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PCR separation of *Mycobacterium intracellulare* and *Mycobacterium chimaera*

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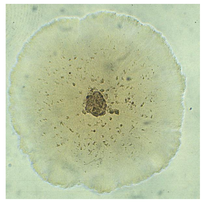
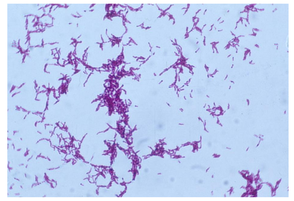
PURPOSE

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



***M. Intracellulare* colony in 7H11 media**



Ziehl-Neelsen stain. *M. intracellulare*

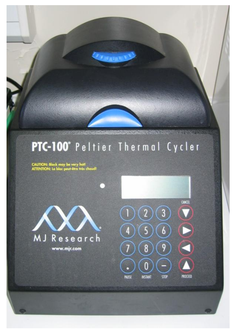
METHODS

❖ <u>DNA isolation</u>: 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.

❖ <u>MIN-33-specific primers</u>:

Forward: (5''-GTGCAGTTCAACCACGAAC-3')

Reverse: (5'-GGCGTTGAACACGTTGGTG-3')



❖ <u>PCR reaction</u>: 1 unit Taq polymerase, 1 µM of each primer, 1 µM dNTP, 5 µL of 5x buffer solution, 1.5 mM of MgCl2, 1 µL of dimethyl sulfoxide, and 25 µL of distilled water and 5 µL of DNA.

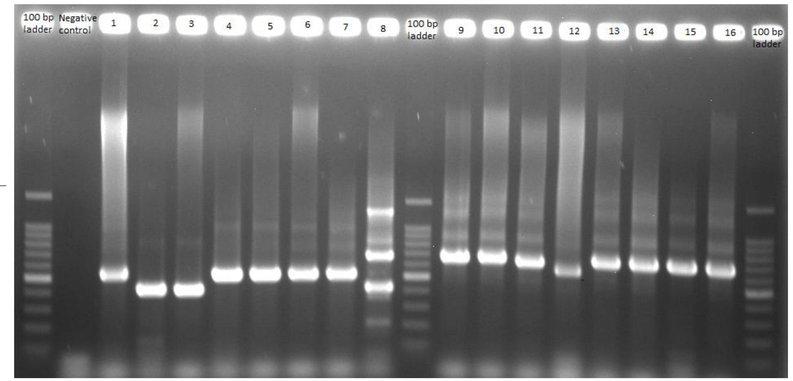
❖ <u>Amplification conditions</u>: 1 cycle of 5 min at 94ºC, 40 cycles of 30 sec at 94ºC, 30 sec at 58ºC, and 30 sec at 72ºC, and 1 cycle of 7 min at 72ºC.

❖ <u>Measurement of copy numbers</u>: 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**.

1. ***Mycobacterium intracellulare* 1406 (CONTROL)**
2. O7-3742, scope, 7 copies
3. A10, patient, 8 copies
4. O7-2654, scope, 9 copies
5. O7-3369, scope, 9 copies
6. A10-4-5-1, soil dust, 10 copies
7. O7-5119, scope, 10 copies
8. O7-8743, scope
9. ***Mycobacterium chimaera* MA 3785-A3 (CONTROL)**
10. P5 SW-3-1, water biofilm, 12 copies
11. P10 SW-4-2, water biofilm, 12 copies
12. A004, patient, 12 copies
13. FMH-SW-25-4, water biofilm, 13 copies
14. o7-5716, scope, 13 copies
15. o7-6177, scope, 13 copies
16. A007, patient, 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 >11 copies, while no *M. chimaera* isolates had copy numbers <12 copies.
- 5 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 %.

CONCLUSIONS

❖ MIN 33 PCR appears to be a simple, rapid and accurate method to distinguish *M. intracellulare* from *M. chimaera* isolates.

❖ 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.

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