The Mycolic Acids of Mycobacterium chitae

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Two-dimensional thin-layer chromatography of whole-organism acid methanolysates of Mycobacterium chitae gives a characteristic pattern composed of a $\alpha$-mycolate, a lower molecular weight $\alpha'$-mycolate and characteristic pairs of polar mycolates. Analysis of alkaline methanolysates confirmed that these polar mycolates were derived from epoxymycolic acids. This pattern of mycolic acids has only been found previously in representatives of Mycobacterium farcinogenes, Mycobacterium fortuitum, 'Mycobacterium peregrinum', Mycobacterium senegalense and Mycobacterium smegmatis.

INTRODUCTION

Mycobacterium chitae (Tsukamura, 1967) was proposed for a number of non-pathogenic fast-growing non-chromogenic mycobacteria isolated from soil. In the second co-operative study of the International Working Group on Mycobacterial Taxonomy (Saito et al., 1977), M. chitae strains were recovered in a homogeneous cluster and considered as an acceptable species and included in the Approved Lists of Bacterial Names (Skerman et al., 1980). M. chitae is related to M. chelonei on the basis of bacteriocin typing (Takeya & Tokiwa, 1972) and to M. thermoresistibile from analysis of lipid patterns seen on chromatograms prepared from ether/ethanol extracts of cells incubated with $^{[35]S}$methionine (Tsukamura & Mizuno, 1975). In the present paper the mycolic acid pattern of M. chitae has been investigated.

METHODS

Strain and cultivation. Mycobacterium chitae (ATCC 19627, type strain) was maintained on Löwenstein-Jensen medium. For the isolation of lipid components, bacteria were cultivated on plates of modified Sauton’s medium (Mordarska et al., 1972) for 7–10 d at 30°C. After checking for purity, organisms were harvested, washed with distilled water and freeze-dried.

Extraction of total fatty acids and preparation of methyl esters. Acid methanolysates of dried bacteria (50 mg) were prepared as described previously (Minnikin et al., 1980). Alkaline methanolysates were made by treating organisms (50 mg) with 2 ml of a methanol solution containing 5% (w/v) potassium hydroxide, 5% (v/v) water and 20% (v/v) benzene, care being taken in the use of the latter toxic solvent. The mixture was heated at 110°C in a water bath for 4 h. After cooling, the mixtures were acidified by the addition of 20% (v/v) sulphuric acid, and the lipids were extracted into diethyl ether. The etheral extracts were washed with water until neutral and dried over anhydrous sodium sulphate. The dried solutions were filtered, concentrated by evaporation, and esterified with diazomethane. For laboratory preparation of diazomethane from N-methyl-N-nitroso-p-toluene sulphonamide (Diazald; Merck), all safety precautions necessary were strictly observed.

Thin-layer chromatography. Analytical two-dimensional TLC was done with glass plates (20 × 20 cm) coated with layers (0.25 mm) of silica gel (Kieselgel 60 F254; Merck). A triple development with petroleum ether (b.p. 60–80°C)/acetone (95:5, v/v) in the first direction was followed in the second direction by a single development with toluene/acetone (97:3, v/v) (Minnikin et al., 1980). The presence of separated components was revealed by spraying with 10% (w/v) molybdo phosphoric acid in ethanol followed by charring at 120°C for 15 min.
RESULTS AND DISCUSSION

The results of TLC analysis of both acid and alkaline methanolysates of \textit{M. chitae} are shown in Fig. 1. The type strain of \textit{M. chitae} gave a characteristic pattern of mycolates. The most mobile components corresponded to non-hydroxylated fatty acid (spot F), \(\alpha\)-mycolic acid (spot A) and \(\alpha'\)-mycolic acid (spot A') methyl esters in both types of methanolysates (Fig. 1a, b). TLC analysis of acid methanolysates of the test strain contained characteristic polar mycolates (spots I, J, N, O; Fig. 1a). These components were absent in the alkaline methanolysates, being replaced by a unique component ‘M’ (Fig. 1b). This component has been identified in previous studies (Minnikin et al., 1982a, 1984a) as an epoxymycolic acid methyl ester which is converted on acid methanolysis to the characteristic polar components present in whole-organism acid methanolysates (Minnikin et al., 1982a). The components N and O are dihydroxy derivates and I and J are hydroxymethoxy compounds (Fig. 1a) formed by acid methanolysis cleavage of epoxymycolate (Minnikin et al., 1982a, 1984a).

This pattern of mycolates is the same as that described in previous studies for \textit{M. fortuitum}, ‘\textit{M. peregrinum}’, \textit{M. smegmatis} (Minnikin et al., 1980, 1984a), \textit{M. farcinogenes} and \textit{M. senegalense} (Ridell et al., 1982). In a recent survey of mycobacterial mycolic acids (Minnikin et al., 1984b) the same pattern was recorded for two strains of ‘\textit{Mycobacterium giae}’, a taxon considered to be a subjective synonym of \textit{M. fortuitum}. Reference was also made to unpublished results stating that \textit{M. chitae} also had the same pattern as that recorded in the present paper.

Other fast-growing mycobacteria have distinct and characteristic patterns of mycolates; \textit{M. chelonei} contains only \(\alpha\)-mycolates and lower molecular weight \(\alpha'\)-mycolates (Minnikin et al., 1982b) but \textit{M. thermoresistibile} has \(\alpha\)– and \(\alpha'\)-, methoxymycolates and ketomycolates (Minnikin et al., 1984b). The mycolic acids of \textit{M. fallax}, a recently described fast-growing mycobacterial species (Lévy-Frébault et al., 1983) are exceptional in that \(\alpha\)-mycolates are the only components present; these \(\alpha\)-mycolic acids are di- and tri-unsaturated acids, a feature that has not been described previously in the mycobacteria.

Lipid analyses are now well established in bacterial taxonomy and have provided good characters for the classification and identification of many bacterial genera (Goldfine, 1972; Lechevalier, 1977; Shaw, 1974; Minnikin & Goodfellow, 1980; Daffé et al., 1983; Athalye et al., 1984). The analysis of mycobacterial mycolic acid methyl esters by two-dimensional TLC provides a sensitive and relatively easy method for systematic studies (Minnikin et al., 1980, 1984b; Daffé et al., 1983), although TLC of mycobacterial mycolates does not necessarily give sufficiently sensitive data for the circumscription of species (Minnikin & Goodfellow, 1980; Ridell et al., 1982; Daffé et al., 1983).
Mycobacterium fortuitum, M. smegmatis and M. chitae form distinct and separate clusters in numerical phenetic surveys (Saito et al., 1977; Tsukamura, 1981) but the lipid data raise the possibility that they might have a fairly close phylogenetic relationship.

REFERENCES


