VARIATION OF POLAR LIPID COMPOSITION OF *BACILLUS SUBTILIS* (MARBURG) WITH DIFFERENT GROWTH CONDITIONS

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1. Introduction

A previous study of the polar lipids of *Bacillus subtilis* (Marburg) has shown that at least five phospholipids in addition to glycolipid may be present [1]. The report shows that by manipulation of growth conditions the lipid composition of this organism can be varied and greatly simplified. In batch cultures, under conditions of apparent phosphate starvation, a close interrelation of phospholipids and phosphate-free polar lipids is observed, the latter increasing in proportion as growth progresses. Certain chemostat cultures (magnesium limitation) are found to contain only one acidic phospholipid, phosphatidylglycerol (PG), and one neutral polar lipid, diglucosyldiglyceride (DG). In phosphate-limited cultures the proportions of phospholipids are reduced and phosphate-free polar lipids are observed in increased proportions; one of these lipids is DG, the other is an acidic peptidolipid.

2. Results

Batches (1 litre) of *Bacillus subtilis* (Marburg N.C.I.B. 3610) were prepared in a medium consisting mainly of Oxoid Nutrient Broth and glucose [2]. Innoculation was made directly from an agar slope and incubation was at 37° on a gyrorotatory shaker; samples (250 ml, pH 7.0 ± 0.2) were taken at intervals (see fig. 1). Continuous cultures were maintained at 37° in a chemostat (600 ml, pH 7.0 ± 0.1) on phosphate-limited and magnesium-limited media [3]. After equilibration for 48 hr at a dilution rate of 0.2 hr^{-1}, samples (300 ml) were removed from the culture vessel. Lipids were extracted from lyophilized cells with chloroform–methanol (2:1, v/v) and investigated by two-dimensional thin-layer chromatography [4] (fig. 2 for details). Qualitative identifications of lipids was made by use of specific spray reagents [2], and densitometry [6] gave an approximate measure of the relative proportions of the lipids.

The lipids of *B. subtilis* (Marburg) grown in batch culture included the phospholipids reported in previous studies [1] i.e. diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), PG and traces of its lysine ester. DG was identified in all cultures, and in some extracts an additional phosphorous-free lipid (X) was found (see fig. 1). Preliminary investigations

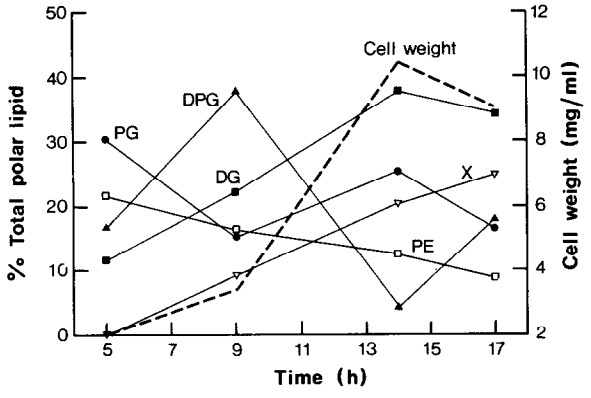

Fig. 1. Polar lipid composition of *B. subtilis* 3610 in exponential and stationary phases of batch culture; results are expressed as a percentage of total polar lipid. Abbrevations:PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; DG, diglucosyldiglyceride; X, acidic peptidolipid.

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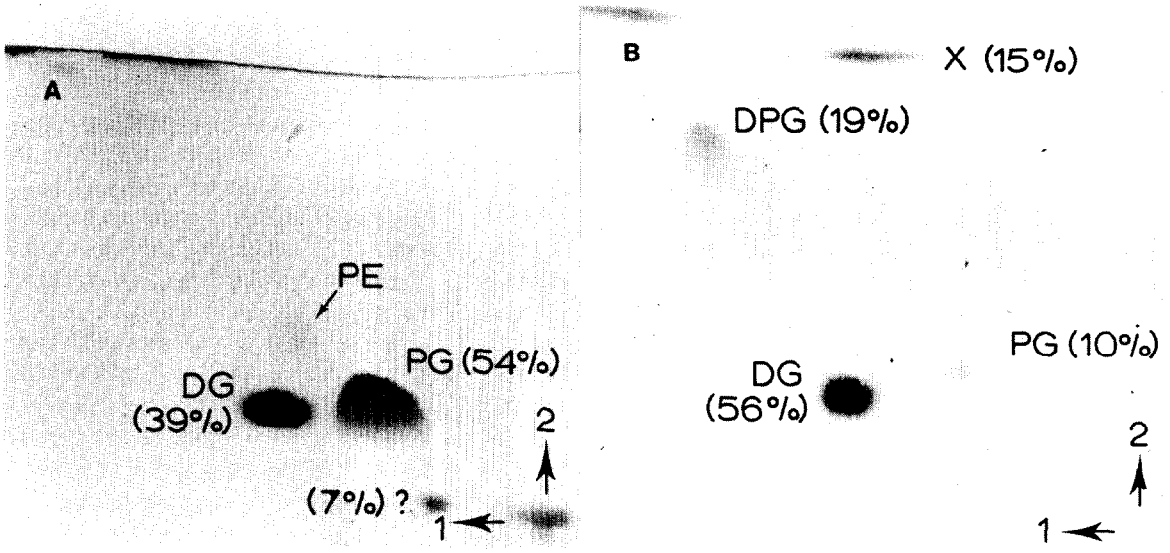

Fig. 2. Two-dimensional thin-layer chromatograms of lipids from magnesium-limited (A) and phosphate-limited (B) cultures of *B. subtilis* 3610. Chloroform–methanol–water (62:25:4) was used in the first direction and chloroform–acetic acid–methanol–water (80:18:12:5) in the second direction. Abbrevations correspond to those in fig. 1 and figures in parentheses are approximate proportions of the lipids as estimated by densitometry after charring.

of this lipid indicate the presence of acidic groups and the absence of free amino functions. Acid hydrolysis (4 M HCl, 32 hr at 100°, sealed tube) yielded leucine, valine aspartic and glutamic acids in the proportions 4:1:1:1. This acidic peptidolipid is distinct from the glucuronosyldiglycerides occurring in *Bacillus cereus* T [5] and pseudomonads [6] but closely resembles a peptidolipid detected in the culture fluids of several strains of *B. subtilis* [7]; preliminary results indicate that it is a normal component of the membrane of these cells of *B. subtilis* (Marburg).

The proportions of the polar lipids of *B. subtilis* found during progression from exponential to stationary phase of batch growth are shown in fig. 1. Within the class of neutral or amphoteric polar lipids, PE appears to balance DG. The total proportion of the acidic phospholipids decreases with age and the amount of the novel acidic lipid (X) increases. This behaviour is generally similar to that reported previously for *B. cereus* T and *B. subtilis* W23 [5].

The polar lipid compositions of phosphate- and magnesium-limited chemostat cultures are shown in fig. 2. It is notable that in contrast to the batch cultures, these chemostat-grown cells are practically devoid of PE. The lipids of some of the magnesium-limited cultures (fig. 2A) comprised almost entirely the two polar lipids (PG and DG) although small amounts (<5%) of cardiolipin have occasionally been encountered in separate experiments. Phosphate-limited cultures (fig. 2B) contain reduced proportions of acidic phospholipids (PG and DPG) and major quantities of DG and the acidic peptidolipid (X).

3. Discussion

The results described here demonstrate that within certain limits the polar lipid composition of *Bacillus subtilis* (Marburg) can be extensively varied by manipulation of the growth conditions. One limit appears to be the presence of one acidic polar lipid (PG) and one neutral polar lipid (DG) as is observed in the case of the magnesium-limited culture (fig. 2). Under such growth limitation PG is the predominant acidic lipid and DPG is absent; PG, therefore, possibly has a more important role that DPG in the assimilation

of Mg^{2+} ions in the membrane. The high proportion of an acidic phospholipid (PG) under conditions of magnesium-limitation correlates with the enhanced amounts of phosphate-containing teichoic acid in the walls of certain bacilli grown under similar conditions [8]. Both wall and membrane teichoic acids have been implicated in the binding of divalent cations [9, 10]; the combined action of wall teichoic acid and membrane teichoic acid contributes towards a controlled cationic evironment necessary for the maximal efficiency of membrane-bound enzymes. Acidic polar lipids, having cation binding capacity, must also be involved in maintaining the cation population necessary for efficient membrane function.

In the batch cultures studied here (fig. 1) and those of *B. subtilis* W23 reported previously [5] it is notable that during exponential phase of growth PG is the most important phospholipid during active growth. Similar batch growths of *Bacillus cereus* T [5] did not show a marked increase in PG during exponential growth but a rapid accumulation of an acidic glucuronosyldiglyceride was observed. *B. subtilis* (Marburg) and W23 do not appear capable, under the batch conditions employed, of producing an acidic glycolipid. The acidic peptidolipid (X) encountered in batch and phosphate-limited growths of *B. subtilis* (Marburg), occurs at the apparent expense of acidic phospholipids. It is uncertain whether such a lipid, normally encountered extracellularly [7], may have a similar function in the membrane as do acidic phospholipids but it may at least assist in maintaining the environment of the membrane.

The absence of PE in phosphate-limited cultures of *B. subtilis* (Marburg) was not unexpected since an interchangeability of this lipid and DG has been proposed [2, 5]. The lack of PE in magnesium-limited cultures indicates that, at least at pH 7, this lipid does not play an important role in divalent cation assimilation. It is notable that a PE-deficient mutant of *B. subtilis* (Marburg), containing increased proportions of DG, has been isolated [11].

The data presented here supports the previous hypothesis [5] of the subdivision of bacterial polar lipids into two main categories, acidic (e.g. PG, DPG, phosphatidylserine, phosphatidylinositol, acidic glycolipids) and neutral or amphoteric (e.g. PE, phosphatidylcholine, glycolipids) polar lipids. To the former category the acidic peptidolipids of bacilli may be provisionally added and the latter group might, as recently suggested [12], include the ornithine-containing lipids occurring in pseudominads and other bacteria. Similar groups have been proposed for the lipids of higher organisms [13] and exact substitution between members of each group elegantly demonstrated in a variety of tissues. Natural membranes, therefore, appear to require a selection of lipids of certain general types; it is important to take note of this when considering the role of polar lipids in membranes.

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