IFS. Pub-49.

Identification of *Mycobacterium* and *Norcadia* with PCR-RFLP assay

Weerasekara DK[1], Magana-Arachchi DN[1] and Madegedara RMD[2]

[1]*Institute of Fundamental Studies, Hantana Road, Kandy.*
[2]*Chest Unit, Teaching Hospital, Kandy.*

Non-tuberculous mycobacteria (NTM) are mycobacteria not belonging to the *Mycobacterium tuberculosis (Mtb)* complex. *Nocardia* currently contains more than 50 species and approximately half are recognized human and/or animal pathogens. This study was performed to optimize a simple assay based on Polymerase Chain Reaction (PCR) and restriction enzyme digestion for identification of mycobacteria isolated from patients with pulmonary disease.

Bronchial washings(n=202) were collected from patients attending the Chest Unit, Teaching Hospital Kandy, who underwent bronchoscopy for pulmonary disease. Specimens were decontaminated using standard NaOH/Na citrate technique and inoculated onto LJ and MB7H10 media. Cultures were observed for 8 weeks at 28°C and 37°C. Genomic DNA was extracted from AFB positive isolates (n=43) using the standard CTAB/NaCl method and the resulting DNA were amplified using known mycobacteria specific Sp1 and Sp2 primers. DNA sequencing was performed for the selected PCR products. The sequenced strains were digested with restriction enzymes of *Hae*III and *Cfo*I.

From PCR analysis, ten isolates were identified as rapid growers (280-320 bp), 13 were slow growers (200-220 bp) and 14 were mixed having both rapid and slow growing mycobacteria. Sequence analysis revealed the presence of *M. intracellulare* (n=3), *M. phocaicum* (n=5), *M. tuberculosis* (n=7), *Nocardia* (n=2) and *Mycobacterium sp* (n=6). *Hae*III digestion of standard/reference MTB $H_{37}Rv$ and *M. bovis* yielded 50 and 110 bp DNA fragments while *M. phocaicum* had 120 and 200 bp bands, *Nocardia* yielded 150 and 160 bp fragments and *M. intracellulare,* a 100 bp DNA fragment. With *Cfo*I, $H_{37}Rv$, *M. bovis, Nocardia* and *M. intracellulare,* DNA remained undigested while *M. phocaicum* yielded 80 and 230 bp fragments. *Hae*III was adequate to differentiate *Mtb* complex and *M. bovis* from others. Other species of *Mtb* complex have not been tested in this study.

Optimized PCR-RFLP assay was able to differentiate *M. tuberculosis* complex bacteria from non-tuberculosis mycobacteria and *Norcardia.* Molecular analysis confirmed the presence of NTM in bronchoscopy specimens and according to the study ~12-13% of the study population had NTM in their bronchial washings. Part of the study was presented as Proceedings of the Sri Lanka Association for the Advancement of Science, 68th Annual Session, 10-12th December 2012. p2).

Key words: Identification, Mycobacterium, Norcadia, PCR-RFLP assay.

Funding: Anti Tuberculosis Campaign Research Grant.