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Note

Chromatographic study of mycolic acid-like substances in lipids of *Listeria monocytogenes*

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Some biological similarities observed between mycobacteria and listeria called for an investigation of their chemical basis. The presence of a considerable amount of lipids in mycobacteria, together with their chemical composition and properties, led to a detailed study of listerial lipids. The most characteristic constituents of mycobacterial lipids are mycolic acids (β-hydroxymonocarboxylic acids with a long aliphatic chain, C_{88}). These fatty acids have been found in other Actinomycetes (Nocardia: C_{55}, nocardomycolic acid) and taxonomically related corynebacteria (C_{32}–C_{36}, corynomycolic acids).

The separation of different mycolic acids is very complicated owing to their very similar chemical structures, based on the length of the aliphatic chain and a hydroxy group in the β-position. The presence of other substituents (hydroxy, methoxy and keto groups) has a substantial influence on the chromatographic mobility, and therefore the length of the aliphatic chain does not have a great effect on the chromatographic behaviour. We have previously developed a method for the separation of free mycolic acids on Silufol[1]. Using reversed-phase paper chromatography, a spot detectable with copper(II) acetate–rubeanic acid reagent[1–3] was formed on the start by hydrolyzates of *Listeria monocytogenes* lipids. Under the same conditions, mycolic acids from mycobacteria have similar chromatographic properties[4]. Because the spots remain unseparated on the start, we have applied this method to the separation and characterization of these substances.

EXPERIMENTAL

Material was obtained from cells of *L. monocytogenes* (strains Brat. 1 and India). Cultivation was carried out by the submersion procedure in 7-l tanks at 24° for 15 h, with controlled stirring at a standard rate of 350 rpm and vortex aeration (8 l/min of air), continuously adjusting the pH to 7.1 (USOL, Prague).

The washed and dried cells were extracted with chloroform–methanol (2:1)

for 3 days at laboratory temperature, giving a fraction of "freely bound lipids". After hydrolysis with hot 2 *N* hydrochloric acid for 8 h and subsequent extraction with cold chloroform, a fraction of "firmly bound lipids" was prepared[5]. Fatty acids from both fractions were extracted with diethyl ether after hydrolysis with a 2.5% (w/v) solution of potassium hydroxide in methanol–benzene (1:1) for 6 h (ref. 6). The residue was dissolved in chloroform and separated on Silufol sheets with *n*-hexane–diethyl ether (85:15)[1].

The spots were detected with acid fuchsin–uranyl nitrate, bromothymol blue–sodium hydroxide and copper(II) acetate–rubeanic acid reagents[1]. The main components of long-chain fatty acids (C_{12}–C_{26}) identified in *L. monocytogenes* lipids by paper and gas chromatography[7,8] form a single spot with high mobility near the solvent front. Further unidentified yellow substances remain on the start. Both fractions were removed mechanically after chromatography. The zone corresponding to mycolic acids of mycobacteria was eluted from the adsorbent layer with diethyl ether and re-chromatographed after evaporation.

RESULTS AND DISCUSSION

By extraction of dry *L. monocytogenes* cells with cold chloroform–methanol (2:1), a 12.7% yield of freely bound lipids was obtained. After acid hydrolysis and subsequent extraction with chloroform, an additional 1.2% of firmly bound lipids was isolated. After alkaline hydrolysis of both lipid fractions in methanol–benzene and extraction with diethyl ether, two volumes of 96% ethanol were added. After standing for several days at 4°, no precipitate was formed. We tried to modify the

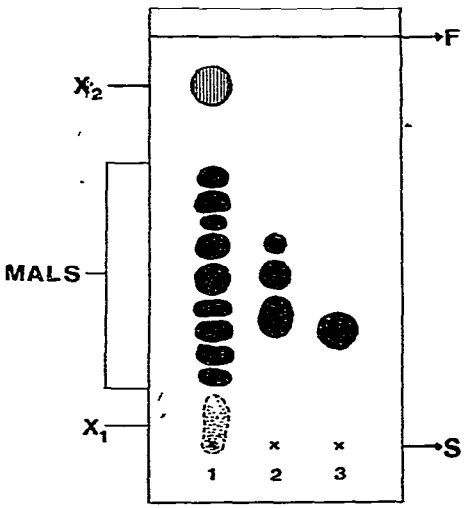

Fig. 1. Separation of mycolic acid-like substances from *Listeria monocytogenes*. Adsorbent: Silufol sheets. Solvent system: *n*-hexane–diethyl ether (85:15). Detection: acid fuchsin–uranyl nitrate. S = start; F = front line of solvent; 1 = mycolic acid-like substances from *L. monocytogenes* isolated by preparative thin-layer chromatography. 2 = mycolic acids from *Mycobacterium tuberculosis* var. bovis-BCG; 3 = nocardomycolic acid; X_1 = unidentified substances; MALS = mycolic acid-like substances; X_2 = higher fatty acids(?).

precipitation procedure using ethanol and distilled water instead of methanol. The precipitation with methanol was unsuccessful and water precipitated all fatty acids. Therefore, the fraction of "mycolic acid-like substances" was isolated by preparative thin-layer chromatography.

Freely and firmly bound lipids give at least five spots detectable with all of the reagents used. The spots have mobilities in the range of those of mycolic acids isolated from mycobacteria, but the R_F values are not exactly the same (Fig. 1). This result suggests differences in the lengths of the aliphatic chains and the presence and positions of substituents. A higher content of these acids was found in firmly bound lipids.

According to our experience, the content of these acids in listerial lipids in comparison with mycobacterial lipids is very low. At present, the possibility of obtaining greater amounts of material for detailed identification by means of other chemical and physical methods is being studied.

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