DprE1, a New Taxonomic Marker in Mycobacteria

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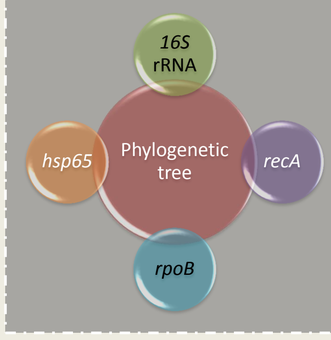
Introduction

Among the 50 species of *Mycobacterium* genus that are now recognized as potential human pathogens, those belonging to *Mycobacterium tuberculosis* complex (MTBC, i.e. *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium canettii*, *Mycobacterium bovis*) are the most known. All these species are the causative agents of tuberculosis (TB) disease. Other important species known for their human pathogenesis are: *Mycobacterium leprae*, the causative agent of leprosy persisting in developing countries, and *Mycobacterium avium-intracellulare* complex (MAC), an important group including nontuberculous mycobacteria responsible for opportunistic infections in immunocompromised individuals. In the *Mycobacterium* genus, the interspecies genetic similarity ranges from 94 to 100% and for some mycobacterial species this is higher than in other bacteria (1). Therefore, it is important to understand the relationships between mycobacterial species.

Current Mycobacteria phylogenetic assays



The *16S* rRNA gene has been the first target for phylogenetic analysis in bacteria. Only recently, multi-gene sequence analysis was applied to mycobacteria, revealing novel insights in the phylogenetic relationships between the various *Mycobacterium* species (1). Alternative phylogenetic markers have been proposed for mycobacteria, such as *hsp*65, *rec*A and *rpo*B genes.

Finding New Phylogenetic/Taxonomic Markers

Is *M. tuberculosis dprE1* a new phylogenetic/taxonomic marker ?

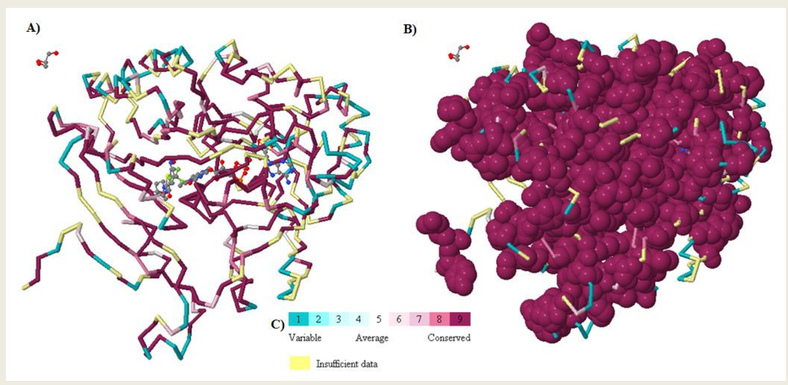
This gene encodes the target of some new antitubercular agents such as the Benzothiazinones (BTZ) (2-6). DprE1 enzyme works in concert with DprE2 and they are involved in the biosynthesis of arabinogalactan, an essential component of the mycobacterial cell wall core (7). Mutations in Cys387 residue of *M. tuberculosis* DprE1 are responsible for BTZ resistance (2). This Cystein is highly conserved in orthologous DprE1 proteins from various BTZ-susceptible Actinobacteria; in *Mycobacterium avium* and *Mycobacterium aurum* the Cys387 residue is replaced by Serine or Alanine, respectively (2); this achievement renders bacteria belonging to these species naturally resistant to BTZ.

Results

1° In order to investigate if DprE1 is a good phylogenetic marker, nine *dprE1* orthologous gene sequences not available in database belonging to *Mycobacterium* species, were amplified by PCR (using STAT-NAT DNA Mix Kit, Sentinel CH SpA, Italy, and *ad hoc* primers), sequenced and deposited at NCBI web site (http://www.ncbi.nlm.nih.gov/) (Table 1).

Species	*dprE1* dimension (bp)	Sequence data submission to GenBank
M. celatum	1383 bp	BankIt1546983 Seq1 JX215332
M. scrofulaceum	1401 bp	BankIt1546983 Seq2 JX215333
M. simiae	1383 bp	BankIt1546983 Seq3 JX215334
M. gastri	1401 bp	BankIt1546983 Seq4 JX215335
M. chelonae	1524 bp	BankIt1546983 Seq5 JX215336
M. africanum	1386 bp	BankIt1603809 Seq1 KC588931
M. xenopi	1383 bp	BankIt1603809 Seq2 KC588932
M. intracellulare	1383 bp	BankIt1603809 Seq3 KC588933
M. avium paratuberculosis	1401 bp	BankIt1603809 Seq4 KC588934

2° To identify DprE1 mycobacterial proteins and putative mycobacterial BTZ susceptible-resistant species, 64 sequences homologous to *M. tuberculosis* DprE1 were retrieved and aligned with the nine obtained ➔ The analysis revealed a high degree of sequence conservation between DprE1 proteins belonging to different mycobacterial species.



A) Conservation scores, obtained with Consurf server (8), of each amino acid at each positions are projected onto the *M. smegmatis* DprE1 protein structure (PDB: 4f4q) (9).
B) Residues having the highest degree of conservation are highlighted.
C) Continuous conservation scores divided into a discrete scale of nine grades.

Of the 12 amino acids analyzed from *M. smegmatis* DprE1 structure and located into the DprE1 active site:
- 11 are conserved in all analyzed sequences (Tyr67, His139, Gly140, Lys141, Lys425, Gln341, Gln343, Leu370, Lys374, Phe376, Asn392);
- Cys394 (Cys387 in *M. tuberculosis*), in some sequences, is replaced by an Alanine.

Since this Cys is clearly associated to BTZ sensitivity, the 11 mycobacterial species having an Ala replacing a Cys in this position are very likely resistant to BTZs.

DprE1 phylogenetic analysis

3° The multialignment of DprE1 amino acid sequences was used to build the neighbor-joining tree (Fig.1):

- Different strains of the same species were clustered together in the tree, except for the *Mycobacterium rhodesiae* and *M. xenopi* strains;
- Each species is clearly separated from each other;
- Discrimination of two different clusters including mycobacterial species having Cys or Ala in position 387 (Fig.1).

The presence of Cys or Ala residue might be a powerful tool to indicate whether a mycobacterial species is sensitive/resistant to BTZs and to the other DprE1 inhibitors.

DprE1 could be used as a taxonomic marker for identifying/clustering strains belonging to the same mycobacterial species.

Fig. 1. DprE1 Phylogenetic tree.C, cysteine; A, alanine.

DprE1 tree and *Mycobacterium* phylogeny

4° A phylogenetic tree was constructed from the alignment of the concatenated amino acid sequences of the products of nine housekeeping genes from 46 *Mycobacterium* strains (Fig.2):

- Strains belonging to the same species were clustered together;
- Nodes separating different species are supported by very high bootstrap values (99-100%);
- There is not separation between the mycobacterial BTZ susceptible and resistant species.

Consequently, this feature is not linked to the phylogeny

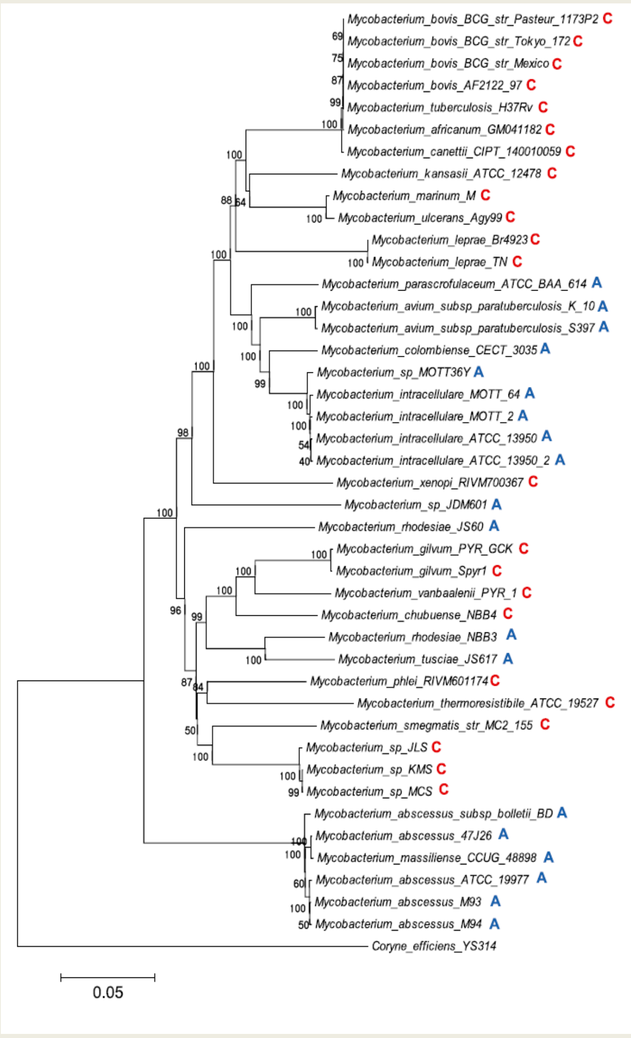


Fig. 2. Phylogenetic tree constructed using the concatenated sequences of nine proteins belonging to 46 different *Mycobacterium* strains. The concatamer was obtained using the orthologs of FusA, IleS, LepA, LeuS, PyrG, RecA, RecG, RplB, and RpoB of *M. tuberculosis* H37Rv strain.

Conclusions

DprE1 represents a good taxonomic marker for the assignment of a mycobacterial isolate to a given species; it also gives insights into sensitivity/resistance to BTZ and to other drugs hitting DprE1 enzyme. Moreover, the concatamer is a good reference phylogeny for the *Mycobacterium* genus (10).

References

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