The Occurrence of Phosphatidylethanolamine and Glycosyl Diglycerides in Thermophilic Bacilli

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Phosphatidylethanolamine, a common constituent of the polar lipids of Gram-negative bacteria, is consistently found in only one genus of Gram-positive bacteria, the bacilli (Goldfine, 1972). Gram-positive bacteria commonly contain diglycosyl diglycerides (Shaw & Baddiley, 1968; Shaw, 1970) which often occur together with phosphatidylethanolamine in the bacilli. An interrelation between phosphatidylethanolamine and glycolipid diglycerides has been demonstrated in the lipids of several bacilli (Minnikin, Abdolrahimzadeh & Baddiley, 1971a, b, 1972). We have now shown that one strain of Bacillus stearothermophilus contains glycosyl diglycerides but no phosphatidylethanolamine, whereas another strain contains phosphatidylethanolamine but no glycolipids.

METHODS

Freeze-dried organisms of a strain of B. stearothermophilus were provided by Professor H. L. Kornberg and Dr R. K. Sundaram and had been grown (55 °C, 50 mM-sodium succinate as carbon source) essentially as described previously (Sundaram, Cazzulo & Kornberg, 1969). Bacillus stearothermophilus NCIB8157 (NCA1518) and the above strain were grown in a broth medium (Minnikin et al. 1971a) at 55 °C for 16 h; harvested bacteria were washed with NaCl (0.85 %) and freeze-dried.

Lipids were extracted with chloroform–methanol (2:1, v/v) and examined by two-dimensional thin-layer chromatography using a modification of the system described by Minnikin & Abdolrahimzadeh (1971); Merck silica gel H and a second development solvent, chloroform–acetic acid–methanol–water (130:27:18:7, by vol.), were used in the present study. Spraying with a saturated solution of potassium dichromate in sulphuric acid followed by charring at 200 °C revealed spots corresponding to all the polar lipids. Specific spray reagents for lipid phosphate (Dittmer & Lester, 1964), α-glycols (Baddiley, Buchanan, Handschumacher & Prescott, 1956) and free amino groups (ninhydrin in butanol) were also used.

Controlled hydrolysis of lipids with alkali was carried out as described by White & Frerman (1967). Acid hydrolysis of lipids was performed by heating in 2 M-HCl at 100 °C for 3 h in a sealed tube; the hydrolysate was evaporated to dryness over KOH in a vacuum desiccator. Water-soluble phosphates resulting from alkaline hydrolysis were examined by descending paper chromatography using the solvent systems propan-1-ol–ammonia (sp.gr. 0.88)–water (6:3:1, by vol.) (Hanes & Isherwood, 1949); butan-1-ol–pyridine–water (6:4:3, by vol.) (Jeanes, Wise & Dimler, 1951) was used for the separation of glycosides and hexoses. Material was detected on paper chromatograms by the periodate–Schiff
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Fig. 1. Two-dimensional thin-layer chromatogram of the polar lipids of *B. stearothermophilus* provided by H. L. Kornberg and T. K. Sundaram. Plates were prepared from a slurry of Merck silica gel H (40 g) in 100 ml aqueous sodium acetate (0.2%). Developing solvents in the first direction were chloroform–methanol–water (65:25:4, by vol.), and in the second direction chloroform–acetic acid–methanol–water (130:27:18:7, by vol.). Lipids were revealed by charring plates sprayed with dichromate–sulphuric acid reagent. PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; MGDG, monoglucosyl diglyceride; DGDG, diglucosyl diglyceride.

RESULTS AND DISCUSSION

A thin-layer chromatogram of the polar lipids from *B. stearothermophilus* provided by H. L. Kornberg & T. K. Sundaram is shown in Fig. 1. Chromatograms treated with specific spray reagents showed no ninhydrin-positive lipids, but two components with the staining properties of glycolipids were observed; the phospholipid components corresponded in chromatographic mobility and staining properties with phosphatidylglycerol and diphosphatidylglycerol. Bacteria of this strain cultivated on a broth medium showed essentially the same lipid pattern. The pattern from *B. stearothermophilus* NCIB8157 showed components corresponding to phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine (Minnikin *et al.* 1972) and an unidentified phospholipid; no glycolipids were detected. Paper chromatography [propan-1-ol–ammonia (sp.gr. 0.88)–water] of the water-soluble deacylated products from the lipids of both strains gave spots which corresponded to di(glycerolphosphoryl)-glycerol and glycerylphosphorylglycerol. The deacylation product from the lipids of *B. stearothermophilus* NCIB8157 contained a component which
co-chromatographed with glycerylphosphorylethanolamine; no spot corresponding to the
decaylation product of the unidentified phospholipid was detected. Examination by paper
chromatography (butan-1-ol–pyridine–water) of the glycosides from the first strain of
B. stearothermophilus showed that these components stained identically to and chromato-
graphed with the glucosylglycerol and diglucosylglycerol standards; acid hydrolysis of the
lipid yielded glucose as the only sugar identified by paper chromatography in the same
system.

The lipids of B. stearothermophilus 2184 (Card, Georgi & Militzer, 1969; Card, 1973) and
B. stearothermophilus B65 (Oo & Lee, 1972) included phosphatidylethanolamine and
phosphatidylglycerol. Long & Williams (1960) suggested that phosphatidylethanolamine
(cephalin) was absent from B. stearothermophilus NCAI518 (NCIB8157), but we found it to
be present. Glycolipids were not investigated in the previous studies, but the results of Oo
& Lee (1972) suggest that they were absent from the lipids of B. stearothermophilus B65.

The apparent inability of a strain of B. stearothermophilus (Fig. 1) to synthesize
phosphatidylethanolamine is remarkable as this lipid has almost invariably been en-
countered in bacilli (Goldfine, 1972). A phosphatidylethanolamine-deficient mutant of
Bacillus subtilis (Marburg) has, however, been obtained (Beebe, 1971) and certain continuous
cultures of B. subtilis (Marburg) lack this lipid (Minnikin et al. 1972). The absence of glyco-
lipids from B. stearothermophilus NCIB8157 is surprising since most bacilli also contain
glycosyl diglycerides (Shaw, 1970). Cultures of Bacillus cereus T have occasionally been
found to contain no detectable amounts of glycolipids (Minnikin et al. 1971 a). The present
results support the proposed interchangeability of phosphatidylethanolamine and glycosyl
diglycerides in the membranes of bacilli (Minnikin et al. 1971 a, b, 1972) and suggest that
polar lipid analysis may be a valuable chemotaxonomic technique for the classification of
thermophilic bacilli.

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