JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2007, p. 3480–3481 Vol. 45, No. 10
0095-1137/07/$08.00+0 doi:10.1128/JCM.01234-07

Presence of Region of Difference 1 among Clinical Isolates of *Mycobacterium tuberculosis* from India▽

Region of difference 1 (RD1) was first described by Mahairas et al. (4) as a region that is present in all virulent laboratory and clinical strains of *Mycobacterium bovis* and *Mycobacterium tuberculosis*. This region comprises nine genes (Rv3871 to Rv3879c) and spans a 9.5-kb region. In *M. bovis* BCG, RD1 deletion completely removes seven genes (Rv3872 to Rv3878) and truncates two others (Rv3871 and Rv3879c) (3). Recently Rao et al. (5) reported the total absence of RD1 in clinical isolates from India. Since this region has genes that are important in immunogenicity (ESAT6 and CFP10 genes) and RD1 deletion mutants of *M. tuberculosis* have been found to be less virulent (3), it was important to study this region in other Indian isolates. In this study, we analyzed 120 *M. tuberculosis* isolates from different parts of Kerala and 23 other isolates, 14 from west India and 9 from north India (mainly from Mumbai and Agra). All these isolates were characterized by biochemical analysis and IS*6110* restriction fragment length polymorphism typing. The IS*6110* copy number ranged from 0 to 15, with the majority (70%) representing the "low-copy-number" group. The presence of the RD1 region was checked with three sets of primers. The locations of the primers on the H37Rv genome and the sizes of the expected amplicon and the reference are indicated in Fig. 1.

Among the isolates from Kerala, *esxB* and *esxA* were conserved in all the isolates (120/120) and Rv3871 and Rv3872 were present in all except 2 (118/120). Rv3878, being present in only 107 of the 120 isolates, was the least conserved among the three regions. In the other 23 isolates, we found that *esxA* and *esxB* were present in all but 1 and that the Rv3878 region was present in 20 isolates. But the Rv3871 and Rv3872 genes were present only in seven isolates.

Thus, PCR analysis revealed that the entire RD1 region is not absent in field strains from India. Our earlier study on the identification of the *moaA3* gene had shown that the RD1 region is present in isolates from Kerala (6). In the other report, Rao et al. (5) had used two sets of primers, one spanning the entire RD1 (9.8-kb) region and the other for amplification of Rv3878. The amplification of the 9.5-kb RD1 region by routine PCR is technically demanding, and truncation of the genes or any mutation at the primer binding site would eliminate the PCR product. But Rv3878 should have been amplified, as our study shows that this gene is reasonably well represented in the isolates from different parts of the country. As all the 30 strains tested by Rao et al. came from a hospital in Hyderabad, it may be possible that a pocket of RD1-deficient strains are concentrated in that area. Beyond that we are unable to speculate on the absence of the RD1 region in all the isolates tested. Rao et al. had downplayed the role of RD1 in virulence and suggested the possibility of alternate virulence mechanisms. But all the isolates that we checked were recovered from patients with active tuberculosis and had a significant portion of the RD1 region intact. In addition, serological analysis of patients from the Mumbai region has shown excellent responses to CFP10 and ESAT6 (Dr. Camilla Rodrigues, personal communication), indicating that these are expressed in infected patients. Thus, in conclusion, while regional differences are present in the RD1 region, there appears to be no universal absence of RD1 in the isolates from the country as a whole.

We gratefully acknowledge Dr. Camilla Rodrigues, Hinduja Hospital, Mumbai, for the strains from western India. We are thankful to all the patients and doctors at the Sanatorium for Chest Diseases, Pulayanarkotta, and the District TB Centre, Trichur, Kerala, for the samples and their cooperation in the study. Technical assistance of Paul K. Laiza and S. Edwin is gratefully acknowledged.

This research was supported by the Department of Biotechnology (DBT), Government of India, through program support to RGCB, and the Bioinformatics facility is supported by the BTIS-NET of DBT. Smitha Soman, Biljo V. Joseph, and Suma Sarojini acknowledge CSIR for a senior research fellowship.

REFERENCES

1. **Behr, M. A., M. A. Wilson, W. P. Gill, H. Salamon, G. K. Schoolnik, S. Rane, and P. M. Small.** 1999. Comparitive genomics of BCG vaccines by whole genome DNA micro array. Science **284:**1520–1523.
2. **Brosch, R., S. V. Gordon, M. Marmiesse, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L. M. Parsons, A. S. Pym, S. Samper, D. van Soolingen, and S. T. Cole.** 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc. Natl. Acad. Sci. USA **99:**3684–3689.
3. **Lewis, K. N., R. Liao, K. M. Guinn, M. J. Hickey, S. Smith, M. A. Behr, and D. R. Sherman.** 2003. Deletion of RD1 from *Mycobacterium tuberculosis* mimics Bacille Calmette-Guerin attenuation. J. Infect. Dis. **187:**117–123.
4. **Mahairas, G. G., P. J. Sabo, M. J. Hickey, D. C. Singh, and C. K. Stover.** 1996. Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis*. J. Bacteriol. **178:**1274–1282.
5. **Rao, K. R., F. Kauser, S. Srinivas, S. Zanetti, L. A. Sechi, N. Ahmed, and S. E. Hasnain.** 2005. Analysis of genomic downsizing on the basis of region-of-

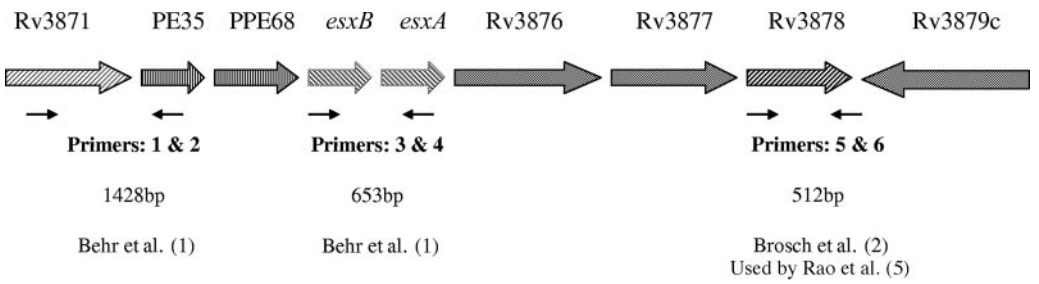

FIG. 1. Genes in the RD1 region and the positions of the primers.

difference polymorphism profiling of *Mycobacterium tuberculosis* patient isolates reveals geographic partitioning. J. Clin. Microbiol. **43:**5978–5982.
6. **Sarojini, S., S. Soman, I. Radhakrishnan, and S. Mundayoor.** 2005. Identification of *moa*A3 gene in patient isolates of *Mycobacterium tuberculosis* in Kerala, which is absent in *M. tuberculosis* H37Rv and H37Ra. BMC Infect. Dis. **5:**81.

Smitha Soman
Biljo V. Joseph
Suma Sarojini
R. Ajay Kumar
Department of Molecular Microbiology
Rajiv Gandhi Centre for Biotechnology
Trivandrum, India

V. M. Katoch
National JALMA Institute for Leprosy and Other Mycobacterial Diseases
Agra, India.

Sathish Mundayoor*
Mycobacterial Research Group
Department of Molecular Microbiology
Rajiv Gandhi Centre for Biotechnology
Thycaud Post
Trivandrum 695014, Kerala, India

*Phone: 91471 2342315
Fax: 91-471-2348096
E-mail: mundayoor_s@yahoo.com

▽ Published ahead of print on 15 August 2007.