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Revisiting a protocol for extraction of mycobacterial lipids

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ABSTRACT

Determination of lipid content of any biological sample is essential for various kinds of studies related to pathogenicity and drug development. Thus, reliable methods for the quantitative extraction of lipids are of critical importance. The mycobacterial cell wall is largely composed of lipids. Commonly used methods to extract lipids, such as the Bligh and Dyer method or the Folch method, yield a low amount of lipids when applied to mycobacterial cells. This study presents an efficient modification of Chandramauli's method, a less known method developed at this institute earlier that is able to yield a considerably higher concentration of mycobacterial lipids.

Introduction

With almost 30% of mycobacterial genes devoted to lipid metabolism *Mycobacterium tuberculosis* is well known for its complex cell wall structure which confers, to the bacilli, resistance to drying, acidic or alkaline conditions, and to chemical disinfectants and therapeutic agents [1]. The most preliminary step for studying the lipid profile is the extraction of cellular lipids. In the search for an efficient method for mycobacterial lipid extraction, three methods were found. These were comprised of two very well known methods given by Folch in 1957 and by Bligh and Dyer in 1959. The Folch's method, which was developed primarily for brain lipids, involved extraction with chloroform/methanol (2:1, v/v) followed by washing with weak salt solutions of NaCl/KCl/$MgCl_2$ in order to retain acidic lipids. All the steps of washing were performed by centrifugation of the suspensions, but it was also stated that in case there is no time constraint, phases may be allowed to separate by prolonged standing [2]. The method as given by Bligh and Dyer [3] involved lipid extraction from animal tissue (fish muscle) and the solvent system used was slightly different. It recommended the use of a different ratio (1:2, v/v) of the same solvent system (chloroform/methanol, v/v) [2,3]. Though these protocols were developed originally for animal tissues and were validated on samples with low lipid content, they have been employed for extraction of lipids from other sources, including bacteria [4,5]. Chandramouli and Venkitasubramanian in 1974 [6] developed

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Abbreviations: MTB, *Mycobacterium tuberculosis*; TLC, thin layer chromatography; HPLC, high pressure liquid chromatography.

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a method (less known method) inspired by Folch's method. They added prolonged mixing of cell pellet with the same ratio of chloroform and methanol and allowed phase separation by standing instead of centrifugation. In this study, a comparative analysis of these three methods was carried out for extraction of mycobacterial lipids in order to choose the best suitable method.

Materials and methods

Bacterial cultures

The different mycobacterial species that were used in the study were comprised of *M. tuberculosis* (H37Rv), *Mycobacterium smegmatis* (ATCC 19420), *Mycobacterium bovis* (ATTCC 19210T), *M. avium* (MTCC, IMTECH, Chandigarh, India), *Mycobacterium fortuitum* (ATTC 6841) and *Mycobacterium kansasii* (ATCC 21982). The cultures were maintained on Middlebrook's 7H9 broth (Difco Laboratories, MI, USA) and were autoclaved prior to lipid extraction to ensure safety during culture handling.

Extraction of lipids

Lipid extraction was initially performed with 200 ml of *M. tuberculosis* H37Rv culture for each protocol. The cultures were harvested when the optical density ($O.D_{600}$ = 1) reached 0.4–0.6. Cultures were centrifuged and pellet obtained was dried. Weight of the dried pellet was noted and equal weight of pellets was taken for lipid extraction. Extraction was carried out using all the three aforementioned protocols in parallel, and the methods were followed as were originally specified (Fig. 1). The solvent/sample ratio was also maintained as per the actual protocol. In order to check any protein contamination, the Bradford assay was performed for each lipid extract.

Thin layer chromatography

The lipid content obtained in each case was dried under nitrogen, weighed and then analysed by thin layer chromatography (TLC) after dissolving the samples in chloroform. Equal volume (2 μl) of each sample was loaded on TLC plates (Merck). Chloroform/methanol/water (60:12:1, v/v) was used as a mobile phase [7]. The spots were visualized using 0.5% α-naphthol dissolved in 50% methanol followed by charring with 50% concentrated sulphuric acid. For detection of phthiocerol dimycocerosates (PDIM), TLC was run using petroleum ether/ethyl acetate (98:2, ×3, v/v) as the mobile phase [8]. The phosphomolybdic acid solution (Sigma) was sprayed for the detection of the spots. After comparison, the selected protocol was used for the extraction of lipids from

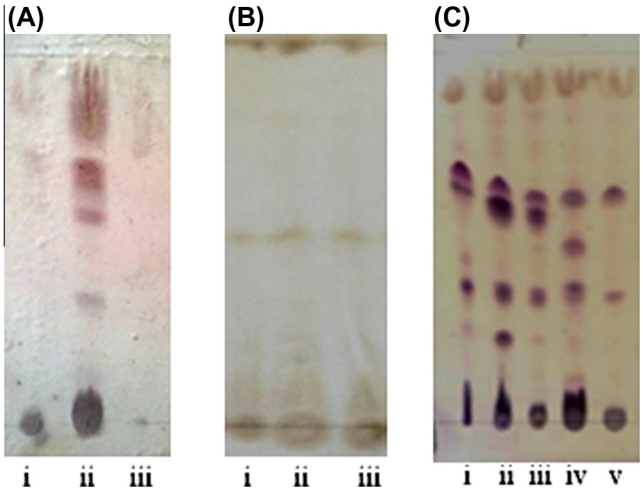

Fig. 2 – (a) TLC of total lipid extract using different protocols. TLC were developed using solvent system (a) and (b) of chloroform/methanol/water 60:12:1(v/v); (b) Solvent system – petroleum ether/ethyl acetate 98:2 (v/v): Lane 1: Folch method, Lane 2: Chandramouli's method, Lane 3: Bligh and Dyer method; (c) TLC of total lipid extracts obtained from different mycobacterial species using Chandramouli's protocol: (i) *M. smegmatis*, (ii) *M. fortuitum*, (iii) *M. avium*, (iv) *M. kansasii*, (v) *M. bovis*. Solvent system used – chloroform/methanol/water (60:12:1).

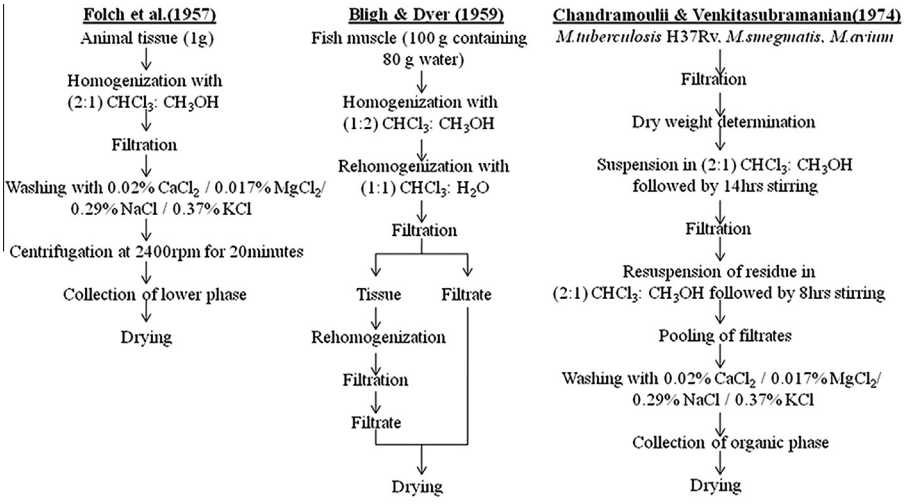

Fig. 1 – Flowchart of three protocols used in this report.