

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 IS6110 restriction fragment length polymorphism typing. The IS6110 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.

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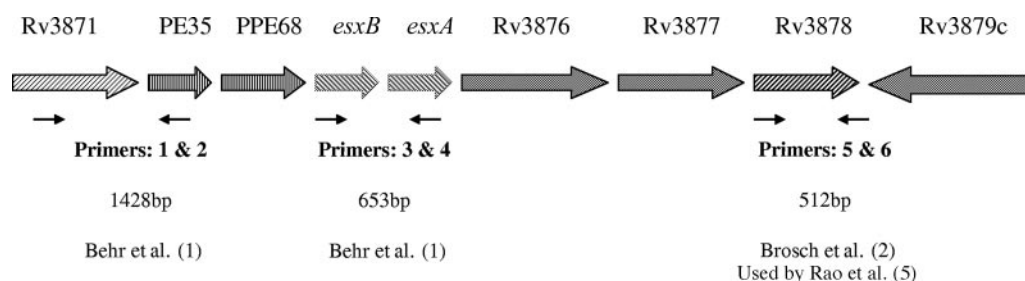


FIG. 1. Genes in the RD1 region and the positions of the primers.

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