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Analysis of *Caulobacter crescentus* Lipids

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The lipids of *Caulobacter crescentus*, a procaryotic species which differentiates into stalked and swarmer cell types, were analyzed. Major lipid classes were purified by chromatography and identified by both chromatographic and chemical methods. Approximately half of the total lipid fraction of this organism consisted of glycolipids, which were primarily monoglucosyldiglyceride and an acylated glucuronic acid. Two of the phospholipids of *C. crescentus* were identified as phosphatidylglycerol and acylphosphatidylglycerol. Commonly occurring bacterial phospholipids, such as phosphatidylethanolamine and cardiolipin (diphosphatidylglycerol), were not detected. Monoglyceride and diglyceride were found in the neutral lipid fraction, which made up 10% of the total lipid. Quantitative lipid compositional studies, performed by the incorporation of [^{14}C]acetate and [^{32}P]orthophosphate into growing cultures, revealed that separated swarmer and stalked cells had similar lipid compositions. However, stationary-phase cultures, compared with logarithmic cultures, had decreased amounts of phosphatidylglycerol and diglyceride and increased amounts of acylphosphatidylglycerol and a glucuronic acid-containing glycolipid, glycolipid X. In addition, two glycolipids were only detected in stationary-phase cultures. These studies indicate that *C. crescentus* has a distinctive lipid composition compared with those of other procaryotic species which have been analyzed.

Caulobacter crescentus, a gram-negative, freshwater bacterium, produces two distinct cell types during growth, motile swarmer cells and nonmotile stalked cells (22). Swarmer cells differentiate into stalked cells, and stalked cells divide asymmetrically into second-generation stalked and swarmer cells. We felt that an identification and analysis of the lipids of a differentiating procaryote might elucidate the role of lipids and therefore membranes in the process of cellular differentiation as well as identify the lipids of a unique bacterial group. Fatty acid analysis of the two cell types had previously indicated minor quantitative differences, although qualitative differences were not detected (5). An analysis of the lipid composition of *C. crescentus* first appeared as a preliminary report (A. J. De Siervo and A. D. Homola, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, K83, p. 140). Since this report, two analyses of the phospholipid composition of *C. crescentus* have been published (6, 18). The analyses reported in this paper agree with the general conclusions of authors of references 6 and 18 concerning the phospholipids of *C. crescentus*, with some notable exceptions, i.e., the presence of cardiolipin and a ninhydrin-positive phospholipid. Moreover, the identification of a major proportion (45 to 62%) of the lipids of this organism as glycolipids, containing glucose and glucuronic acid, is reported in this paper.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *C. crescentus* strain CB2 (ATCC 15252) was used for these experiments. The lipids of *C. crescentus* strain CB15 were analyzed for comparison. Cultures were grown in modified M36 medium containing 2.0 g of peptone, 1.0 g of yeast extract, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 2.0 g of glucose (M36 + G) made up to 1 liter with distilled water. The pH of the sterile medium was 6.6. Cultures were grown at room temperature for 48 h with aeration and stirring by inoculating two 5-liter fermentors, each containing 3.5 liters of medium and 1 to 2 ml of Antifoam A emulsion (Sigma Chemical Co., St. Louis, Mo.), with a 10-ml culture grown for 24 h at 30°C with shaking from stock cultures. Alternatively, cells were grown in a Microferm fermentor (New Brunswick Scientific Co., New Brunswick, N.J.) Twenty-two liters of M36 + G was inoculated with 200 ml of a 24-h CB2 culture and 5 ml of sterile Antifoam A emulsion. Cells were grown at 30°C, aerated at 22 liters/min and stirred at 200 rpm for 24 h to an optical density of 2.25. The pH of logarithmic- and stationary-phase cultures did not exceed 7.0. Cells were collected at 4°C by centrifugation at 27,000 × *g*. Cultures were monitored for purity by phase microscopy and Gram staining before use.

Preparation of labeled cells. Phospholipids were labeled by adding 75 μCi of $^{32}P_i$ to 75 ml of an 8-h culture grown at 30°C with shaking. The stationary-phase cells were harvested by centrifugation. Lipids labeled with ^{14}C were obtained by growing *C. crescentus* in M36 + G containing 0.5 μCi of sodium [1-^{14}C]acetate per ml to log or stationary phase. In cultures grown with [^{14}C]acetate, the label was present

through at least six generations in the log phase. The generation time was approximately 3 h. Autoradiograms were made with washed and stained chromatograms, using DuPont Cronex CD2 medical X-ray film (E. I. Dupont De Nemours & Co., Inc., Wilmington, Del.).

Lipid extraction and analysis. Lipids were extracted according to the method of Bligh and Dyer (3), and the samples were dried under N_2 at 45°C, weighed, suspended in chloroform, and then stored at −20°C. Lipid extracts were chromatographed by two-dimensional chromatography on Whatman SG-81 silica gel-loaded paper as previously described (9). Neutral lipids were chromatographed in two dimensions on silica gel paper, using the solvent system of Steiner and Lester (29): benzene-chloroform-acetic acid, 90:10:1 (vol/vol), in the first dimension and benzene-chloroform-pyridine, 90:10:1 (vol/vol), in the second. Chromatograms were stained with rhodamine 6G to locate the lipid spots. Free amino groups were detected with ninhydrin (20), phosphate-containing lipids were detected by ammonium molybdate reagent (2), vicinal hydroxyl groups were detected by the periodate test (33), and glycolipids were detected by alkaline silver nitrate (32).

Chemical tests. Lipids were deacylated by methanolysis, using the method of White and Frerman (34), and the water-soluble derivatives were chromatographed descendingly on Whatman no. 1 paper previously washed with 2 N HCl. Solvent systems used were phenol-water-acetic acid-ethanol 50:22:3:3 (vol/vol) (12) and ethanol–1 M ammonium acetate (pH 7.5) 65:35 (vol/vol) (4). Some purified lipids were subjected to acetolysis, and the derivatives were chromatographed as previously described (21).

Phosphate was determined by the method of Ames and Dubin (1), glycerol was determined by the method of Renkonen (24), fatty acid esters were determined by the method of Snyder and Stephens (28), and glucose was determined by anthrone (16). Glycolipids were hydrolyzed with 2 N HCl at 100°C for 3 h in a screw-capped tube, and the sugars were analyzed by ascending chromatography three times on Whatman no. 1 paper (17) in butanol-pyridine-water 45:25:40 (vol/vol) (14) and by gas-liquid chromatography of trimethylsilyl (31) and alditol acetate (15) derivatives, using an F & M Scientific Corp. research chromatograph, model 810, on a column (6 feet by ⅛ inch [ca. 1.8 m by 0.3 cm]) of 3% OV-225 on 80/100 Supelcoport (Supelco, Inc., Bellefonte, Pa.).

Isolation of cell types. Swarmer and stalked cells were separated by a procedure with Ludox HS-40 similar to that reported by Evinger and Agabian (11). A portion of *C. crescentus* culture (20 ml) was mixed with 20 ml of a 46% Ludox HS-40 solution (46 ml of Ludox HS-40 and 54 ml of water, adjusted to pH 7.4, plus 2.9 g of dextran) to give a final concentration of 23% Ludox. The mixture was centrifuged at 43,000 × *g* for 15 min. Two bands were obtained. The top band contained stalked cells, and the bottom band contained primarily swarmer cells. The bottom band was made up to a volume of 20 ml with culture medium and mixed with an equal volume of 82% Ludox HS-40 solution (82 ml of Ludox HS-40, 18 ml of water, 5.9 g of dextran) to produce a final concentration of 41% Ludox. The mixture was centrifuged at 43,000 × *g* for 15 min, and the upper band, which contained swarmer cells, was removed. This band and the previously separated stalked cell band were washed twice by centrifugation to remove the Ludox reagent by mixing with fresh culture medium. The resulting pellets were extracted for total lipids.

Cardiolipin synthetase assay. Cell envelope fractions of *C. crescentus* were prepared and assayed for cardiolipin synthetase activities as previously described for marine bacterial isolates (8).

Materials. A cardiolipin standard was purified from *Micrococcus lysodeikticus* (*M. luteus*). A monogalactosyl diglyceride standard was obtained from spinach leaf lipids. Tripalmatin and acetylation kits were obtained from Supelco, and hexamethyldisilazane in pyridine was obtained from Analabs, Inc., North Haven, Conn. $^{32}P_i$ and [1-^{14}C]acetate were obtained from New England Nuclear Corp., Boston, Mass., and chromatography papers SG-81 and no. 1 were obtained from Whatman, Inc., Clifton, N.J. Ludox HS-40 was a generous gift of E. I. DuPont De Nemours & Co., Inc. (Industrial Chemical Department).

RESULTS

Lipid identification. Autoradiograms of two-dimensional chromatograms were made of ^{32}P-labeled lipids (Fig. 1) and of ^{14}C-labeled lipids (Fig. 2). An autoradiogram of separated neutral lipids is shown in Fig. 3. Identifications were made by cochromatography with standards and chemical tests performed on the chromatograms and on purified lipids. Monoglucosyldiglyceride, phosphatidylglycerol (PG), acylphosphatidylglycerol (APG), monoglyceride, diglyceride, and free fatty acid were identified in the total lipids of stationary-phase cells. Glycolipid X was tentatively identified as diacylglucuronic acid. The hydrolysis of unknown glycolipids 2 and 3 yielded a sugar that cochromatographed with glucuronic acid and whose alditol acetate derivative was indistinguishable from known glucitol acetate. A summary of the results of tests performed to identify glycolipid X, monoglucosyldiglyceride, and acylphosphatidylglycerol is given in Table 1.

Neither cardiolipin nor ninhydrin-positive lipids were detected when cells were extracted by either the Bligh and Dyer (3) or the Folch (13) method. Lipid analysis of *C. crescentus* strain CB15 yielded a lipid profile qualitatively identical to that of strain CB2. The identification of an acidic phospholipid as APG was suspected because of its chromatographic behavior in two solvent systems. This lipid accounted for 2% of the total lipid. Tests done directly on chromatograms indicated it did not have vicinal hydroxyl, free amino, or sugar groups. A positive phosphomolybdate reaction and ^{32}P labeling of this spot confirmed the presence of phosphate, and mild alkaline hydrolysis yielded a derivative

which cochromatographed in two solvent systems with glycerophosphorylglycerol. Quantitative glycerol, phosphate, and fatty acid ester determinations, using PG as a standard, produced ratios of glycerol-phosphate-fatty acid ester of 2.0:0.9:3.1, which are similar to the expected 2:1:3 theoretical ratios of APG. In addition, the procedure of acetolysis produced two major acylated derivatives, as expected from APG. PG and cardiolipin produce only one major acylated product, diacylglycerol monoacetate.

Monoglucosyldiglyceride represented 35% of the total lipid. Its identity was confirmed by cochromatography with monogalactosyldiglyceride extracted from spinach. Tests for phosphate and amino groups were negative, and positive permanganate-periodate and alkaline silver nitrate tests indicated the presence of carbohydrate. The carbohydrate released by hydrolysis at 100°C in 2 N HCl cochromatographed with glucose on an ascending chromatogram, and trimethylsilyl and alditol acetate derivatives produced peaks in gas-liquid chromatography that correspond to known glucose. Quantitative tests revealed the presence of glycerol and fatty acid esters in the ratio of 1:2, and the anthrone test gave a glucose-fatty acid ester ratio of 1:2. The structure of monoglucosyldiglyceride is consistent with these findings.

Another glycolipid, which constituted about 10% of the total extracted lipid, proved more

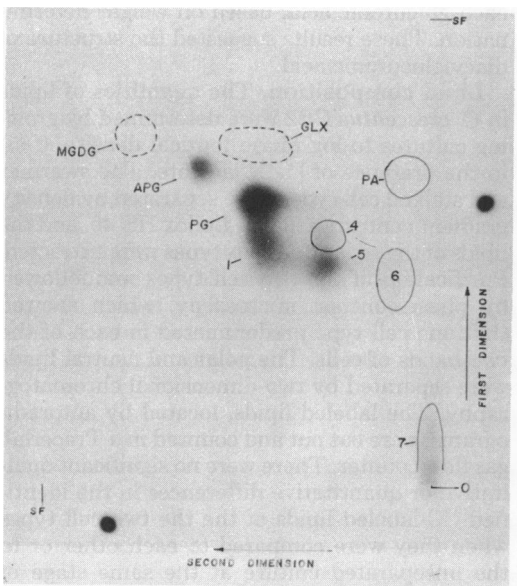

FIG. 1. *Autoradiogram of a two-dimensional chromatogram of ^{32}P-labeled phospholipids of C. crescentus. Minor phospholipids which were detected on the autoradiogram but which may not appear in the photograph are circled. Monoglucosyldiglyceride (MGDG) and acylated glucuronic acid (GLX), indicated by dashed lines, were located on the chromatogram by staining, but were unlabeled. Abbreviations: PA, phosphatidic acid; O, origin; SF, solvent front. Phospholipids 1, 4, 5, 6, and 7 were unidentified.*

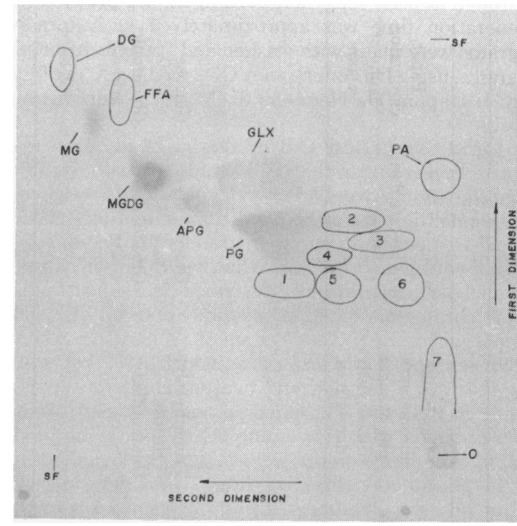

FIG. 2. *Autoradiogram of a two-dimensional chromatogram of ^{14}C-labeled lipids of C. crescentus. Minor lipids which were detected on the autoradiogram but which may not appear in the photograph are circled. Abbreviations: DG, diglyceride; MG, monoglyceride; FFA, free fatty acids (see legend to Fig. 1 for other abbreviations). Lipids 1 through 7 were unidentified.*

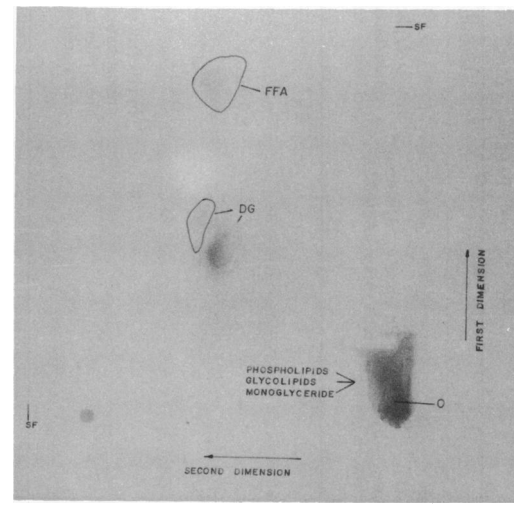

FIG. 3. *Autoradiogram of a two-dimensional chromatogram of ^{14}C-labeled neutral lipids. Lipids which were detected on the autoradiogram but which may not appear in the chromatogram are circled. See legends to Fig. 1 and 2 for abbreviations.*

TABLE 1. *Lipid identification*

Lipid component	Identifying characteristic									
	Rhodamine 6G staining reaction	Free amino group-ninhydrin test	Vicinal hydroxyl groups	Phosphorous spray	Alkaline $Ag(NO_3)_2$	Glycerol/phosphate/fatty acid ester ratio	Deacylated derivative	Gas-liquid chromatography of sugar trimethylsilyl derivatives	Alditol acetate derivatives	Paper chromatography of sugars
PG	Blue	–	+	+	–	2:1:2 (standard)	Glycerophosphorylglycerol			
APG	Blue	–	–	+	–	2.0:0.9:3.1	Glycerophosphorylglycerol			
Monoglucosyldiglyceride	Yellow	–	+	–	+	1.0:0:1.9	ND[a]	Trimethylsilyl-glucose	Glucitol acetate	Glucose
Glycolipid X	Orange	–	+	–	+	No glycerol or phosphate, 1.5 equivalents of fatty acid per mg	ND	Unidentified	Glucitol acetate	Glucuronic acid

[a] ND, Not determined.

difficult to identify. It gave an orange spot under UV light after rhodamine 6G staining, a positive Schiff test, negative ninhydrin and phosphate tests, and positive permanganate-periodate and alkaline silver nitrate reactions. Paper chromatography showed several hydrolysis products, one of which had an R_f close to that of known rhamnose. However, gas-liquid chromatography of trimethylsilyl derivatives of this compound gave several peaks that were not identified by comparison to lists of retention times. The alditol acetate derivative of glycolipid X cochromatographed with the glucose derivative. Paper chromatography of additional standards revealed that glucuronic acid produced two spots very similar to those found for our unknown. The chemical behavior of glucuronic acid is consistent with these gas-liquid chromatographic findings. Glucuronolactone, readily formed from glucuronic acid, and its decarboxylation product could have been present and could have resulted in several trimethylsilyl derivatives. Glucitol acetate would have been formed from glucuronic acid as well as from glucose in the sodium borohydride reduction step in the alditol acetate procedure. Glycolipid X contained no glycerol, but had approximately two fatty acid esters for each glucuronic acid, based on weight determination. These results suggested the structure of diacylglucuronic acid.

Lipid composition. The quantities of lipids in *C. crescentus* CB2 were determined by growing cultures to log phase (optical density, 0.65) in the presence of [1-^{14}C]acetate. The swarmer and stalked cell types were separated by density gradient centrifugation in Ludox HS-40, and the lipids of the separated cells types were extracted. Purification of the two cell types was followed by phase-contrast microscopy, which showed that one cell type predominated in each of the two bands of cells. The polar and neutral lipids were separated by two-dimensional chromatography. The labeled lipids, located by autoradiograms, were cut out and counted in a Tracerlab gas flow counter. There were no significant qualitative or quantitative differences in the identified ^{14}C-labeled lipids of the the two cell types when they were compared to each other or to the unseparated culture at the same stage of growth (Table 2). A large difference was found for unidentified lipid 1. However, the separation between this lipid and PG was not always satisfactory in repeated chromatograms. Smaller differences were also noted for lipids 4 and 5. Whether these differences between stalked and swarmer cells are significant or reflect difficulties in chromatographic separation or result from manipulations involved in the separation of the

two cell types will require further investigation to ascertain. Free fatty acids were detected only in the separated cell types and were presumably formed during the separation procedure.

When the ^{14}C-labeled lipids of logarithmic-phase cells (optical density, 0.5) were compared with those of stationary-phase cells (optical density, 3.0), qualitative and quantitative differences were found (Table 3). Large increases in the percentages of APG, glycolipid X, and monoglyceride were found in the stationary phase compared with the log phase, and the percentages of PG, diglyceride, and unknown lipid 1 decreased. The unidentified glycolipids (lipids 2 and 3) were detected only in stationary-phase cells.

C. crescentus was also grown in the presence of $^{32}P_i$ to label phospholipids and determine their relative amounts (Table 4). PG and unidentified lipid 1, which was most likely a form of PG, as it did not consistently separate from PG and was positive for vicinal hydroxyl groups, constituted 63.9% of the phospholipids. APG constituted 7.8% of the total ^{32}P incorporation.

DISCUSSION

This study concerns the identification of the lipids of *C. crescentus* and the lipid composition in the two cell types and in cells from cultures of different ages. Under the conditions of growth and cell separation there were only minor quantitative differences between the identified lipids of stalked and swarmer cells, consistent with the results of Jones and Smith (18). However, there may be differences between the amounts of some unidentified lipids in the two cell types, and further analyses will be necessary to substantiate these observations. Both qualitative and quantitative differences between log- and stationary-phase cell lipids were found. PG, unidentified component 1, and diglyceride decreased in stationary-phase cells, whereas APG, diacylglucuronic acid, and monoglyceride increased. Two unidentified glycolipids (2 and 3) were only found in stationary-phase cultures. Changes in the phospholipid composition of a closed culture as it ages have been reported in many bacterial species (7, 10, 23). Cell types of *C. crescentus* were not analyzed separately in older cultures; it was observed, and has been reported in cultures under phosphate limitation (25), that the percentage of swarmer cells is decreased in stationary-phase cultures. The use of stationary-phase cultures to characterize stalked cell lipids was not attempted, because the lipid composition of separated log-phase stalk cells did not resemble that of stationary-phase cultures. Quantitative changes in the phospholipids of *C.*

TABLE 2. *Percentages of total lipids of C. crescentus labeled with [^{14}C]acetate*[a]

Lipid component	% of total lipids in:		
	Unseparated cells	Swarmer cells	Stalked cells
PG	26.1	30.4	27.8
APG	2.0	2.2	1.8
Monoglucosyldiglyceride	35.0	35.1	36.7
Glycolipid X	10.1	10.6	10.6
Monoglyceride	2.7	2.3	2.3
Diglyceride	7.5	5.4	5.1
Free fatty acid	ND[b]	0.7	0.2
Unidentified			
1	10.1	1.7	9.7
2[c](glycolipid)	ND	ND	ND
3[c](glycolipid)	ND	ND	ND
4	1.6	2.5	1.3
5	2.4	6.5	2.4
6	0.7	1.0	0.6
7	0.4	0.2	0.3
Phosphatidic acid and other minor lipids	1.2	1.3	1.0

[a] Total counts per minute for the three samples ranged from 25,000 to 87,000. Cells were grown to an optical density of 0.65 (pH 6.9).

[b] ND, Labeled lipid not detected.

[c] The presence of the unidentified glycolipids, 2 and 3, were noted on rhodamine 6G-stained chromatograms as part of the cold stationary-phase carrier lipid, but labeled lipid was not present on visual examination of autoradiograms.

TABLE 3. *Comparison of total lipids of logarithmic- and stationary-phase cells of C. crescentus by percentage of [^{14}C]acetate incorporation*[a]

Lipid component	% [^{14}C]acetate incorporation in:	
	Log-phase cells	Stationary-phase cells
PG	36.1	23.4
APG	1.0	3.5
Monoglucosyldiglyceride	42.4	46.8
Glycolipid X	6.4	15.0
Monoglyceride	2.1	3.9
Diglyceride	5.1	1.2
Free fatty acid	0.3	0.4
Unidentified		
1	2.9	1.3
2 (glycolipid)	ND[b]	2.1
3 (glycolipid)	ND	1.4
5	1.2	ND
4, 6, 7	ND	ND
Phosphatidic acid and other minor lipids	2.5	1.0

[a] Log-phase culture, optical density of 0.54; stationary-phase culture, optical density of 3.0.

[b] ND, none detected.

TABLE 4. *Percentages of ^{32}P-labeled lipids of C. crescentus*[a]

Lipid component	%
PG	41.2
APG	7.8
Phosphatidic acid	0.5
Unidentified	
1	22.5
4	3.8
5	13.5
6	2.4
7	8.5

[a] See Fig. 1 for autoradiogram.

crescentus in cultures of different ages were reported by Contreras et al. (6).

The identifications of some of the phospholipids in *C. crescentus* in this laboratory do not agree with those of other reports. Other investigators (6, 18) have found a ninhydrin-positive phospholipid which was identified as lysyl-PG in one laboratory (18). Numerous extractions in this laboratory of cells grown at different times and under different conditions have failed to reveal the presence of ninhydrin-positive lipid. In addition to the Bligh and Dyer lipid extraction procedure we routinely use, we also extracted *C. crescentus* by the Folch (13) method. Identical lipid profiles were obtained. The lipids of strain CB15, used by one group of investigators (6), were also analyzed and compared with those of the CB2 strain used in this study. Again, similar lipid profiles were obtained. However, we did obtain a trace amount of a ninhydrin-positive lipid which cochromatographed with lipid 5 when we ran the second dimension before the chromatographic paper was completely free of the acidic solvent. The same lipid sample showed no ninhydrin-positive spots when the chromatogram had dried after the first dimension. This ninhydrin-positive lipid only appeared in log-phase cells and either is extremely labile under our extraction conditions or can be produced by experimental conditions which are presently undefined. It should be noted that when the lipids of *Staphylococcus aureus* cells are extracted and chromatographed in our laboratory in the same manner as those of *C. crescentus*, a ninhydrin-positive, aminoacyl-PG lipid described by White and Frerman (34) is detected. Incidentally, this ninhydrin-positive lipid did not correspond chromatographically to the lipid sometimes detected in *C. crescentus*. In light of these observations, we are unable to explain the differences between our results and those previously published.

The identification of a minor acidic lipid as cardiolipin (6, 18) also does not agree with our data. Cardiolipin was never found in any *C. crescentus* extracts. However, the phospholipid we have identified as APG does compare quantitatively with the amounts of cardiolipin reported by the authors of references 6 and 18. Considering the similar R_f values of cardiolipin, APG, and the related bisphosphatidic acid, as well as the difficulties inherent in interpreting chromatograms of deacylated derivatives of phospholipids, it is conceivable that the more common phospholipid, cardiolipin, could be confused with the less common APG or even bisphosphatidic acid. All of our data point to the presence of APG and not cardiolipin in *C. crescentus*, i.e., this phospholipid has a glycerol-phosphate-fatty acid ester ratio of 2.0:0.9:3.1; does not cochromatograph with cardiolipin; produces the same deacylation product as PG, glycerophosphorylglycerol; forms two acylated products from acetolysis; and cochromatographs with a lipid in marine bacteria which we had tentatively identified as APG (8). Further, several marine bacterial strains investigated had cardiolipin, bisphosphatidic acid, and APG, all of which are chromatographically separable by the same chromatographic systems described in this paper. In addition, we could find no indication of cardiolipin synthetase activity in cell envelope fractions of *C. crescentus*. Although this negative enzymatic data would not normally be reliable, we had shown a very high correlation between the chromatographic detection of cardiolipin and cardiolipin synthetase activity in marine bacteria (8). These data, along with our chemical evidence, indicate to us that *C. crescentus* contains APG and no detectable cardiolipin.

There are several striking features of the lipid composition of *C. crescentus* which are not typical of the lipid composition of other well-studied gram-negative bacteria. We found a high percentage of the total extractable lipids to be glycolipid, in fact as high as 62% in stationary-phase cells, based on [^{14}C]acetate incorporation. The major glycolipids were monoglucosyldiglyceride and glucuronic acid-containing glycolipid. Glycolipids containing glucuronic acid have been reported in a halotolerant bacterium (30), *Pseudomonas diminuta*, and *P. rubescens* (26, 35). In addition, we have observed lipids chromatographically similar to the glucuronic acid-containing glycolipid of *C. crescentus* in several marine isolates (unpublished data) on which we had previously reported phospholipid analyses (8). One minor glycolipid identified in the halotolerant bacterium by Stern and Tietz (30) was glucuronosyldiglyceride, which we eliminated as the structure of glycolipid X due to the absence of glycerol. However, they also reported a major glucuronic acid-containing glycolipid which in-

creased substantially in older cultures, as did glycolipid X in *C. crescentus*. The glucuronic acid-containing glycolipids of *Pseudomonas* identified by Wilkinson also contained diglyceride (35). The minor glucuronic acid-containing glycolipids of *C. crescentus* may be of the diglyceride type, but we have not analyzed these glycolipids, as they only appeared in small quantities in stationary-phase cells.

According to Shaw (27), monoglucosyldiglycerides are rare except in genera such as *Pseudomonas*, *Arthrobacter*, and *Mycoplasma*. Further, monoglycosyldiglycerides do not usually accumulate in cells, since they are reported to be the precursors of diglycosyldiglycerides. In *C. crescentus* there was no indication of the presence of diglycosyldiglycerides.

The absence of phosphatidylethanolamine, which was noted for *C. crescentus*, is rare in gram-negative bacteria, although phosphatidylethanolamine is lacking many gram-positive species (19). It therefore follows that phosphatidylethanolamine and cardiolipin, which is also absent from *C. crescentus*, are not essential for the functioning or structure of some biological membranes, including the outer membrane of some gram-negative bacteria.

The unique lipid composition of *C. crescentus*, in conjunction with its complex morphology and life cycle, suggests that this procaryote has evolved quite independently from other widely studied bacterial groups.

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LITERATURE CITED

1. **Ames, B. N., and D. J. Dubin.** 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. **235**:769–775.
2. **Ansell, G. B., and J. N. Hawthorne.** 1964. Phospholipids—chemistry, metabolism, and function, p. 50–51. Elsevier Publishing Co., Amsterdam.
3. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
4. **Cho, K. S., G. Benns, and P. Proulx.** 1973. Formation of acyl phosphatidyl glycerol by *Escherichia coli* extracts. Biochim. Biophys. Acta **326**:355–360.
5. **Chow, T. C., and J. M. Schmidt.** 1974. Fatty acid composition of *Caulobacter crescentus*. J. Gen. Microbiol. **83**:369–373.
6. **Contreras, I., L. Shapiro, and S. Henry.** 1978. Membrane phospholipid composition of *Caulobacter crescentus*. J. Bacteriol. **135**:1130–1136.
7. **De Siervo, A. J.** 1969. Alterations in the phospholipid composition of *Escherichia coli* B during growth at different temperatures. J. Bacteriol. **100**:1342–1349.
8. **De Siervo, A. J., and J. W. Reynolds.** 1975. Phospholipid composition and cardiolipin synthesis in fermentative and nonfermentative marine bacteria. J. Bacteriol. **123**:294–301.
9. **De Siervo, A. J., and M. R. J. Salton.** 1971. Biosynthesis of cardiolipin in the membranes of *Micrococcus lysodeikticus*. Biochim. Biophys. Acta **239**:280–292.
10. **De Siervo, A. J., and M. R. J. Salton.** 1973. Changes in phospholipid composition of *Micrococcus lysodeikticus* during growth. Microbios **8**:73–78.
11. **Evinger, M., and N. Agabian.** 1977. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. J. Bacteriol. **132**:294–301.
12. **Fischer, W., I. Ishizuka, H. R. Landgraf, and J. Herrmann.** 1973. Glycerophosphoryl diglucosyl diglyceride, a new phosphoglycolipid from *Streptococci*. Biochim. Biophys. Acta **296**:527–545.
13. **Folch, J., M. Lees, and G. H. Sloane-Stanley.** 1957. A simple method for the isolation and purification of the total lipids from animal tissues. J. Biol. Chem. **226**:497–509.
14. **Glegg, R. E., and D. Eidinger.** 1954. Hydrolysis of polysaccharides by a cation exchange resin and identification of monosaccharide components by paper chromatography. Anal. Chem. **26**:1365–1367.
15. **Griggs, L. J., A. Post, E. R. White, J. A. Finkelstein, W. E. Moeckel, K. G. Holden, J. E. Zarembo, and J. A. Weisbach.** 1971. Identification and quantitation of alditol acetates of neutral and amino sugars from mucins by automated gas-liquid chromatography. Anal. Biochem. **43**:369–381.
16. **Hassid, W. Z., and S. Abraham.** 1957. Chemical procedures for analysis of polysaccharides. Methods Enzymol. **3**:34–50.
17. **Jeanes, A., C. S. Wise, and R. J. Dimler.** 1951. Improved techniques in paper chromatography of carbohydrates. Anal. Chem. **23**:415–420.
18. **Jones, D. E., and J. D. Smith.** 1979. Phospholipids of the differentiating bacterium *Caulobacter crescentus*. Can. J. Biochem. **57**:424–428.
19. **Lechavalier, M. P.** 1977. Lipids in bacterial taxonomy—a taxonomst's view. Crit. Rev. Microbiol. **5**:109–210.
20. **Marinetti, G. V.** 1964. Chromatographic analysis of polar lipids on silicic acid impregnated paper, p. 339–377. *In* A. T. James and L. J. Morris (ed.), New biochemical separation. D. Van Nostrand Co., Ltd., London.
21. **McAllister, D. J., and A. J. De Siervo.** 1975. Identification of bisphosphatidic acid and its plasmalogen analogs in the phospholipids of a marine bacterium. J. Bacteriol. **123**:302–307.
22. **Poindexter, J. S.** 1964. Biological properties and classification of the *Caulobacter* group. Bacteriol. Rev. **28**: 231–295.
23. **Randle, C. L., P. W. Albro, and J. C. Dittmer.** 1969. The phospholipid composition of gram-negative bacteria and the changes in composition during growth. Biochim. Biophys. Acta **187**:214–220.
24. **Renkonen, O.** 1962. Determination of glycerol in phosphatides. Biochim. Biophys. Acta **56**:367–369.
25. **Schmidt, J. M., and R. Y. Stanier.** 1966. The development of cellular stalks in bacteria. J. Cell Biol. **28**:423–436.
26. **Shaw, J. M., and R. A. Pieringer.** 1972. Biosynthesis of glucuronsyl diglyceride by particular fractions of *Pseudomonas diminuta*. Biochem. Biophys. Res. Commun. **46**:1201–1205.
27. **Shaw, N.** 1970. Bacterial glycolipids. Bacteriol. Rev. **34**: 365–377.
28. **Snyder, F., and N. Stephens.** 1959. A simplified spectrophotometric determination of ester groups in lipids. Biochim. Biophys. Acta **34**:244–245.
29. **Steiner, M. R., and R. L. Lester.** 1972. In vitro studies of phospholipid biosynthesis in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **260**:222–243.
30. **Stern, N., and A. Tietz.** 1973. Glycolipids of a halotolerant, moderately halophilic bacterium. Biochim. Biophys. Acta **296**:130–135.
31. **Sweeley, C. C., R. Bently, M. Makita, and W. W.**

Wells. 1963. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. **85**:2497–2507.

32. **Trevelyan, W. E., D. P. Proctor, and J. S. Harrison.** 1950. Detection of sugars on paper chromatograms. Nature (London) **166**:444–445.
33. **Vorbeck, M. L., and G. V. Marinetti.** 1965. Separation of glycosyl diglycerides from phosphatides using silicic acid column chromatography. J. Lipid Res. **6**:3–6.
34. **White, D. C., and F. E. Frerman.** 1967. Extraction, characterization, and cellular localization of the lipids of *Staphylococcus aureus*. J. Bacteriol. **94**:1854–1867.
35. **Wilkinson, S. G.** 1969. Lipids of *Pseudomonas diminuta*. Biochim. Biophys. Acta **187**:492–500.