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REVIEW

Virulence, biochemistry, morphology and host-interacting properties of detergent-free cultured mycobacteria: An update

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SUMMARY

The culturing of mycobacteria is a standard procedure that is consistent world-wide, with little variation in the growth media constituents, particularly those found in liquid and solid media. Before the 1940s however, the aggregating nature of mycobacteria as well as the characteristic slow growth-rate saw mycobacterial research delay considerably. Dubos and colleagues addressed both these issues and observed that a very small volume of Tween detergent was sufficient to greatly improve the culturing of mycobacteria. Over the years however, evidence of the unfavourable effects of this detergent on a number of morphological, biochemical, pathogenic and host-interacting properties of mycobacteria surfaced. For the first time we bring together literature, past and present to comprehensively review the mycobacterial properties which are, and are not affected by the use of this detergent. We also address other detergents and methods which may circumvent the need to include Tween compounds in mycobacterial culture media.

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1. Introduction

After the emergence of tuberculosis in the early years, experimental progress into investigations concerning mycobacteria and its disease causing mechanisms were unproductive. This was mostly due to the slow growth-rate and the characteristic aggregating nature of mycobacterial species, making it a challenge to work with. In light of this, researchers were compelled to explore various growth medium supplements in an attempt to address both these issues so that investigations concerned with pathogenesis, immunity and chemotherapy could be expedited.

Almost 80 years ago Dubos and colleagues were successful in developing an efficient and effective method of growing mycobacterial cultures for use under various experimental conditions [1]. They discovered that the addition of egg yolk to the growth medium significantly enhanced the rate of growth, and after much

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investigation attributed this increased growth rate to the abundance of phospholipids that were readily utilised by the mycobacteria [2]. Other natural sources of phospholipids, such as those from soy beans were also used, however due to the laborious task of preparing such compounds, alternatives were required. Synthetic phospholipids in the form of polyoxyethylene sorbate compounds (Tweens) were introduced and it was observed that not only did the rate of growth increase considerably, but the tendency of the mycobacteria to aggregate was greatly reduced, resulting in diffuse homogenous cultures. From that point forward, the introduction of Tween 80 into mycobacterial growth media became standard laboratory practice. Over the years, a number of investigations were aimed at dissecting and elucidating the mechanisms responsible for the advantageous effects achieved when mycobacteria were grown in liquid culture. During the process however, a substantial amount of evidence surfaced which suggested that Tween significantly alters a number of biochemical and physiological properties in mycobacteria.

For the first time we bring together 70 years of literature documenting the various effects that Tween use has on mycobacterial structural, biochemical and host-interacting properties. In doing so, we assess when the effects of this culture additive is negligible and in what context it is necessary to reassess its suitability within liquid culture media. In addition, we discuss other detergents and methods which may circumvent the need to include Tween compounds in mycobacterial culture media.

1.1. Tween-containing liquid media and associated mycobacterial growth and morphological properties

After the introduction of Tween into standard culturing protocols and procedures, it was determined that these detergents provide a reservoir of oleic acid that serves as a potent carbon and energy source [3], thus enhancing the growth-rate of mycobacteria. Cellular enzymes hydrolyze Tween 80 into free oleic acid and polyoxyethylated sorbitol, which are then used as metabolic substrates (Figure 1). This becomes a disadvantage since the free oleic acid is toxic to mycobacteria, particularly when the medium is acidified. This issue was circumvented by the addition of bovine serum albumin to the medium [4], which, depending on the type of investigation being carried out, presented with challenges of its own. The additional albumin represents an unwanted extrinsic protein which complicates the detection of secreted mycobacterial antigens, however under standard culturing procedures is not an issue since albumin and oleate complex with one another and this successfully abrogates the toxic effects of the oleic acid (mycobacteria are still able to metabolize this complex). To date, it has been established that Tween compounds enhance the growth of several species of mycobacteria [5–8]. A comprehensive study on the effects of Tween compounds on mycobacterial growth observed that *in vitro* growth rates were stimulated in a concentration-dependent manner with optimal growth achieved after the addition of 0.1 and 1.0% Tween 80 [9]. In fact, the effect of Tween on mycobacteria is so distinct, that species of mycobacteria may be differentiated based on their Tween hydrolyzing abilities [10,11]. It was this test which allowed researchers to rapidly distinguish between various species of slow-growing mycobacteria for classification and diagnostic purposes.

Indeed, Tween-containing growth media is acceptable for studies concerning growth rates and kinetics, as well as the preparation of bacteria for chemical extraction, however it is important to take note that the majority of data associated with the growth kinetics of mycobacteria are based on liquid cultures supplemented with Tween compounds and may therefore not be entirely accurate. With the above said, the enhancement of mycobacterial growth rate may be beneficial for various studies, however since Tween is utilized as a carbon source, studies focussing on metabolomics and other related fields should be aware that the shift in metabolism is distinct.

1.2. Tween-induced mycobacterial cell wall aberrations and potential effects on pathogenicity and virulence

Mycobacterium tuberculosis, like many other pathogenic bacteria, lose virulence with prolonged culture in artificial media supplemented with Tween. For the detergent to have an effect on virulence, it must affect the ability of *Mtu* to enter host cells, counter host cell defences and multiply intracellularly [12]. *In vivo*,

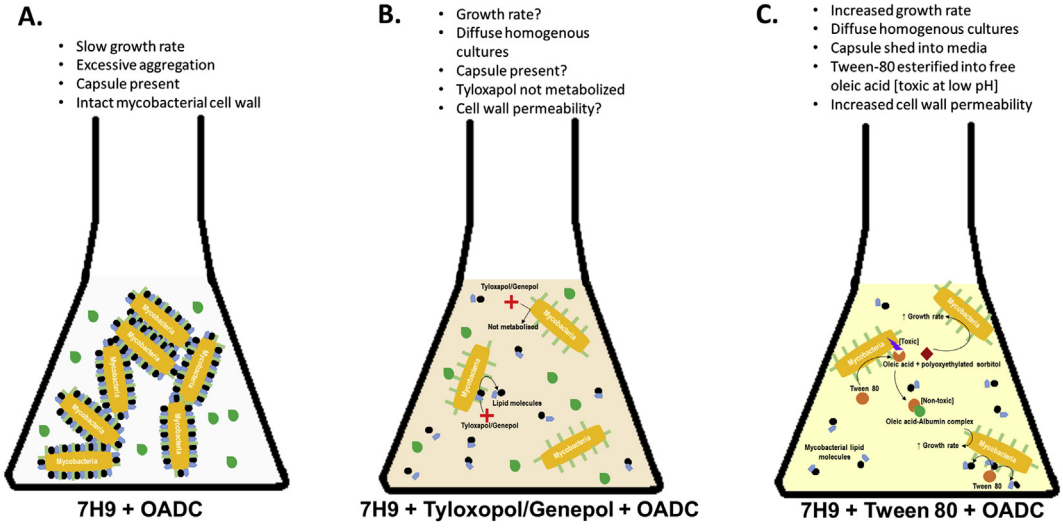

Figure 1. Liquid media compositions commonly used for culturing mycobacterial species. A. Mycobacteria grown in 7H9 media (supplemented with Oleic Albumin Dextrose Catalase (OADC) without any detergent cause the characteristic aggregation of the bacilli into serpentine cords. Although handling of the bacteria is impossible at this stage, the mycobacterial capsule and cell wall remain intact and cell wall permeability is not affected. B. The addition of Tyloxopol or Genepol to liquid culture media effectively disperses the mycobacteria but possibly affects mycobacterial capsular structures thus possibly affecting virulence. Both detergents are non-hydrolyzable by mycobacteria and therefore do not affect the metabolism of the organism. C. Tween 80-supplemented 7H9 also effectively disperses mycobacteria, however its powerful detergent function causes the capsule to be shed into the media along with other important lipid-based antigens. Its hydrolyzable nature causes free oleic acid to accumulate in the growth media which, without the presence of albumin is toxic to mycobacterial species, especially in acidified media. Polyoxyethylated sorbitol and the oleic acid-albumin complex are both readily metabolized by mycobacteria which contribute to an increase in growth rate.