalose, respectively).

As the *aec-35–aec-37* gene cluster is well conserved in several pathogenic bacteria of the family *Enterobacteriaceae* (*Erwinia carotovora*, *Shigella sonnei*, and *Yersinia* spp., Table 4), we hypothesized that the ability to efficiently metabolize the seven carbohydrates identified above would give an adaptive advantage to *E. coli* for the colonization of several tissues and body fluids. This was first tested by comparing the growth of

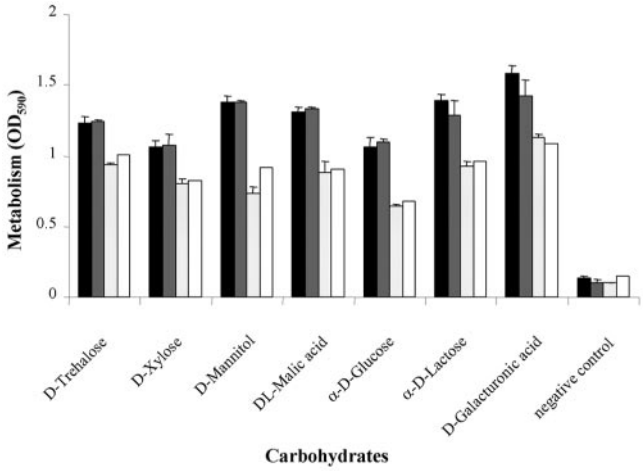

FIG. 2. Role of the *aec-35–aec-37* gene cluster in carbohydrate metabolism. Data for the seven carbohydrates that were differently metabolized by strain BEN2908 and its derivatives are reported as well as data obtained for the negative control (a well without carbohydrate). Strains BEN2908, BEN2929/pBEN182, BEN2929, and BEN2929/pCR2.1 (from left to right within each group) were seeded into Biolog PM1 and PM2 microplates containing a panel of 190 carbohydrates. The microplates were incubated aerobically at 37°C for 24 h and the optical density at 590 nm (OD_{590}) was measured. Experiments were done in triplicate (except for strain BEN2929/pCR2.1); means and standard deviations are indicated.

the wild-type strain BEN2908 and its mutant derivative BEN2929 in chicken serum. Both strains were able to multiply equally well in serum (Fig. 3). We then compared the virulence of both strains in experimental colibacillosis of chicken. Compared to the wild-type strain, the mutant derivative was significantly ($P = 0.0016$) less able to induce specific lesions of colibacillosis such as aerosacculitis, perihepatitis, and pericarditis (Fig. 4). The mutant was also less bacteremic at 24 h ($P = 0.0457$) and 48 h postinoculation ($P = 0.0328$), and less able to colonize the liver ($P = 0.0175$) than the wild-type strain (Fig. 4). These results indicate that cluster *aec-35–aec-37* is involved in the virulence mechanism of *E. coli* BEN2908 for chicken.

Prevalence of *aec-35*, *aec-36*, and *aec-37* in a collection of pathogenic and nonpathogenic *E. coli* strains. The prevalence

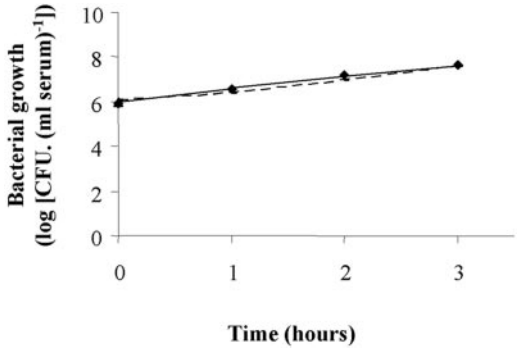

FIG. 3. Bacterial growth in chicken serum. Strain BEN2908 (◆), its derivative BEN2929 (dotted line) were grown in 90% normal chicken serum for 3 h. Viable cells were estimated by plating serial dilutions of the cultures at 1-hour intervals.

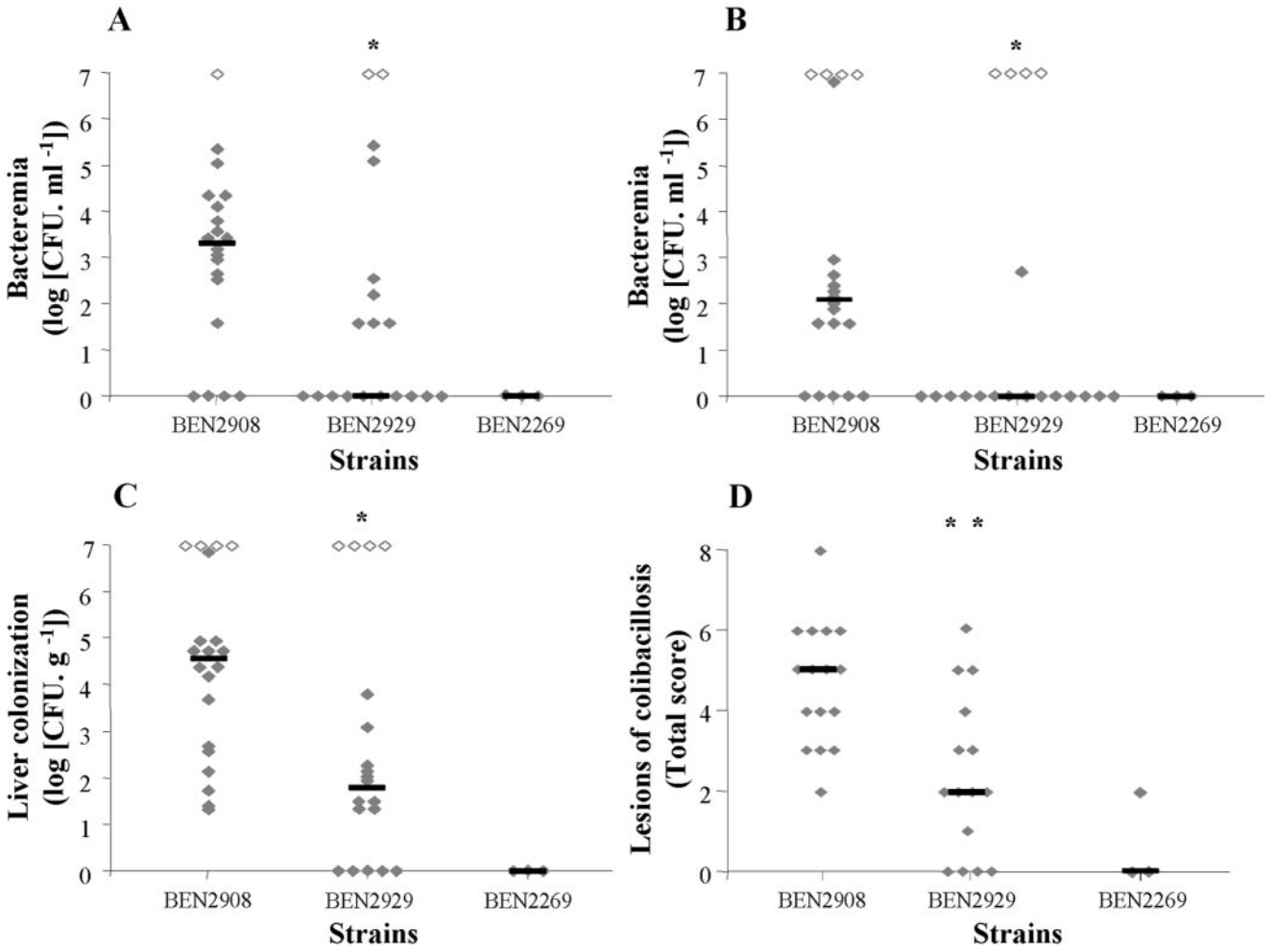

FIG. 4. Role of the *aec-35–aec-37* gene cluster in the colonization of liver and blood and in the development of colibacillosis lesions in chickens. Twenty and 19 25-day-old specific-pathogen-free White Leghorn chickens were inoculated in the right air sac with 5×10^6 CFU of strains BEN2908 or BEN2929, respectively. Three chickens were inoculated with the nonpathogenic avian strain BEN2269. Each animal is represented by a diamond symbol (◆, ◇). Bacteremia was determined 24 h (A) and 48 h (B) postinoculation. Animals were euthanized 48 h postinoculation by injection of Nesdonal and then necropsied. Liver bacterial colonization (C) and colibacillosis lesions (D) were recorded. The lesion score was not determined for dead animals. In each group infected by either BEN2908 or BEN2929, four chickens died and were given the highest rank. They are represented by an open diamond symbol (◇). Bars indicate the median for each group of chickens. A Wilcoxon-Mann-Whitney test was used to analyze the differences between groups of chickens inoculated with the wild-type and the mutant strains (*, $P < 0.05$; **, $P < 0.01$).

of the *aec-35–aec-37* gene cluster was examined among 36 nonpathogenic strains of avian origin and 249 ExPEC strains: 205 strains of avian origin, 7 strains isolated from animals other than poultry, and 37 strains of human origin. For that aim, PCRs were performed on each strain to amplify a 948-bp internal fragment of *aec-35* (primers IC79 and IC36, Table 2), a 1,225-bp DNA fragment of *aec-36* (primers IC39 and IC64, Table 2), and a 1,574-bp DNA fragment of *aec-37* (primers IC45 and IC50, Table 2). These three ORFs were amplified in 33 (30 strains of avian origin and 3 strains of human origin) of the strains analyzed (11.6%), whereas in two strains (one of avian origin and one of an animal other than avian origin) only the *aec-35* ORF could be amplified (Table 5).

The locations of the three ORFs (*aec-35* to *aec-37*) were determined in the 33 positive strains by amplification of a fragment overlapping *aec-35* and *aec-36* (primers IC37 and

TABLE 5. Distribution and genetic link to *selC* of genes *aec-35, aec-36*, and *aec-37* in a collection of pathogenic and nonpathogenic *E. coli* strains[a]

DNA region amplified	No. of strains positive		
	Avian pathogenic strains ($n = 205$)	Avian nonpathogenic strains ($n = 44$)	Human and animal pathogenic strains ($n = 36$)
aec-35	27	4	4
aec-36	26	4	3
aec-37	26	4	3
aec-35 to *aec-37*	26	4	3
selC to *aec-35*	25	4	3

[a] Pathogenic strains of avian origin were isolated from chickens or ducks with signs of colibacillosis and were able to kill at least four of five day-old chicks inoculated subcutaneously with 10^8 CFU. Nonpathogenic strains of avian origin were isolated from the gut of healthy birds and were unable to kill any chick in the day-old chick lethality model (16). Other pathogenic strains were isolated from pathological samples.

IC60, Table 2) and a fragment overlapping *aec-36* and *aec-37* (primers IC43 and IC44, Table 2). In all 33 of these strains the *aec-35*, *aec-36*, and *aec-37* genes were linked in one cluster and in the same order as in BEN2908. A PCR amplifying a fragment of 4,143 bp covering the region between *selC* and *aec-35* (primers SelC1 and IC80, Table 2) was then performed to investigate if the *aec-35–aec-37* cluster was linked to *selC*. In 32 strains of the 33 strains carrying the *aec-35–aec-37* cluster, it was linked to *selC* (Table 5). Among the 33 strains carrying *aec-35–aec-37* genes, 29 belonged to the pathogenic group and 4 to the nonpathogenic group (Table 5). The prevalence of the region was therefore not significantly different (around 12%) between the pathogenic and the nonpathogenic strains tested. It is interesting that the *aec-35–aec-37* cluster was present in all 15 avian pathogenic strains tested belonging to the O5 serogroup.

DISCUSSION

Genomic islands have been described as horizontally acquired DNA regions that are frequently chromosomally inserted in the vicinity of tRNA genes (28). They encode various functions which are related to virulence, symbiosis, metabolism, resistance to antibiotics or degradation of xenobiotic compounds. A typical genomic island is flanked by direct repeat structures and carries several genes encoding traits that may increase bacterial adaptability or fitness under certain growth conditions. Typically, GEIs carry multiple functional and fragmented insertion sequence elements and other mobility-related genes, as well as a functional integrase (*int*) gene, the product of which is involved in insertion and deletion of the DNA region that is flanked by direct repeat structures (19).

We have characterized a novel genomic island (AGI-3) which fulfills most of the criteria described above. AGI-3 is inserted at the *selC* tRNA gene (a hot spot insertion site for PAIs) in the avian ExPEC strain BEN2908. The presence of a putative active integrase gene (*aec-33*) the product of which is highly similar (86%) to the integrase of the retronphage ϕR73, the vicinity of the *selC* tRNA locus, integration site of ϕR73 phage, and the presence of direct repeats of 16 bp corresponding to the half site of the *attP* site of ϕR73 strongly suggest that AGI-3 or a primitive core of AGI-3 was acquired by horizontal gene transfer via a bacteriophage of the ϕR73 family. These elements are also in favor of putative mobility of the AGI-3 island as it was observed for PAI-I of the uropathogenic *E. coli* strain 536 (18).

AGI-3 shows a modular structure composed of five modules bound by IS elements. Parts of modules 4 and 5 (*aec-61* to *aec-78*, with the exception of *aec-65* and *aec-66*, which encode transposases) are also found with the same organization in the 111-kb PAI of the enterohemorrhagic *E. coli* strain RW1374 inserted at the *pheV* locus (34). Moreover, from *aec-64* (located at the right end of module 4) to *aec-81*, very similar regions exist in the *she* pathogenicity island of *Shigella flexneri* 2a strain 301 inserted at the *pheV* locus, in genomic island I of *E. coli* strain Nissle 1917 inserted at the *serX* locus, and in two different islands of *E. coli* strain CFT073, inserted at the *serX* and *pheV* loci (1, 27, 65). The *aec-65* and *aec-66* ORFs could not be found in these PAIs. Taken together, these observations indicate that module 4 and module 5 initially consisted of one module that was later disrupted by the *aec-65–aec-66* transposase genes in strain BEN2908 or modified by the insertion of transposase-encoding genes at various locations in the other cited PAIs.

Various ORFs of modules 4 and 5 (*aec-64*, *-67*, *-68*, *-73*, *-74*, *-75*, *-76*, and *-77*) are also similar to the putative prophage CP4-44, suggesting that they could have a phage origin (6, 10). Modules 1 and 3 are also found in other pathogenic *E. coli* strains and in other species of pathogenic bacteria, whereas the organization of module 2 has never been described before. The presence of modules with a similar organization in other bacteria as well as the presence of IS elements and transposase genes strongly argue in favor of stepwise acquisition of the modules from other strains into an AGI-3 primitive core, resulting in a mosaic structure.

The *aec-35–aec-37* gene cluster of module 1 was shown to be implicated in the uptake of seven carbohydrates and faster growth of the strain in minimal medium containing galacturonate or trehalose. The *aec-35* gene was predicted to encode a regulator of the LacI family. As the bacterial LacI family has been described to repress transcription of genes involved in carbohydrate transport and utilization, deletion of the *aec-35* gene would lead to abolition of the repression of such genes and to an increase in the transport of carbohydrates (64). Such results have not been observed in our phenotype microarray assays since we only demonstrated the lesser utilization of seven carbohydrates by the mutant strain BEN2929. We can thus rule out the hypothesis that the observed decrease in the assimilation of seven carbohydrates could be due to a pleiotropic effect resulting from the deletion of *aec-35*.

The *aec-36* gene was predicted to encode a major facilitator superfamily sugar permease similar to the hexuronate transporter ExuT of *E. coli* (52). Among the seven carbohydrates identified only the D-galacturonic acid is a hexuronate, the others are pentose (D-xylose), tetrose (D,L-malic acid), hexoses (α-D-glucose, D-xylose and D-mannitol) or disaccharides (D-lactose and D-trehalose). We can thus hypothesize that the Aec-36 sugar permease encoded by strain BEN2908 could be able to transport different kind of carbohydrates. However all these carbohydrates can be also transported by other permeases as we do not have a complete abolition of their assimilation in the mutant strain (Fig. 2). Furthermore, as only 190 different carbohydrates were tested with phenotype microarrays, we cannot completely exclude that the main substrate of the system is another carbohydrate that has not been identified yet.

The *aec-37* gene product, a putative glycosylhydrolase, has been assigned to family 31 in Henrissat's classification based on amino acid sequence (30). Enzymes in this family cleave only α-glucosidic linkages (1–1, 1–2, 1–3, 1–4, and 1–6) from various di- and/or oligosaccharides. Among the seven identified carbohydrates, only D-lactose and D-trehalose are disaccharides but only D-trehalose is formed by two glucose molecules bound in αα-1,1 linkage and might be a substrate for Aec-37. To better understand the activity of the enzyme encoded by *aec-37*, it would be interesting to clearly determine its substrate.

The mutant strain deleted of the *aec-35–aec-37* gene cluster was also found to be less able to induce specific lesions of colibacillosis, bacteremia, and colonization of the liver of chickens compared to the wild-type strain. These results

strongly suggest that the *aec-35–aec-37* gene cluster is involved in the virulence of strain BEN2908 for chickens. Avian colibacillosis generally starts as a respiratory infection that evolves to a generalized infection resulting in fibrinopurulent lesions of internal organs. Passage into the bloodstream is an important step for the dissemination of the bacteria in avian colibacillosis and allows further multiplication in blood and colonization of internal organs.

Pourbakhsh et al. have shown that the invasion of the vascular system via the lung and the damaged air sac interstitium may be an important portal of entry for bacteria (53). We have observed that the mutant was less bacteremic than the wild-type strain in experimental colibacillosis. However, in in vitro tests, the mutant strain showed the same capacity to multiply in chicken serum as the wild-type strain. We thus hypothesize that the deletion of the *aec-35–aec-37* gene cluster affects the early steps of colibacillosis, such as colonization of the air sacs and/or the lung, due to reduced fitness, leading to a reduced ability to invade the bloodstream and to disseminate in internal organs.

Indeed, the importance of hexuronate and other sugar catabolism in the fitness of *E. coli* has recently been stressed. It has been shown that hexuronate catabolism plays a role in the maintenance of *E. coli* strain MG1655 in the mouse intestine. Furthermore, several genes, such as *exuT*, that are involved in catabolism of hexuronate are significantly induced by mucus (12). Analysis of the transcriptome of the uropathogenic *E. coli* strain CFT073 during urinary tract infection also revealed that genes involved in hexuronate metabolism were upregulated. The authors suggested that in the urinary tract metabolism shifted so that hexuronates could be used to support growth (59).

Recently, in the APEC strain IMT5155, which harbors the same serotype (O2:K1:H5) as BEN2908, it was shown that metabolic functions play a role in host infectivity (38). Using signature-tagged mutagenesis, Li et al. (38) identified several genes, mutation of which results in reduced septicemia, that are also involved in metabolism and nutrient uptake. Of these gene products, one is the transketolase TktA, involved in the catabolism of pentose sugars, the formation of D-ribose 5-phosphate and in the provision of D-erythrose 4-phosphate, a precursor of aromatic amino acids, aromatic vitamins and of pyridoxine. Another is a β-cystathionase involved in methionine biosynthesis, and the latter is involved in pyrimidine pathway regulation.

Taken together, all of these observations indicate that metabolism in ExPEC is probably closely linked to virulence. In addition to the *aec-35–aec-37* gene cluster of module 1, we have also identified module 2 of AGI-3, which is potentially involved in carbohydrate uptake and assimilation and could also be linked to virulence.

In conclusion, we reported here the description of a new APEC genomic island associated with the *selC* locus and we demonstrated its role in carbohydrate assimilation and virulence. As three of the five modules described in AGI-3 of the APEC strain BEN2908 are also present in other pathogenic *E. coli* and in other pathogenic bacterial species, it is tempting to hypothesize that these modules also play a similar role in other pathogenic bacteria. As the AGI-3 genomic island possesses all the potential to be mobile, future work will be directed towards evaluation of its ability to be transferred between *E. coli* strains and between various bacterial species.

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