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Short communication

A rapid and simple method for identifying *Mycobacterium tuberculosis* W-Beijing strains based on detection of a unique mutation in *Rv0927c* by PCR-SSCP

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Abstract

Recently we have found that W-Beijing *Mycobacterium tuberculosis* strains have a unique in-frame trinucleotide (AGC) deletion at position 421 of *Rv0927c* and a −127G → A mutation in *Rv0927c-pstS3* intergenic region. Based on detecting the 421 trinucleotide deletion of these two mutations which can alter the ssDNA conformation more extensively than the other, we developed a PCR-SSCP method for rapid identification of W-Beijing strains among non-Beijing strains. Altogether, 104 clinical isolates were analyzed, including 68 W-Beijing strains and 36 non-Beijing strains. We found that PCR-SSCP successfully differentiated all the W-Beijing strains from the non-Beijing strains. In addition, we unexpectedly discovered that SDS-PAGE protein gels had better resolving power than conventional TBE polyacrylamide gel in detecting the AGC deletion mutation in the SSCP analysis.

Keywords: *Mycobacterium tuberculosis*; W-Beijing strain; Strain identification; *Rv0927c*; PCR-SSCP

1. Introduction

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide, especially in Asia and Africa. Globally, 9.2 million new TB cases with 1.7 million deaths occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive people [1]. In the early 1990s, a multidrug-resistant "W" strain, a member of the Beijing family of *Mycobacterium tuberculosis* strains, was the cause for a major outbreak in New York City [2]. In 1995, a particular genotype of tuberculosis, named Beijing strain, was first recognized [3]. These strains can be characterized on the basis of a unique IS*6110* insertion in the origin of replication (*oriC*) and a specific spacer oligotype lacking spacers 1–34 in the direct repeat (DR) chromosomal locus [20]. *M. tuberculosis* strains of the Beijing genotype have been shown to be widespread globally and are particularly prevalent in East Asia [3]. Subsequently, this genotype is increasingly reported in other parts of the world [6], and is strongly associated with drug resistance [7,8], high virulence [9] and insufficient immunological defense induced by BCG [3,10]. The W-Beijing strains have been also associated with tuberculosis (TB) treatment

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failures and relapse cases [21]. Given the prevalence of the W-Beijing strains in the global TB epidemic, the availability of a simple and rapid method for identification of this family of strains is important for TB epidemiology and disease control.

Polymerase Chain Reaction-Single-Strand Conformational Polymorphism (PCR-SSCP) analysis is an important tool for detecting point mutations underlying genetic diseases since it was established in 1989 [11,12]. In non-denaturing conditions, single-stranded DNA (ssDNA) has a folded structure that is determined by intramolecular interactions, and therefore, by its sequence. Even a single point mutation can alter the conformation of ssDNA, so that the altered conformation affects the migration of ssDNA which can be detected as abnormal bands on a non-denaturing gel. Thus SSCP analysis has a high resolving power to distinguish most conformational changes caused by subtle sequence differences even in a several-hundred-base fragment [12]. Usually, the most important factor in the sensitivity of this method is the temperature during electrophoresis, since the formation of intrastrand bonds and thereby the conformation of ssDNA is temperature dependent. In addition to temperature, the presence of glycerol in the gel can also affect the conformation of ssDNA significantly.

Up to now, PCR-SSCP has been successfully used to detect drug-resistant TB strains with concordant results obtained by drug susceptibility testing [13,14]. However, no published study is available in the application of PCR-SSCP analysis for identifying *M. tuberculosis* strains, including W-Beijing strains. Although several genotyping methods are available for identifying W-Beijing strains, time and cost as well as technical demand of these methods still limit wide application of these techniques [15]. Thus there is a need to develop a more rapid and convenient method such as PCR-SSCP for identifying W-Beijing strains.

In our previous work, we compared protein patterns between the W-Beijing and non-Beijing strains and found a unique protein Rv0927c that is absent in W-Beijing strains but present in non-Beijing strains [16]. *Rv0927c* gene encodes a putative short dehydrogenase/reductase. Subsequently, we analyzed sequence difference of *Rv0927c* between W-Beijing and non-Beijing strains. Compared with non-Beijing isolates, all W-Beijing strains had two characteristic mutations, a unique in-frame trinucleotide (AGC) deletion at position 421 of the *Rv0927c* gene, causing deletion of a serine codon at amino acid position 141 and a −127G → A mutation in *Rv0927c-pstS3* intergenic region, which can cause failure to express *Rv0927c* [16]. Based on these findings, we decided to take advantage of the trinucleotide deletion and design a PCR-SSCP based test for rapid identification of Beijing family strains. In this study we developed a PCR-SSCP method and evaluated this method for rapid identification of W-Beijing strains among non-Beijing strains on a panel of 104 *M. tuberculosis* clinical isolates. The results clearly demonstrated that the PCR-SSCP method based on detection of the AGC trinucleotide deletion in *Rv0927c* can be used to rapidly distinguish Beijing family strains from non-Beijing family strains.

2. Materials and methods

2.1. Reference strains and clinical isolates of M. tuberculosis

The reference strain H37Rv and 64 *M. tuberculosis* clinical isolates were obtained from Shanghai Pulmonary Hospital, China. These isolates were collected from five provinces of eastern China, including Jiangsu, Zhejiang, Anhui, Fujian, and Jiangxi. The other 40 clinical isolates were obtained from Portugal. Bacteria were grown without shaking in Middlebrook 7H9 medium supplemented with 0.2% (v/v) glycerol and 10% albumin–dextrose–catalase (ADC) at 37 °C.

2.2. Spoligotyping

Spoligotyping was performed as previously described [16]. Briefly, the direct repeat (DR) region was amplified by PCR with oligonucleotide primers derived from DR sequence. Mycobacterial genomic DNA was extracted from cultured cells. Twenty-five microliters of the following reaction mixture were used for PCR. The amplified PCR product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. After hybridization, the membrane was washed twice for 10 min in 2 × SSPE (1 × SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA) −0.5% sodium dodecyl sulfate (SDS) at 60 °C and then incubated in 1:4000 diluted streptavidin peroxidase conjugate (Isogen Bioscience) for 45–60 min at 42 °C. The membrane was washed twice for 10 min in 2 × SSPE-0.5% SDS at 42 °C and rinsed with 2 × SSPE for 5 min at room temperature. Hybridizing DNA was detected by the enhanced chemiluminescence method (Amersham) using Hyperfilm ECL (Amersham) as specified by the manufacturer.

2.3. PCR and DNA sequencing of Rv0927c gene

The *Rv0927c* gene from 104 clinical isolates was amplified by PCR using high-fidelity KOD polymerase (Toyobo Co.) according to the manufacturer's instructions with the following primers: forward primer, 5′-GGTTGACCCCTGATGATGGACACG-3′; reverse primer, 5′-CAACGTCTTGCCGGTCAGGAAGCT-3′. Amplification was performed for 30 cycles in each run as follows: 30 s at 94 °C, 30 s at 63 °C, 45 s at 68 °C, and a final extension for 10 min at 68 °C. The PCR products from all 104 isolates were purified by electrophoresis on a 1% agarose gel using the QIAquick PCR purification kit (Qiagen) and sequenced using an ABI 377 automated DNA sequencer (Applied Biosystems, Inc.).

2.4. PCR-SSCP analysis

The target DNA for PCR-SSCP was a 249-bp fragment with the mutation positioned in the middle of the fragment for optimal mutation detection. Based on the published genome