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IbeB is involved in the invasion and pathogenicity of avian pathogenic *Escherichia coli*

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ABSTRACT

The *ibeB* gene in neonatal meningitis *Escherichia coli* (NMEC) contribute to the penetration of human brain microvascular endothelial cells (HBMECs). However, whether IbeB plays a role in avian pathogenic *E. coli* (APEC) infection remains unclear. Thus, this study was conducted to investigate the distribution of the *ibeB* gene in Chinese APEC strains and examine whether IbeB is involved in APEC pathogenicity. The *ibeB* gene was found in all 100 detected *E. coli* isolates with over 97% sequence homology. These results indicated that *ibeB* is a conserved *E. coli* gene irrelevant of pathotypes. To determine the role of *ibeB* in APEC pathogenicity, an *ibeB* mutant of strain DE205B was constructed and characterized. The inactivation of *ibeB* resulted in reduced invasion capacity towards DF-1 cells and defective virulence in animal models as compared to the wild-type strain. Animal infection experiments revealed that loss of *ibeB* decreased APEC colonization and invasion capacity in brains and lungs. These virulence-related phenotypes were partially recoverable by genetic complementation. Reduced expression levels of invasion- and adhesion-associated genes in *ibeB* mutant could be major reasons as evidenced by reduced *ibeA* and *ompA* expression. These results indicate that IbeB is involved in APEC invasion and pathogenicity.

1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) predominantly belongs to phylogenetic group B2, which is currently categorized as newborn meningitis *E. coli* (NMEC), uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* (APEC), and septicemia-associated *E. coli* (SEPEC) based on their original hosts and clinical symptoms (Dho-Moulin and Fairbrother, 1999; Ewers et al., 2003; Johnson and Russo, 2002; Kim, 2000). ExPEC causes urinary tract infections (UTIs) and meningitis in humans (Blondeau, 2004). Similarly, APEC enters and colonizes the avian respiratory tract by inhalation of fecal dust leading to localized infections such as airsacculitis and pneumonia. In some cases, APEC can spread to various internal organs, causing pericarditis, perihepatitis, peritonitis, salpingitis and other extraintestinal diseases, thereby resulting in systemic infections that are economically devastating to the poultry industry (Dho-Moulin and Fairbrother, 1999; Ewers et al., 2003; Rodriguez-Siek et al., 2005b).

Although certain ExPEC subset strains isolated from different host organisms exhibit considerable genomic diversity, they possess a broad range of similar virulence factors (Ewers et al., 2007; Johnson et al., 2008; Moulin-Schouleur et al., 2006; Rodriguez-Siek et al., 2005a). Virulence determinants common to UPEC, APEC, NMEC or/and SEPEC, have been identified, such as the aerobactin

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iron transport system, Ibe proteins (IbeA, IbeB, IbeC), the K1 capsule, and types 1 and P fimbriae (Bahrani-Mougeot et al., 2002; Dho-Moulin and Fairbrother, 1999; Gunther et al., 2002; Hoffman et al., 1999; Mobley et al., 1993; Pourbakhsh et al., 1997; Torres et al., 2001). Mounting evidence has revealed that poultry may be a vehicle or even a reservoir for *E. coli* capable of causing UTIs and newborn meningitis (Ewers et al., 2007). Thus, it is necessary to study APEC zoonotic potential.

Recently, several virulence determinants were identified by transposon mutagenesis, including the Ibe proteins, OmpA, AslA, and the K1 capsule that contribute to NMEC invasion of brain microvascular endothelial cells (BMECs) (Hoffman et al., 2000; Prasadarao et al., 1999; Wang et al., 1999; Wang and Kim, 2002). In a previous study, we showed that IbeA contributes to the invasion of APEC to DF-1 cells and brain during animal infection (Wang et al., 2011a). However, whether the IbeB protein contributes to APEC pathogenicity remains unclear.

This study attempted to examine the distribution of the *ibeB* gene in Chinese APEC strains. An *ibeB* mutant and complementary strains were constructed, and the virulence, invasion capacity, and virulence factor expression associated with invasion and adhesion were evaluated to aid in deciphering the role of the *ibeB* gene in APEC pathogenicity.

2. Materials and methods

2.1. Bacterial strains, plasmids, and growth conditions

Strains and plasmids used in this study are listed in Table 1. The APEC strain DE205B was isolated from the brain of a duck with septicemia and neurological symptoms, which belonged to the phylogenetic *E. coli* reference (ECOR) group B2 as determined by phylogenic analysis using multiplex PCR (Clermont et al., 2000; Wang et al., 2011a). This strain harbored virulence-associated genes *tsh, mat, fyuA, irp2, iucD, iutA, iss, aatA, vat, malX, fimC, ompA, ibeA, ibeB, yijp, gimB* and *aslA*, but was negative for *papC* and *hlyA* by PCR analysis (Wang et al., 2011a,b). Strain DE205B was used for mutant construction, infection studies and functional assays.

In addition, a total of 100 APEC strains, isolated from ducks with clinical signs of colibacillosis at different times, and from different areas of eastern China, were used to determine the prevalence of *ibeB*. *E. coli* strain DH5α was used for cloning procedures, and BL21 (DE3) cells were used for protein expression (Davanloo et al., 1984; Studier and Moffatt, 1986). All *E. coli* strains were grown in Luria–Bertani (LB) medium at 37 °C with aeration. When necessary, LB medium was supplemented with appropriate antibiotics: ampicillin (Amp; 100 μg mL^{-1}), kanamycin (Kan; 50 μg mL^{-1}) or nalidixic acid (Nal; 50 μg mL^{-1}) unless otherwise specified.

2.2. DNA and genetic manipulations

DNA manipulations and transformations were performed using standard methods. All restriction enzymes were purchased from TaKaRa (Dalian, China). Plasmid DNA was isolated using the High Pure Plasmid Miniprep Kit (Invitrogen, Shanghai, China). PCR products and DNA extraction from agarose gels were purified using the Agarose Gel DNA Fragment Recovery Kit (TaKaRa) according to the manufacturer's guidelines. For *ibeB* sequencing and expression, TaKaRa PrimeSTAR® HS DNA Polymerase was used for PCR, whereas the 2× PCR premix (TIANGEN Biotech, Beijing, China) was used in screening assays according to the manufacturer's instructions. The primers used in this study are shown in Table 2. The prevalence of *ibeB* was assessed by PCR using primers ibeB-F and ibeB-R. DNA and amino acid sequence analyses were performed using the DNASTAR Lasergene 7 software (http://www.dnastar.com/t-products-lasergene.aspx) and the National Center for Biotechnology Information (NCBI) online BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Expression of IbeB, antibody production and immunoblotting

The *ibeB* open reading frame (ORF), except for the sequence encoding the signal peptide (1–63 bp), was amplified with primers WSH91F and WSH92R adding *Nhe*I and *Xho*I recognition sites (Table 2). The obtained PCR

Table 1
Bacterial strains and plasmids used in this study.

Strain or plasmid	Characteristics	Reference
Strain		
DE205B	O2:K1 Nal^R	
DE205B4	DE205BΔ*ibeB*	This study
DE205B5	DE205B4 with plasmid pUC18	This study
DE205B6	DE205B4 with plasmid pUC18-*ibeB*	This study
DH5α	F-, *Δ(lacZYA-argF)U169, recA1, endA1, hsdR17*(*rk−, mk+*), *phoA, supE44,* λ-	TIANGEN
BL21 (DE3)	F-, *ompT, hsdS* ($r_B^-m_B^-$)*gal, dcm* (DE3)	TIANGEN
Plasmid		
pET28a (+)	Kan, F1 origin, His tag	Novagen
pET28a-*ibeB*	pET28a (+) carrying *ibeB* gene	This study
pMD® 18-T Vector	Amp, lacZ	Takara
pUC18	Amp, lacZ	Takara
pUC18-*ibeB*	pUC18 carrying *ibeB* ORF and its putative promoter	This study
pKD46	Amp; expresses λ red recombinase	Datsenko and Wanner (2000)
pKD4	*kan* gene, template plasmid	Datsenko and Wanner (2000)
pCP20	Cm, Amp, yeast Flp recombinase gene, FLP	Datsenko and Wanner (2000)