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Role of hypothetical protein YicS in the pathogenicity of Avian Pathogenic *Escherichia coli in vivo* and *in vitro*

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ABSTRACT

Avian Pathogenic *Escherichia coli* (APEC) strains belong to the extra-intestinal pathogenic group of *E. coli* (ExPEC) that causes colibacillosis in poultry. A variety of putative virulence factors of APEC are recognized as potent causes of pathogenicity, the mechanisms underlying their pathogenicity are still not fully understood. The role of *yicS* in the virulence of pathogenic *E. coli* is still unclear. Thus, *yicS* may be related to biofilm formation, which in some bacteria plays a role in pathogenicity. Therefore, the fact that this gene appears to be under positive selection pressure suggests that *yicS* may be associated with the pathogenicity of APEC. To better understand the role of *yicS* protein in APEC biological characteristics and pathogenicity, we deleted *yicS* in an APEC Swollen Head Syndrome strain (APEC strain SCI-07) and studied its effects by comparing wild type and isogenic mutants through comprehensive *in vitro* and *in vivo* assays. We demonstrated that *yicS* plays a role in pathogenicity of APEC. We suggest that the *yicS* gene, which encodes an exporter protein, has a significant role in biofilm formation, motility, invasion of CEC-32 and Hep-2 cells and APEC pathogenicity in a day-old chick model.

1. Introduction

Avian Pathogenic *Escherichia coli* (APEC) strains belong to the extra-intestinal pathogenic group of *E. coli* (ExPEC) that causes colibacillosis in poultry. These strains cause high morbidity and mortality rates in chickens and are considered an economic threat to the poultry industry worldwide (Barnes et al., 2003). A variety of bacterial factors, such as adhesins, toxins, iron acquisition systems, two-component regulatory systems, miscelleanous related virulence genes, colicin V plasmid, serum resistance proteins, vacuolating autotransporter toxin (Vat), capsule and lipopolysaccharide complexes, as well as exporter or transporter proteins, have been known to act as putative virulence factors of APEC (Barnes et al., 2003; de Paiva et al., 2015a; Dho-Moulin and Fairbrother, 1999; Janben et al., 2001; Parreira and Gyles, 2003; Guabiraba and Schouler, 2015; Verma et al., 2015). Although these virulence factors are recognized as potent causes of pathogenicity, the mechanisms underlying their pathogenicity are still not fully understood.

In recent years, genome-wide analyses have led to a better knowledge about the gene content and genome organization of many different groups of bacteria (Li et al., 2005). Although these techniques provide valuable information for analyses of bacterial virulence, they do no shot a direct relationship with genes and pathogenicity mechanisms.

Positive selection analyses can identify genes that contain a significantly higher than expected frequency of non-synonymous mutations, indicating selection for the emergence of new alleles in place of the old gene copy. If these new alleles are under positive selection, they may be responsible for enhanced fitness, which can indicate participation of this gene in the parasite-host relationship in pathogenic bacteria (Aguileta et al., 2010; Aguileta et al., 2009). In previous work, our group together with the Multiuser Bioinformatics Analysis Laboratory, from EMBRAPA, Brazil, performed positive selection analyses of APEC genomes and identified several genes, including *yicS* that are under positive selection pressure (Rojas et al., 2017).

The role of *yicS* in the virulence of pathogenic *E. coli* is still unclear. The hypothetical protein YicS is highly conserved in *E. coli*, *Salmonella* spp., *Shigella* spp., and *Citrobacter rodentium* (Morin et al., 2013). In addition, *yicS* is located next to the gene *csgD* (Keseler et al., 2013), which is important for the control of biofilm matrix production in *E. coli*. Thus, *yicS* may be related to biofilm formation, which in some bacteria (Wang et al., 2011) plays a role in pathogenicity. Therefore,

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Table 1
List of Strains and Plasmids used in this work.

Strains or Plasmid	Relevant genotype	Source or reference
Strains		
SCI-07	APEC strain isolated from lesions of a laying hen presenting clinical signs of Swollen Head Syndrome	From our laboratory
DH10β	K-12 *E.coli* strain	From our laboratory
HB101	K-12 *E. coli* strain	From our laboratory
DH5α	K-12 *E. coli* strain	From our laboratory
Plasmids		
pKD3	*cat* gene	(Datsenko and Wanner, 2000)
pKD46	Amp^R express λ red recombination	(Datsenko and Wanner, 2000)
pACYC177	Cloning vector	New England Biolabs

Table 2
List of Primers and Sequences used in PCR and qRT-PCR.

Primers	Sequences (5′ → 3′)
Primers used for mutagenesis	
Forward Primer (F Δ*yicS*)	CAAACTACACTTATGCTTCATTCGATGCACATTTGCAGAAGGTTGCCGCT GTGTAGGCTGGAGCTGCTTC
Reverse Primer (R Δ*yicS*)	GAAATCCTGATATCAGGTAAAGAAGCGTTGGTCAGTGGTTGTTGCCGTTC CATATGAATATCCTCCTTAG
Primers used for mutant confirmation	
Internal primer	
Forward primer (F *yicS*-1)	ATGAAGCCAACGATGCTACT
Reverse primer (R *yicS*-2)	TTACATATCCGGGCATTCTA
Primers used for mutant confirmation	
External primer	
Forward primer (F *yicS*-3)	ATTTGCAGAAGGTTGCCGCT
Reverse primer (R *yicS*-4)	GTCAGTGGTTGTTGCCGTTC
Primers used for genetic complemented	
Forward Primer (F-*C.*Δ*yicS*)	TCTT ***aagctt***ATGAAGCCAACGATGCTACT
Reverse Primer (R-*C.* Δ*yicS*)	TCTT ***cacgttgtg***TTACATATCCGGGCATTCTA
Primers used in qRT-PCR	
Forward Primer *yicS*	ACAGTCGGCAAAAGAGAAAACC
Reverse Primer *yicS*	TGTGGCGTGCAAATTTTACG
Forward Primer *rpoA*	GCGCTCATCTTCTTCCGA
Reverse Primer *rpoA*	CGCGGTCGTGGTTATGTG
Forward Primer *fliC*	GCCGTTGAACTGGGTCTGA
Reverse Primer *fliC*	CAAATCCCGTCTCGACGAA
Forward Primer *flhD*	TCTTGCGCAGCGCTTCT
Reverse Primer *flhD*	GCTCTCAACTCGCTTGCTGAA
Forward Primer *ompR*	GCGTTCCATTGCGGAATATT
Reverse Primer *ompR*	CCCGCGATAAGCTGATGAA
Forward Primer *flgE*	CCTCAACTCCATGCAGCAAA
Reverse Primer *flgE*	TTCTGGGTGGTTGCCACAA
Forward Primer *flgN*	GACCGTTCGCCCCATACA
Reverse Primer *flgN*	GGCGCTGGAAATGTTGAAAC
Forward Primer *marB*	AGGGCGTTGCGGAACA
Reverse Primer *marB*	CGACATTGCCACAGGAAGTAAC
Forward Primer *yehR*	ATGCCGCCAGGACACTTG
Reverse Primer *yehR*	TCTTCAACACCCGCGATGT
Forward Primer *wcaM*	GCCCTCCTCCCCTGTCA
Reverse Primer *wcaM*	AATGGCAGAGGGCGATTTG
Forward Primer *nlpA*	AAAGGGTCGATGCTGGAAGA
Reverse Primer *nlpA*	CGAACGATGCGACAAATCAT
Forward Primer *ppdC*	CGATGTCTGCATTCGGTTGA
Reverse Primer *ppdC*	CTGCGCGCGATTTCG
Forward Primer *ynfC*	AAAACAGCCTTCTTCCGACAAA
Reverse Primer *ynfC*	TGAGCAAGGTGAAGTGACGAA
Forward Primer *yccT*	GAGACTGCGGCGGAATAGC
Reverse Primer *yccT*	AGATGACAGTACGCTGCTTTCG
Forward Primer *ydcK*	GCTCCTCGCCGTTAATGACT
Reverse Primer *ydcK*	CCTTTGATGGCGACACGAT
Forward Primer *rstA*	GTGCACTGATTGCCGCATAC
Reverse Primer *rstA*	GCGCGGCTCTACGGTAAC
Forward Primer *rstB*	CAGGGATTGCCCCAGAAA
Reverse Primer *rstB*	CGAGGCGAACAAAAGGTTCA
Forward Primer *iucA*	ACTGCTGACAGGACACGCTTT
Reverse Primer *iucA*	GGCGGTTAAACGGTTCATGA

Bold italic letters indicate restriction enzymes sites in the complement primer.

the fact that this gene appears to be under positive selection pressure suggested that *yicS* may be associated with the pathogenicity of APEC strains.

To better understand the role of *yicS* protein in APEC biological characteristics and pathogenicity, we deleted *yicS* in an APEC Swollen Head Syndrome strain (APEC strain SCI-07) (de Paiva et al., 2015b; Verma et al., 2015) and studied its effects by comparing wild type and isogenic mutants through comprehensive *in vitro* and *in vivo* assays.