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BRIEF COMMUNICATION

Polymerase chain reaction-restriction fragment length polymorphism method for differentiation of *uropathogenic specific protein* gene types

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Abstract The putative pathogenicity island (PAI) containing the *uropathogenic specific protein* (*usp*) gene and three small open reading frames (*orfU1*, *orfU2*, and *orfU3*) encoding 98, 97, and 96 amino acid proteins is widely distributed among uropathogenic *Escherichia coli* (UPEC) strains. This PAI was designated as PAI*usp*. Sequencing analysis of PAI*usp* has revealed that the *usp* gene can be divided into two types — *usp*I and *usp*II — based on sequence variation at the 3′ terminal region and the number and position of *orfUs* differ from strain to strain. Based on *usp* gene types and *orfU* sequential patterns, PAI*usp* can be divided into four subtypes. Subtyping of PAI*usp* is a useful method to characterize UPEC strains. In this study, we developed a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to differentiate *usp* gene types. This method could correctly identify the *usp* gene type in *usp*-positive UPEC strains in our laboratory.

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Introduction

Kurazono et al[1] discovered a putative pathogenicity island (PAI) containing the gene encoding uropathogenic specific protein (*usp*) in uropathogenic *Escherichia coli* (UPEC) strain Z42. Located downstream of the *usp* gene are three small open reading frames (*orfU1*, *orfU2*, and *orfU3*) putatively encoding 98, 97, and 96 amino acid proteins, respectively. It has been demonstrated that the plasmid containing the *usp* gene enhances the infectivity of host *E. coli* strains in a mouse pyelonephritis model suggesting that the *usp* gene contributes to the pathogenesis of urinary tract infection (UTI) and the *usp* gene may encode the putative virulence factor of UPEC.[2] The *usp* gene is widely distributed in *E. coli* strains isolated from urinary tract infection as well as other extraintestinal infections.[2–4] This PAI is different from a PAI previously reported in UPEC strains and designated as PAI*usp*. Based on sequence homology, it is thought that uropathogenic specific protein (usp) encoded by the *usp* gene is a bacteriocin and the proteins encoded by small open reading frames downstream of the *usp* gene (designated as OrfU proteins) are immunity proteins for Usp.[5] A recent study has demonstrated that Usp is a non-specific DNase belonging to H-N-H nuclease family.[6]

Sequencing analysis of PAI*usp* in representative *E. coli* strains revealed that the *usp* gene can be classified into two types — *usp*I and *usp*II — based on sequence variation in 3′ end. In addition, the number of *orfUs* and position of *orfU* in relation to each other vary from strains to strains.[7] Detailed investigation of the PAI*usp* showed that *usp*I is linked with *orfU1* and *usp*II is linked with *orfU2*.[5]

PAI*usp* can be subtyped into four subtypes (Ia, Ib, IIa, and IIb) depending on *usp* gene type and sequential pattern of *orfUs* (Figure 1). Kanamaru et al[8] performed the subtyping of PAI*usp* in UPEC strains isolated in Japan using a polymerase chain reaction (PCR) method. Type IIa was found to be the most common subtype (42.4% of UPEC strains subtyped) followed by Type Ia (28.4%), Type Ib (9.8%), and Type IIb (2.7%). In their report, the method for differentiation of *usp* gene types was not described. However, identification of *usp* gene type is the first step in subtyping of PAI*usp*. So, in our study, we developed a PCR-restriction fragment length polymorphism (PCR-RFLP) method for differentiation of *usp* gene types.

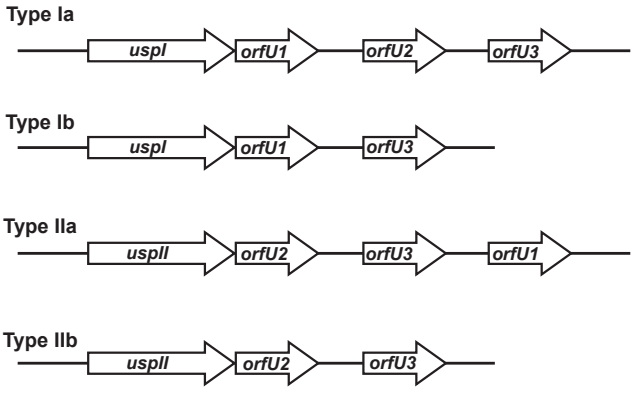

Figure 1. Structures of the *usp* gene and *orfUs* in each subtype of PAIusp. The arrows represent the length and transcriptional direction of the genes in PAIusp (Modification from Kanamaru et al., 2006).[8]

Materials and methods

Bacteria strains and media

Forty *usp*-positive UPEC isolates stocked in Microbiology Laboratory, School of Medicine, Universiti Malaysia Sabah, Malaysia were used in this study. Bacteria were grown on MacConkey agar and subsequently in tryptic soy broth at 37°C overnight.

Preparation of DNA template

Bacteria grown in tryptic soy broth overnight were harvested by centrifugation at 6149 *g* for 10 minutes. Bacterial cells were suspended in sterile distilled water. DNA extracted by the boiling method was used as the template for PCR.

PCR

Each PCR reaction was prepared as 50 μL reaction mixture containing 5 μL of template DNA, 1 μL of dNTPs (10 mM), 5 μL of 10× buffer, 2 μL of each primer (10μM), 34.5 μL of distilled water and 0.5 μL of *Taq* polymerase (5 U/μL) (Takara Bio Inc, Shiga, Japan). The sequence of primers is shown in Figure 2. PCR was performed with a Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, USA) and began with initial denaturation at 95°C for 5 minutes followed by 30 cycles of amplification (95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds) and final extension at 72°C for 10 minutes. PCR products were checked by electrophoresis in 1% agarose gel and ethidium bromide staining. ExactMark 100 bp DNA ladder (1st BASE Singapore Ltd, Singapore) was used as the molecular size marker.

RFLP analysis

PCR products (6 μL) were mixed with 1 μL *Hpa*II (Thermo Fisher Scientific Inc., Waltham, MA, USA), 2 μL 10× buffer and 11 μL distal led water and incubated at 37°C for 3 hours. The digested DNA was analyzed by 1.5% agarose gel electrophoresis and ethidium bromide staining.

DNA sequencing

DNA sequence analysis was performed at First BASE Laboratories Sdn. Bhd. using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, USA).

Results

Nakano et al[7] reported that the *usp* gene can be divided into two subtypes, *usp*I and *usp*II, depending on sequence variations at the 3′ terminal 230 bp region. To develop the PCR-RFLP method for differentiation of *usp* gene types, *usp*I and *usp*II sequences were retrieved from the database.