Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3399–3403, May 1989
Microbiology

Differences in fatty acid composition between vegetative cells and N_2-fixing vesicles of *Frankia* sp. strain CpI1

ANDERS TUNLID*†, NANCY A. SCHULTZ‡, DAVID R. BENSON‡, D. BERNIE STEELE*, AND DAVID C. WHITE*

*Institute for Applied Microbiology, University of Tennessee, Knoxville, TN 37932; and ‡Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06268

Communicated by John G. Torrey, December 9, 1988 (received for review September 28, 1988)

ABSTRACT **When growing on N_2, actinomycetes from the genus *Frankia* form multicellular structures that contain nitrogenase. The structures are referred to as vesicles and are indistinguishable from vesicles formed when *Frankia* sp. are in root-nodule symbioses. Vesicles isolated from N_2-grown cells of *Frankia* sp. strain CpI1 had a significantly higher amount and different composition of fatty acids than did vegetative cells recovered from NH_4^+-containing medium. Lipids from vesicles, whole cells grown on N_2, and whole cells grown on NH_4^+ were fractionated by silicic acid chromatography into neutral lipids, glycolipids, and polar lipids. The fatty acids were transesterified by methanolysis and analyzed by gas chromatography and mass spectrometry. Vesicles had considerably higher amounts of fatty acids in the neutral and glycolipid fractions but lower amounts of polar lipid fatty acids than did vegetative cells. Polar lipids from vesicles had a higher proportion of monounsaturated and cyclopropane fatty acids and a lower proportion of isobranched fatty acids than did polar lipids from NH_4^+-grown or N_2-grown cells. The neutral lipid and glycolipid fractions contained several long-chain compounds with molecular ions at m/z 408 and 410. The proportions of these compounds were significantly higher in the lipids from vesicles than from vegetative cells. These results suggest that lipids in vesicles might be involved in the protection of nitrogenase from O_2 and suggest a parallel with the glycolipids involved in protecting nitrogenase from O_2 in the cyanobacterial heterocysts.**

Frankia sp. are N_2-fixing actinomycetes that induce root nodules on several nonleguminous plants including species of *Alnus, Myrica,* and *Casuarina* (1). When fixing N_2 in symbiosis and in culture, most *Frankia* sp. form specialized cellular structures called vesicles. Vesicles contain nitrogenase as shown by direct assay after isolation (2, 3) and by immunogold-labeling using antibodies against nitrogenase (4).

In culture, *Frankia* sp. fix N_2 at atmospheric partial pressures of O_2 (p_{O_2}) (5–7). Since *Frankia* sp. grow and respire slowly and since their nitrogenase is as sensitive to O_2 inactivation as other nitrogenases (8), some mechanisms must exist that protect the enzyme from O_2. Part of this protection has been proposed to reside in the cell envelope of vesicles, which may function as a diffusion barrier (6, 9, 10). Evidence for this function includes the observation that nitrogenase activity occurs without vesicles in *Frankia* cultures grown under microaerobic conditions; such activity is sensitive to inactivation by O_2 (11). A parallel occurs in cyanobacteria, where cultures lacking heterocysts can fix N_2 only under microaerobic conditions, while aerobic fixation occurs only when heterocysts are present (12).

Frankial vesicles have a cell envelope or capsule that completely surrounds the vesicle stem and head regions (4, 9, 10, 13). In freeze-fracture and in freeze-substituted preparations, the envelope material appears as repeated lipid monolayers with as many as 50 or more layers observed (10, 13). Recent studies have shown that the number of monolayers increases in proportion to the level of O_2 under which frankial cultures are induced for nitrogenase activity (10). The multilayered structure of the vesicle envelope is similar to the heterocyst envelope structure (12). The inner laminated layer of the heterocyst envelope is composed of glycolipids with long-chain polyhydroxyl alcohols and hydroxy acids not found in vegetative cells (14–16). Mutant studies on *Anabaena vulgaris* have suggested that these lipids are essential for protecting cyanobacterial nitrogenase from O_2 (17), and gas-diffusion studies using gas vesicle collapse have shown the diffusion resistance of intact heterocysts to gases to be quite high (18), primarily because of the glycolipid laminae in the heterocyst envelope.

In the present paper we have taken advantage of a newly described technique for isolating vesicles from N_2-fixing cultures of *Frankia* (2). We have examined the fatty acid profiles of NH_4^+-grown cells lacking vesicles, cells from N_2-fixing cultures, and vesicles isolated from N_2-fixing cultures to identify differences in fatty acid composition of polar lipids, glycolipids, and neutral lipids.

MATERIALS AND METHODS

Organism and Culture Media. *Frankia* sp. strain CpI1, a *Comptonia peregrina* isolate (19), was grown in defined succinate/NH_4^+ or succinate/combined nitrogen-free liquid medium as described (2).

Vesicle Isolation. Nitrogen-fixing cells grown in 3-liter batch cultures were harvested by vacuum filtration and were homogenized in 50% (wt/wt) sucrose (2). The homogenate was transferred to 50-ml polycarbonate centrifuge tubes. The sucrose was overlaid with 10% (vol/vol) glycerol in distilled H_2O, and the tubes were centrifuged for 1 hr at 16,000 × *g*. The vesicle band was removed with a Pasteur pipet. Both the isolated vesicles and gradient pellet were washed with 20 mM imidazole/HCl buffer (pH 6.9) containing 2 mM $MgCl_2$ and 1% glycerol.

Cells grown on NH_4^+ or on N_2 were collected by vacuum filtration and stored at −80°C. The frozen cell pastes from NH_4^+-grown and N_2-grown cultures and the gradient pellet and vesicle fractions from the sucrose density gradient centrifugation were lyophilized with a Virtis lyophilizer (Gardiner, NY). The freeze-dried samples were held under N_2 atmosphere until analysis.

Lipid Extraction and Fractionation. Approximately 10 mg (dry weight) of the lyophilized samples was extracted with the single-phase mixture chloroform/methanol/water (1:2:0.8, vol/vol) as described by Bligh and Dyer (20). The extracts were split into two phases by the addition of 1

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†Present address: Laboratory of Ecological Chemistry, Lund University, Lund, Sweden.

volume each of chloroform and water. The organic phase was recovered, and the extracted lipids were fractionated on columns of silicic acid (Unisil, 100–200 mesh; Clarkson, Williamsport, PA) containing 1 g (dry weight) of adsorbent. The lipids were applied to the columns in a minimal volume of chloroform and were eluted with solvents of increasing polarity: 10 ml of chloroform, 40 ml of acetone, and 10 ml of methanol. For lipids of most bacteria, the chloroform fraction contains neutral lipids; the acetone fraction, glycolipids; and the methanol fraction, polar lipids (21, 22). The lipid fractions were dried under a stream of N_2.

Mild Alkaline Methanolysis. The lipid fractions were subjected to a mild alkaline methanolysis, which yields the methyl esters of the ester-linked fatty acids (23).

Gas Chromatography (GC). The methyl esters were analyzed by capillary gas GC with a Hewlett–Packard 5880 gas chromatograph equipped with a flame-ionization detector. The esters were separated on a 50 m × 0.2 mm i.d. fused silica column coated with a cross-linked methylsilicone stationary phase (Hewlett–Packard). The initial oven temperature was held at 80°C for 1 min and then was increased at a rate of 10°C/min to 240°C and finally at 5°C/min to 300°C. The injector temperature was 250°C, and the detector temperature was 270°C. Hydrogen at a linear gas flow rate of 80 cm/sec was used as the carrier gas. Injections were performed in the splitless mode.

Tentative peak identification, prior to mass spectrometric (MS) analysis, was based on comparison of retention times with data from standards obtained from Supelco, Sigma, Nu Chek Prep (Elysian, MN), and from compounds previously identified in our laboratory. Peak areas were quantified by adding an internal standard, methyl nonadecanoate, prior to the GC injections. The GC data were acquired and manipulated by a Nelson chromatography data system (Nelson, Paramus, NJ).

GC/MS. GC/MS analyses were performed on a VG TRIO-3 GC/MS/MS instrument equipped with a HP 5890 gas chromatograph. The GC conditions were those described as above, but helium was used as the carrier gas. The electron energy in electron-impact ionization was 70 eV, and the source temperature was 200°C. Isobutane (purity, 99.99%) was used as the reagent gas in chemical ionization. The source temperature in chemical ionization was 150°C.

MS identifications of the methyl esters were based on comparison with spectra from standards or with spectra reported in the literature (24–26). The positions of double bonds were determined by preparing the dimethyldisulfide adducts (27). Positions of cyclopropane rings were determined after hydrogenation of the methylesters in the presence of Adam's catalyst (PtO_2) (28).

Fatty Acid Nomenclature. Fatty acids are designated by the total number of carbon bonds: number of double bonds, with the position closest to the aliphatic end of the molecule indicated (ω) along with the geometry (*c* for cis and *t* for trans). The prefixes "i" and "a" refer to iso- and anti-isobranching, respectively. Cyclopropane fatty acids are designated "cy."

RESULTS

Fatty Acid Content. The data in Figs. 1–4 are presented as the amount, by weight, of the most abundant fatty acids in each cell type. The data in Table 1 present all fatty acids identified in the polar lipids in the methanol fraction from the silicic acid column and are expressed in mol %. Major differences were observed in the amount and composition of fatty acids between the various *Frankia* cell types. Vesicles had the highest proportion of their dry weight as fatty acids; N_2-grown cells had less, and NH_4^+-grown cells had only half the amount found in vesicles (Fig. 1).

There were also significant differences in the relative proportions of fatty acids recovered in the methanol, acetone, and chloroform fractions between the three cell types (Fig. 1). Sixty percent by weight of the total fatty acids in vegetative cells from NH_4^+-containing medium were from polar lipids extracted in the methanol fraction (Fig. 1). This fraction was considerably smaller in N_2-grown cells (26%) and much smaller in the vesicles (10%); the latter samples had higher proportions of their fatty acids in the glycolipid and neutral lipid fractions. Approximately 70% by weight of the fatty acids in vesicles and 55% in N_2-grown cells were found in the neutral lipid fraction compared with 30% in vegetative cells.

Polar Lipid Fatty Acids. Ester-linked fatty acids from the methanol fraction had 14–18 carbon atoms (Table 1, Fig. 2). The predominant fatty acid in NH_4^+-grown cells, N_2-grown cells, and vesicles was il6:0, which comprised between 41% and 51% by weight of the total polar fatty acids. Other common fatty acids in these profiles were monounsaturated fatty acids with double bonds localized at the Δ9 position, including 17:1ω8, 15:1ω6, il6:1ω6, 16:1ω7*c*, and 18:1ω9*c*, and normal saturated fatty acids with 15, 16, and 17 carbons. Among the minor compounds were two cyclopropane fatty acids. The most prominent of these had 18 carbon atoms and was identified as cy18:0(ω8, 9) (Table 1).

The composition of fatty acids in the polar lipids of NH_4^+-grown cells and N_2-grown cells were quite similar. However, by comparing the relative proportions expressed in mol %, it was shown that polar lipids from the vesicles had a significantly lower mol % of isobranched fatty acids but a higher proportion of monounsaturated and cyclopropane fatty acids than did polar lipids from vegetative cells (Table 2).

Fatty Acids in Glycolipids. The acetone eluent from the silicic acid column contained the isobranched, monounsaturated, and normal saturated fatty acids as identified in the polar lipids. The two major fatty acids were il6:0 (17–25% by weight of the glycolipid fatty acids) and 17:1ω8 (18–27%) (Fig. 3). Furthermore, the acetone fraction contained several longer chain components. The electron impact ionization spectra of these showed extensive fragmentation of the molecules with no molecular ions present. The molecular weight of the most common long-chain compound was determined by chemical-ionization MS to be *m/z* 408. This compound was designated *C*. Two other compounds (*B* and

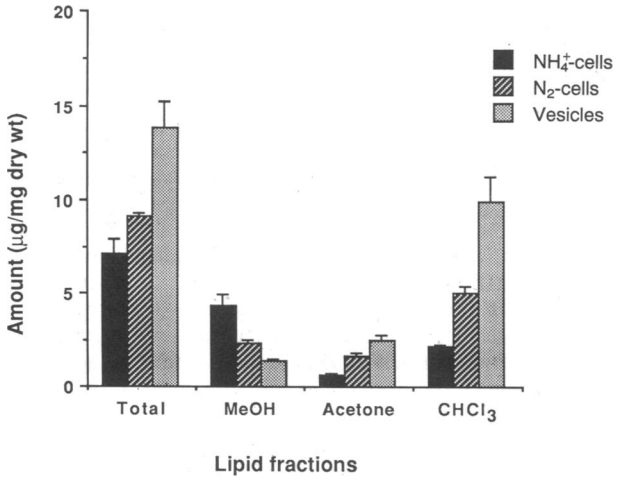

FIG. 1. Total amount of fatty acids in NH_4^+-grown cells, N_2-grown cells, and vesicles of *Frankia* sp. strain CpI1. The lipid extracts were fractionated on a silicic acid column. The methanol fraction contains polar lipids, the acetone fraction contains glycolipids, and the chloroform fraction contains neutral lipids. Vertical bars represent 1 SD (n = 3).

Table 1. Fatty acid composition in the polar lipids (methanol fraction) from NH_4^+-grown cells, N_2-grown cells, and vesicles of *Frankia* sp. strain CpI1

Fatty acid	Fatty acid composition, mean mol % (SD, n = 3)		
	NH_4^+-cells	N_2-cells	Vesicles
i14:0	1.05 (0.15)	0.55 (0.02)	0.80 (0.18)
14:1ω5*	0.44 (0.07)	0.27 (0.01)	0.29 (0.12)
14:0	0.99 (0.10)	0.43 (0.02)	0.88 (0.18)
15:1	0.85 (0.05)	0.65 (0.02)	0.90 (0.09)
15:1ω6	2.32 (0.12)	1.98 (0.11)	1.96 (0.21)
15:0	2.34 (0.09)	3.18 (0.11)	2.04 (0.14)
i16:1ω6	1.78 (0.04)	2.64 (0.20)	4.10 (0.30)
i16:0	51.82 (0.41)	49.86 (2.19)	41.07 (0.96)
16:1ω9c*	0.62 (0.01)	0.40 (0.03)	0.73 (0.04)
16:1ω7c	4.42 (0.05)	3.42 (0.23)	5.78 (0.22)
16:0	6.48 (0.13)	7.99 (0.22)	6.04 (0.26)
17:1ω8	18.97 (0.48)	20.38 (1.08)	23.76 (0.81)
cy17:0	0.07 (0.00)	0.18 (0.01)	0.36 (0.01)
17:0	0.88 (0.03)	1.25 (0.05)	0.78 (0.04)
i18:1	0.54 (0.01)	0.34 (0.03)	0.74 (0.03)
18:1ω9c	5.60 (0.24)	3.99 (0.40)	7.88 (0.51)
18:1 ω7c/ω9t*†	0.23 (0.01)	1.29 (0.16)	0.33 (0.03)
cy18:0 (ω8, 9)	0.28 (0.01)	0.82 (0.07)	0.98 (0.05)
18:0	0.32 (0.01)	0.38 (0.02)	0.58 (0.04)

*Position and/or geometry determined by GC only.
†The peaks for 18:1ω7c and 18:1ω9t were coeluted.

D) with electron-impact ionization spectra similar to each other were separated by GC.

Vesicles had significantly higher amounts of both the identified fatty acids and *B*, *C*, and *D* than did the vegetative cells grown on NH_4^+ (Fig. 3). The relative proportions of the fatty acids in the glycolipid fraction also differed between the vesicles and the vegetative cells. For example, vesicles had a higher proportion by weight of i16:0, 17:1ω8, and of *C* in their glycolipids than those of NH_4^+-grown cells.

Fatty Acids in Neutral Lipids. The major components detected in the chloroform fractions for the lipids from *Frankia* CpI1 were a series of long-chain compounds (Fig. 4). The chemical-ionization spectra of these compounds revealed the molecular ions at m/z 408 (*B2* and *C2*) and m/z 410 (*A* and *D2*). The electron impact ionization MS spectra and the GC retention time of *B2*, *C2*, and *D2* were similar to those of *B*, *C*, and *D* found in the glycolipid fraction. The MS information suggests that these compounds are long-chain fatty acids with one or several hydroxyl groups and/or fatty alcohols with 22–26 carbon atoms.

Table 2. Patterns of polar lipid fatty acids in NH_4^+-grown cells, N_2-grown cells, and vesicles of *Frankia* sp. CpI1

Fatty acid group	Polar lipid fatty acids, mean mol % (SD, n = 3)		
	NH_4^+-cells	N_2-cells	Vesicles
Normal saturated	11.01 (0.25)	13.24 (0.23)	10.32 (0.34)
Iso-branched saturated	52.87 (0.59)	50.41 (2.67)	41.87 (1.02)
Monounsaturated	35.76 (0.84)	35.35 (2.35)	46.48 (0.06)
Cyclopropane	0.35 (0.01)	0.99 (0.10)	1.33 (0.06)

The amount and proportion of long-chain components were higher in lipids from vesicles and N_2-grown cells than in lipids from vegetative cells. For example, *D2* was approximately 39% by weight of the total amount of fatty acids in vesicles, 17% in N_2-grown cells, and 12% in vegetative cells. The remaining fatty acids in the profiles from each cell type were similar in amount and in composition to those seen in the acetone and methanol fractions (Fig. 4).

DISCUSSION

Isolated vesicles and N_2-grown cells of *Frankia* sp. strain CpI1 had quantitatively more fatty acids in their lipids than did NH_4^+-grown cells. In addition, the composition of fatty acids differed significantly between cells grown on NH_4^+, cells grown on N_2, and vesicles. In previous work, polar lipid and glycolipid compositions of seven *Frankia* strains were studied by thin-layer chromatography (29). No obvious differences in lipid composition were found between cells grown on N_2 or NH_4^+. However, no quantitative analysis of individual fatty acids was done, nor were techniques for isolating vesicles available at the time.

The major fatty acid in the polar lipids and glycolipids in CpI1 was i16:0, which agrees with the results from two *Frankia* isolates analyzed by Lechevalier *et al.* (30). In addition, the profiles of CpI1 contained several monounsaturated fatty acids. The double bonds in these acids, including 17:1ω8, 15:1ω6, i16:1ω6, 16:1ω7c, and 18:1ω9c, were localized at the Δ9 position, indicating the presence of the aerobic pathway for unsaturated fatty acid biosynthesis (31). This pathway is common among actinomycetes, in contrast to most other prokaryotes, which use the anaerobic pathway (32).

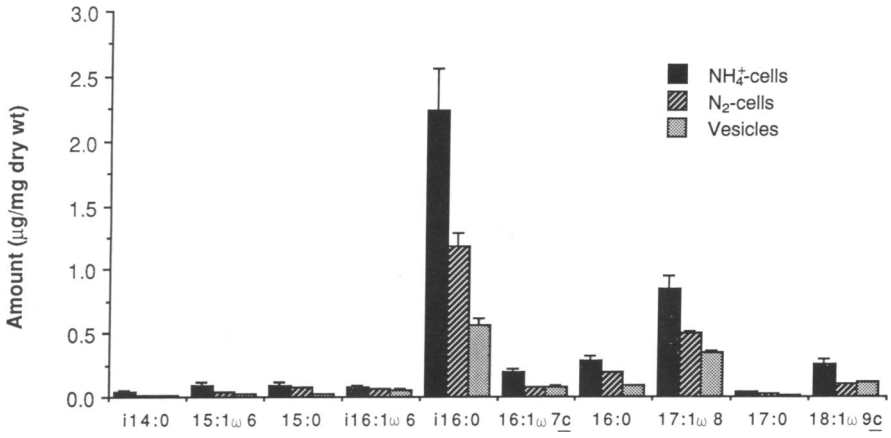

FIG. 2. Amount of fatty acids in the methanol fraction (containing polar lipids) from lipid extracts of *Frankia* CpI1 NH_4^+-grown cells, N_2-grown cells, and vesicles. Data of the 10 most common fatty acids are shown (compare with Table 1). Vertical bars show 1 SD (n = 3).

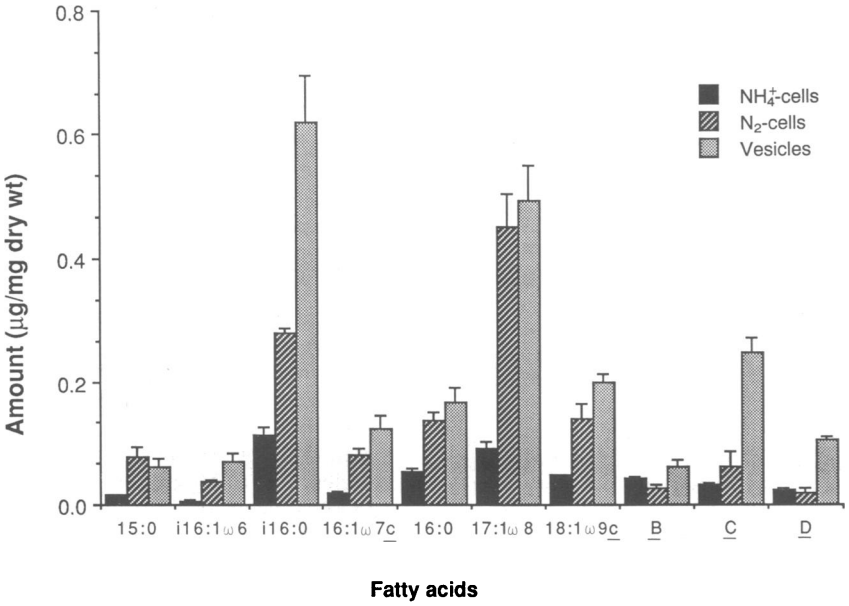

FIG. 3. Amount of fatty acids in the acetone fraction (containing glycolipids) from lipid extracts of *Frankia* CpI1 NH_4^+-grown cells, N_2-grown cells, and vesicles. Data of the 10 most common fatty acids are shown. *B*, *C*, and *D* are unidentified long-chain components. Vertical bars show 1 SD (n = 3).

Polar lipids are important components of the plasma membrane in actinomycetes, and a functional membrane requires a suitable selection of various types of such lipids, particularly phospholipids (33). Phosphatidylinositol and diphosphatidylglycerol are the major polar lipids identified in *Frankia* (29, 30). The higher proportion of monounsaturated and cyclopropane fatty acids in the polar lipids of vesicles than in hyphae from CpI1 would be expected to influence the physical properties and thereby the physiological function (in as yet unidentified ways) of the membranes (34).

The chemical structures of the long-chain compounds detected in the neutral lipid and glycolipid fractions have not been identified. Preliminary characterization, based on MS, suggests that they are polyhydroxy fatty acids or alcohols with 22–26 carbon atoms. Therefore, they have some similarity with the C_{26} to C_{28} hydroxy alcohols and fatty acids that have been identified in the glycolipids from heterocysts of cyanobacteria (16). Further work is needed to finalize the structure of the long-chain compounds in *Frankia* CpI1.

The cellular location of the long-chain compounds within the vesicles is not known, but light and electron microscopic studies suggest that they could be constituents of the laminated layers seen in the cell envelope. In freeze-fracture electron microscopy, up to 50 lipid monolayers have been observed in envelopes, with each layer having a thickness from 3.5 to 4.0 nm (9, 10), which is between the thickness of a monolayer and a bilayer of palmitic acid. In addition, studies with Nile red fluorescence staining indicate that the envelope of the *Frankia* vesicle is composed largely of lipid (35). These observations, together with the abundance of the long-chain compounds, strongly suggest that they are localized in the laminae. Ultrastructurally similar laminae are occasionally seen in vegetative hyphae of CpI1, but never with the abundance noted in vesicles (36). Thus, lipids associated with vesicle laminae may be normal cell components that accumulate during vesicle differentiation.

The apparent similarity in structure with the long-chain compounds of the heterocysts as well as the significantly

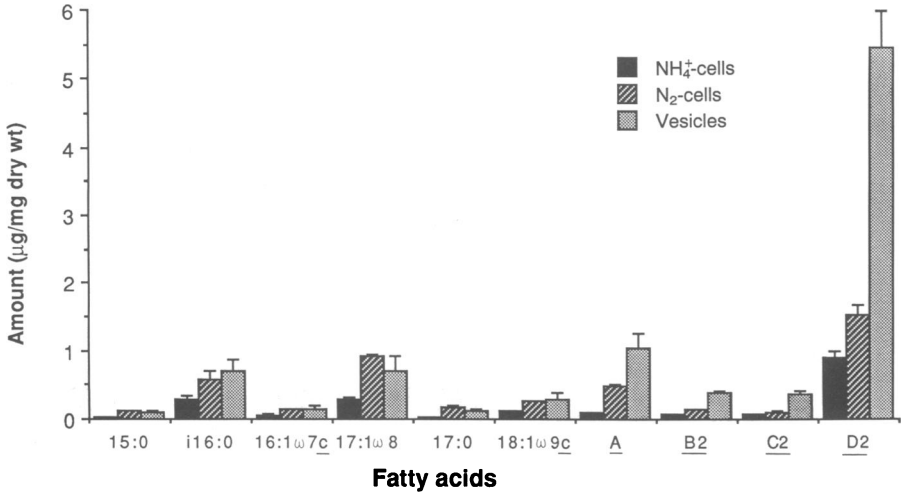

FIG. 4. Amount of fatty acids in the chloroform fraction (containing neutral lipids) from lipid extracts of *Frankia* CpI1 NH_4^+-grown cells, N_2-grown cells, and vesicles. Data of the 10 most common fatty acids are shown. *A*, *B2*, *C2*, and *D2* are long-chain compounds with unknown structure. Vertical bars show 1 SD (n = 3).

higher amounts of these compounds in vesicles than in hyphae suggest that long-chain fatty acids or alcohols can be an important part of the cell envelope and can function as a diffusion barrier to O_2 in *Frankia* vesicles.

This research has been supported by U.S. Department of Agriculture Grant 85-CRCR-1-1657 from the Competitive Research Grants Office (to D.R.B.) and by Swedish Natural Research Council Grant B-BU 8564-300 (to A.T.).

1. Torrey, J. G. (1978) *Bioscience* **28,** 586–592.
2. Noridge, N. A. & Benson, D. R. (1986) *J. Bacteriol.* **166,** 301–305.
3. Tisa, L. S. & Ensign, J. C. (1987) *J. Bacteriol.* **169,** 5054–5059.
4. Meesters, T. M. (1987) *Arch. Microbiol.* **146,** 327–331.
5. Gauthier, D. L., Diem, H. G. & Dommergues, Y. (1981) *Appl. Environ. Microbiol.* **41,** 306–308.
6. Murry, M. A., Fontaine, M. S. & Tjepkema, J. D. (1984) *Arch. Microbiol.* **139,** 162–166.
7. Tjepkema, J. D., Ormerod, W. & Torrey, J. G. (1980) *Nature (London)* **287,** 633–635.
8. Benson, D. R., Arp, D. J. & Burris, R. H. (1978) *Science* **205,** 688–689.
9. Torrey, J. G. & Callaham, D. (1982) *Can. J. Microbiol.* **28,** 749–757.
10. Parsons, R., Silvester, W. B., Harris, S., Gruijters, W. T. M. & Bullivant, S. (1987) *Plant Physiol.* **83,** 728–731.
11. Murry, M. A., Zhongze, Z. & Torrey, J. G. (1985) *Can. J. Microbiol.* **31,** 804–809.
12. Stewart, W. D. P. (1980) *Annu. Rev. Microbiol.* **34,** 497–536.
13. Lancelle, S. A., Torrey, J. G., Hepler, P. K. & Callaham, D. A. (1985) *Protoplasma* **127,** 64–72.
14. Nichols, B. W. & Wood, B. J. B. (1968) *Nature (London)* **217,** 767–768.
15. Winkenbach, F., Wolk, C. P. & Jost, M. (1972) *Planta* **107,** 69–80.
16. Lambein, F. & Wolk, C. P. (1973) *Biochemistry* **12,** 791–798.
17. Haury, J. F. & Wolk, C. P. (1978) *J. Bacteriol.* **136,** 688–692.
18. Walsby, A. E. (1985) *Proc. R. Soc. London Ser. B* **226,** 345–366.
19. Callaham, D., DelTredici, P. & Torrey, J. G. (1978) *Science* **199,** 899–902.
20. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **31,** 911–917.
21. Kates, M. (1986) *Techniques of Lipidology, Isolation, Analysis, and Identification of Lipids* (Elsevier, Amsterdam), 2nd Ed., p. 194.
22. King, J. D., White, D. C. & Taylor, C. W. (1977) *Appl. Environ. Microbiol.* **33,** 1177–1183.
23. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. (1979) *Oecologia (Berlin)* **40,** 51–62.
24. Ryhage, R. & Stenhagen, E. (1960) *J. Lipid Res.* **1,** 361–390.
25. Campbell, I. M. & Naworal, J. (1969) *J. Lipid Res.* **10,** 589–592.
26. Boon, J. J., van de Graaf, B., Schuyl, P. J. W., de Lange, F. & de Leeuw, J. W. (1977) *Lipids* **12,** 717–721.
27. Nichols, P. D., Guckert, J. B. & White, D. C. (1986) *J. Microbiol. Methods* **5,** 49–55.
28. McCloskey, J. A. & Law, J. H. (1967) *Lipids* **2,** 225–230.
29. Lopez, M. F., Whaling, C. S. & Torrey, J. G. (1983) *Can. J. Bot.* **61,** 2834–2842.
30. Lechevalier, M. P., Baker, D. & Horriere, F. (1983) *Can. J. Bot.* **61,** 2826–2833.
31. Scheuerbrandt, G. & Bloch, K. (1962) *J. Biol. Chem.* **237,** 2064–2068.
32. Fulco, A. J. (1983) *Prog. Lipid Res.* **22,** 133–160.
33. Minnikin, D. E. & O'Donnell, A. G. D. (1983) in *The Biology of Actinomycetes,* eds. Goodfellow, M., Mordarski, M. & Williams, S. T. (Academic, London), pp. 337–388.
34. Cronan, J. E. & Gelmann, E. P. (1975) *Bacteriol. Rev.* **39,** 232–256.
35. Lamont, H. C., Silvester, W. B. & Torrey, J. G. (1988) *Can. J. Microbiol.* **34,** 656–660.
36. Newcomb, W. & Wood, S. M. (1987) *Int. Rev. Cytol.* **109,** 1–88.