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Poster Session III
Recent advances in molecular diagnosis of M. tuberculosis

PCR SEPARATION OF MYCOBACTERIUM INTRACELLULARE AND MYCOBACTERIUM CHIMAERA

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OBJECTIVES

To distinguish *Mycobacterium intracellulare* from *Mycobacterium chimaera* by copy number of the mycobacterial interspersed repetitive unit (MIRU) MIN 33.

METHODS

DNA was isolated from a total of 134 isolates identified by rRNA internal transcribed segment (ITS) as either *M. intracellulare* or *M. chimaera* and used as substrate for amplification of the MIRU, MIN-33 by PCR using the forward (5''-GTGCAGTTCAACCACGAAC-3') and reverse (5'-GGCGTTGAACACGTTGGTG-3') MIN-33-specific primers. Each PCR reaction was composed of 1 unit Taq polymerase, 1 µM of each primer, 1 µM dNTP, 5 µL of 5x buffer solution, 1.5 mM of $MgCl_2$, 1 µL of dimethyl sulfoxide, and 25 µL of distilled water and 5 µL of DNA. The amplification conditions were 1 cycle of 5 min at 94º C, 40 cycles of 30 sec at 94º C, 30 sec at 58 º C, and 30 sec at 72 º C, and 1 cycle of 7 min at 72 º C. To measure copy numbers, PCR products were separated by electrophoresis in 1 % (wt/vol) agarose gel and copy number calculated by dividing PCR product size in bp by 54, the size of the MIN 33 repeat unit. Values were rounded up to the next whole copy number.

RESULTS

There was a distinct difference in MIN-33 copy number of the two species; *M. intracellulare* isolates had 7-11 copies, while *M. chimaera* isolates had 12-14 copies. Most of the isolates had one band; *M. chimaera* (72) and *M. intracellulare* (64). No isolates of *M. intracellulare* (by rRNA ITS sequence) had MIN-33 copy numbers above 11 copies, while no *M. chimaera* isolates had copy numbers of less than 12 copies. Five isolates exhibited two bands; 2 had 2 *M. intracellulare*-size bands and 3 had one *M. intracellulare*-size and one *M. chimaera*-size bands. Excluding the 5 isolates with multiple MIN-33 bands, the sensitivity of MIN-33 differentiation of *M. intracellulare* from *M. chimaera* was 100 %.

CONCLUSION

MIN 33 PCR appears to be a simple, rapid and accurate method to distinguish *M. intracellulare* from *M. chimaera* isolates. Heretofore, only rRNA ITS sequencing has proven of use in distinguishing these two closely related species. Distinction between these two species is important as other work has shown that *M. intracellulare* is not found in water or pipe biofilm samples, only soil. In contrast, *M. chimaera* originates from water and pipe biofilm samples.