

SHORT COMMUNICATION

Mycolic Acid Patterns of Four Vaccine Strains of *Mycobacterium bovis* BCG

By D. E. MINNIKIN,^{1*} S. M. MINNIKIN,¹ G. DOBSON,^{1,2}
M. GOODFELLOW,² F. PORTAELS,³ L. VAN DEN BREEN³ AND
D. SESARDIC⁴

¹ Department of Organic Chemistry, The University, Newcastle upon Tyne, NE1 7RU, U.K.

² Department of Microbiology, The University, Newcastle upon Tyne, NE1 7RU, U.K.

³ Department of Microbiology, Institute of Tropical Medicine, Nationalestraat 155,
B-2000 Antwerpen, Belgium

⁴ MRC Unit for Laboratory Studies of Tuberculosis, Royal Postgraduate Medical School,
Hammersmith Hospital, Ducane Road, London, W12 0HS, U.K.

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Thin-layer chromatography of methanolysates of four widely used vaccine strains of *Mycobacterium bovis* BCG showed that only one organism had the expected pattern of mycolic acid methyl esters characteristic of *Mycobacterium bovis* and *Mycobacterium tuberculosis*. The remaining three BCG strains lacked methoxy mycolic acid.

INTRODUCTION

Mycolic acids are characteristic high molecular weight 3-hydroxy 2-alkyl branched fatty acids whose discontinuous distribution is of considerable value in both the classification and identification of mycobacteria (Minnikin & Goodfellow, 1980). TLC of acid (Minnikin *et al.*, 1980) or alkaline (Minnikin *et al.*, 1982) methanolysates provides an efficient procedure for the recognition of characteristic patterns of the general types of mycolic acids. In this report it will be shown that only one of four strains of *Mycobacterium bovis* BCG had the expected pattern of mycolates characteristic of *M. bovis* and *M. tuberculosis*. The remaining three strains were unusual in lacking mycolic acids having a methoxy function.

METHODS

Strains. BCG strains Moreau (Brazil), Prague (Czechoslovakia) and Institut Pasteur no. 942 (Paris) (International Union against Tuberculosis, 1978) were grown in flasks containing 2 litres of 7H9 broth (Difco) with continuous magnetic stirring at 37 °C for 40 d. After checking for purity, cultures were killed with 1% (v/v) formalin, harvested by centrifugation at 10000 g for 10 min, washed three times with distilled water and freeze-dried. BCG Glaxo strain was grown as a surface pellicle on Sauton's medium for 46 d at 37 °C and supplied as wet cell paste by J. A. Carman, Public Health Laboratory Services, Centre for Applied Microbiology and Research, Porton Down, Salisbury, U.K.

Analysis of mycolic acids. Dried biomass was degraded by both acid (Minnikin *et al.*, 1980) and alkaline (Minnikin *et al.*, 1982) methanolysis and the methanolysate examined by two-dimensional TLC (Minnikin *et al.*, 1980). Mycolic acid methyl esters were isolated by preparative TLC using 10 × 10 cm pieces of Merck (5735) plastic-backed TLC sheets and three developments with petroleum ether (b.p. 60–80 °C)/acetone, 95:5 (v/v). Separated bands were located by spraying with 0.01% ethanolic rhodamine 6G and viewing the dried sheets with long-wave (366 nm) UV light. The bands were cut out and mycolic acid methyl esters eluted with diethyl ether (2 × 1 ml) and examined by mass spectrometry using an AEI MS9 instrument (70 eV, 230–250 °C).

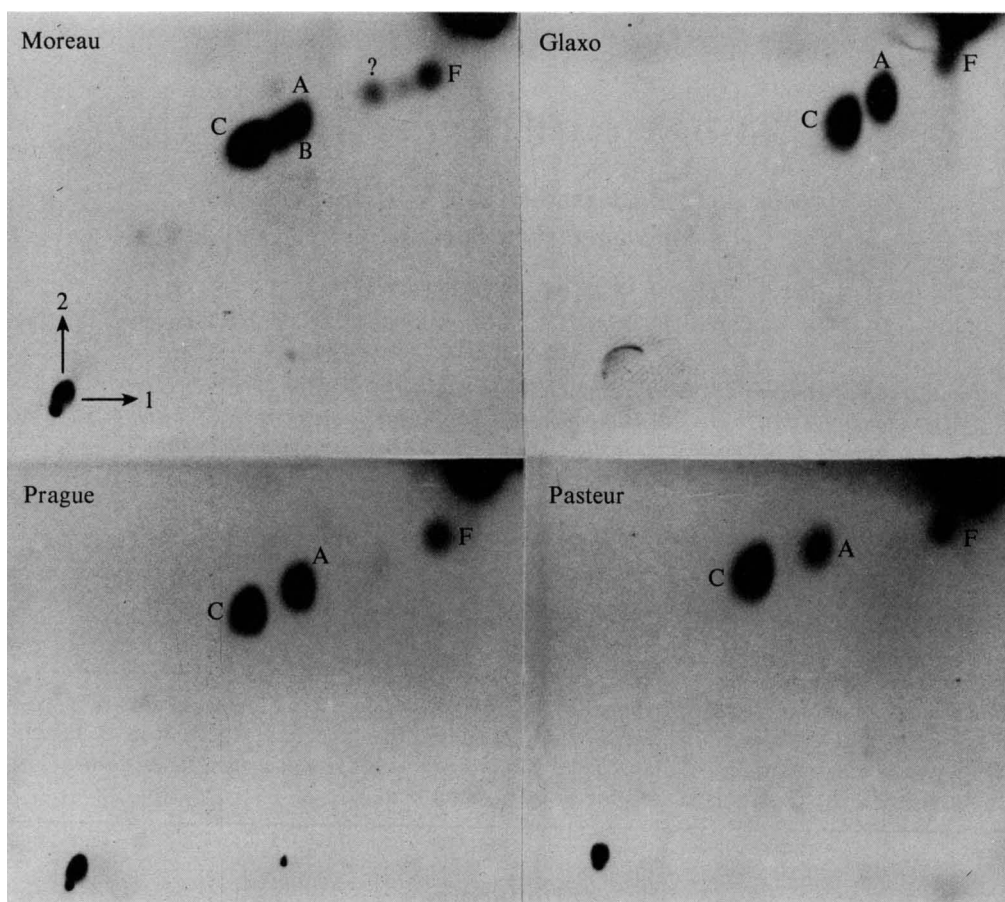


Fig. 1. Two-dimensional TLC of alkaline methanolysates of BCG strains. A triple development with petroleum ether (b.p. 60–80 °C)/acetone (95:5, v/v) in the first direction was followed by a single development with toluene/acetone (97:3, v/v) in the second direction. Abbreviations: F, non-hydroxylated fatty acid methyl esters; A, α -mycolate; B, methoxymycolate; C, ketomycolate; ?, unknown.

RESULTS AND DISCUSSION

The patterns obtained on TLC of alkaline methanolysates of the test materials are shown in Fig. 1; essentially similar patterns were also obtained for acid methanolysates. Components corresponding to so-called α -mycolates and ketomycolates (Minnikin & Goodfellow, 1980) were present in all cases but only one sample contained an ester corresponding in mobility to a methoxymycolate (Fig. 1). A superficially similar TLC pattern would be seen for alkaline methanolysates of organisms producing α - and epoxy mycolates but on acid methanolysis the epoxy mycolates are converted to more polar derivatives (Minnikin *et al.*, 1982). Mass spectrometry of the mycolic acid methyl esters from all the test strains showed that the branch in the 2-position contained mainly 24 carbons with minor amounts of a 22-carbon component.

All mycobacteria examined to date produce α -mycolic acids and, if ketomycolic acids are also present, either methoxy or ω -carboxymycolates are also usually found (Minnikin & Goodfellow, 1980). All representatives of the *M. tuberculosis* and *M. bovis* group of mycobacteria, studied so far, contained methoxymycolates (Minnikin & Goodfellow, 1980) so it is surprising that only the Moreau BCG strain produced a methoxymycolate (Fig. 1). The only other example of a mycobacterial mycolic acid pattern consisting of only α - and ketomycolates was provided by

Mycobacterium leprae (Etémadi & Convit, 1974; Young, 1980; Draper *et al.*, 1982) though in another study the presence of significant amounts of methoxymycolates was suggested (Asselineau *et al.*, 1981). The alkyl branch in the 2-position of the mycolic acids from *M. leprae* was composed of similar amounts of 20- and 22-carbon chains (Draper *et al.*, 1982) in contrast to the 24-carbon branch found in the BCG mycolates.

Extensive systematic studies of mycobacterial mycolic acids are showing that the qualitative patterns obtained are relatively stable for a given strain (Minnikin & Goodfellow, 1980). The complete absence of methoxymycolates in three of the test strains, therefore, is probably a highly reliable chemotaxonomic marker for these mycobacteria. The present study was stimulated by the finding that the BCG Glaxo strain, being investigated for a different purpose, lacked the expected methoxymycolate (Fig. 1). The significance of this finding was reinforced by the results obtained (Fig. 1) for the three additional recognized BCG vaccine strains available for immediate study. It would also be of value to examine the composition of other mycobacterial complex lipids having taxonomic potential (Minnikin & Goodfellow, 1980), and to extend such studies to a wider selection of the considerable number of existing BCG daughter strains. A study of the free lipids of eight BCG daughter strains showed a number of quantitative differences (Asselineau & Portelance, 1974) and it is particularly interesting that the Moreau strain was distinguished by the presence of relatively high proportions of trehalose mycolates, the so-called 'cord-factor'.

Routine vaccination with BCG strains is practised world-wide to provide protection against tuberculosis and it is therefore of great importance to have reliable methods for checking the authenticity of seed lot cultures used for this purpose. Unfortunately BCG strains have been maintained separately in many different countries using different conditions. As a result, a number of variants different in their biological properties and immunizing potency have been selected (International Union against Tuberculosis, 1978; Osborn, 1980). The presence or absence of methoxymycolic acids provides another example of such variations: they are uniformly present in the 'parent' species, *M. bovis*, and in the closely related *M. tuberculosis*. It would be interesting to investigate a possible relationship between lipid composition and immunogenic and other biological properties. The absence of methoxymycolic acids from three of the BCG strains and from *M. leprae* may also be of some value in understanding the biochemistry of the leprosy bacillus.

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