

TITLE: CHOLESTEROL CONSUMPTION OF *Mycobacterium smegmatis* PROMOTES BACILLUS RESISTANCE

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**ABSTRACT:** Pathogenic mycobacteria, such as the etiological agent of tuberculosis (*Mycobacterium tuberculosis*), are known to overcome the microbicide mechanisms of phagocytosis, surviving in the microenvironment of stress and nutritional scarcity within pulmonary macrophages. For this, the cholesterol supplied by the host cell itself has been studied as the main alternative source of energy and carbon that the bacillus needs to maintain its necessary physiological functions during the infection and then to escape of phagocytosis. Our group has shown that changes in constituents of the mycobacterial cell wall are induced after the consumption of cholesterol that was inducible by culture in minimal medium (MM), instead of *Middlebrook broth* 7H9, allowing the resistance of the bacillus to stress induced by H<sub>2</sub>O<sub>2</sub>. The aim of present work is to study the relevance of cholesterol consumption in MM to generate bacteria resistant to different others stressors, eventually developed during the infection. For this, we cultured the *Mycobacterium smegmatis* (saprophytic specie) in *Middlebrook broth* 7H9, supplemented with glycerol, and MM, supplemented with glycerol and/or cholesterol, up to early stationary growth phase, to submit these bacilli to the treatment with different pH and different concentrations of H<sub>2</sub>O<sub>2</sub> and SDS (sodium dodecyl sulfate). After those treatments, the mycobacteria were spotted on plates containing *Middlebrook agar* 7H10 for 3 days at 37 °C for analysis of the growth of resistant bacteria. We verified that the in MM, independently of glycerol and/or cholesterol supplementation, provided resistance to *M. smegmatis* against 20mM H<sub>2</sub>O<sub>2</sub> and 0.005% SDS. However, we observed that the bacillus after consuming cholesterol in MM showed sensitivity to pH 3, whose *M. smegmatis* is resistant after culture in *Middlebrook* 7H9. Therefore, we suggest that the development of resistance against H<sub>2</sub>O<sub>2</sub> and SDS, in addition to pH sensitivity, may be related to the new architecture of the bacterial cell wall after culture in MM, which may be able to induce significant behavior changes of the bacillus to the endogenous macrophage stressors, like the ones we studied in the present study.

**Keywords:** tuberculosis, cholesterol, bacillus resistance

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