

TITLE: MYCOBACTERIUM MOBILOME: *IN SILICO* AND *IN VITRO* EVIDENCE OF A DIVERSITY OF PLASMIDS

AUTHORS: MORGADO, S.M.; VICENTE, A.C.P.

INSTITUTION: INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO, RJ (AVENIDA BRASIL, 4365, CEP 21040-900, RIO DE JANEIRO - RJ, BRAZIL)

ABSTRACT:

The *Mycobacterium* genus presents hundreds of species with agricultural, biotechnological, clinical, and ecological importance. Despite the huge diversity of species, the mobilome (the set of all mobile genetic elements) of this genus is underexplored. Few plasmids have been described so far in this genus, and, notably, they have been associated with the evolution and radiation of the type VII secretion system (T7SS) among species from this genus. Thus, here, we explored the mycobacteria mobilome focusing on plasmids and performing *in silico* and *in vitro* analyses. We look for putative plasmids in 7670 *Mycobacterium* genomes from Genbank, besides 17 *Mycobacterium* genomes from the Atlantic Forest soil strains (CBMA strains) generated in this study. Applying multiple *in silico* strategies to plasmid detection, such as topology determination; blastn analyzes; and searches, based on hmm profiles, for protein-coding genes associated with DNA transfer and replication. We included known *Mycobacterium* plasmids as a positive control. As results, 2019 putative plasmids (including CBMA genomes) were selected from these search strategies. From the control group, 90% of them were assigned as plasmids. The CBMA strains were submitted to a pulsed-field gel electrophoresis analysis in order to raise evidence of the presence of medium and large size plasmids. This *in vitro* analysis corroborated with the *in silico* plasmid prediction concerning these strains. Two plasmids (20 and 270 kb sizes) were observed *in vitro* and contigs with these sizes had been identified *in silico*. Overall, the *in silico* prediction showed putative plasmids with sizes ranging from 1 to 625 kb length, most of them between 10-50 kb. Around 40% of the plasmids presented circular topology, and most of them had a GC content between 60-70%, that is the genus GC content. The taxonomic distribution of these putative plasmids encompassed 79 species and 46 sp. genomes, most of them belonging to *Mycobacterium abscessus*, *Mycobacterium chimaera*, and *Mycobacterium avium* species. A gene content analysis revealed that most of them harbor a relaxase gene, and some of them presented the set of genes resembling the T7SS locus. Therefore, plasmids seem to be an abundant element in the underexplored *Mycobacterium* mobilome.

Keywords: *Mycobacterium*, plasmids, mobilome, type VII secretion system, relaxase, *in silico*

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