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In silico analysis of *tkt1* from avian pathogenic *Escherichia coli* and its virulence evaluation in chickens

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Abstract

Extraintestinal pathogenic *Escherichia coli* (ExPEC) contain *tktA* and *tktB* which code for transketolases involved in the pentose phosphate pathway. Recent studies demonstrated that a third gene coding for transketolase 1 (*tkt1*) was located in a pathogenicity island of avian and human ExPEC belonging to phylogenetic group B2. In the present study, in silico analysis of *tkt1* revealed 68% and 69% identity with *tktA* and *tktB*, respectively, of ExPEC and 68% identity with *tktA* and *tktB* of *E. coli* MG1655. The translated *tkt1* shared 69% and 68% identity with TktA and TktB proteins, respectively, of ExPEC and *E. coli* MG1655. Phylogenetically, it is shown that the three genes (*tktA*, *tktB* and *tkt1*) cluster in three different clades. Further analysis suggests that *tkt1* has been acquired though horizontal gene transfer from plant-associated bacteria within the family *Enterobacteriaceae*. Virulence studies were performed in order to evaluate whether *tkt1* played a role in avian pathogenic *E. coli* CH2 virulence in chickens. The evaluation revealed that mutant virulence was slightly lower based on LD50 when compared to the wild type during infection of chickens, but there were no significant differences when the two strains were compared based on the number of deaths and lesion scores.

Keywords: In silico; Horizontal gene transfer; Transketolase; Avian pathogenic *Escherichia coli*; Extraintestinal pathogenic *E. coli*

1. Introduction

Escherichia coli colonizes a variety of environments as a harmless commensal, but also causes a repertoire of intestinal and extraintestinal infectious diseases in man and animals. The extraintestinal pathogenic *E. coli* (ExPEC) group includes pathogens causing urinary tract infections (uropathogenic *E. coli*, or UPEC), neonatal meningitis *E. coli* (NMEC) and septicemia, and also those which are pathogenic to poultry (avian pathogenic *E. coli*, or APEC) (Barnes et al., 2008; Kaper et al., 2004).

The enzyme transketolase is found in animals, plants and bacteria, catalyzing reversible transfer of a ketol group in the pentose phosphate pathway (PPP) (Josephson and Fraenkel, 1969, 1974). The PPP is responsible for production of essential cell constituents such as amino acids, NADPH and several sugar phosphate intermediates (Iida et al., 1993; Zhao and Winkler, 1994). Among other functions, this pathway plays a protective role during oxidative stress by production of reducing power via NADPH (Kletzien et al., 1994; Pandolfi et al., 1995). *E. coli* K-12 contains two transketolase isoenzymes encoded by the *tktA* and *tktB* genes on its chromosome (Harinarayanan et al., 2008; Iida et al., 1993; Josephson and Fraenkel, 1969). TktA is the major isoenzyme and is responsible for about 70–90% of transketolase activity in cells grown under the limited physiological conditions examined to date. The cellular activity of the TktB isoenzyme was reported to be very low, but each of the two isoenzymes contributes to the strength of transketolase activity in the cell (Harinarayanan et al., 2008; Zhao and Winkler, 1994). The *tktA*

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gene was shown to be induced in the exponential growth phase, while the *tktB* gene was induced in the stationary growth phase (Jung et al., 2005).

Previously, a DNA fragment containing a putative gene coding for transketolase 1 (*tkt1*) was identified as being virulence-associated in APEC using the genomic subtractive hybridization technique. The fragment was reported to be absent in *E. coli* MG1655 and diarrheagenic *E. coli*, but present in ExPEC where *tktA* is also present (Schouler et al., 2004). Moreover, recent studies demonstrated that *tkt1* is contained in a novel pathogenicity island in avian and human ExPEC strains (Johnson et al., 2007; Li et al., 2012) and it was suggested that *tkt1* contributes to the fitness and/or virulence of these strains. The *tkt1* gene was also identified as an APEC virulence-associated gene in a study performed using signature-tagged mutagenesis (STM) (Li et al., 2005) and also as a gene induced in vivo during APEC infections in chickens using recombinase-based in vivo expression technology (RIVET) (Tuntufye et al., 2012). In the present study, a comparative in silico analysis was performed to obtain an overview of the differences among the different transketolase isoenzyme genes present in APEC. Moreover the APEC *tkt1* was evaluated in vivo for virulence in chickens by the use of deletion mutant.

2. Material and methods

2.1. Bacteria strains, plasmids, media and growth conditions

Bacteria strains and plasmids used in this study are mentioned in Table 1. The APEC CH2 strain (O78) was isolated from an infected chicken and was shown to be virulent in experimentally infected chickens (Vandemaele et al., 2005). Lysogeny broth (LB broth and agar) medium and SOC medium were prepared as described elsewhere (Sambrook et al., 1989). All bacteria were routinely grown in LB broth or agar media at 37 °C unless stated otherwise. Antibiotics for plasmids and/or recombinants selection were added at 100 μg ml^{-1} for ampicillin (Amp) and at 35 μg ml^{-1} for chloramphenicol (Cm). *E. coli* strain Top10F′ (Invitrogen, Paisley, Scotland) was used as the cloning strain.

2.2. DNA manipulations

DNA manipulations were performed as described elsewhere (Sambrook et al., 1989). Electrocompetent cells were prepared using standard procedures unless stated otherwise (Sambrook et al., 1989). Electroporation was carried out as previously described (Tuntufye and Goddeeris, 2011). APEC CH2 genomic DNA was extracted using the Wizard® Genomic DNA purification kit (Promega BNL B.V.), while plasmid DNA and DNA fragments were purified using commercial kits purchased from Fermentas (St. Leon-Rot, Germany). DNA restriction and modification enzymes were purchased from Fermentas and used as recommended by the manufacturer. PCR amplifications were performed using AccuPrime™ *Taq* High Fidelity polymerase (Invitrogen) or SuperTaq polymerase (SphaeroQ, Leiden, The Netherlands). Oligonucleotides were obtained from Sigma–Aldrich (Bornem, Belgium).

2.3. PCR and sequencing of a putative gene tkt1

A purified APEC CH2 genomic DNA template was used to amplify the *tkt1* gene using primer pair HT107 (Table 2) which was designed based on the sequenced APEC O1 genome in the database (Accession number: NC_008563.1). Initial denaturation was performed at 94 °C for 2 min followed by 30 cycles of [denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and elongation at 68 °C for 2 min], and a final elongation at 68 °C for 5 min. The reactions were performed using AccuPrime™ *Taq* High Fidelity polymerase (Invitrogen) in a Biometra T3000 thermocycler (Westburg, The Netherlands). PCR products were purified and stored at −20 °C. Samples were prepared according to the Macrogen EZ-sequencing manual and sequenced using automatic DNA sequencer 3730XL under BigDye™ terminator cycling conditions (Macrogen Europe, The Netherlands).

2.4. Comparative in silico analysis of tkt1

DNA sequenced reads were assembled using the CLC Main Workbench 5.5 software program which is used for analysis of DNA and protein sequences. The sequence was subjected to the basic local alignment search tool (BLAST), an online program for searching in a nucleotide database (BLASTN) and BLASTX for searching in the protein database, obtained from the National Centre for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov) (Altschul et al., 1997). Pairwise alignment of APEC CH2 *tkt1* was performed using the FASTA software package (Pearson and Lipman, 1988). The translated sequence of APEC CH2 *tkt1* was used to obtain similar sequences from the UniProt Knowledgebase (http://www.

Table 1
Bacteria and plasmids used in this study.

	Relevant feature(s)	References
Strains		
APEC CH2	O78 serotype isolated from infected chicken	(Vandemaele et al., 2005)
E. coli BL21 DE3	B strain, T7 expression strain	Invitrogen
APEC CH2/pKD46	APEC CH2 derivative containing pKD46 (oriR101 *bla* P_{BAD}-λ *gam bet exo*)	This study(Datsenko and Wanner, 2000)
CH2Δ*tkt1*	APEC CH2 derivative with deleted *tkt1*	This study
Plasmids		
pKD3	*cat*, template plasmid	(Datsenko and Wanner, 2000)