TITLE: CHARACTERIZATION OF SPORE-LIKE STRUCTURES IN RAPIDLY GROWING MYCOBACTERIA.

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ABSTRACT:

Bacterial spores are structures with particular biological properties such as resistance to high temperatures, desiccation, radiation and exposure to chemicals. The sporulation usually occurs when there is environmental stress or when the bacterial population reaches a critical density, being normally carried out by several soil bacteria. For a long time, it was believed that bacteria belonging to the genus Mycobacterium were not able to form spores. However, due to the ability of mycobacteria to adapt and colonize different environments, they may have adequately developed mechanisms of dormancy and / or persistence in order to survive under the most varied stress conditions. In recent years, several studies have suggested the hypothesis that some species of mycobacteria could sporulate. However, the occurrence of these structures by these microorganisms may provide indications for better understanding facts such as persistence of pathogenic mycobacteria for a long time in the host without causing any symptoms. Thus, in order to contribute to the knowledge on the mechanisms of dormancy / persistence of mycobacteria, in this present study, strains of different species of mycobacteria will be evaluated for possible production of spore-like structures. For this purpose, 20 strains of rapidly growing mycobacteria will be cultured up to nutritional exhaustion on 7H10 medium at periods of 7, 15, 20, 30 and 45 days. The remaining cells will be submitted to spore enrichment, quantification of dipicolinic acid, as well as morphology, morphological heterogeneity and cell viability, metabolic activity, physical and chemical resistance-related characteristics, expression of the genes necessary for their formation. For these aims, this study will include the following methods fluorescence and phase contrast microscopy, scanning electron microscopy and transmission electron microscopy; flow cytometry; determination of intracellular ATP level and respiratory rate; heat treatment and exposure to stress, and gene expression through RNAseq. Until now, the spore selection process has been applied to the strains M. marinum, M. nonchromogenicum and M. insubicum and through the results of optical microscopy it was possible to observe the presence of spore-like structures in the strains M. marinum and M. nonchromogenicum after different incubation times. Thus, these results provide us with the first indication that mycobacteria may be able to sporulate.

Key-words: rapidly growing mycobacteria, spores, spore-like structures, dormancy, morphology, gene expression.

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