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Sporulated the Mycobacteria!

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Mycobacteria are hardy organisms and resist a number of stresses. It is this property that has made Mycobacteria very famous amongst researchers. Ever since 2009 when a group of scientists accidentally discovered spores in two strains of Mycobacteria, sporulation in Mycobacteria has been the most debated research topic, as the other groups have failed to reproduce the results obtained the first time. However, my aim in this study was to identify other strains of Mycobacteria that sporulated and, three strains were identified to be sporulating. These three strains of Mycobacteria were grown in different media and incubated at different temperatures to identify the condition that produced refractile-spore particles. The cells with refractile bodies were differentially stained to view the different parts of the cell. Four different stains were used after optimizing their temperature and duration of incubation after addition of the dye. The stained cells were subject to fluorescent microscopy and images were taken. Cells of different ages were stained to check if there was any compartmentalization inside the cell similar to *Bacillus subtilis*, as compartmentalization is a pre-requisite to endospore formation in *B. subtilis*. Two out of the four strains of Mycobacteria considered in this study showed beautiful compartmentalization similar to *B. sublitilis*, and thus further biochemical analysis such as the heat-sensitivity test, Dipicolinic acid assay (a hallmark assay for spores), spore enrichment and transmission electron microscopy (TEM) was done for the samples that showed compartmentalization with shiny refractile bodies. In order to be sure that the colonies observed were not some contamination, they were subject to 16s rRNA PCR amplification with primers specific to a conserved region in the Mycobacterial chromosome and were sequenced.

It has been speculated that sporulation could explain the latency in Mycobacterial infections and this study was started with a view to find out other sporulating Mycobacteria. I was able to identify three more strains that sporulated , however, further study would be required to ascertain the results obtained. Future perspectives include further biochemical analysis with more samples and necessary controls and sequencing of the samples after every positive result in order to totally exclude the possibility of contamination.

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