Revisiting the specific molecular identification of non-tuberculous mycobacteria through a panel of gene targets

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Background
The identification of non-tuberculous mycobacteria remains a challenge for clinical laboratories. The most widely used gene target is the 16S rRNA. However, several studies have signaled that this molecular target underestimates the diversity within the genus and does not distinguish between some mycobacterial species.

Objective
The objective of this study was to compare the usefulness of other gene targets for species identification of mycobacteria.

Methods
We performed sequencing analysis of 7 housekeeping genes (16S rRNA, hsp65, recA, dnaJ, sodA, rpoB, dnaA), on a collection of 14 mycobacterial type strains available at the laboratory of Microbiology (UGent-Belgium). Sequencing was performed using an ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems). The sequencing output was analyzed and assembled using BioNumerics v5.1. All targets were subjected to BLAST recognition in the NCBI database. Phylogenetic trees were constructed using the neighbour-joining method and Kimura’s two-parameter substitution model, and visualized using the MEGA software package.

Results
The table shows the results of sequence comparisons for each gene by BLAST analysis using sequences in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov) by referring to published data.

Table. BLAST recognition for each gene target

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<th>Gene</th>
<th>Mycobacterium_parafortuitum</th>
<th>Mycobacterium_marinum</th>
<th>Mycobacterium_abscessus_subsp._abscessus</th>
<th>Mycobacterium_nonchromogenicum</th>
<th>Mycobacterium_incunabilis</th>
<th>Mycobacterium_tuberculosis</th>
<th>Mycobacterium_thermoresistibile</th>
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<th>Mycobacterium_bovis</th>
<th>Mycobacterium_septicum</th>
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Conclusions
• Preliminary analysis show that rpoB gene provided a higher number of species-level identification than the 16S rRNA and dnaJ genes. recA and sodA genes were more variable than 16S rRNA and dnaJ, but rpoB allowed the discrimination of all strains at species level.
• Sequencing analysis of these 7 housekeeping genes using clinical isolates from Argentina is ongoing.
• Definitive species identification remains problematic without the use of multiple DNA targets.

Fig 1. Phylogenetic tree of 921bp region of 16S rRNA gene of 14 mycobacteria type strains. As expected, rapid (RGM) and slow-growing (SGM) mycobacteria, were separated in two different branches. Branch support is recorded at nodes, as a percentage of 100 bootstrap repetitions. Six SGM formed a cluster supported with 69% bootstrap value. 16S rRNA sequence did not discriminated mycobacteria belonging to the Mycobacterium abscessus complex.

Fig 2. Phylogenetic tree of 360 bp region of partial dnaJ sequence of 14 mycobacteria type strains. SGM and M. abscessus complex are in the same branch supported by a 18% bootstrap value. M. parafortuitum and M. vaccae are in two independent branches supported by 56% bootstrap value. As with the 16S rRNA tree M. abscessus complex could not be separated.

Fig 3. Phylogenetic tree of 650 bp region of recA gene of 14 mycobacteria type strains. In this tree SGM formed a cluster supported by 53% bootstrap value. M. abscessus, M. bolletii and M. immunogenenum also formed a cluster with 99% bootstrap value. We found a lack of interspecies variability in the recA gene for M. wolinskyi and M. peregrinum. M. parafortuitum is located in an independent branch supported by 41% bootstrap value.

Fig 4. Phylogenetic tree of 764 bp region of rpoB gene of 14 mycobacteria type strains. We could not separate SGM and RGM in two branches. Only M. terrae and M. marinum are present in a separated branch with 100% of bootstrap value. Each strain could be differentiated in the phylogenetic tree as unique.

Fig 5. Phylogenetic tree of 411 bp region of sodA gene of 14 mycobacteria type strains. This phylogenetic tree could not separate between RGM and SGM. Only two strains, M. terrae and M. nonchromogenenium, were placed in a separated branch supported by 87% bootstrap value. The other SGM; M. marinum, M. szulgai and M. celatum were placed in the same general branch as RGM supported by 100% bootstrap value. Each strain could be differentiated as unique.

References