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Original article

IS6110 element distribution in Tunisian clinical strains and phylogenetic relationship generated by its mobility

S. Thabet[a,*], W. Tombari[b]

[a]*Laboratory of Microorganisms and Active Biomolecules, Department of Biology Faculty of Mathematical, Physical and Natural Sciences of Tunis. University of Tunis El Manar1, 2092 Tunis, Tunisia.*
[b]*Epidemiology and Veterinary Microbiology Laboratory, Institut Pasteur de Tunis, Tunis- Belvédère, Tunisia.*

*Corresponding author; Laboratory of Microorganisms and Active Biomolecules, Department of Biology Faculty of Mathematical, Physical and Natural Sciences of Tunis. University of Tunis El Manar1, 2092 Tunis, Tunisia.

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ABSTRACT

The dynamics of IS6110 transposition is a valuable epidemiological tool in tuberculosis studies. In the present work, we studied IS6110 distribution along the genome of 58 unrelated clones and go over phylogenetic relationship between them by performing a molecular study with GL-PCR methodology. A total number of 309 insertion sites have been detected showing that IS6110 integration in samples studied crossed randomly several positions through the Mycobacterium tuberculosis genome. These sites were divided into 232 sequences having homologous in reference strains mainly in M. tuberculosis H37Rv strain and 77 other unique regions that did not have counterparts in reference strains fully sequenced and available in global databases. Most insertions occurred in coding regions. 114 insertions took place in the direction of the replication fork and 118 insertions were oriented against the direction of the replication fork, carried by the complementary strand of the chromosome. No clustered patterns have been illustrated. Several genetic statistical tests have been performed with language R package software to study the distribution and the symmetry of IS6110 insertion events. Our data confirm that IS6110 element prevalence and effect on genome function demonstrate the potential of this transposon as an evolutionary marker and as the best indicator of essential/virulence genes. In fact, survey of patterns of IS6110 insertions in circulating

isolates may provide useful information for populational studies mainly in epidemic situations.

1. Introduction

A recent discovery showed that the earliest evidence of human tuberculosis dates back to about 500,000 years ago. Despite being such an ancient human pathogen, Mycobacterium tuberculosis remains the second leading cause of death due to a single infectious agent (Aryan et al., 2010; Dalla Costa et al., 2013). Rapid and efficient diagnosis of infectious pulmonary tuberculosis is one of the crucial issues in the global fight to control the disease and to arrest further transmission (Haldar et al., 2009).

In Several cases, it has been shown that repetitive DNA sequences display species specificity. For example, the insertion sequence IS900 was shown to be specific to Mycobacterium paratuberculosis although it shows significant homologies to IS110, an insertion element of Streptomyces coelicor A3 (Green et al., 1989). The repetitive sequence IS6110 was isolated from a M. tuberculosis cosmid library and was detected only in species belonging to the M. tuberculosis complex (MTBC) (Thierry et al., 1990; Thorne et al., 2010). As IS6110 has been described as an efficient epidemiological marker of tuberculosis, oligonucleotides derived from this sequence were used to detect M. tuberculosis in clinical specimens following in vitro DNA amplification and several existing strategies relying on PCR amplification have been performed to map its location within the mycobacterial genome.

In general, the genome of MTBC bacteria is highly conserved (Fleischmann et al., 2002), however, IS6110 is associated with a high level of DNA polymorphism (Brosch et al., 1999). In fact, the numbers and chromosomal positions of IS6110 elements have been shown to be highly variable among unrelated strains (Thorne et al., 2007), while IS6110 RFLP analysis of strains isolated from patients who developed tuberculosis showed identical patterns over a 2- to 3-year period. So the presence of IS6110 does not induce in vivo major genomic rearrangements over a 2- to 3-year period and confirms its use as a valuable epidemiological marker in tuberculosis epidemiological studies.

A cloning-based method combined with selective PCR amplification that targets both termini of IS6110 insertion sites, referred to as GL-PCR, has been described previously (Namouchi et al., 2006). GL-PCR allows for the isolation of the majority of IS6110 insertion sites in MTBC isolates and is able to discriminate between strains differing by a single IS6110 band. Besides being highly discriminatory methodology, it produces data that can be easily interpreted, compared and transported to be used for epidemiological investigations and control of epidemic outbreaks. A molecular epidemiological investigation previously conducted in Tunisia provided a global picture of the current TB epidemiology and described major genotypes circulating in Tunisian territory (Namouchi et al., 2008). In the current work we characterized Tunisian clinical strains through IS6110 distribution along the genome of unrelated clones and go over phylogenetic relationship between them by performing a molecular study with GL-PCR methodology. As GL-PCR assay is based on the determination of IS6110 insertion sites, it derives its interest because of IS6110 specificity for the MTBC and the multicopy number. Thus GL-PCR approach offers suitable tool to further elucidate the dynamics of transposition and IS6110 distribution versus hotspots registered.

2. Materials and methods

2.1. M. tuberculosis strains

The present study was performed on 58 M. tuberculosis strains isolated from 60 pulmonary and extrapulmonary patients and cultured on Lowenstein-Jensen medium. The DNA extraction from all isolates was performed by phenol chloroform method (Namouchi et al., 2006; Van Embden et al., 1993).

2.2. Mapping of IS6110 transposition sites

Genomic addresses of IS6110 were determined by GL-PCR (Namouchi et al., 2010).

2.3. Sequencing and data analysis

The GL-PCR products were agarose-gel purified with the Geneclean Kit (BIO 101) according to the recommandations of the manufacturer. Automated sequencing was performed with the BigDye Terminator Cycle Sequencing Kit and IS6110 outward primers (IS1 and IS2) on an ABI PRISM 377 DNA sequencer (Applied Biosystems Inc., CA, USA). Sequence data were analyzed with BioEdit v5.0.9 (Hall, 1999) and used for a BLAST search in the TubercuList website (http://genolist.pasteur.fr/TubercuList/) or at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) (Namouchi et al., 2006).

2.4. Statistical analysis

Statistical analyses were performed with R language.

3. Results

3.1. IS6110 insertion sites distribution and orientation

As IS6110 insertions represent “in vivo” transposition events that provide information regarding genes required for human infection and disease and because the highly variable copy number of IS6110 element provides valuable epidemiological interest, we examined its prevalence and distribution in 58 Tunisian circulating strains. Upon IS6110 flanking regions determining with GL-PCR methodology and analysis with BioEdit v5.0.9 (Hall, 1999), a BLAST search in the TubercuList website (http://genolist.pasteur.fr/TubercuList/) or at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) allowed to recognition of a total number of 309 insertion sites showing that IS6110 integration in samples studied crossed randomly several positions through the genome according to H37Rv reference strain. These sites were divided into 77 (25%) sites corresponding to unique regions that did not have counterparts in the reference strains fully sequenced and available in global databases, and 232 (75%) sites having homologous sequences in reference strains mainly in M. tuberculosis H37Rv strain. Among these sequences, 176 (76%) were located in coding regions of the genome and 56 (24%) rather corresponded to non-coding intergenic regions. 114 insertions took place in the direction of the replication fork and 118 insertions were oriented against the direction of the replication fork, carried by the complementary strand of the chromosome. The rate of insertions in coding and noncoding regions and the location of corresponding sites in the H37Rv reference strain genome were represented in Fig. 1(a) and Fig. 1(b).

3.2. Phylogenetic study and clustering rate

Alignments of all sequences with genomes of reference isolates and comparative analysis enabled screening of three to nine IS6110 insertions per strain. Some sites were shared by two or three strains. Phylogenies constructed and visualized with MEGA 5 software showed that no clustered patterns have been illustrated (Fig. 2). All profiles were different confirming that even some transposition sites are shared by several strains; each of the latter has its own specific fingerprint for the distribution of IS6110 in different positions across the chromosome. Such result confirms the potential of IS6110 insertion as important source of genomic variability for M. tuberculosis.

3.3. Statistical studies

Several genetic statistical tests have been performed with language R package software. Descriptive statistics were used to calculate statistical values informing on distribution and correlation between variables. The mean of insertions in coding regions was of 3.03 with a standard deviation of 1.90 (3.03+/- 1.90). The mean of insertions in noncoding regions was of 0.96 with a standard deviation of 0.72 (0.96+/- 0.72). The coefficient of variation (CV) representing the dispersion rate of insertions in coding and noncoding regions was 62.63 and 75.05 respectively. So our data were highly dispersed thought they are tighter in the case of insertions in coding regions. The distribution and the symmetry of insertions were illustrated in a box-plot showing only one extreme value in noncoding insertion that may be an influential value (Fig. 3).

4. Discussion

As already shown in previous works, a high degree of polymorphism was observed between strains of the MTBC isolated from different patients. The mycobacterial insertion sequence IS6110, through its integration in

multiple copies in the chromosome, has the potential to provide more genotypic data than would have been possible by conventional approaches (Alonso et al., 2011). Furthermore, insertions of IS6110 sequence copies in M. tuberculosis were assumed, for a long time, to be random (Fang & Forbes, 1997). Nevertheless IS preferential loci (ipl) or insertional hotspots have been observed (Casart et al., 2008; Kamerbeek et al., 1997; Thorne et al., 2010).

Molecular typing of M. tuberculosis isolates has led to important insights about the epidemiology and pathophysiology of tuberculosis, which has implications for prevention strategies (Durmaz et al., 2007; Lari et al., 2007). Many different assays have been developed for the molecular diagnosis of mycobacterial infections such as techniques based on primers and probes designed for IS6110 of M. tuberculosis and the 16S rRNA genes (Karen et al., 1992; Blaschitz et al., 2011). This validates IS6110 and mycobacterial 16S rRNA genes as targets for DNA-based detection and identification of MTBC (Lazzeri et al., 2012) and thus contributes to the surveillance of subsequent cases. IS6110 based studies have contributed large knowledge on essential/dominant genes (Alonso et al., 2011) involved in transmission pathways, tuberculosis epidemiology and bacillus dynamics especially in high-burden countries. Therefore use of IS6110 as genetic markers allows the sensitive and specific detection of MTBC (Pérez-Osorio et al., 2012).

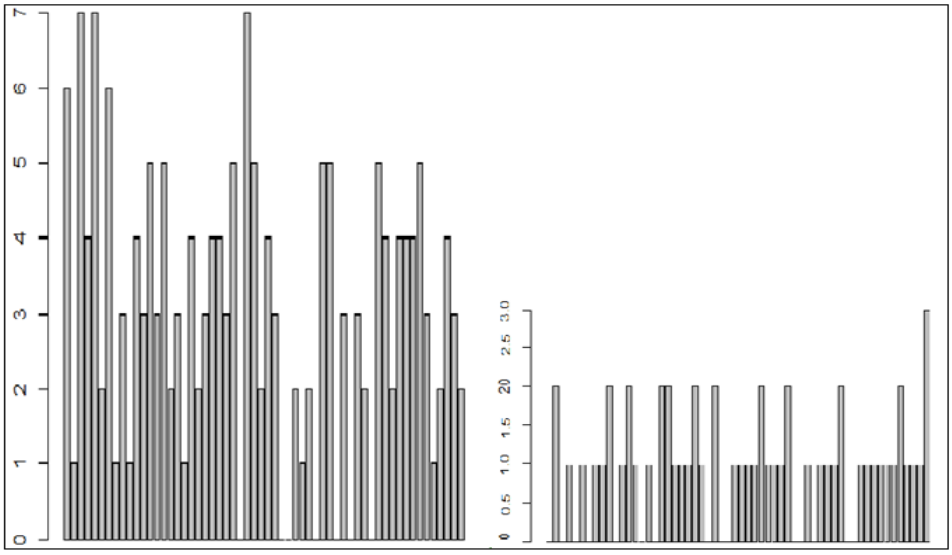

Fig. 1a. Rate of insertions in coding and noncoding regions.

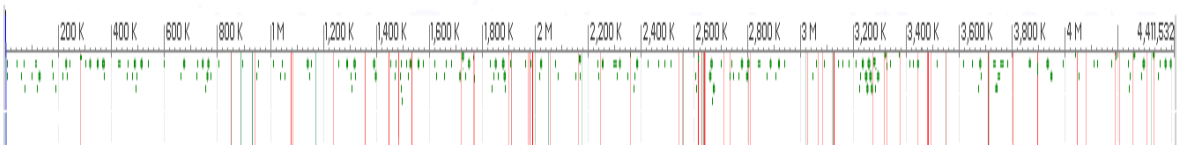

Fig. 1b. Location of IS6110 insertion sites according to H37Rv reference strain. Insertions in coding regions are shown with red color and insertions in noncoding regions are shown with green color.

The aim of the present work was to study IS6110 distribution along the genome of Tunisian clinical strains and go over phylogenetic relationship between them. The multiple alignment of IS6110 integration sites in all strains studied showed a high specificity of each strain for number of copies and positions. IS6110 insertions can affect gene expression by interrupting protein-coding genes, by mediating recombination events that result in fragments deletions and/or inversions, and by up-regulating the expression of nearby genes due to a promoter located within the transposable element (Reyes et al., 2012). Isolates that are unique on M. tuberculosis typing are presumed to result from reactivation disease, while patterns that are part of a cluster are more likely to represent recent transmission. As no identical or similar patterns have been identified in our collection, GL-PCR profiles were not grouped into families which have been correlated with the genotypic specificity of IS6110 distribution acquired by M. tuberculosis isolates in the same geographic area. Incidence rate of IS6110 integrations in coding regions (76%) were higher than those in non-coding regions (24%); which may be rational inasmuch as the mycobacterial

coding capacity is nearly 91% of the total genome corresponding to about 4000 genes encoding proteins and 50 genes encoding ribonucleic acids (Cole & Barrell, 1998).

IS6110 element determination may be of great advantage for tubercle bacillus survey and is effective for detecting latent infection by demonstrating the presence of mycobacterial DNA in pulmonary and multiple extrapulmonary tissues from people who died from causes other than tuberculosis and had no history of this disease (Barrios et al., 2012). The ability to disseminate and establish latent infection in multiple organs is a fundamental strategy used by M. tuberculosis for survival and not an unusual property of a particular strain seeing that the bacterial strains in different individuals were of different genotypes. In another component, the comparison of multiple parts of different genomes from different isolates demonstrates that the M. tuberculosis genome is currently undergoing an active process of gene decay, analogous to the adaptation process of obligate bacterial symbionts. Such observation opens new perspectives into the evolution and the understanding of the pathogenesis of this bacterium.

In conclusion, our data confirm that IS6110 element prevalence and effect on genome function demonstrate the potential of this transposon as a valuable epidemiological tool. Thus, conducting a broader survey of patterns of IS6110 insertions in circulating isolates may provide useful information for populational studies mainly in epidemic situations. IS6110 continues to be promising marker for genotyping of M. tuberculosis in epidemiologic investigations that could be used to elucidate the dynamics of tuberculosis transmission in populations.

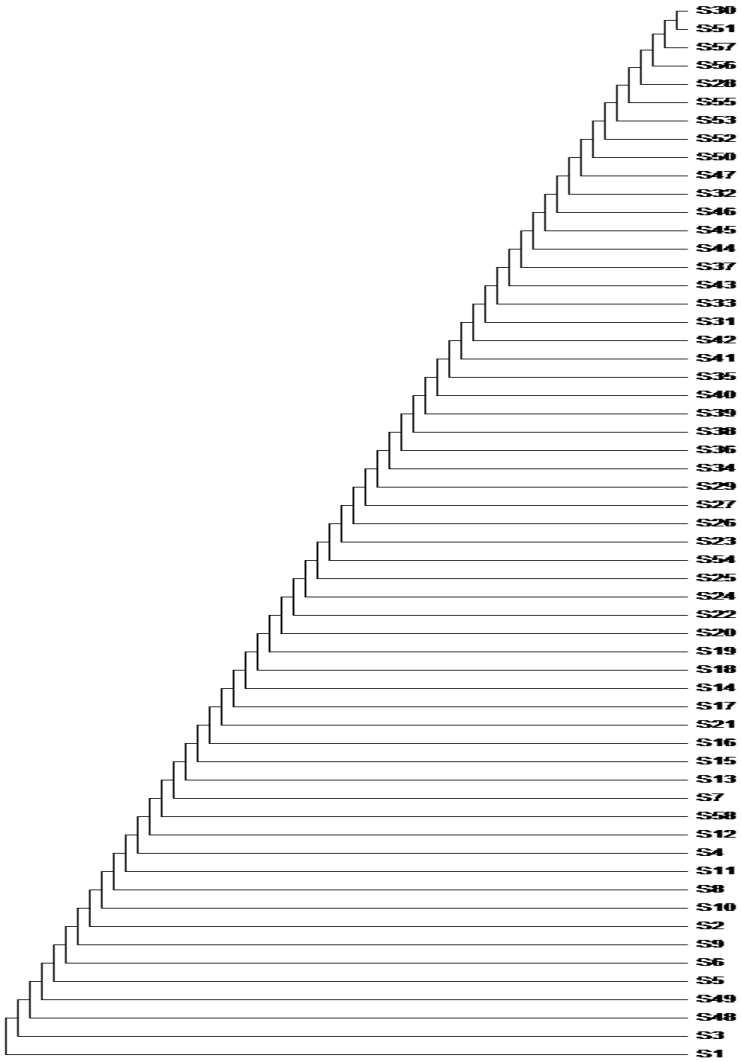

Fig. 2. Phylogenetic tree construced with MEGA5 sofware representing phylogenetic relationship between strains studied.

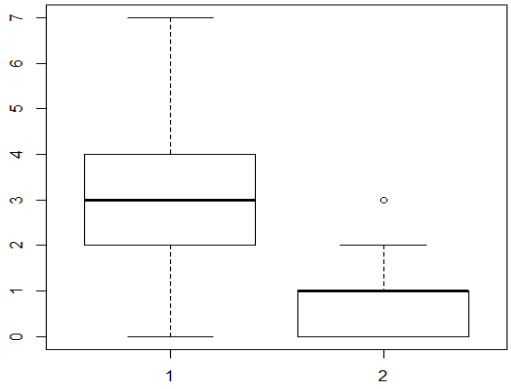

Fig. 3. Distribution and symmetry of IS6110 insertions. 1: insertions in coding regions. 2: insertions in noncoding regions.

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