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Quantitative and Structural Characteristics of Lipids in *Chlorobium* and *Chloroflexus*

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Abstract. The lipid compositions of *Chlorobium limicola* (4 strains) and *Chloroflexus aurantiacus* (2 strains) have been compared. Both species contained straight-chain, saturated and monosaturated fatty acids as their main fatty acid constituents but the patterns were distinctly different. *Chlorobium* contained acids of chain-length essentially in the range $C_{12}-C_{18}$ with *n*-tetradecanoate, hexadecenoate and *n*-hexadecanoate predominating. *Chloroflexus* was characterized by the presence of significant amounts of C_{17} and $C_{18}-C_{20}$ fatty acids not detected in *Chlorobium*. The latter, on the other hand, contained hydroxylated and cyclopropane-substituted acids not detected in *Chloroflexus*. Simple wax esters ($C_{28}-C_{38}$) were found solely in *Chloroflexus* and accounted for 2.5–3.0% of the cell dry weight. Their fatty acid constituents ranged from $C_{12}-C_{19}$ (both saturated and monounsaturated isomers) whereas the alcohols were generally saturated and of chain-length $C_{16}-C_{19}$. Waxes in the range $C_{34}-C_{36}$ accounted for more than 60% of the total.

The polar lipid patterns of the two genera also showed marked differences. All strains contained phosphatidylglycerol, monogalactosyl diglyceride and sulfoquinovosyldiglyceride. *Chlorobium* contained in addition cardiolipin, phosphatidylethanolamine, the unidentified "glycolipid II" and several other unidentified glycolipids, whereas phosphatidyl inositol and a diglycosyl diglyceride were specific for *Chloroflexus*. The latter lipid contained equimolar amounts of glucose and galactose.

Phenol-water extraction yielded material comprising 14% of the dry cell weight for *Chlorobium* but only 2.5% for *Chloroflexus*. The *Chlorobium* material contained two 3-hydroxy fatty acids and several uncommon sugars (not identified). The analytical results were inconclusive regarding occurrence of 2-keto-3-deoxyoctonate. No typical lipopolysaccharide constituents were found in *Chloroflexus*.

Key words: Phototrophic bacteria – *Chlorobium* – *Chloroflexus* – Lipids – Taxonomy

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Non-standard Abbreviations: CL, cardiolipin; cy, cyclopropane; DGDG, digalactosyl diglyceride; g.l.c., gas-liquid chromatography; LPS, lipopolysaccharide; MGDG, monogalactosyl diglyceride; OH, hydroxy; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; SQDG, sulfoquinovosyl diglyceride; TFA, trifluoroacetyl; t.l.c., thin-layer chromatography; TMS, trimethylsilyl

The anoxygenic phototropic bacteria can be divided into three groups that are distantly related to each other: the purple bacteria (Rhodospirillineae), the green sulfur bacteria (Chlorobiaceae) and the green gliding bacteria (Chloroflexaceae) (Fox et al. 1980). Each of these taxa has previously been shown to exhibit specific lipid patterns (see Kenyon 1978, for review). Thus, all green bacteria contain monogalactosyl diglyceride (MGDG) which is absent from purple bacteria but present in cyanobacteria and chloroplasts (Constantopoulos and Bloch 1967; Cruden and Stanier 1970; Kenyon and Gray 1974). In addition, *Chloroflexus* has been shown to contain a specific diglycosyl diglyceride which apparently contains galactose and another sugar (Kenyon and Gray 1974). Furthermore, *Chloroflexus* is the only phototrophic bacterium known to contain phosphatidyl inositol (PI) as a major phospholipid (Kenyon and Gray 1974), and *Chlorobium* is unique in having *n*-tetradecanoic acid as a major lipid constituent (Kenyon 1978). Quantitative data on the lipid composition of green phototrophic bacteria are lacking, however, and the present investigation is intended to remedy this.

Materials and Methods

Organisms and Cultivation. Chlorobium limicola f. *thiosulfaticum* strains 8327 and 2230 were obtained from Dr. N. Pfennig. Strain 8327c is a bacteriochlorophyll c-containing mutant derived from strain 8327 (Broch-Due and Ormerod 1978). The Tassajara strain was obtained from Dr. B. B. Buchanan. The two strains of *Chloroflexus aurantiacus*, OK-70-fl and J-10-fl were received from Dr. R. Castenholz. Each strain was subjected to purification by the agar shake technique. Purity was checked by microscopy of inoculated cultures to which had been added small quantities of sterile yeast extract and sodium succinate or acetate. These cultures were incubated anaerobically in the light and and then in the dark, first anaerobically, then aerobically. No evidence of contamination was found.

All organisms were grown anaerobically with a light intensity of 2,000 lux (60 W tungsten lamps) in completely filled 300 ml screw-capped bottles. *Chlorobium* was grown at 26–28° C in Pfennig's (1961) medium as modified by Sirevåg (1974).

For *Chloroflexus* the "Roux" modification of D-medium (Pierson and Castenholz 1974) was used at 52° C. *Chloroflexus* was also grown on the defined medium of Madigan et al. (1974) with acetate as the sole organic carbon source. Inositol was omitted from the vitamin mixture, and cells

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for inoculation were subcultured several times in the same medium before use.

Cultures were harvested in early stationary phase by centrifugation at 3,900 × *g* for 20 min at 4° C and washed twice with cold distilled water before lyophilization and storage under N_2 at −20° C.

Chemicals. All solvents were of analytical grade and distilled before use. 2 M HCl in methanol was obtained by bubbling dry HCl gas (Fluka AG, Buchs, Switzerland) into dried methanol until saturated, followed by dilution. Fatty acid methyl ester, long-chain alcohol and wax ester standards were obtained from Applied Science Laboratories Inc., State College, PA, USA. Monosaccharides, lipids (CL, DGDG, MGDG, PE and PI), BCl_3 in methanol and phospholipase D (E.C. 3.1.4.4) were purchased from Sigma Inc., Saint Louis, MO, USA. Trifluoroacetic anhydride, bis-trimethylsilyl-acetamide, ethanolamine and silica thin layer plates (0.2 mm DC Alufolien 60 F_{254} and 0.5 mm Fertigplatten 60 F_{254}) were obtained from Merck Inc., Darmstadt, FRG. Cellulose thin layer sheets (MN-polygram CEL 300 UV_{254}) were purchased from Macherey-Nagel & Co., Düren, FRG. A reference sample of *Salmonella abortus equi* LPS was obtained from Dr. O. Lüderitz, Max-Planck-Institut für Immunbiologie, Freiburg, FRG.

Extraction of Lipids. Lipids were extracted by a modified version of the method of Bligh and Dyer (1959). Lyophilized cells (100 mg) were magnetically stirred in 300 ml glass centrifuge tubes for 1 h at room temperature with 32 ml chloroform/methanol/water (5:8:3 v/v) and then centrifuged. This treatment was repeated and chloroform (44 ml) and water (12 ml) were added to the combined extracts. After centrifugation, the lower phase was recovered and washed with 10 ml chloroform/methanol/water (3:48:47 v/v). Subsequently the solvents were removed on a rotary evaporator and the residue was redissolved in chloroform/methanol (2:1 v/v), flushed with N_2 and kept at −20° C until further analysis.

Isolation of Wax Esters. A crude lipid fractionation was obtained by chromatography on a column (1 × 23 cm) containing 20 g silicic acid, 100 mesh (Mallinckrodt Chemical Works, Saint Louis, MO, USA). A sequential elution with chloroform (175 ml), acetone (700 ml) and methanol (175 ml) gave 3 main fractions. The first contained pigments and wax esters, the 2 others mainly polar lipids. The crude wax fraction was purified on silica sheets as described below, using octadecanoyl-octadecanoate (Sigma Inc., Saint Louis, MO, USA) as standard. The wax esters were eluted from the silica by chloroform.

Extraction of Lipopolysaccharides. The hot phenol method of Westphal and Jann (1965) was used. Lyophilized cells (10−15 mg) were mixed with 1.5 ml 90% w/v phenol (68° C) followed by 1.5 ml water (68° C) and stirred for 15 min. After cooling the aqueous phase was recovered and the phenol phase reextracted. The combined aqueous phases were freed from phenol by dialysis followed by lyophilization. The extracted material from *Chlorobium* was purified further by ultracentrifugation (105,000 × *g*, 3 h)

Methanolyis. Whole cells and fractions of extracted lipids were methanolyzed by 2 M HCl in dry methanol (3 ml) at 85° C for 18 h under N_2 as previously described (Jantzen et al. 1978). Subsequently, excess HCl was removed by a stream of N_2, 3 ml half saturated NaCl was added and the mixture extracted twice with 4.5 ml hexane. The combined hexane phases were evaporated to near dryness, the volume was adjusted to 100 µl with hexane and samples were stored in glass capillaries at −20° C.

Saponification. Since cyclopropane fatty acids are destroyed by acidic methanolysis, a saponification-reesterification procedure was also used (Jantzen et al. 1978). Samples were tested with 1 M NaOH in methanol/benzene (6:1 v/v) at 100° C for 15 min under N_2. After cooling, the solution was acidified (2 M HCl in methanol) to pH 2. Completion of methyl ester formation was obtained by adding 0.5 ml 14% w/v BCl_3 in methanol and heating at 100° C for 5 min. Extraction was performed as described above.

Wax esters were cleaved according to Dittmer and Wells (1969) by heating with 2 M KOH/ethanol (1:1 v/v) under N_2 at 100° C for 2 h. After adjusting the pH to 7, the alcohols were extracted twice with 3 ml hexane, washed with 1 ml water and concentrated under a stream of N_2. After lowering the pH to 2, the fatty acid constituents were subsequently extracted and esterified as described above.

Hydrolysis and Deacylation. Glycolipids were hydrolyzed with 2 M aqueous HCl at 100° C for 2 h or, alternatively, 4 M HCl at 100° C for 6 h. Enzymic hydrolysis was achieved by incubation with phospholipase D. To 5−6 µmol of PE, obtained by preparative thin layer chromatography (see below), 0.5 ml 0.2 M acetate buffer (pH 5.6), 0.1 ml 1 M aqueous $CaCl_2$, 0.4 ml diethylether and 0.5 mg phospholipase D were added. The mixture was magnetically stirred at room temperature for 4 h. Phase separation was obtained by adding 1.25 ml chloroform and 1.25 ml water followed by centrifugation. The aqueous phase was taken to dryness, TFA derivatized and analysed by g.l.c. as described below.

Mild alkaline deacylation of isolated lipids was done according to Kates (1975). To 30−600 µg dry lipid, 1.0 ml 0.1 M NaOH in $CHCl_3$/methanol (1:4) was added. After 15 min at room temperature phase separation was achieved by the addition of 0.2 ml methanol, 0.8 ml chloroform and 0.5 ml water. After centrifugation, the aqueous methanolic phase was neutralized by cationic exchanger (Amberlite 120). The remaining chloroform phase was extracted twice with 0.5 ml with methanol/water (10:9 v/v) and the combined methanol/water phases were then made slightly alkaline with methanolic NH_4OH, concentrated to dryness and redissolved in methanol/water (10:9 v/v).

Thin-layer Chromatography. Neutral lipids (fatty acid methyl esters, fatty alcohols and wax esters) were analysed and purified on silica sheets developed by hexane/diethylether (85:15 or 90:10 v/v). Polar lipids were also chromatographed on silica sheets; both one- and two-dimensional development were routinely used with the following solvent systems: A: chloroform/methanol/water (65:25:4 v/v); B: acetone/benzene/water (91:30:8 v/v); C: chloroform/methanol/conc. ammonia (65:35:5 v/v); D: chloroform/methanol/acetone/acetic acid/water (5:1:2:1:0.5 v/v). Systems C (1st dimension) and D (2nd dimension) were combined in the two-dimensional chromatographic system.

Deacylated lipids and monosaccharides were chromatographed on cellulose sheets and developed by phenol/wa-

ter (100:38 w/v) and ethylacetate/pyridine/acetic acid/water (5:5:1:3 v/v), respectively.

Lipids were detected by sulfuric acid charring (10% H_2SO_4 in ethanol), free amino groups with ninhydrin (0.2% w/v in ethanol), phospholipids with molybdate reagent, glycolipids with α-naphthol, and sugars by alkaline $AgNO_3$ reagent (Kates 1975). For preparative purpose lipid material was detected with UV light or by iodine vapor.

Gas-liquid Chromatography. Chromatographs (Hewlett-Packard 5750 and 5710) equipped with hydrogen flame ionization detectors were used. Two standard glass columns (200 × 0.2 cm) were used routinely; *non-polar:* 10% SE-30 (methyl silicone) on Gas-Chrom Q, 100–120 mesh; *polar:* 10% SILAR-10C (cyanopropyl silicone) on Gas-Chrom Q, 100–120 mesh (Applied Science Laboratories Inc.). Additional analyses were performed on a 25 m WCOT SE-30 glass capillary column (Chrompack Inc., Middelburg, The Netherlands). The two standard columns were operated from 90°C (TFA derivatized sugars), 120°C (fatty acid methyl esters, fatty alcohols), 150°C (TMS derivatized sugars) to 250°C increasing at 2°C min^{-1} with a carrier gas (N_2) flow of 20 ml min^{-1}. Wax esters were analysed at 250°C–350°C with a rate of 1°C min^{-1}. Ethanolamine (TFA derivatized) was analysed isothermally at 80°C.

The injection port and detector temperatures were usually set to 250°C and 300°C, respectively. The glass capillary column was operated in splitless mode using a Hewlett-Packard 18740B capillary column control and with a carrier gas (He) flow of 1.5 ml min^{-1}. The column oven was programmed from 170–250°C at a rate of 4°C min^{-1}.

Peak areas were recorded by a Hewlett-Packard 3380A digital integrator as percentage of total peak area. The response of underivatized hydroxy fatty acids is generally poor and variable. These constituents and fatty alcohols were therefore quantitated after trifluoroacetylation (see below).

The amounts of lipids were calculated from the fatty acid content (Christie et al. 1970; Radwan 1978). For unidentified phospholipids and "glycolipid II", K values of 1.5 and 2 were used, respectively.

Chemical Identification. Fatty acid methyl esters and fatty alcohols were primarily identified by comparing their retention times on both the polar and non-polar column with those of the standards. The identity of the hydroxylated lipid constituents was confirmed by determination of their retention times before and after conversion to TFA derivatives (Jantzen et al. 1978). The presence of unsaturation and/or cyclopropane substitution in the alkyl chain was examined by the reduction method of Brian and Gardner (1968). Since methanolic HCl largely degrades cyclopropane fatty acids, a comparison of fatty acid profiles obtained after alkaline and acidic methanolysis, respectively, provides another test for the presence of cyclopropane fatty acids.

Trifluoroacetyl derivatives were formed by heating to boiling for 5 min with 200 µl of trifluoroacetic anhydride and acetonitrile (1:1). Trimethylsilyl derivatives were prepared by adding equal volumes (100 µl) of bis-trimethylsilylacetamide and acetonitrile to the dried sample followed by heating at 120°C for 5 min in an oil-bath.

Wax esters were primarily identified by comparing their g.l.c. retention times and R_f values in t.l.c. with those of standards (hexadecanoyl hexadecanoate and octadecanoyl octadecanoate). Further characterization was obtained by hydrolysis and subsequent analysis of their fatty acid and alcohol constituents by g.l.c. and mass spectrometry.

Polar lipids were identified by their chromatographic and staining properties in comparison to standards, and by chemical or enzymic hydrolysis followed by analysis by g.l.c. and mass spectrometry.

2-Keto-3-deoxy-octonic acid was analysed colorimetrically by the thiobarbituric assay (Drøge et al. 1970) and by g.l.c. (Bryn and Jantzen 1982).

Structural elucidations were verified by g.l.c.-mass spectrometry using a Hewlett-Packard 5992A quadropole instrument equipped with a glass capillary column (25 m × 0.5 mm CP^{tm}Sil 5, methyl silicone; Chrompack Inc.).

Results

Cellular Fatty Acids and Alcohols. Both *Chlorobium* and *Chloroflexus* contained straight-chain, saturated and monounsaturated fatty acids as their main fatty acid constituents, but the patterns were distinctly different in the two groups of organisms (Table 1). The ratios of saturated to unsaturated fatty acids were 1.1 and 3.7 for *Chlorobium* and *Chloroflexus*, respectively. Similarly, the ratios of C_{16}/C_{18} fatty acids differed considerably with ranges of 15–47 and 0.6–0.7, respectively. Thus, *Chlorobium* contained acids of chain-length essentially in the range $C_{12}-C_{18}$ with *n*-tetradecanoate (14:0), hexadecenoate (16:1), and *n*-hexadecanoate (16:0) as the most ubundant, whereas the *Chloroflexus* pattern was characterized by the presence of significant amounts of C_{17} and $C_{18}-C_{20}$ fatty acids mostly absent in *Chlorobium*. The latter, on the other hand, contained hydroxylated- and cyclopropane substituted acids, not

Table 1. Cellular fatty acid and alcohol composition of *Chlorobium* and *Chloroflexus*

Fatty acids	*Chlorobium*				*Chloroflexus*	
	8327	8327c	2230	Tassajara	OK-70fl	J-10fl
12:0	2.1	2.9	2.9	1.1	tr	tr
14:0	22.4	26.5	23.8	27.1	tr	tr
3-OH −14:0	1.4	1.8	1.4	1.1	–	–
15:0	–	–	–	–	1.0	0.9
16:1	42.8	46.9	43.4	37.3	2.5	2.2
16:0	23.0	15.8	22.1	20.3	23.7	20.3
3-OH −16:0	2.1	2.1	1.6	1.4	–	–
17:1	–	–	–	–	3.1	2.3
17:cy	0.7	1.1	2.6	7.3	–	–
17:0	–	–	–	–	12.3	9.1
18:1	1.2	1.4	3.1	3.1	9.0	9.1
18:0	3.3	0.5	tr	tr	28.6	29.5
19:1	–	–	–	–	1.3	1.6
19:0	–	–	–	–	3.6	3.6
20:1	–	–	–	–	2.5	2.9
20:0	–	–	–	–	0.8	1.1
Fatty alcohols						
16:0	–	–	–	–	2.0	2.7
17:0	–	–	–	–	0.5	1.7
18:0	–	–	–	–	8.8	12.6
19:0	–	–	–	–	0.3	0.4

The amounts are given as percentages (w/w) of the total; tr (trace), less than 0.5%

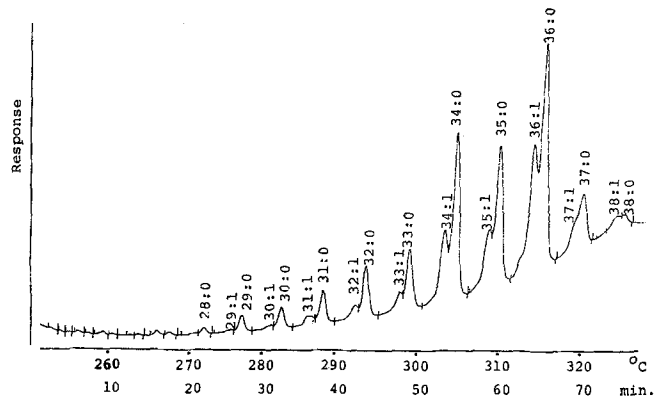

Fig. 1. A g.l.c. wax ester profile of *Chloroflexus* strain OK-70-fl

Table 2. Wax ester composition of *Chloroflexus* OK-70-fl

Wax ester	Amount	Wax ester	Amount
28:0	tr	34:1	7.0
29:1	tr	34:0	12.9
29:0	1.0	35:1	5.0
30:1	tr	35:0	9.6
30:0	1.7	36:1	13.3
31:1	tr	36:0	20.7
31:0	3.1	37:1	3.5
32:1	0.5	37:0	7.1
32:0	4.9	38:1	1.2
33:1	0.9	38:0	1.0
33:0	6.5		

The amounts of wax esters are given as percentages (w/w) of the total; tr (traces), less than 0.5%

detected in *Chloroflexus*. Trifluoroacetylation of the fatty acid fractions rendered several components eluting at shorter retention times. This indicated the presence of hydroxyl substituted substances. Comparison of retention data and mass spectrometric fragmentation with those of standards revealed that the *Chlorobium* strains contained 3-hydroxy-tetradecanoic acid and 3-hydroxy-hexadecanoic acid. The derivatizable compounds of *Chloroflexus* showed g.l.c. retention data uncharacteristic of fatty acids, whereas comparison with fatty alcohol standards gave complete matching. The mass spectra confirmed this result; all the peaks gave an abundant M-15 fragment (as TMS derivative) and otherwise a fragmentation pattern as described by Sharkey et al. (1957). Thus, the four non-fatty acid components of the *Chloroflexus* chromatograms were found to be *n*-hexadecanol, *n*-heptadecanol, *n*-octadecanol and *n*-nonadecanol, respectively.

Wax Esters. The fatty alcohols found in the cellular fatty acid fraction of *Chloroflexus* indicated the presence of wax esters, and t.l.c. revealed material of R_f-value corresponding to octadecanoyl-octadecanoate (wax_{18-18}). As shown in Fig. 1, high temperature gas chromatography of this fraction provided a typical wax ester profile (Bryn et al. 1977) consisting of peaks corresponding to saturated and mono-unsaturated wax esters of chain-length $C_{28}-C_{38}$ (Table 2). The total amount of this lipid fraction was found to be 2.5–3.0% of cell dry weight.

Table 3. Extractable lipids of *Chlorobium* and *Chloroflexus*

Lipid	Amount (μg lipid/mg dry cell)	
	Chlorobium	*Chloroflexus*
Cardiolipin (CL)	6–12	–
Phosphatidylglycerol (PG)	2–3	16–35
Phosphatidylethanolamine (PE)	4–9	–
Lyso-PE	tr	–
Phosphatidylinositol (PI)	–	12–26
Total phospholipid	12–24	28–61
Monogalactosyldiglyceride (MGDG)	21–42	28–60
Glycolipid II	1–2	–
Digalactosyldiglyceride (DGDG)	–	10–21
Lipid 1	1–3	–
Lipid 2	12–24	–
Sulfoquinovosyldiglyceride (SQDG)	+	+
Total glycolipids	35–71	38–81
Wax esters	–	25–30
Others	2–4	2–5

The amount of the respective lipids were calculated from their fatty acid content, see text. The two figures for each concentration refer to two separate experiments including growth. tr, trace; +, presence noted but not quantitated; –, not detected

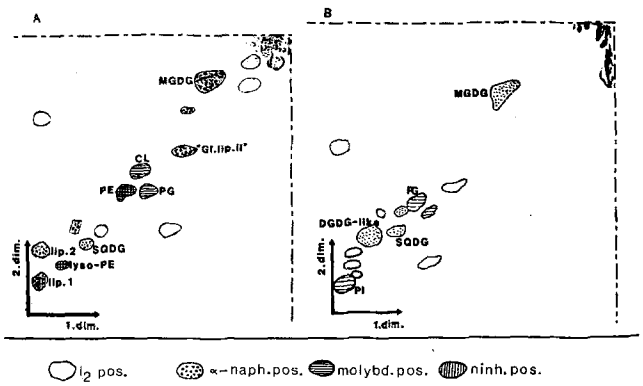

Fig. 2A and B. Two-dimensional t.l.c. of the polar lipids of **A**: *Chlorobium* 8327D and **B**: *Chloroflexus* OK-70-fl. See text for abbreviations and the chromatographic conditions

Hydrolysis and subsequent g.l.c. and mass spectrometry of the corresponding fatty acid and fatty alcohol fractions showed a fatty acid spectrum within the range $C_{12}-C_{19}$ (both saturated and mono-unsaturated) whereas the corresponding fatty alcohols were generally saturated and of chain-length $C_{16}-C_{19}$.

Polar Lipids. Table 3 shows the contents of polar lipids (phospholipids, glycolipids, sulfolipids) of the two genera, and Fig. 2 illustrates the qualitative differences as revealed by two-dimensional t.l.c. Quantitatively, this fraction seemed to be higher in *Chloroflexus* than in *Chlorobium*.

Since PE was not detected in a previous study of *Chlorobium* lipids (Kenyon and Gray 1974), the chromatographie spot corresponding to PE was eluted and purified by preparative t.l.c. for further structural studies. This constituent was both phosphate- and amino group positive. Deacylation and subsequent t.l.c. analysis gave a spot of

the same R_f as the PE standard treated in the same way. Enzymic hydrolysis by phospholipase D and subsequent g.l.c. analysis provided a peak of identical retention time to that of ethanolamine. I.R. analysis was also strongly indicative of PE since all major absorptions were shared by the PE standard. In *Chlorobium* strain 8327 small amounts of another lipid of lower R_f value than PE, giving phosphate and amino group reactions, could also be detected. This compound, which has not been analysed further, is believed to be lysophosphatidyl ethanolamine.

One carbohydrate-positive lipid found in both bacterial groups was identified as MGDG. The second major carbohydrate-containing lipid of *Chlorobium* marked "Gl.lip. II" in Fig. 2 is assumed to correspond to "Glycolipid II" of Constantopoulos and Bloch (1967). In addition to fatty acids and glycerol this lipid was found to contain rhamnose, galactose, glucose and an unidentified sugar in approximate molar ratios 3:3:1:0.6. The unidentified sugar eluted in the "hexose region" as TMS and TFA derivatized methylglycoside, but has not been characterized further.

Chlorobium also contained two other carbohydrate-containing lipids of low R_f values, designated "lipid 1" and "lipid 2". "Lipid 1" contained small amounts of glucose, and "lipid 2", mannose and two additional unidentified sugars. One of these showed the same chromatographic properties as the unidentified monosaccharide of "Glycolipid II". Lipid 1 was weakly ninhydrin positive. As shown in Fig. 2, an additional α-naphthol positive (weakly) substance was found in *Chlorobium* and tentatively identified as SQDG on the basis of its chromatographic and staining properties.

Chloroflexus contained a lipid of R_f value identical to that of the DGDG standard, but both glucose and galactose were shown to be present in equal amounts. Thus, the polar lipid patterns of the two genera showed marked differences. Both contained PG and MGDG while *Chlorobium* contained in addition CL, PE, the still unidentified "glycolipid II", and several other unidentified glycolipids. *Chloroflexus*, on the other hand, contained PI and a diglycosyl diglyceride. PI was found in cells cultured both with and without yeast extract in the medium.

Strain 8327 of *Chlorobium* and OK 70-fl of *Chloroflexus* were examined for typical LPS constituents after extraction of carbohydrate containing material. The yields of such material were 14% for *Chlorobium* and only 2.5% for *Chloroflexus*. No typical LPS constituents could be found in *Chloroflexus* while *Chlorobium* contained 3-hydroxy-tetradecanoate and 3-hxdroxy-hexadecanoate in equimolar amounts. The chromatographic monosaccharide profiles of the *Chlorobium* fraction were very complex with two unidentified, uncommon sugars dominating. Several less abundant compounds eluted in the hexose and heptose regions but their retention times did not match any of the ordinary sugar standards. No 2-keto-3-deoxy-octonate peaks were observed by g.l.c. (Bryn and Jantzen 1982), and colorimetric determination of this sugar was hampered by interferring unknown substances.

Discussion

The whole-cell fatty acid patterns of the present study are mainly in agreement with previous reports (Kenyon and Gray 1974; Kenyon 1978). Both the high degree of saturation, and the prevalence of longer fatty acids in *Chloroflexus* is in accordance with the thermophilic character of this organism and the notion of regulation of membrane fluidity by fatty acid composition.

The high content of *n*-tetradecanoic acid in *Chlorobium* confirmed in this study, is interesting. This fatty acid is present in only small amounts in purple bacteria which contain instead a large proportion of *n*-octadecanoic acid. The latter is almost absent from *Chlorobium*.

Methylene-hexadecanoic acid (17:cy) was present in all of the *Chlorobium* strains but not in *Chloroflexus*. The amount of this acid present in *Chlorobium* (strain Tassajara) was much higher in stationary phase than in log phase cultures (10 and 0.5%, respectively) and correspondingly 16:1 decreased from 52 to 43%, suggesting a biosynthesis similar to that described for *Escherichia coli* (Cronan et al. 1979).

Hydroxy fatty acids were found in *Chlorobium* whole cells and in the phenol-water extracted carbohydrate material but not in the extractable lipid fraction indicating that this organism contains LPS. No commonly occurring hexoses or heptoses seemed to be present. Colorimetry of 2-keto-3-deoxy-octonate was inconclusive due to interference, and since the g.l.c. analysis was negative, this sugar, if present, must be phosphorylated (Bryn and Jantzen 1982).

Chloroflexus lacks hydroxylated fatty acids (Table 1), and the phenol-water extraction gave very little carbohydrate material and no saccharides typical of outer membrane LPS. On the other hand, ultrastructural studies have shown that a typical outer membrane is present (Staehelin et al. 1978).

Wax esters have only rarely been reported in bacteria (Bacchin et al. 1974; Gallagher 1971; Bryn et al. 1977) and never in phototrophic bacteria. The major wax species in *Chloroflexus* (Table 3) is the fully saturated C_{36}. The exact location and physiological significance of wax esters in bacteria is uncertain although a correlation between wax ester content and adhesion to oil droplets has been suggested for *Acinetobacter* (Rosenberg et al. 1981).

The phospholipid patterns in *Chlorobium* and *Chloroflexus* are essentially similar to those reported earlier with the exception of PE which was notably absent in previous data (Kenyon and Gray 1974; Kenyon 1978). The presence of this lipid was verified in a culture of *C. limicola* strain 8327 which had been resubjected to purification followed by a thorough check for contamination.

Chloroflexus contained PI irrespective of whether the growth medium contained inositol or not. This lipid is unusual and may be a useful taxonomic marker for this organism.

The glycolipids of green bacteria are the class of lipids about which there is most uncertainty. *Chlorobium* contains at least five glycolipids: MGDG, "glycolipid II", lipid 1 (may contain glucose and an amino group), lipid 2 (with mannose and two other carbohydrate residues), and a lipid tentatively identified as SQDG. *Chloroflexus* contains MGDG, SQDG and a diglycosyl diglyceride.

The "glycolipid II" of *Chlorobium* was shown to contain glucose in addition to the previously reported rhamnose, galactose and a third unidentified sugar (Constantopoulos and Bloch 1967; Cruden and Stanier 1970; Kenyon and Gray 1974) which was also detected in "lipid 2" and in the phenol-water extracted material. The structure of "glycolipid II" is not established, but on the basis of its chromatographic properties it seems unlikely that it is a diglyceride with two or three carbohydrate constituents, unless the

saccharides are unusually unpolar (e.g., acylated or O-methylated).

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