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Detecting genetic variability among different *Mycobacterium tuberculosis* strains using DNA microarrays technology

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Summary Recent advances in functional and comparative genomics have improved our understanding of genetic diversity among the *Mycobacterium tuberculosis* complex. In this study, we investigated the genetic polymorphism of *M. tuberculosis* using whole-genome microarray analysis. Amplified fragments of 15 *M. tuberculosis* strains (from two different geographical origins) and the reference strain H37Rv were produced by random amplification of polymorphic DNA (RAPD) using three different primers. The RAPD products were labeled with fluorescent dyes (Cy3 and Cy5) and hybridized to a TB DNA microarray representing nearly all open reading frames (ORFs) of H37Rv. The final results were analyzed using bioinformatic tools. Some genetic variability was found among the 16 *M. tuberculosis* strains. The majority of the highly polymorphic DNA sequences were observed in ORFs representing non-essential genes of the bacterium. The future use of comparative genomics based on DNA microarray technology should prove a powerful tool for understanding phenotypic variability among *M. tuberculosis* isolates of similar genetic composition. It is also a promising approach to provide important insights into evolution, virulence and pathogenesis of *M. tuberculosis*.

Introduction

The availability of the complete genome sequence of *Mycobacterium tuberculosis* has deeply increased the knowledge about this important pathogen. In

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spite of large-sequence polymorphisms (LSPs) and single nucleotide polymorphisms (SNPs) that have been recently found in *M. tuberculosis* isolates, the molecular basis of genotypic variation in virulence and transmissibility of the bacillus is unclear. More recently, new developments in functional and comparative genomics, using DNA microarrays and bioinformatics, have fostered major advances in our understanding of genetic variability among *M. tuberculosis*, providing a whole-genome perspective on genomic content, gene regulation and metabolism of *M. tuberculosis*.[1,2] The application of DNA microarray technology among natural populations of mycobacteria is a promising approach for understanding their evolution, virulence and pathogenesis. However, the resolution of such methods for detecting chromosomal variation is limited to insertions and deletions of sufficient size to be detected by microarrays. These methods often fail to detect small insertion/deletion events and are unlikely to find single nucleotide polymorphisms (SNPs).

Here we describe a different method to detect genetic polymorphisms in *M. tuberculosis*. We combine random amplified polymorphic DNA (RAPD)[3] with DNA microarray hybridization.[2]This allows us to detect chromosomal alterations that cannot be easily seen using either method alone.

Materials and methods

Mycobacterial strains

Sixteen *M. tuberculosis* strains were used in this study: *M. tuberculosis* H37Rv, two from Russia provided by Megan Murray (Harvard School of Public Health, Massachusetts, USA) and 13 from Massachusetts, USA, provided by Alexander Sloutsky (Massachusetts State Laboratory Institute, USA). DNA was purified by a standard protocol.[4] All DNA samples had been genotyped previously by restriction fragment length polymorphism (RFLP)[4] and spoligotyping.[5]

Polymerase chain reaction (PCR) by random amplified polymorphic DNA (RAPD) analysis

A RAPD method using 20 different primers, OPA1-20 (OPA A Kit, Operon Technologies, California, USA), were performed to obtain a large set of amplified fragments. DNA of *M. tuberculosis* H37Rv was evaluated to select the best combination of primers. The DNA amplification reaction (25 μL) contained 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 10% (vol/vol) DMSO, 25 pmoles primer, 0.2 mM dNTPs, 50 ng DNA, and 1.25 units of Taq DNA polymerase (Takara Bio Inc., Shiga, Japan). After selection of adequate primers the volume, the primer concentration and the quantity of polymerase were increased to 100 μL, 125 pmoles and 5 units, respectively. PCR conditions in a PTC-200 Thermocycler (MJ Research, Massachusetts, USA) or a Mastercycler Gradient Thermocycler (Eppendorf, California, USA) were 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 30 °C for 1 min and 72 °C for 2 min, and a final extension of 72 °C for 5 min. Ten microliters of each PCR product was electrophoresed in 1% agarose with Tris-acetate-EDTA buffer at 70 V for 2 h.

DNA microarray

Fluorescent labeling

PCR amplicons were purified by QIAquick Nucleotide Removal Kit (Qiagen, California, USA). The Klenow reaction (to incorporate amino allylmodified dNTPs for labeling) was performed in 25 μL total volume using 1 μg of template (300 ng each OPA 2, 4, 20 PCR products), 2 μg of 9-mer random primer 0.2 mM aadNTPs and 27500 unit of Klenow DNA polymerase (New England Biolabs, Massachusetts, USA). Around 6 μg of Klenow product were labeled with fluorescent dyes (Cy3 and Cy5, Amersham, New Jersey, USA) at room temperature in the dark for 2 h.

Microarray hybridization

A total of 4–5 μg of labeled Klenow products with Cy3 (and or Cy5) were hybridized against a tuberculosis microarray slide containing 3855 open reading frames (ORFs) from *M. tuberculosis* H37Rv,[6] (nearly all ORFs of this bacterium) using an automated hybridizing workstation (TECAN, Maennedorf, Switzerland) and a hybridization program according to Sassettti et al.[6,7] The hybridized slides were scanned using an Axon 4000B scanner 1 (Axon Instruments, California, USA) and analyzed using: GenePix software 5.1 (Axon) and GeneSpring 7.0 software (Silicon Genetics, California, USA).

Results

RAPD analysis

A set of 20 primers was evaluated in RAPD experiments using *M. tuberculosis* H37Rv DNA.