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Assessing the propensity of drug resistant tuberculosis to enter and exit the differentially culturable state

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Abstract

Tuberculosis (TB), one of the oldest and most contagious infectious diseases, continues to be a global health concern. TB is caused by members of the *Mycobacterium tuberculosis* complex (MTBC) which comprises of several species. These species are further subdivided into strains based on subtle genetic differences. The success of *M. tuberculosis* as a pathogen can be attributed to its ability to survive various stresses by adopting different growth states. Previous studies have shown that sputum from TB infected patients harbours a large proportion of drug-tolerant bacteria that are unable to form colonies on agar plates but can grow in liquid media. This population of organisms, termed differentially culturable tubercle bacilli (DCTB), could be resuscitated to grow by supplementing liquid media with cell free culture filtrates from axenic cultures of wild type *M. tuberculosis* H37Rv or mutant H37Rv lacking all five resuscitation promoting factors (*Rpf*s). Laboratory models that induce this differentially culturable state are critical for studying the physiology and metabolism of these bacteria in order to develop new TB diagnostic tests. In this study, five Beijing and five LAM drug resistant strains of *M. tuberculosis* were selected and used to robustly generate DCTB through an *in vitro* stress model using carbon starvation. The most probable number (MPN) assay and colony forming units were used to determine the amount of DCTB. Furthermore, the phenotype of these cells was studied using microscopy as well as metabolic probes that target the peptidoglycan (PG) component of the bacterial cell wall. Our findings demonstrated that applying the carbon starvation model to clinical *M. tuberculosis* strains (Beijing and LAM) resulted in robust levels of DCTB, as evidenced by limited growth on agar plates and enhanced growth in liquid media supplemented with culture filtrate from LAM and Beijing strains. Comparison of cell length between carbon starved cells to those grown in routine laboratory media suggested that DCTB appeared to be non-replicating and significantly shorter. The metabolic activity of the starved cultures was restored when they were supplemented with H37Rv, LAM and Beijing culture filtrate. Our results also demonstrated that Beijing strains had a higher propensity to produce DCTB compared to LAM strains and that the supplementation with Beijing culture filtrate resuscitated more DCTB. Collectively, our findings allow for the advancement of experimental systems that enable further investigation of DCTB and the properties of the Beijing strain that facilitate better adoption of the differentially culturable state.