YqeH contributes to avian pathogenic *Escherichia coli* pathogenicity by regulating motility, biofilm formation, and virulence

Lei Yin¹, Baoyan Cheng², Jian Tu², Ying Shao², Xiangjun Song², Xiaocheng Pan¹* and Kezong Qi²*

**Abstract**

Avian pathogenic *Escherichia coli* (APEC) is a pathotype of extraintestinal pathogenic *E. coli* and one of the most serious infectious diseases of poultry. It not only causes great economic losses to the poultry industry, but also poses a serious threat to public health worldwide. Here, we examined the role of YqeH, a transcriptional regulator located at *E. coli* type III secretion system 2 (ETT2), in APEC pathogenesis. To investigate the effects of YqeH on APEC phenotype and virulence, we constructed a *yqeH* deletion mutant (APEC40-Δ*yqeH*) and a complemented strain (APEC40-CΔ*yqeH*). Compared with the wild type (WT), the motility and biofilm formation of APEC40-Δ*yqeH* were significantly reduced. The *yqeH* mutant was highly attenuated in a chick infection model compared with WT, and showed severe defects in its adherence to and invasion of chicken embryo fibroblast DF-1 cells. However, the mechanisms underlying these phenomena were unclear. Therefore, we analyzed the transcriptional effects of the *yqeH* deletion to clarify the regulatory mechanisms of YqeH, and the role of YqeH in APEC virulence. The deletion of *yqeH* downregulated the transcript levels of several flagellum-, biofilm-, and virulence-related genes. Our results demonstrate that YqeH is involved in APEC pathogenesis, and the reduced virulence of APEC40-Δ*yqeH* may be related to its reduced motility and biofilm formation.

**Keywords:** Avian pathogenic *Escherichia coli*, *yqeH*, motility, biofilm formation, virulence, pathogenicity

**Introduction**

Avian pathogenic *Escherichia coli* (APEC), a pathotype of extraintestinal pathogenic *E. coli* (ExPEC), causes serious infectious diseases in poultry [1]. Different APEC serotypes cause local or systemic infections in poultry, including respiratory infections, sepsis, polyserositis, coligranuloma, cellulitis, yolk sac infection, omphalitis, and swollen head syndrome, resulting in significant economic losses to the poultry industry [2]. Understanding the underlying molecular mechanisms of APEC pathogenicity is crucial for controlling avian colibacillosis.

There are many transcriptional regulators in Gram-negative pathogenic bacteria, which play important roles in regulating bacterial metabolism and the expression of virulence genes. More than 250 transcription factors are known to regulate gene expression in *E. coli*, and coordinate the expression of numerous promoters in response to specific environmental cues [3–5]. Although many virulence factors are known to be associated with APEC pathogenicity, the regulation of their expression is still not fully understood.

In *E. coli*, two distinct type III secretion systems (T3SSs) have been identified and characterized. The locus of enterocyte effacement (LEE), which encodes T3SS in intestinal *E. coli*, such as enteropathogenic *E. coli*.
coli and enterohemorrhagic E. coli (EHEC), is essential for the formation of attaching and effacing (A/E) lesions. A second T3SS, E. coli type III secretion system 2 (ETT2), plays an important role in the invasion, intracellular survival, and virulence of ExPEC, including APEC and newborn meningitis E. coli [6, 7]. We speculated that the transcriptional regulators located in the ETT2 cluster play an important role in APEC pathogenicity. Many reviews have described the various transcriptional regulator genes located in the ETT2 cluster, including yqeK/etrB, etrA, and eivF, which encode transcription factors that regulate the expression of virulence genes in intestinal pathogenic E. coli [8]. For example, EtrA and EivF of EHEC O157 negatively affect LEE expression and bacterium adherence to epithelial cells [9]. In contrast, the regulator YqeK/EtrB activates LEE expression and promotes A/E lesion formation by directly interacting with the Ler regulatory region [10]. Identifying new transcriptional regulators and their functions will clarify ETT2 pathogenesis.

In this study, we investigated the role of the transcriptional regulator YqeH located at ETT2 locus in the pathogenesis of APEC infections. The deletion of yqeH significantly reduced the virulence of APEC in vivo and in vitro. This was associated with reductions in the expression of many virulence-associated genes regulated by YqeH, which reduced bacterium motility, biofilm formation, adhesion, and invasion, suggesting that YqeH is involved in the pathogenicity of APEC.

Materials and methods

Bacterial strains, plasmids, and growth conditions

The strains and plasmids used in this study are listed in Table 1. The nucleotide sequences of the primers (GenBank: HG738867.1) are listed in Additional file 1. APEC strain APEC40 [11] (serotype O18:K1) of the B2 and ST95 phylogenetic groups, was isolated from a chicken with clinical septicemic symptoms of colibacillosis in Anhui, China. All E. coli strains were grown in Luria–Bertani (LB) medium at 37 °C with aeration. LB medium was supplemented with ampicillin (Amp, 100 mg/mL) and chloramphenicol (Cm, 30 mg/mL), unless specified otherwise.

Ethics statement

The animal experiments were approved by the Institutional Animal Care and Use Committee at the Institute of Animal Husbandry and Veterinary Science, Anhui Academy of Agricultural Sciences (Permit No: AAAS-IHVS-Po-2020-0034). The chickens were employed in experiments that complied with the ARRIVE guidelines of laboratory animal welfare and ethics [12].

| Table 1 Strains and plasmids used in this study |
|-------------------------------|-----------------|----------------|
| Strains or plasmids | Description | Reference |
| Strains | | |
| APEC40 | wild-type strain, isolated from sick chicken | This study |
| APEC40-ΔyqeH | yqeH deletion mutant in APEC40 | This study |
| APEC40-ΔyqeH pSTV28 | APEC40-ΔyqeH with plasmid pSTV28-yqeH | This study |
| DH5a | F- Δ(lacZYA-argF) U169, recA1, endA1, hsdR17 (rk-,mk+) | TIANGEN |
| pSTV28 | Cm, lacZ | Takara |
| pSTV28-yqeH | pSTV28 derivative harboring yqeH gene | This study |
| Plasmids | | |
| pKD46 | Amp, expresses γ red recombinase | This study |
| pKD3 | Cm gene, template plasmid | This study |
| pCP20 | Cm, Amp, yeast Flp recombinase gene, FLP | This study |
| pSTV28 | Cm, lacZ | This study |
| pSTV28-yqeH | pSTV28 derivative harboring yqeH gene | This study |

Mutant construction and complementation plasmids

A yqeH deletion mutant strain was generated with the lambda Red recombinase system, as described previously [13]. Linear PCR products were transformed into APEC40 carrying the pKD46 plasmid and the yqeH gene was replaced with a chloramphenicol resistance cassette. The chloramphenicol-resistance cassette was then eliminated by the helper plasmid pCP20 and a chloramphenicol-sensitive mutant strain was selected. The mutant strain was confirmed with PCR and DNA sequencing, and designated APEC40-ΔyqeH. To generate a complemented strain, the yqeH gene (including its putative promoter) was amplified and subcloned into plasmid pSTV28 with the primer pair yqeH Co-F and yqeH Co-R (Additional file 1). The mutant strain APEC40-ΔyqeH was then transformed with recombinant plasmid pSTV28-yqeH to generate the complemented strain APEC40-CΔyqeH.

Determination of bacterial growth, motility, and biofilm formation

Bacterial growth was measured as described previously [14]. Briefly, strains APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH were cultured in LB medium until the optical density at 600 nm (OD600) reached 1.0. An equal amount of each bacterial culture was then transferred into 100 mL of LB medium in a volumetric ratio of 1:100 (v/v) and incubated at 37 °C with shaking. OD600 was monitored at 2 h intervals with a spectrophotometer (Bio-Rad, USA). The experiment was repeated three times and all samples were measured in triplicate.
The swarming motility of strains APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH was assessed on soft-agar plates (0.25% agar), as previously described [15]. Briefly, bacteria were grown to an OD_{600} of 1.0 in LB medium and pelleted by centrifugation. The resulting pellet was washed and suspended in phosphate-buffered saline (PBS). The bacterial suspension (2 μL) was spread by washing and suspended in PBS for 1 h. Swarming motility was assessed by measuring the diameter of migration. The experiment was repeated three times and all samples were measured in triplicate. In addition, the strains APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH were negatively stained using 2% phosphotungstic acid and observed under a FEI T12 transmission electron microscope (FEI, Ltd, Hillsboro, OR, USA).

An assay involving crystal violet staining was used to quantify biofilm formation by APEC, as previously described [11]. Briefly, the strains were grown over night in 5 mL of LB medium at 37 °C with rotation at 200 rpm. The cultures were suspended in M9 minimal medium supplemented with 0.2% fructose, and diluted to an OD_{600} of 0.1. An aliquot (200 μL) of each cell suspension was transferred to a 96-well plate (Corning, Corning, NY, USA) and incubated at 37 °C for 36 h. The wells were washed gently three times with PBS and stained with 0.1% crystal violet for 30 min at room temperature. The wells were then rinsed three times with PBS, air-dried, and 100 μL of 95% ethanol was added to dissolve the crystal violet. OD_{595} was measured with a Synergy 2 micro-plate reader (BioTek, USA).

**Bacterial adhesion and invasion assays**
Bacterial adhesion and invasion assays were performed as described previously [16]. Chicken embryo fibroblast (DF-1) cell monolayers were washed with Dulbecco's modified Eagle's medium (DMEM) without fetal bovine serum, and infected with bacteria at a multiplicity of infection (MOI) of 100 for 2 h at 37 °C under 5% CO_{2}. After the cells were washed with PBS, they were lysed with 0.5% Triton X-100, and the bacteria were counted by plating them on LB agar plates. For the invasion assay, cell cultures were inoculated with bacteria as described for the bacterial adhesion assay. After incubation for 1 h, the cells were washed and treated with DMEM containing gentamicin (100 μg/mL) for 1 h to kill any extracellular bacteria. The monolayers were then washed and lysed with 0.5% Triton X-100. Serial dilutions of the cell suspensions were spread onto LB agar plates, and after overnight growth at 37 °C, the colony-forming units (CFUs) were counted. The input dilution of the bacteria was also plated to determine the CFU count for each inoculum.

**Animal infection experiments**
The virulence of strains APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH was determined in chicks. The APEC strains were grown to the exponential phase and collected, washed three times in PBS, and then adjusted to the appropriate concentration. For each group, eight 7-day-old Roman chicks were inoculated intratracheally with 10^{8} CFUs of bacteria (APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH, respectively), or with PBS as negative control. Mortality was monitored daily up to 7 days post infection.

Bacterial colonization was determined during systemic infections, as described previously [17]. At 24 h post-infection, the chicks were bled, euthanized, and dissected. The bacterial loads in the blood were determined by plating the bacteria onto LB agar plates. The chick livers, spleens, and lungs were collected, weighed, and homogenized. The homogenates were diluted and plated onto LB agar to determine the bacterial numbers.

**RNA-Seq transcriptomic assay**
The transcriptional levels of APEC40 and APEC40-ΔyqeH cells were determined, as described previously [18], with some modification. To evaluate the effects of YqeH on the transcriptional levels in APEC40 and APEC40-ΔyqeH, the cells were collected by centrifugation at 12 000 rpm for 5 min from cultures in LB medium when OD_{600} attained 0.80. The collected cells were washed with PBS (pH 7.4) and centrifuged. Total RNA was extracted from the cells with TRIzol Reagent (Invitrogen, Thermo Fisher Scientific, USA), according to the manufacturer’s instructions, and the genomic DNA was removed with DNase I (TaKaRa, Japan). cDNA was synthesized with the SuperScript™ Double-Stranded cDNA Synthesis Kit (Invitrogen, Thermo Fisher Scientific) and sequenced by Shanghai Major BioBiopharm Technology Co. Ltd. (Shanghai, China) with the Illumina HiSeq2500 System (Illumina, CA, USA). The differential expression analysis was performed with the EdgeR software. Differences in expression levels between groups were considered significant after adjustment was made for multiple testing, based on a q-value < 0.05. The genes were first filtered, based on a q < 0.05 and an absolute difference > two-fold, when |log_{2} fold change| > 1.0. The enrichment of the differentially expressed genes was analyzed with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed with KOBAS.
RNA isolation and reverse transcription–quantitative PCR (RT–qPCR)

RNA isolation and RT–qPCR (primers listed in Additional file 1) were performed as described previously [19]. The bacterial strains were grown to post-exponential phase (OD$_{600}$ = 0.80) in the absence of antibiotics. Total RNA was isolated with the EasyPure RNA Kit (TransGen Biotech Co., Ltd, China), according to the manufacturer's instructions. The quality of the RNA was determined, cDNA was synthesized, and a microarray was performed. SYBR Green detection was used for the RT–qPCR. Each reaction was conducted in triplicate in two-step multiplex qPCR assays with SYBR® Premix Ex Taq™ (TransGen Biotech Co., Ltd), and bacterial dnaE was used as the internal reference gene. The relative expression levels were measured with the $2^{-\Delta\Delta CT}$ method [19].

Detection of transcription level of type 1 fimbriae genes by RT–qPCR

The transcription level of type 1 fimbriae genes (fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI) was detected using RT–qPCR. The total volume of the reaction system was 20 μL with one μg total RNA, and the reverse transcription was conducted using SYBR green PCR master mix. The standard cycling parameters were performed on the ABI StepOne Plus instrument, and each target gene was examined three times. The primers of the target genes were listed in Additional file 1.

Statistical analyses

Statistical analyses were performed with the GraphPad software package (GraphPad Software, LaJolla, CA, USA). One-way analysis of variance (ANOVA) was used to analyze the adhesion and invasion assay data, and two-way ANOVA was used to analyze the survival assay, and RT–qPCR results. The animal infection data were analyzed by measuring OD$_{600}$ from 0 to 24 h. After the deletion of the yqeH gene, APEC40-ΔyqeH showed a growth rate similar to that of WT strain APEC40 (Figure 2A). These data suggest that the deletion of the yqeH gene had no significant effect on the growth of APEC40.

Characterization of APEC40-ΔyqeH mutant strain

To determine whether the deletion of yqeH influenced the growth of APEC40, bacterial growth was evaluated by measuring OD$_{600}$ from 0 to 24 h. After the deletion of the yqeH gene, APEC40-ΔyqeH showed a growth rate similar to that of WT strain APEC40 (P < 0.05) (Figure 2B). The influence of yqeH on the bacterial morphology was examined by transmission electron microscopy. The results revealed remarkable differences in the flagella between the APEC40 and APEC40-ΔyqeH strains. Many long flagella were distributed at APEC40 periphery, but APEC40-ΔyqeH flagella were few and impaired. A few broken flagella appeared on the surface of APEC40-CΔyqeH (Additional file 2). Moreover, APEC40-ΔyqeH formed significantly less biofilm than WT strain APEC40 (Figure 2C).

Deletion of yqeH reduces bacterial adherence and invasion

As shown in Figure 3, the APEC40-ΔyqeH mutant showed reduced adherence to and invasion of DF-1 cells compared with those of WT strain APEC40.

Virulence of mutant strain APEC40-ΔyqeH

To investigate the effects of YqeH on bacterial pathogenicity, the virulence of the APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH strains was compared in a chick model. As shown in Figure 4A, the mortality rate was significantly lower after infection with strain APEC40-ΔyqeH (50%, 4/8) than after infection with strain APEC40 (100%, 8/8). The mortality rate after infection with strain APEC40-CΔyqeH was restored to 75% (6/8) of the WT level. These results indicate that YqeH contributes to APEC virulence.

To measure the influence of YqeH on APEC bacterial numbers in blood and tissues or organs in vivo, a systemic infection experiment was performed to assess bacterial proliferation in chicks. The bacterial loads in the blood, livers, spleens, and kidneys of infected chicks were determined at 24 h post-infection. With the removal of yqeH, the bacterial loads of APEC40-ΔyqeH were significantly lower than those of WT strain APEC40 in all
samples. Moreover, the virulence of APEC40-CΔyqeH was restored (Figure 4B).

**Effects of YqeH on the transcriptional profile of virulence-related genes**

A transcriptional analysis showed that 112 genes were upregulated and 101 genes were downregulated in APEC40-ΔyqeH relative to their expression in WT strain APEC40 (Figure 5A). The up-and downregulated genes (Additional file 3) were analyzed for their enrichment in KEGG pathways. The differentially expressed genes were enriched in pathways functions related to ABC transport, quorum sensing, bacterial chemotaxis, two-component system and flagellar assembly (Figure 5B). We selected several differentially expressed genes related to biofilm formation, bacterial mobility, and virulence for further study, as shown in Figure 5C.

**Verification of differentially expressed genes with RT–qPCR**

The RNA-seq results were validated with RT–qPCR. Eight genes representing a wide range of gene expression ratios in the yqeH mutant were selected for a comparative RT–qPCR analysis. The gene expression patterns determined with RT–qPCR were highly concordant with the RNA-seq results (Figure 6).

**Expression variations of type 1 fimbriae genes**

The expression of type 1 fimbriae genes fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH and fimI showed
Figure 2 Characteristics of strains APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH. A Growth curves of APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH in LB broth. Growth rates of APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH were monitored by measuring their OD₆₀₀ values in LB medium at 2 h intervals for 24 h. During culture for 24 h, there was no significant difference in the growth rates of APEC40, APEC40-ΔyqeH, and APEC40-CΔyqeH grown in LB broth (P > 0.05). Each value is the average of three independent experiments. B Bacterial motility. Swimming motility of strain APEC40-ΔyqeH was significantly lower than that of the wild-type APEC40 strain, whereas the swimming motility of complemented strain APEC40-CΔyqeH was restored. The experiment was repeated three times and all samples were measured in triplicate. C Biofilm formation by APEC strains as determined with crystal violet (CV) staining. APEC40-ΔyqeH showed significantly lower biofilm formation than wild-type strain APEC40 after crystal violet staining (**P < 0.01 for the WT vs. mutant).

Figure 3 Deletion of yqeH reduced the adherence and invasion abilities of APEC. Adherence to and invasion of DF-1 cells was significantly lower in mutant strain APEC40-ΔyqeH than in WT strain APEC40. (**P < 0.01 for WT vs. mutant).
no significant difference between the wild-type strain APEC40 and the yqeH mutant strain (Additional file 4).

**Discussion**

The ETT2 locus is present in most *E. coli* strains. However, the transcriptional regulators located in the ETT2 locus are considered key factors in the pathogenicity of *E. coli* [20]. Genomic studies have identified several transcriptional regulators, including YqeK/EtrB, EtrA, and EivF, located at the ETT2 locus, which regulate the expression of virulence genes in pathogenic *E. coli* [8]. In this report, we propose for the first time that YqeH is a transcriptional regulator located at the ETT2 locus of APEC, and describe it. We constructed a YqeH deletion mutant to identify the role of YqeH in APEC pathogenesis. However, the rescue assays for expression of YqeH in YqeH-mutant strain resulted in partial recovery. It is possibly due to deficient expression of YqeH by using a low-copy plasmid, pSTV28, as the complementation plasmid.

Biofilm formation is an important process by which bacteria establish infections, leading to host diseases [21]. In this study, APEC biofilm formation was significantly reduced after yqeH deletion compared with that of WT strain APEC40. Biofilm formation is a complex process, during which the expression of specific genes is required to produce specific substances that promote and regulate biofilm formation [22]. The effects of YqeH on the expression of biofilm-related genes in APEC were investigated by using a transcriptional analysis. In total, seven downregulated genes related to biofilm formation were enriched in differentially expressed genes of YqeH-deletion mutant compared with WT strain. Among them, mlrA, yoaD, and bdM were reported for their role in c-di-GMP signaling pathway. Moreover, it was reported that lsrB, lsrD, lsrF and lsrG mediated the biosynthesis of AI-2 protein in the quorum sensing signaling pathway. As a signaling molecule, AI-2 promotes the transformation of bacteria from suspension to biofilm modes, and promotes the transcription of bacterial genes that facilitate biofilm formation [23]. The second messenger c-di-GMP regulates biofilm formation by changing the concentrations of bacteria within host cells [24]. Our results show that after the deletion of yqeH, the expression levels of genes related to the synthesis of AI-2 and c-di-GMP were lower than in WT strain APEC40, which ultimately affected the formation of the APEC biofilm.

The flagellum is a motor organelle and a protein export apparatus that controls bacterial motility and behavior [25]. In this study, bacterial motility was significantly reduced after yqeH deletion compared with that in WT

---

**Figure 4** Animal systemic infection in vivo. A Determination of bacterial virulence. Seven-day-old chicks were inoculated intratracheally with $10^8$ colony-forming units (CFUs) of APEC40, APEC40-ΔyqeH, or APEC40-CΔyqeH. Chicks that were administered PBS were used as negative control. Survival was monitored for 7 days post-infection. B Bacterial colonization and proliferation in chicks. Groups of eight 7-day-old chicks were intratracheally infected with bacteria ($10^8$ CFUs). Bacteria were recovered from the lung, blood, liver, and spleen at 24 h post-infection. The nonparametric Mann–Whitney U-test was used to determine statistical significance (**P < 0.01, ***P < 0.001 for WT vs. mutant).
Transcriptomic profiling showed that the transcription of *flgC*, *flgD*, *fliT*, and *fliR* was similarly reduced. FlgC is the flagellar basal-body protein; FlgD is essential for flagellar hook formation; FliT is the flagellar synthesis and assembly chaperone protein; and FliR is the flagellar export pore protein [26, 27]. Therefore, we speculate that the reduced expression of these genes impaired flagellum synthesis in the bacteria, resulting in reduced bacterial motility.

The bacterial colonization and invasion of host cells are crucial steps in APEC infection process [28, 29]. Type 1 fimbriae contribute to bacterial adhesion and invasion of host cells, which create conditions for a series of processes, such as pathogen colonization and infection [30, 31]. However, our results (Additional file 4) showed that the transcript expression of type 1 fimbriae genes had no significant differential changes after the deletion of *yqeH*. The flagellum has been shown to mediate bacterial adhesion and invasion, and is implicated in the virulence of strain APEC40.
pathogenic bacteria. The motility of the flagellum is an important virulence feature of many bacterial pathogens and is necessary for the establishment of infection [32]. For example, the FlgC protein plays an important role in the binding of Salmonella enteritidis to host epithelial cells [33], and the deletion of the flagellar flIR gene affects the adhesion and pathogenicity of bacteria to host cells [34]. Biofilm formation is also strongly related to the ability of bacteria to adhere to and invade cells. Several studies have shown that the biofilm-synthesis-related gene pgaC, encoding N-glycosyltransferase, not only mediates biofilm formation, but also plays an important role in bacterial adhesion to its host cell [35]. The quorum-sensing transcriptional activator SdiA regulates the expression of the rck gene, which mediates E. coli adherence to and invasion of epithelial cells [36]. Our results show that the loss of yqeH reduced bacterial motility and biofilm formation, which may have accounted for the diminished adhesion to and invasion of DF-1 cells by mutant APEC40-ΔyqeH. The adhesion and colonization abilities of APEC in the host blood and tissues are key factors in its pathogenesis [37]. The numbers of viable cells of deleted strain APEC40-ΔyqeH in the host blood and various tissues were significantly lower than those in the WT strain. Our experimental results demonstrate that YqeH is involved in APEC virulence.

In summary, we have demonstrated for the first time that the transcriptional regulator YqeH is involved in the motility, biofilm formation, and virulence of APEC strains. This study provides a basis for further functional research into the pathogenic role of YqeH in APEC.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13567-022-01049-6.

Acknowledgements

This work was supported by grants from the National Science Foundation of China (grant no. 31772707, 31972644), Natural Science Foundation of Anhui Province (grant no. 1080085QC137).

Author contributions

Conceptualization, KQ and LY; methodology, BC; software, XS; validation, LY; XP and KQ; formal analysis, YS; investigation, XS; resources, JT; data curation, BC; writing—original draft preparation, LY; writing—review and editing, LY; visualization, KQ; supervision, YS; project administration, XP; funding acquisition, KQ; JT and LY. All authors read and approved the final manuscript.

Availability of data and materials

The sequence was deposited in the GenBank database (Accession number PRJNA805014).

Declarations

Ethics approval and consent to participate

The animal experiments were approved by the Institutional Animal Care and Use Committee at the Institute of Animal Husbandry and Veterinary Science, Anhui Academy of Agricultural Sciences (Permit No: AAAS-IAHVS-Po-2020-0034).

Competing interests

The authors declare that they have no competing interests.

Received: 26 November 2021 Accepted: 10 March 2022
Published online: 18 April 2022

References

1. Apostolakos I, Laconi A, Mughini-Gras L, Yapicier O, Piccirillo A (2021) Occurrence of colibacillosis in broilers and its relationship with avian pathogenic Escherichia coli (APEC) population structure and molecular characteristics. Front Vet Sci 8:737720. https://doi.org/10.3389/fvets.2021.737720

2. Paudel S, Fink D, Abdelhamid MK, Zäggele A, Liebhart D, Hess M, Hess C (2021) Aerosol is the optimal route of respiratory tract infection to induce pathological lesions of colibacillosis by a lux-tagged avian pathogenic Escherichia coli in chickens. Avian Pathol 50:417–426. https://doi.org/10.1080/03079457.2019.1798392

3. Pérez-Rueda E, Collado-Vides J (2000) The repertoire of DNA-binding transcriptional regulators in Escherichia coli K-12. Nucleic Acids Res 28:1838–1847. https://doi.org/10.1093/nar/28.8.1838

4. Marbaniang CN, Gowrishankar CN (2012) Transcriptional cross-regulation between Gram-negative and gram-positive bacteria, demonstrated using ArgP-argO of Escherichia coli and LysG-lye of Corynebacterium glutamicum. J Bacteriol 194:5657–5666. https://doi.org/10.1128/jb.00947-12

5. Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176:269–275. https://doi.org/10.1128/JB.176.2.269-275.1994

6. Slater SL, Ságors AM, Pollard DJ, Ruano-Gallego D, Frankel G (2018) The type III secretion system of pathogenic Escherichia coli. Curr Top Microbiol Immunol 416:51–72. https://doi.org/10.1007/82_2018_116

7. Zhou M, Guo Z, Duan Q, Hardwidge PR, Zhu G (2014) Escherichia coli type III secretion system 2: a new kind of T3SS? Vet Res 45:32. https://doi.org/10.1186/1297-9176-45-32

8. Wang S, Xu X, Liu X, Wang D, Liang H, Wu X, Tian M, Ding C, Wang G, Yu S (2017) Escherichia coli type III secretion system 2 regulator EtrA promotes virulence of avian pathogenic Escherichia coli. Microbiology 163:1515–1524. https://doi.org/10.1099/mic.0.000525

9. Zhang L, Chaudhuri RR, Constantinidou C, Hobman JL, Patel MD, Jones AC, Sari D, Roe AJ, Vlisidou I, Shaw RK, Falciani F, Stevens MP, Gally DL, Knutton S, Frankel G, Penn CW, Falciani F, Shaw UK, Roe AJ, Vlisidou I, Steven M (2004) Regulators encoded in the Escherichia coli type III secretion system 2 gene cluster influence expression of genes within the locus for enterocyte effacement in enterohemorrhagic E. coli O157:H7. Infect Immun 72:7282–7293. https://doi.org/10.1128/iai.72.12.7282-7293.2004

10. Luzader DH, Willsley GC, Wargo MJ, Kendall MM (2016) The type three secretion system 2-encoded regulator EtrB modulates enterohemorrhagic Escherichia coli virulence gene expression. Infect Immun 84:2555–2565. https://doi.org/10.1128/iai.00407-16

11. Yin L, Li Q, Wang Z, Tu J, Shao Y, Song X, Pan X, Qi K (2021) The role of Escherichia coli type III secretion system 2 chaperone protein ygeG in pathogenesis of avian pathogenic Escherichia coli. Res Vet Sci 140:203–211. https://doi.org/10.1016/j.resv.2021.09.011

12. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010) Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160:1577–1579. https://doi.org/10.1111/j.1476-5381.2010.00872.x
13. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci USA 97:6640–6645. https://doi.org/10.1073/pnas.120163297
14. Wang S, Liu X, Xu X, Yang D, Wang D, Han X, Shi Y, Tian M, Ding C, Peng D, Yu S (2016) Escherichia coli type III secretion system 2 ATPase EvoC is involved in the motility and virulence of avian pathogenic Escherichia coli. Front Microbiol 7:1387. https://doi.org/10.3389/fmicb.2016.01387
15. Yin L, Shen X, Zhang D, Zhao R, Dai Y, Hu X, Zhou X, Hou H, Pan X, Qi K (2021) Flagellar rotor protein FlgK is involved in the virulence of avian pathogenic Escherichia coli. Microb Pathog 160.105198. https://doi.org/10.1016/j.micpath.2021.105198
16. Zuo J, Tu C, Wang Y, Qi K, Hu J, Wang Z, Mi R, Yan H, Chen Z, Han X (2019) The role of the wzy gene in lipopolysaccharide biosynthesis and pathogenesis of avian pathogenic Escherichia coli. Microb Pathog 127:296–303. https://doi.org/10.1016/j.micpath.2018.12.021
17. Zuo J, Yin H, Hu J, Miao J, Chen Z, Qi K, Wang Z, Gong J, Phouthapane V, Jiang W, Mi R, Huang Y, Wang C, Han X (2019) Lsr operon is associated with AI-2 transfer and pathogenicity in avian pathogenic Escherichia coli. Vet Res 50.109. https://doi.org/10.1186/s13567-019-0725-0
18. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616
19. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
20. Shulman A, Yair Y, Biran D, Sura T, Otto A, Gophna U, Becher D, Hecker M, Ron EZ (2018) The Escherichia coli type III secretion system 2 has a global effect on cell surface. mBio. https://doi.org/10.1128/mBio.01701-18
21. Yin L, Li Q, Xue M, Wang Z, Tu J, Song X, Shao Y, Han X, Xue T, Liu H, Qi K (2019) The role of the phoP transcriptional regulator on biofilm formation of avian pathogenic Escherichia coli. Avian Pathol 48:362–370. https://doi.org/10.1080/03079457.2019.1605147
22. Wang Y, Zhang W, Wu Z, Zhu X, Lu C (2011) Functional analysis of luxS in Streptococcus suis reveals a key role in biofilm formation and virulence. Vet Microbiol 152:151–160. https://doi.org/10.1016/j.vetmic.2011.04.029
23. Zeng B, Xu K, Zhang X, Zhang Y, Chen G, Chen F, Zeng W, Li J, Zhu L, He Q (2019) The AI-2/luxS quorum sensing system affects the growth characteristics, biofilm formation, and virulence of Haemophilus parasuis. Front Cell Infect Microbiol 9:62. https://doi.org/10.3389/fcimb.2019.00062
24. Moreira RN, Dressaire C, Barahona S, Galego L, Kaever V, Jenal U, Arraijo CM (2017) BoIA is required for the accurate regulation of c-di-GMP, a central player in biofilm formation. mBio. https://doi.org/10.1128/mBio.00443-17
25. Chaban B, Hughes HV, Beeby M (2015) The flagellum in bacterial pathogens: motility and a whole lot more. Semin Cell Dev Biol 49:91–103. https://doi.org/10.1016/j.semcdb.2015.10.032
26. Denne JC, Johnson S, Vickey C, Aron A, Monkhouse H, Griffiths T, James RH, Berks BC, Coulton JW, Stansfeld PJ, Lea SM (2020) Structures of the stator complex that drives rotation of the bacterial flagellum. Nat Microbiol 5:1553–1564. https://doi.org/10.1038/s41564-020-0788-8
27. Saijo-Hamano Y, Uchida N, Namba K, Oosawa K (2004) In vitro characterisation of FlgB, FlgC, FlgF, FlgG, and FlIE, flagellar basal body proteins of Salmonella. J Mol Biol 339:423–435. https://doi.org/10.1016/j.jmb.2004.03.070
28. Newman DM, Barbieri NL, de Oliveira AL, Willis D, Nolan LK, Logue CM (2021) Characterising avian pathogenic Escherichia coli (APEC) from coli bacillosis cases, 2018. PeerJ 9:e11025. https://doi.org/10.7717/peerj.11025
29. Mellata M, Dho-Moulin M, Dozois CM, Curtis R 3rd, Lehoux B, Fairbrother JM (2003) Role of avian pathogenic Escherichia coli virulence factors in bacterial interaction with chicken heterophils and macrophages. Infect Immun 71:494–503. https://doi.org/10.1128/iai.71.1.494-503.2003
30. Lillington J, Geibel S, Waksman G (2014) Bio genesis and adhesion of type 1 and P pili. Biochim Biophys Acta 1840:2783–2793. https://doi.org/10.1016/j.bbagen.2014.04.021
31. Spauld CN, Schreiber HL, Zheng W, Dodson KW, Hazen JE, Conover MS, Wang F, SvenmarkkerLuna-RicoFrancetse PACO, Andersson M, Hultgren S, Egelman EH (2018) Functional role of the type 1 pilus rod structure in mediating host-pathogen interactions. Elife 7:e31662. https://doi.org/10.7554/eLife.31662
32. Sevrin G, Massier S, Chassaing B, Agus A, Delmas J, Denizot J, Billard E, Barnich N (2020) Adaptation of adherent-invasive E. coli to gut environment: impact on flagellum expression and bacterial colonization ability. Gut Microbes 11:364–380. https://doi.org/10.1080/19490976.2017.1421886
33. Shippy DC, Eakley NM, Mikhail DM, Fadl AA (2014) Role of the flagellar basal-body protein, FlgC, in the binding of Salmonella enterica serovar Enteridis to host cells. Curr Microbiol 68:621–628. https://doi.org/10.1007/s00284-014-0521-z
34. Awan P, Wang XY, Sun AH, Li SJ, Yan J (2008) Association of fliC gene in Lepotopisa interorganis with adhesion and pathogenicity to host cells. Zhejiang Da Xue Xue Bao Yi Xue Ban 37:572–578
35. Chen KM, Chiang MK, Wang M, Ho HC, Lu MC, Lai YC (2014) The role of pgC in Klebsiella pneumoniae virulence and biofilm formation. Microb Pathog 77:89–99. https://doi.org/10.1016/j.micpath.2014.11.005
36. Smith JL, Fratamico PM, Yan X (2011) Eavesdropping by bacteria: the role of SdiA in Escherichia coli and Salmonella enterica serovar Typhimurium quorum sensing. Foodborne Pathog Dis 8:169–178. https://doi.org/10.1089/fpd.2010.0651
37. Zhang Y, Wang Y, Zhu H, Yi Z, Afaybo DIA, Tao C, Li T, Tian M, Qi J, Ding C, Yu S, Wang S (2021) DctR contributes to the virulence of avian pathogenic Escherichia coli through regulation of type III secretion system 2 expression. Vet Res 52:101. https://doi.org/10.1186/s13567-021-00970-6

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.