JOURNAL OF BACTERIOLOGY, June 1982, p. 1192–1201
0021-9193/82/061192-107$02.00/0

Vol. 150, No. 3

Polar Lipids in Phototrophic Bacteria of the *Rhodospirillaceae* and *Chromatiaceae* Families

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The polar lipids of photosynthetic purple bacteria of the genera *Chromatium*, *Thiocapsa*, *Thiocystis*, *Ectothiorhodospira*, *Rhodopseudomonas*, *Rhodospirillum*, and *Rhodomicrobium* were analyzed. Characteristic compositions of the polar lipids were found for most of the *Rhodospirillaceae* and *Chromatiaceae* species. Phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin were the major phospholipids in most species. Phosphatidylcholine was present as a major component in all species of the genus *Ectothiorhodospira*, but was not detected in the remaining *Chromatiaceae*. It was also present in most of the *Rhodospirillaceae* species. No glycolipids were found in any of the *Ectothiorhodospira* species. In the *Rhodospirillaceae*, the glycolipids mono- and digalactosyl diglycerides were generally absent. Sulfoquinovosyl diglyceride was present in significant amounts in at least three species of the *Rhodospirillaceae* and may have been present in most of them, but only in traces. All of the *Chromatiaceae* species contained several glycolipids, one of which was similar to monogalactosyl diglyceride. Ornithine lipids were found in large amounts in most *Rhodospirillaceae*, but were absent in *Ectothiorhodospira* and in the other *Chromatiaceae*. The species examined could be divided into three groups on the basis of their lipid composition: (i) the genus *Ectothiorhodospira*; (ii) the remaining *Chromatiaceae*; and (iii) the *Rhodospirillaceae*. The data presented are compared with those available in the literature, and differences from other phototrophic organisms are discussed.

Most of the studies reported on the lipid composition of phototrophic bacteria deal with relatively few *Rhodospirillaceae* species, namely, *Rhodospirillum rubrum*, *Rhodopseudomonas sphaeroides*, and *Rhodopseudomonas capsulata*, but little information is available on the lipids of other *Rhodospirillaceae* or families of phototrophic bacteria (for review, see reference 20). The *Chromatiaceae* and *Rhodospirillaceae* species examined so far were found to contain phosphatidylglycerol (PG), cardiolipin (CL), and phosphatidylethanolamine (PE) as major polar lipids; some of the *Rhodospirillaceae* species also contained phosphatidylcholine (PC). Glycolipids, which are the major and characteristic lipid components of higher plants, green algae, and cyanobacteria, appeared to be absent in nearly all of the *Rhodospirillaceae* species examined; they were found in *Chloroflexus*, in several species of the *Chlorobiaceae* (21), and in the two species of the *Chromatiaceae* examined, *Chromatium vinosum* (32) and *Thiocapsa roseopersicina* (33).

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In the present study, we undertook a comparative survey of the lipid composition of representative species of the *Chromatiaceae* and *Rhodospirillaceae* families, including species of the genera *Chromatium* (3 species), *Thiocapsa* (1 species), *Thiocystis* (1 species), *Ectothiorhodospira* (5 species, 11 strains), *Rhodospirillum* (3 species), *Rhodopseudomonas* (7 species, 9 strains), and *Rhodomicrobium* (1 species).

MATERIALS AND METHODS

Organisms. The following strains of *Chromatiaceae* and *Rhodospirillaceae* were studied: *Chromatium vinosum* D (DSM180), *C. minus* 1211, *C. warmingii* (DSM173), *Thiocapsa roseopersicina* 6311 (DSM219), *Thiocystis gelatinosa* 2611, *Rhodopseudomonas palustris* $1a_1$, *R. acidophila* 7050 (DSM137), *R. capsulata* Kb_1 (DSM155), *R. sulfidophila* W4 (DSM1374) and BN193, *R. globiformis* 7950 (DSM161), *R. gelatinosa* BN151, *Rhodopseudomonas* sp. BN126 and BN127, *Rhodomicrobium vannielii* 17100 (DSM122), *Rhodospirillum rubrum* S_1 (DSM467), *R. tenue* BN230, *R. molischianum* DSM120, *Ectothiorhodospira abdelmalekii* BN9840 (16), *E. halochloris* BN9851, *E. vacuolata* BN9512 (14), *E. halophila* BN9626, BN9630, BN9624, BN9625, BN9627, BN9631, and SL_1 (DSM244), and *E. mobilis* BN9903.

Most of the strains were obtained either from N. Pfennig (University of Göttingen) or from the Deutsche Sammlung von Mikroorganismen; strain Kb_1 was obtained from J. H. Klemme (University of Bonn), and strain la_1 was obtained from G. Drews (University of Freiburg); strains with "BN" numbers were isolated by J. F. Imhoff and were maintained in culture in the Institut für Mikrobiologie in Bonn.

Media and culture conditions. All *Ectothiorhodospira* strains were grown at their optimal salt concentrations in the medium described by Imhoff and Trüper (15). The basic mineral medium was supplemented with vitamins, sodium acetate (0.1%), sodium sulfide (0.1%), and sodium thiosulfate (0.2%). All other *Chromatiaceae* were grown in Pfennig's medium (24). *Rhodopseudomonas globiformis* was grown in the medium given by Pfennig (25); all other *Rhodospirillaceae* were grown in a medium for nonsulfur purple bacteria based on the solutions I, II, and III of Pfennig's medium and supplemented with malate (10 mM) and sulfate (5 mM). Strains of *R. sulfidophila* and the unidentified *Rhodopseudomonas* species were grown in the presence of 3% NaCl. The pH was 6.9 for all *Rhodospirillaceae* except *R. acidophila*, which was grown at pH 5.6. Yeast extract was omitted from all media. All bacteria were grown under low light intensity (1,000 lx) and at 30°C.

Extraction and analysis of lipids. Freeze-dried cell material of each of the investigated strains was extracted by a modification (17) of the method of Bligh and Dyer (4): a suspension of the cells in 1 M NaCl (8 ml/g [dry weight]) was diluted with methanol and chloroform in the ratio 2:1:0.8 (chloroform-methanol-water); the mixture was blended and centrifuged; and the pellet was repeatedly extracted with the same solvent mixture until the supernatant was colorless. The combined extracts were filtered through a glass fiber filter and made into two phases by addition of appropriate amounts of chloroform and water to give a final ratio of 1:1:0.9 (chloroform-methanol-water). The phases were allowed to separate overnight, and the lower chloroform phase was collected and brought to dryness on a rotary evaporator; the residue was redissolved in a known volume of chloroform-methanol (4:1), and a portion of this solution, referred to as "total lipid" fraction, was taken for dry weight determination. The ratio of polar to nonpolar lipids was determined by weighing the precipitate of the polar lipids obtained by addition of 20 volumes of ice-cold acetone to a solution of the total lipids in a minimal volume of chloroform (17). To avoid any possible enzymatic degradation of the lipids, cells were also extracted with 2-propanol as described elsewhere (17).

Total lipids were separated by thin-layer chromatography (TLC) on silica gel plates (Brinkmann Instruments Inc.; precoated TLC plates SIL G-25 activated at 100°C for 1 h) in the solvent system chloroform-methanol-acetic acid-water (85:15:10:3.5) or chloroform-methanol-7 N ammonia (60:35:5). Identification was achieved by cochromatography with reference standards of PE, PC, phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), CL, PG, lyso-PE, and lyso-PC and by specific stains (17): for general detection of lipids, by iodine vapor or spraying with 40% aqueous H_2SO_4 followed by charring; for amino group-containing lipids, by ninhydrin reagent (0.2% in acetone); for phospholipids, by the molybdate spray reagent of Vaskovsky and Kostetsky (34); for PC, by the Dragendorff reagent (3); and for sugar-containing lipids, by the α-naphthol stain (31).

For the determination of amino acids and fatty acids, total lipids were methanolyzed by heating under reflux in 2.5% methanolic hydrochloride for 2 h and, after dilution with 10% water, the fatty acid methyl esters were extracted with petroleum ether (17). The methanol-water phase was concentrated and used for the determination of amino acids. Amino acids were separated on silica gel TLC plates in butanol-acetic acid-water (5:3:1) or chloroform–methanol–17% ammonia (2:2:1). They were identified by cochromatography with standards and by spraying with ninhydrin reagent or the ethanolamine-specific reagent 0.5 g benzoquinone in 10 ml of pyridine plus 40 ml of butanol. Ethanolamine gives brown spots at room temperature, whereas serine reacts only slowly at 100°C (8).

RESULTS

Total and polar lipid content. Photosynthetic bacteria contain extensive intracellular membrane systems, and it is well known that under low light intensity these membrane systems are even more extensive than under high illumination (19). Thus, a higher lipid content would be expected in these bacteria than in bacteria without internal membrane systems, and the highest lipid content would be expected after growth at low light intensities. In our study, the average total lipid content of phototrophic purple bacteria was about 13% (range, 5 to 21%) (Table 1). Three groups of organisms could be distinguished among these bacteria on the basis of their relative proportions of polar lipids (see Table 1): the *Chromatiaceae* species (with the exception of *Ectothiorhodospira*), with 23 to 48% (average, 33%); the *Rhodospirillaceae* species, with 56 to 77% (average, 66%); and the *Ectothiorhodospira* species, with 69 to 79% (average, 74%). Some species (*Rhodospirillum molischianum*, *Rhodopseudomonas globiformis*, and *E. vacuolata*) had significantly lower contents of polar lipids compared with the respective average values in their group. In these cases, the lower polar lipid content could have been attributed to enzymatic degradation, but no difference in lipid composition was observed when cells were extracted with 2-propanol. For comparison, the lipid content of the nonphotosynthetic bacterium *Paracoccus denitrificans* was found to be 3% of the cell dry weight with 80% polar lipids (data not shown in Table 1).

Lipids of the *Chromatiaceae*. All of the *Chromatiaceae* species examined here (*C. vinosum*, *C. minus*, *C. warmingii*, *Thiocapsa roseopersicina*, and *Thiocystis gelatinosa*) contained PG, CL, PE, and lyso-PE, but no PC (Fig. 1; Table 2). Seven different glycolipids were found in these strains (Fig. 1B), of which glycolipid 1 (GL 1) had chromatographic properties similar to

TABLE 1. Lipid contents of phototrophic purple bacteria

Species	Strain	Total lipids (% of dry weight)	Polar lipids (% of total lipids)
Chromatiaceae			
Thiocapsa roseopersicina	6311	12	23
Thiocystis gelatinosa	2611	16	33
Chromatium vinosum	D	16	36
C. minus	1211	19	24
C. warmingii	173	21	48
Ectothiorhodospira halochloris	BN9851	ND[a]	71
E. halophila	SL_1	ND	79
E. halophila	BN9624	ND	73
E. halophila	BN9630	ND	71
E. halophila	BN9625	ND	78
E. halophila	BN9626	ND	69
E. vacuolata	BN9512	ND	54
Rhodospirillaceae			
Rhodomicrobium vannielii	17100	16	66
Rhodopseudomonas globiformis	7950	10	42
R. gelatinosa	BN151	10	62
Rhodopseudomonas sp.	BN126	8	65
Rhodopseudomonas sp.	BN127	11	77
R. acidophila	7050	19	71
R. palustris	$1a_1$	10	ND
R. capsulata	Kb_1	7	56
R. sulfidophila	W4	17	66
Rhodospirillum tenue	BN230	5	67
R. rubrum	S_1	9	66
R. molischianum	DSM120	12	38

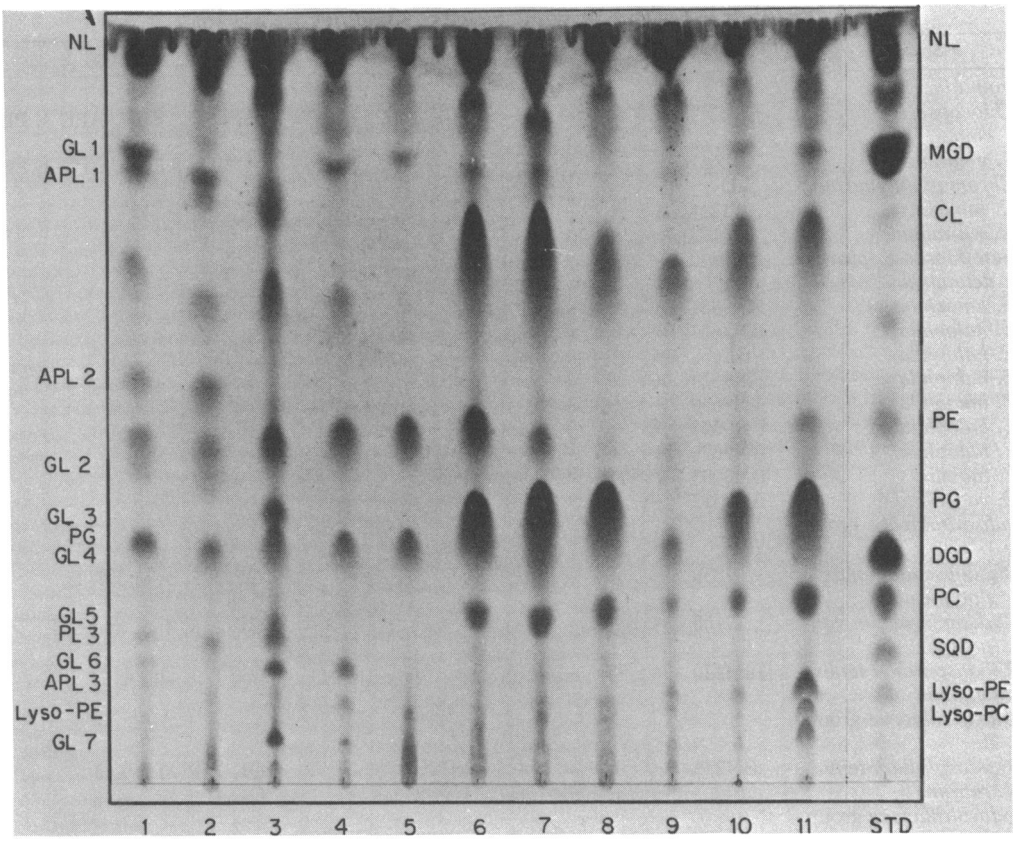
[a] Not determined.

those of monogalactosyl diglyceride (MDG), GL 4 was similar to digalactosyl diglyceride (DGD), and GL 6 was similar to sulfoquinovosyl diglyceride (SQD). All species contained the MGD-like lipid (GL 1), GL 2, GL 7, and one to three other glycolipids. *Thiocapsa roseopersicina* and *Thiocystis gelatinosa* were virtually identical in lipid composition, containing the same glycolipids as well as the same phospholipids, including two unidentified aminophospholipids with mobilities below that of GL 1 (APL 1) and above that of PE (APL 2) (Table 2; Fig. 1). The *Chromatium* species lacked these aminophospholipids and instead of GL 4 contained a different glycolipid, GL 3 (Table 2). Our results are in general agreement with earlier studies on the lipids of *C. vinosum* (32) and *T. roseopersicina* (33) which reported the presence of PG, CL, PE, lyso-PE, and some glycolipids. However, we found a larger number of glycolipids in all of our strains. Three different glycolipids containing mannose and glucose have been found in *C. vinosum* (32), compared with five glycolipids found in our study. It may be of interest that the glycolipids of *T. roseopersicina* were reported to contain glucose and rhamnose (33).

Lipids of *Ectothiorhodospira*. The genus *Ectothiorhodospira* is at present considered to belong to the *Chromatiaceae* family, but it is treated separately here because of the striking differences in relative amounts and qualitative composition of its polar lipids. The average percentages of polar lipids (74%) in representative strains of the species *E. mobilis*, *E. halophila*, *E. halochloris*, *E. abdelmalekii*, and *E. vacuolata* were higher than in the *Rhodospirillaceae* (66%) and in the other *Chromatiaceae* species (33%) (Table 1). In all 11 strains, PG, CL, and PC were present as major constituents (Fig. 1; Table 2). The content of PE was higher in strains with low salt requirements (5% optimal) (e.g., *E. mobilis* BN9903) than in strains requiring higher salt concentrations (10 to 25% optimal, according to the strain) (e.g., *E. halophila* BN9624), and in some of these latter strains PE was present only in traces. All *Ectothiorhodospira* strains contained only small to trace amounts of lyso-PE. Ornithine lipids were absent in *Ectothiorhodospira*, since chromatography of the methanol-water phase of acid hydrolysates of the total lipids showed only one ninhydrin-positive spot corresponding to ethanolamine. None of the lipids in *Ectothiorhodospira* showed a positive reaction with the α-naphthol reagent for glycolipids. In some of the strains, traces of PA, lyso-PC, and two unidentified phospholipids were present. Phosphatidylsulfocholine, which is chromatographically indistinguishable from PC and has been found in the nonphotosynthetic diatom *Nitzschia alba* (1, 2), was not detected in *E. halophila* BN9631 by autoradiography of the lipids from [^{35}S]sulfide-grown cells.

Lipids of *Rhodospirillaceae*. The *Rhodospirillaceae* species in general showed a very heterogeneous lipid composition (Fig. 2). Species of subgroup 1, *Rhodomicrobium vannielii*, *Rhodopseudomonas globiformis*, and *Rhodospiril-*

FIG. 1. TLC of the total lipids of *Chromatiaceae* species in the acidic solvent system. (A) Charred TLC plate; (B) tracing of TLC plate stained with specific stains for: phosphate (hatched spots), amino groups (dotted lines), and sugars (black spots). Identity of the strains: 1, *Thiocapsa roseopersicina*; 2, *Thiocystis gelatinosa*; 3, *C. minus*; 4, *C. vinosum*; 5, *C. warmingii*; 6, *E. mobilis* BN9903; 7, *E. halophila* DSM244; 8, *E. halophila* BN9624; 9, *E. vacuolata* BN9512; 10, *E. abdelmalekii* BN9840; 11, *E. halochloris* BN9851. STD, spinach standard; NL, nonpolar lipids and pigments; GL, unidentified glycolipids; APL, unidentified aminophospholipids; PL, unidentified phospholipid.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GL 1
APL 1
APL 2
GL 2
GL 3
PG
GL 4
GL 5
PL 3
GL 6
APL 3
Lyso-PE
GL 7
NL
MGD
CL
PE
PG
DGD
PC
SQD
Lyso-PE
Lyso-PC
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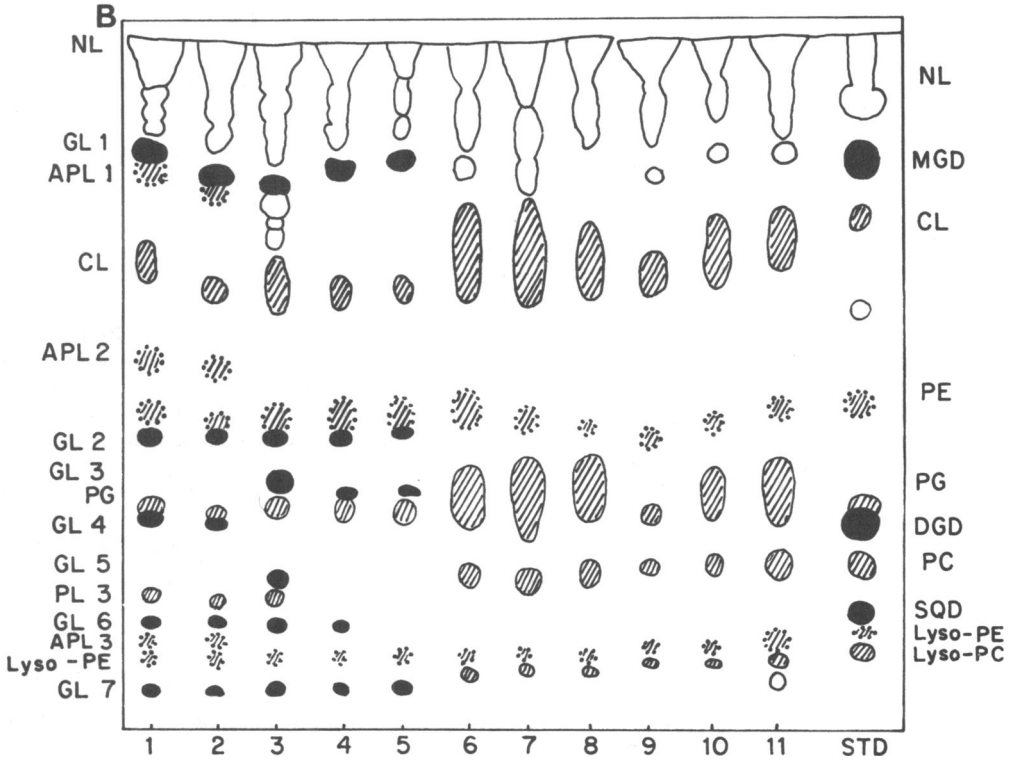
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APL 1
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PG
GL 4
GL 5
PL 3
GL 6
APL 3
Lyso-PE
GL 7
NL
MGD
CL
PE
PG
DGD
PC
SQD
Lyso-PE
Lyso-PC
1 2 3 4 5 6 7 8 9 10 11 STD

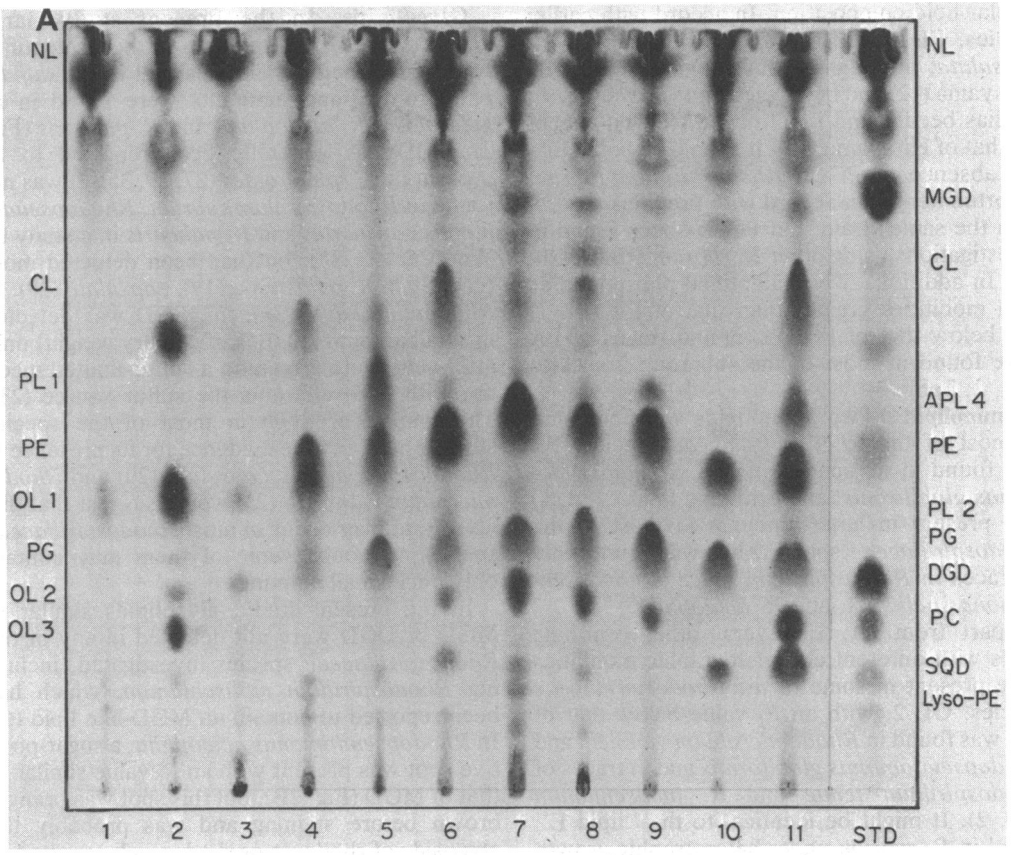
TABLE 2. Polar lipid composition of phototrophic purple bacteria[a]

Species	Strain	PC	PG	CL	PE	OL 1	GL	Others
Chromatiaceae								
Thiocapsa roseopersicina	6311	−	+	+	+	−	1, 2, 4, 6, 7	APL 1, APL 2, APL 3, PL 3
T. gelatinosa	2611	−	+	+	+	−	1, 2, 4, 6, 7	APL 1, APL 2, APL 3, PL 3
Chromatium vinosum	D	−	+	+	+	−	1, 2, 3, 6, 7	
C. minus	1211	−	+	+	+	−	1, 2, 3, 5, 6, 7	PL 3
C. warmingii	173	−	+	+	+	−	1, 2, 3, 7	
Ectothiorhodospira abdelmalekii	BN9840	+	+	+	(+)	−	−	
E. halochloris	BN9851	+	+	+	(+)	−	−	
E. halophila	BN9627	+	+	+	(+)	−	−	
E. halophila	SL_1	+	+	+	+	−	−	
E. halophila	BN9624	+	+	+	(+)	−	−	
E. halophila	BN9630	+	+	+	+	−	−	
E. halophila	BN9625	+	+	+	(+)	−	−	
E. halophila	BN9626	+	+	+	+	−	−	
E. mobilis	BN9903	+	+	+	+	−	−	
E. vacuolata	BN9512	+	+	+	(+)	−	−	
Rhodospirillaceae group 1								
Rhodopseudomonas globiformis	7950	−	−	−	−	+	−	OL 2, 3, 5, and 6, L_x
Rhodomicrobium vannielii	17100	−	−	−	(+)	+	−	OL 2, 3, 4, 5, and 6, L_x
Rhodospirilium molischianum	DSM120	−	−	−	(+)	+	−	OL 2, 3, and 4
Rhodospirillaceae group 2a								
Rhodospirillum tenue	BN230	−	+	+	+	(+)	−	OL 2, PL 1, PL 2
R. rubrum	S_1	−	+	+	+	+	−	OL 4
Rhodospirillaceae group 2b								
Rhodopseudomonas capsulata	Kb_1	+	+	−	+	+	2	PL 1
R. sulfidophila	W4	−	+	−	+	+	6	
R. gelatinosa	BN151	+	+	+	+	+	6	OL 5, PL 1, PL 2
Rhodopseudomonas sp.	BN126	+	+	+	+	+	6	OL 5, PL 1, PL 2, APL 4
Rhodopseudomonas sp.	BN127	+	+	+	+	+	6	OL 5, PL 1, PL 2, APL 4
R. acidophila	7050	+	+	+	+	(+)	6	PL 1, PL 2
R. palustris	$1a_1$	+	+	+	+	(+)	(6)	OL 5, PL 1, AL 1

[a] Symbols: +, present; (+), present in traces; −, absent. Abbreviations as in the legends to Fig. 1 and 2.

lum molischianum, were found to have ornithine lipids as major polar lipid components, but no PC, CL, or PG, and only traces of PE (no PE in *R. globiformis*). In *R. vannielii* and *R. globiformis*, an unidentified phosphate- and ninhydrin-negative lipid (L_x) was present with mobility similar to that of CL. Species of subgroup 2a, *Rhodospirillum rubrum* and *R. tenue*, had CL, PE, ornithine lipid 1 (OL 1), and PG as their main polar lipids, but no PC. Subgroup 2b species, *Rhodopseudomonas gelatinosa*, *R. acidophila*, *R. palustris*, *R. capsulata*, and the unidentified *Rhodopseudomonas* species had in general the same lipids present in subgroup 2a with the addition of varying amounts of PC. Some exceptions are noted below. *R. sulfidophila* lacked both PC and CL, but could also be assigned to subgroup 2b because of its otherwise

FIG. 2. TLC of total lipids of *Rhodospirillaceae* species in the acidic solvent system. (A) Charred plate; (B) tracing of TLC plate with stains as in Fig. 1. Identity of the strains: 1, *Rhodospirillum molischianum*; 2, *Rhodomicrobium vannieli*; 3, *Rhodopseudomonas globiformis*; 4, *Rhodospirillum rubrum*; 5, *R. tenue*; 6, *Rhodopseudomonas gelatinosa*; 7, *R. acidophila*; 8, *R. palustris*; 9, *R. capsulata*; 10, *R. sulfidophila* DSM1374; 11, *Rhodopseudomonas* sp. BN126. STD, spinach standard; Rg, rhodopinglucoside; OL, unidentified ornithine lipids; APL, unidentified aminophospholipids; AL, unidentified aminolipid; PL, unidentified phospholipids; L_x, unidentified lipid.


A
NL
CL
PL 1
PE
OL 1
PG
OL 2
OL 3
NL
MGD
CL
APL 4
PE
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PG
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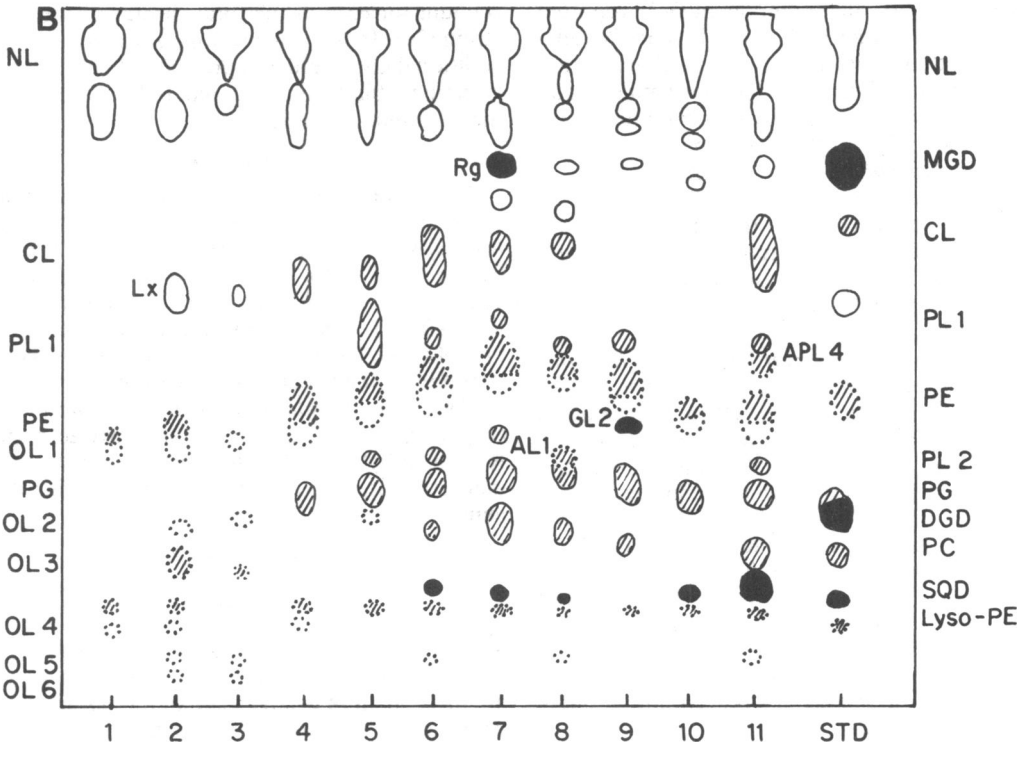
SQD
Lyso-PE
1 2 3 4 5 6 7 8 9 10 11 STD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OL 6
Rg
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AL1
GL2
APL 4
NL
MGD
CL
PL 1
PE
PL 2
PG
DGD
PC
SQD
Lyso-PE
1 2 3 4 5 6 7 8 9 10 11 STD

similar lipid composition. In accord with earlier studies, CL was also found to be absent in *R. capsulata*, although it was found in traces by Hirayama (12) and by Russell and Harwood (29); PC has been found in all of the studies, except for that of Hirayama (12). It should be noted that the absence of PC in *Rhodospirillum rubrum* reported here is in accord with previous reports with the same strain, but PC has been found in investigations using other *R. rubrum* strains (20, 29). In addition, two unidentified phospholipids with mobilities greater than that of PE (PL 1) and below that of PG (PL 2) and traces of PA were found in most of the subgroup 2 species (Fig. 2; Table 2).

Aminolipids. Two aminolipids were common to most of the *Rhodospirillaceae* species: PE was found in all species except *Rhodopseudomonas globiformis*, and ornithine lipid 1 (OL 1) was present in large amounts in most of the *Rhodospirillaceae* species, but was found only in traces in *Rhodospirillum tenue*, *Rhodopseudomonas palustris*, and *R. acidophila*.

Apart from OL 1, several other ornithine lipids with different chromatographic mobilities were present in some of the *Rhodospirillaceae* species. OL 2, with an R_f value below that of PG, was found in *Rhodomicrobium vannielii* and *Rhodopseudomonas globiformis* and in traces of *Rhodospirillum tenue* and *R. molischianum* (Fig. 2). It might be identical to the "lipid E" found in *R. rubrum* under heterotrophic growth conditions (5) or the "component D-2" reported by Park and Berger (23). OL 3 had an R_f value similar to that of PC and gave a positive reaction both with the phosphate and the amino group stains and was most probably ornithine-PG. It was found only in *Rhodomicrobium vannielii* and *Rhodopseudomonas globiformis* and in traces in *Rhodospirillum molischianum*. OL 4 had an R_f value close to that of lyso-PE and might be the "lipid F" found by Brooks and Benson (5) in *R. rubrum* or "component E-2" found by Park and Berger (23) in *R. vannielii*. It was found in the present study in *Rhodomicrobium vannielii*, *Rhodospirillum molischianum*, and *R. rubrum*. OL 5 and OL 6 had very low chromatographic mobilities. OL 5 was found in *Rhodomicrobium vannielii*, *Rhodopseudomonas globiformis*, *R. gelatinosa*, *R. palustris*, and the unidentified *Rhodopseudomonas* species; OL 6 was found in *R. globiformis* and *R. vannielii*.

An unidentified aminophospholipid (APL 4) with chromatographic mobility similar to that of the APL 2 (found in *Thiocystis gelatinosa and Thiocapsa roseopersicina*) was found in the unidentified *Rhodopseudomonas* species. In *R. palustris*, an unidentified aminolipid, running slightly faster than PC, was present in considerable amounts (Fig. 2).

Glycolipids. In the present study, large amounts of SQD were found in the unidentified *Rhodopseudomonas* species and in *R. sulfidophila*; only minor amounts were found in *R. gelatinosa*, *R. acidophila*, and *R. palustris* (Fig. 2). SQD has generally been reported to be present in *R. sphaeroides* (27, 29, 35). It was not found in *Rhodospirillum rubrum*, *Rhodopseudomonas capsulata*, and *R. palustris* in a study by Wood et al. (35), but has been detected more recently in *R. palustris* and *R. capsulata* (29). In *Rhodomicrobium vannielii*, SQD was detected in small amounts (0.01% of cell dry weight) only after cells were grown on a sulfur-limited medium with [^{35}S]sulfate as the sulfur source (23). The finding of SQD in most of the species studied here and the evidence for its presence in *Rhodopseudomonas capsulata* (29) and *Rhodomicrobium vannielii* (23) would suggest that this glycolipid may occur in most *Rhodospirillaceae* species, although some of them may contain only very small amounts.

In the present study, glycolipids similar to MGD or DGD were not detected in any of the *Rhodospirillaceae* species investigated, including *Rhodospirillum molischianum*, which has been reported to contain an MGD-like lipid (6). In *Rhodopseudomonas acidophila*, a sugar-positive spot was present with an R_f value similar to that of MGD (Fig. 2B), but this spot was orange-brown before staining and was probably the glycoside of rhodopin or rhodopinal, reported to account for 65% of the total carotenoids in this strain (30). In *Rhodopseudomonas capsulata* an additional glycolipid was present with chromatographic mobility similar to that of GL 2, found in the *Chromatiaceae* species.

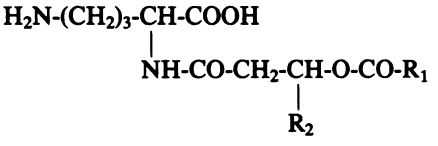
DISCUSSION

Several ornithine lipids have been detected in strains of *Rhodospirillum rubrum* (5, 9, 29, 35), *Rhodopseudomonas sphaeroides* (10, 29, 35), *R. capsulata* (29, 35), *R. palustris* and *R. gelatinosa* (35), *R. viridis* (26), and *Rhodomicrobium vannielii* (23). The chemical structures for only two of these compounds have been established, as follows: (i) esters of α-*N*-acylornithine (amide I) found in *Rhodopseudomonas sphaeroides* (11), with R_1 as an alkyl chain of a fatty acid and R_2 as an alkyl group of a higher alcohol with a C-methyl group or a cyclopropane ring, having the general structure

$$\begin{array}{l} H_2N\text{-}(CH_2)_3\text{-}CH\text{-}COO\text{-}R_2 \\ \qquad\qquad\quad | \\ \qquad\qquad NH\text{-}CO\text{-}R_1 \end{array}$$

and (ii) α-*N*-(acyloxy)acylornithine (amide II) found in *Rhodospirillum rubrum* (5), with R_1 as an alkyl chain of a fatty acid and R_2 as an alkyl chain of a 3-hydroxy fatty acid, having the

general structure



In *Rhodopseudomonas sphaeroides*, another aminolipid has been reported to be "a fatty acid amide of ornithine and of aminopropanol or some other diaminocompound" (22). These three ornithine lipids all have chromatographic mobilities only slightly lower than that of PE and are barely separated from PE. The OL 1 reported here might be either of these compounds or a mixture of them. The failure to separate these lipids completely from PE might account for their misidentification as *O*-ornithylphosphatidyl-glycerol (23, 35). In fact, ornithine-PG has R_f values similar to those of PC (13).

Past work has shown that major groups of photosynthetic organisms may be distinguished by their polar lipid composition (Table 3). If one compares the lipid composition of higher plants and algae with that of cyanobacteria, which also carry out oxygenic photosynthesis, it is apparent that they all contain large amounts of the glycolipids MGD, DGD, and SQD as their main polar lipids. The phospholipids PG, PE, CL, and PC are present as minor components in the plant-type cells, and only PG is present in cyanobacteria.

Among the phototrophic green bacteria, *Chlorobiaceae* and *Chloroflexaceae*, glycolipids are present in significant amounts, but compared with plants and cyanobacteria, relatively higher amounts of phospholipids are present (Table 3). In various *Chlorobiaceae* species, SQD, MGD, and a "glycolipid II" with galactose, rhamnose, and a third sugar have been identified; the only phospholipids found were PG and CL (6, 21). In *Chloroflexus aurantiacus* PG, large amounts of PI, SQD (?), and two unidentified glycolipids were found (21). As in the cyanobacteria, PE has not been found in either of the families of photosynthetic green bacteria.

The present work shows that among phototrophic purple bacteria, three major groups can be distinguished (Table 2). (i) The *Chromatiaceae* (except *Ectothiorhodospira*) contain several glycolipids in each species, which contain different sugars, but, as shown for *C. vinosum* and *T. roseopersicina*, are not galactolipids (32, 33). The phospholipids PG, PE, and CL were found in all of the investigated species, but PC appeared to be absent. (ii) The genus *Ectothiorhodospira* has as the main polar lipid components the phospholipids PG, CL, PC, and PE; PG and CL constitute more than 50% of the total lipids in most of the strains (data not shown), whereas PE is present only in traces in some of them. The lipid composition of *Ectothiorhodospira* species differs from that of the other *Chromatiaceae* not only by the lack of glycolipids and different proportions of PG and CL, but also by the presence of PC and the general lower content of nonpolar lipids. Within the *Chromatiaceae*, differences in the glycolipid composition were observed on the genus level, though all investigated strains (except *Ectothiorhodospira*) showed similar proportions of the different phospholipids. (iii) The *Rhodospirillaceae* family has the most heterogeneous lipid composition of all photosynthetic organisms. This group shows a characteristic species specific lipid composition. The subgroup 1 (see Table 2) lacks PG, PC, and CL, but has different ornithine lipids as main polar lipid components. Within the species of subgroup 2a, CL, PG, PE, and OL 1 are present, whereas PC is apparently absent. Within the species of subgroup 2b, PC is present in addition to the lipids present in subgroup 2a. Although

TABLE 3. Comparison of the general lipid composition of photosynthetic organisms[a]

Organism	PC	PG	PE	CL	OL 1	MGD	GL 2	DGD	SQD	PI
Higher plants and algae[b]	+	+	+	?	?	+++	−	+++	+	+
Cyanobacteria[b,c]	−	++	−	−	?	+++	−	++	++	−
Chlorobiaceae[c]	?	+	−	+	−	++	++	−	+	?
Chloroflexaceae[c]	?	+	−	?	−	+[d]	−	+++[d]	+	+++
Chromatiaceae	−	++	++	+	−	+[e]	−	+[e]	+	−
Ectothiorhodospira	++	+++	+	+++	−	−	−	−	−	−
Rhodospirillaceae										
Subgroup 1	−	−	(+)	−	+++	−	−	−	−	−
Subgroup 2a	−	++	++	+	++	−	−	−	−	−
Subgroup 2b	++	++	++	+	++	−	−	−	+	−

[a] Symbols: −, absent; (+), traces present; +, small amounts; ++, large amounts; +++, predominant lipid; ?, presence uncertain.

[b] Data from Kates (18).

[c] Data from Kenyon (20).

[d] Galactose and another sugar present (21).

[e] No galactolipids (32, 33).

each of the above subgroups contains species with the most similar qualitative lipid composition (Table 2), exceptions are found. Even species with similar qualitative lipid composition may differ quantitatively in this respect.

The variations observed on genus and species levels for the phototrophic purple bacteria are in contrast to the rather uniform lipid composition in the plant kingdom and in the cyanobacteria so far examined.

Despite the great variation in the lipid compositions in different phototrophic organisms, all photosynthetic membranes carry out similar photosynthetic primary reactions. In plants, algae, and cyanobacteria, which have two different light reactions, the glycolipids predominate, and dienoic and trienoic fatty acids specifically accumulate in the galactolipids (28). These findings led to the conclusion that these lipids might be necessary for the function of the oxygenic photosynthetic process. The fact that all photosynthetic bacteria lack polyunsaturated fatty acids (20) and show a great variability in their lipid composition indicates that neither glycolipids nor highly unsaturated fatty acids are essential for the photosynthetic process in general. Because glycolipids, ornithine lipids, or one of the phospholipid components are absent in at least one of the groups of the photosynthetic bacteria (Table 3), it may be concluded that there is no specific lipid associated with photosynthesis that is common to all of the phototrophic bacteria.

In the phototrophic green bacteria, in which accessory reactions and reaction centers are morphologically separated in chlorosomes and cell membranes, glycolipids are found mainly in the chlorosomes, and phospholipids occur in the cell membranes (7). It would certainly be of interest to see if different lipids are associated with the photosynthetic reaction centers in different phototrophic bacteria and how they influence the functional features of the photosynthetic reaction.

ACKNOWLEDGMENTS

One of us (J.F.I.) was supported by a NATO postdoctorate fellowship. The work was supported in part by grants to M.K. and D.J.K. from the Natural Sciences and Engineering Research Council of Canada.

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