Occurrence of sulfoquinovosyl diacylglycerol in some members of the family *Rhizobiaceae*

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Abstract A radiolabeled component of a membrane extract of *Rhizobium meliloti* 2011 cells grown in the presence of ^{35}S-labeled sulfate was isolated by silica flash chromatography and purified by high performance liquid chromatography (HPLC). Based on 1- and 2-dimensional nuclear magnetic resonance (NMR) spectroscopic and mass spectrometric analyses, the structure of the compound was determined to be sulfoquinovosyl diacylglycerol (SQDG). NMR analyses indicated substantial heterogeneity in the fatty acid composition and that an important group was the cyclopropyl fatty acids. This first report of the occurrence of SQDG outside of the plant kingdom, photosynthetic bacteria or diatoms deserves special attention as, in this case, the bacterium is one that can fix nitrogen in symbiosis with plants. The origins of the bacterium's ability to synthesize this class of membrane lipids is an important question. Membrane extracts of other strains of the family *Rhizobiaceae* were screened for the presence of SQDG. The occurrence of SQDG in the symbiotic organisms was confirmed, while no SQDG was detected in either the *Agrobacterium tumefaciens* or the *Escherichia coli* strains tested. The current function of these lipids in symbiosis and the commonality of the ability of bacteria that function as plant symbionts to synthesize such molecules are all germane to studies of the *Rhizobium*/legume symbiosis.—**Cedergren, R. A., and R. I. Hollingsworth.** Occurrence of sulfoquinovosyl diacylglycerol in some members of the family *Rhizobiaceae*. *J. Lipid Res.* 1994. **35:** 1452–1461.

Supplementary key words *Rhizobium* • *Bradyrhizobium* • *Agrobacterium* • membrane • symbiosis • lipid • sulfolipid • ^{1}H, ^{13}C, and 2-D NMR spectroscopy • mass spectrometry

The cell surface of *Rhizobium* has always been considered to be an essential factor in our attempts at understanding the interactions between these bacterial symbionts and their legume partners. Much debate, and admittedly much controversy, has revolved around the roles of capsular polysaccharides (1–5). The role of lipopolysaccharides in ensuring a successful infection process is much more generally accepted. It is well established that bacterial mutants with defective lipopolysaccharide structures are incapable of infections that progress into viable, occupied nodules that are capable of supporting nitrogen fixation (6–9). The bacterial cell envelope, in which the lipopolysaccharide resides, has still not been rigorously characterized, despite many years of study. For instance, it was shown only comparatively recently that 27-hydroxyoctacosanoic acid, a 28-carbon fatty acid, was a major component of the lipopolysaccharide of virtually all *Rhizobia* (10, 11). There have been several studies attempting to elucidate the total lipid composition of the membranes of some of the membranes of the family *Rhizobiaceae*. A major consistent finding in these studies is the presence of phosphatidylcholine (PC), a typical plant lipid, in the membrane of all of the species investigated thus far (12–14). This finding may suggest either a role for this lipid in the infection process or the possibility of gene exchange between the bacterium and the plant in the distant past. However, although PC is not common amongst gram-negative bacteria, it is also not exclusive to the *Rhizobiaceae* (14).

Over the past few years, the Nod factors have been advanced as the determinants of host specificity in the *Rhizobium*/legume symbiosis. The Nod factors are a class of closely related mono-N-fatty acid acylated chitin oligomers substituted with various side groups. The type of fatty acid and the nature of the side group are thought to account for the host specificity (15). In the case of *R. meliloti* it has been shown that the critical feature that determines host specificity is the sulfate ester on the reducing terminal glucosamine residue (16–18). The emergence of sulfated lipid-linked carbohydrates as mediators of the infection process in the *R. meliloti*/alfalfa symbiosis raised the question in our minds of a possible link of this phenomenon to the bacterial surface chemistry. We then

Abbreviations: PC, phosphatidylcholine; HPLC, high performance liquid chromatography; TLC, thin-layer chromatography; NMR, nuclear magnetic resonance; DEPT, distortionless enhancement by polarization transfer; SQDG, sulfoquinovosyl diacylglycerol; FID, free induction decay; HOHAHA, homonuclear Hartmann-Hahn spectroscopy; HMQC, ^{1}H-detected heteronuclear multiple-quantum coherence spectroscopy; DFQ-COSY, double quantum filtered-correlated spectroscopy; GC–MS, gas chromatography–mass spectrometry; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol.

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began a systematic study of the bacterial membrane with a focus on finding sulfur-containing lipid components.

Bacteria were grown in the presence of ^{35}S-labeled sulfate and labeled membrane components were purified chromatographically and characterized. In the first part of this work, we describe the isolation, purification, and characterization of a sulfolipid whose occurrence was thought to be restricted to plants, algae, and photosynthetic bacteria (19). The questions that arise due to this finding are: Was this a trait that was captured from plants but has no symbiotic significance? Or, is the ability of the bacteria to make plant-like lipids a prerequisite for the close interaction (possibly membrane fusion) between bacteria and plants during symbiosis? In the second part of the present study we began to address these questions by screening various members of the *Rhizobiaceae* for the presence of this sulfolipid. These experiments were conducted in an attempt to probe the relatedness of the various genera and to, perhaps, provide a clue to the possibility of a requisite membrane compatibility in rhizobial infection.

MATERIALS AND METHODS

Bacteria and growth conditions

The various bacterial strains used are as follows: *Rhizobium meliloti* 2011 (from Ethan Signer, Massachusetts Institute of Technology), *R.* sp. strain NGR234 (from Frank Dazzo, Michigan State University), *R. leguminosarum* bv. *trifolii* ANU843, *R. leguminosarum* bv. *trifolii* ANU845 (from Barry Rolfe, Australian National University), *Bradyrhizobium japonicum* USDA110 (from Frank Dazzo, Michigan State University), *Agrobacterium tumefaciens* C58 (from Frans DeBruijn, Michigan State University), and *Escherichia coli* TG-1 (from Gregory Zeikus, Michigan State University). All strains of bacteria (with the exception of *E. coli*) were grown at 30°C in liquid cultures containing modified Bergensen's (BIII) media as previously described (20) except that, in addition, ^{35}S-labeled sulfate at a level of 100 μCi/liter of culture was added in the form of carrier-free sulfuric acid (New England Nuclear). The *E. coli* strain was grown at 37°C in MSX4 media with ^{35}S-labeled sulfate added as above.

Lipid isolation and purification

Lipids were extracted by stirring the bacterial cells (after harvesting by centrifugation) with 100 ml of a mixture of chloroform–methanol–n-butanol–water 2:1:1:4 at room temperature for 24 h. The cell debris was removed by centrifugation and the supernatant was allowed to partition into two layers. The lower organic layer was recovered and the aqueous layer was recombined with the cell debris and the mixture was re-extracted with 200 ml chloroform–methanol–n-butanol 2:1:1. The organic layer was again recovered and combined with the one from the first extraction. The combined organic layer was concentrated to dryness and chromatographed on a silica column (4 cm × 2 cm) with a mixture of chloroform–acetone–methanol–acetic acid–water 10:4:2:2:1. Fractions of 4 ml were collected and a 100-μl aliquot of each was analyzed for radioactivity by scintillation counting. One major fraction (from a plot of fraction number vs. dpm) was recovered and subjected to further purification by high performance liquid chromatography (HPLC) on an ALTEX ultrasphere C-8 reverse phase column (1 cm × 25 cm). A linear gradient was used starting with a combination of acetonitrile–water 1:1 (40%) and n-propanol (60%). This ratio was maintained for 10 min at a flow rate of 1 ml/min. The flow rate was then increased to 1.2 ml/min and the gradient was run to a final composition of the two solvent systems of 10 and 90%, respectively. The column effluent was monitored at 230 nm and the peaks were collected and counted. The entire isolation and purification was repeated using unlabeled sulfate to obtain material for use in structural characterization. Purity of the product was assessed by thin-layer chromatography (TLC) on silica layers using a mixture of chloroform–acetone–methanol–acetic acid–water 10:4:2:2:1.

Chemical characterization

Fatty acids were released as methyl esters from the purified lipid by methanolysis of a sample (~50 μg) at 75°C with 1 ml of methanol containing 2% HCl. The methanolysate was concentrated to dryness and partitioned between 2 ml hexane and 1 ml water. The upper hexane layer was recovered and subjected to gas chromatography and gas chromatography–mass spectrometry.

The glycosyl component of the purified lipid was obtained by treating a sample (3 mg) of the component with ethanolic potassium hydroxide (0.5 M) at 100°C for 30 min. The mixture was treated with 10 μl acetic acid and then concentrated to dryness. The fatty acids were removed by partitioning the residue between 4 ml of water–hexane 1:1. The aqueous layer was chromatographed by gel filtration on a Bio-Gel P2 column (20 cm × 0.5 cm) in pure water. One-ml fractions were collected and assayed for the presence of carbohydrates by spotting on silica plates, spraying with orcinol/sulfuric acid, and heating at 120°C for 5 min. Fractions with a positive reaction were pooled and lyophilized. The optical rotation was measured in water at the sodium D line on a Perkin Elmer Model 141 polarimeter.

Nuclear magnetic resonance (NMR) spectroscopy

All NMR spectra were measured in CD_3OD solution at 500 MHz for the ^{1}H or 125 MHz for the ^{13}C nucleus. The ^{13}C-DEPT spectrum (21) was measured at 75 MHz for ^{13}C. Double quantum filtered J-correlated 2-dimensional spectra (phase sensitive mode) (22) were

obtained using a total of 512 data sets (32 transients) over a spectral width of 3000 Hz. The total number of points used was 2048. The FIDs were multiplied by a gaussian function in the f_2 dimension and by a shifted gaussian in f_1. The final data set was symmetrized by triangular folding. Data for the HOHAHA experiments (23) were obtained using similar acquisition and processing conditions. A mixing time of 80 msec was used. Data were not symmetrized. For the HMQC experiment (24), a spectral width of 19,000 was used for the ^{13}C dimension. A total of 80 transients was acquired for each of a total of 512 data sets. A total of 1024 points was used in these analyses.

Mass spectrometry

Fast atom bombardment mass spectra were recorded on a JEOL HX-110 HF mass spectrometer using xenon neutrals (6 keV) as the primary beam. Triethanolamine was used as the matrix and spectra were obtained in the negative ion mode. Gas chromatography–mass spectrometry was performed on a JEOL 505 mass spectrometer using a DB1 capillary column. The gas chromatography program used was: 150° to 300°C at 3°C/minute with a 10-min hold at 300°C.

Screening for sulfoquinovosyl diacylglycerol (SQDG)

Cells were harvested by centrifugation and lipids were extracted using the procedure described above. The crude organic extracts were subjected to TLC on silica gel on isolated lanes to prevent cross-contamination and using the solvent system described above. Sample extracts were chromatographed with a standard of SQDG, the isolation and characterization of which is presented in this paper. Bands containing glycosyl components were visualized by spraying with an orcinol/sulfuric acid mixture and heating at 120°C for approximately 10 min. Radioactive bands were detected by exposure of the 2,5-diphenyloxazole-impregnated TLC plate to a Kodak X-omat X-ray plate at −80°C for 3 weeks. Additionally, TLC plates were developed in which the radioactive glycosyl-containing bands were scraped and the lipids were extracted from the silica with chloroform–methanol 1:1. Verification of the presence of SQDG was obtained by FTIR spectroscopy using a Nicolet 710 Fourier Transform Infrared Spectrometer. The samples were analyzed as thin films on a NaCl substrate.

RESULTS

Analysis of purified lipid

1-D NMR analysis. TLC analysis of the membrane lipids of *Rhizobium meliloti* 2011 on silica layers followed by autoradiography revealed the presence of two major radiolabeled components (R_f 0.22 and 0.18). These com-

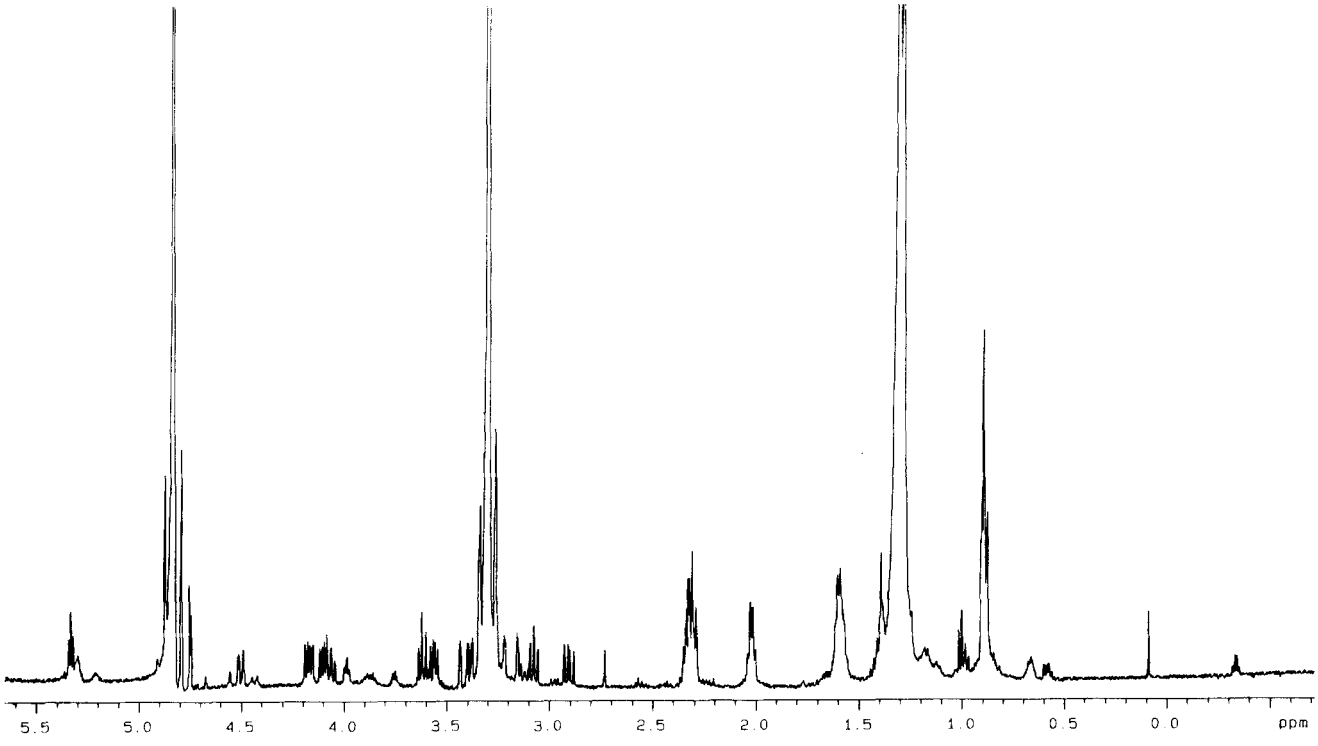


Fig. 1. ^1H-NMR spectrum of the purified ^{35}S-labeled major component (R_f 0.22) from the membrane extract of *R. meliloti* 2011. Signals between 2.8 and 4.8 ppm are due to a carbohydrate and a glyceryl component. The other signals are due to fatty acids. Note the presence of a high degree of unsaturation (vinyl proton resonances at 5.35 ppm) and the presence of cyclopropyl group (most upfield signals). Assignments are made in the text. The strong resonances at 3.30 and ~4.8 ppm are due to residual CHD_2^- and OH signals, respectively, of the solvent. The signals between 0.5 and 2.4 ppm are due to resonances in the fatty acids.

ponents were eventually isolated using a combination of flash chromatography on silica and reverse phase chromatography. The ^{1}H-NMR spectra of the two components were very similar except for slight differences due to fatty acid distribution. The spectrum of the faster migrating component on TLC (second eluting on reverse phase HPLC) is shown in **Fig. 1.** It contained signals between 2.8 and 4.7 characteristic of carbohydrate groups as well as signals between 0.8 and 2.4 ppm characteristic of fatty acid groups. A triplet at 5.35 ppm was assigned to the vinyl protons of an unsaturated fatty acid. A multiplet at 2.02 ppm was assigned to the methylene groups adjacent to the unsaturation. Signals for the methylene groups adjacent to the carbonyl carbons of fatty acid groups appeared as a multiplet at 2.32 ppm. The signals for the terminal methyl groups of fatty acid chains appeared at 0.89 ppm. One interesting feature was the presence of two sets of very upfield signals. These were assigned to signals for protons on cyclopropyl groups. The most upfield signals (−0.33 ppm) were assigned to the methylene group of the cyclopropyl function. A broad multiplet at 5.32 ppm indicated the presence of a glyceryl moiety. This signal was assigned to H-2 of the glyceryl moiety. The other signals between 2.8 and 4.8 ppm were generally too complex to assign directly. The ^{13}C-DEPT spectrum of the same faster migrating component (**Fig. 2**) contained signals between 10 ppm and 40 ppm, consistent with the aliphatic resonances of fatty acid chains. The presence of unsaturation was confirmed by the presence of resonances at 130 ppm. A resonance at 99.2 ppm was assigned to an anomeric proton of a glycosyl residue. Based on the chemical shift of a proton (assignable to an anomeric proton) at 4.76 in the ^{1}H-NMR spectrum, the α-configuration was assigned. This proton displayed a coupling constant of 2–3 Hz indicating that the neighboring proton was axial. Signals between 50 and 110 ppm were sufficient for only 1 glycosyl unit. The ^{13}C-DEPT spectrum indicated that the molecule contained three primary carbons bearing heteroatoms. One of these at 53.5 ppm was assigned to carbon linked to sulfur. The other two were assigned to a glyceryl moiety. Five carbons bearing oxygen atoms gave rise to signals 62 and 76 ppm, giving a total of 9 carbon attached to heteroatoms.

2-D NMR analysis. The HOHAHA spectrum of the faster migrating major lipid component (**Fig. 3**) conveniently broke the spectrum down into spin systems for the glyceryl, carbohydrate, and fatty acid components. Based on this spectrum, the signals at 5.32 ppm, 4.50 ppm, 4.17 ppm, and 3.58 ppm were assigned to the glyceryl moiety. The other signals between 2.8 and 4.8 ppm were

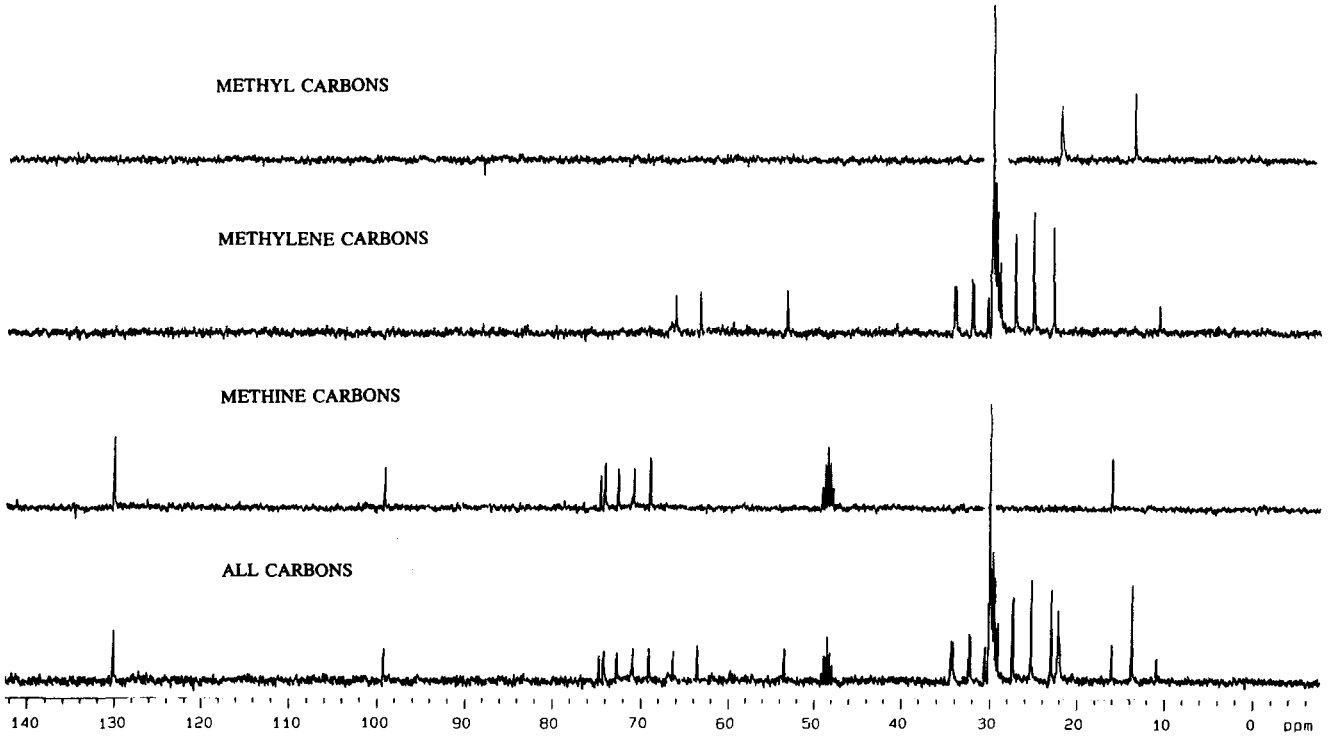


Fig. 2. ^{13}C-DEPT-NMR spectrum of the purified ^{35}S-labeled component (R_f 0.22). The anomeric carbon of a glycosyl residue appears at 99.2 ppm. The number of signals in the range of 50–110 ppm and the number of anomeric carbons are consistent with the presence of only one glycosyl unit. The signal at 130 ppm is due to vinyl carbons. Note the very upfield primary carbon resonance at 53.5 ppm in the carbohydrate region of the spectrum, indicative of a carbon-sulfur linkage.

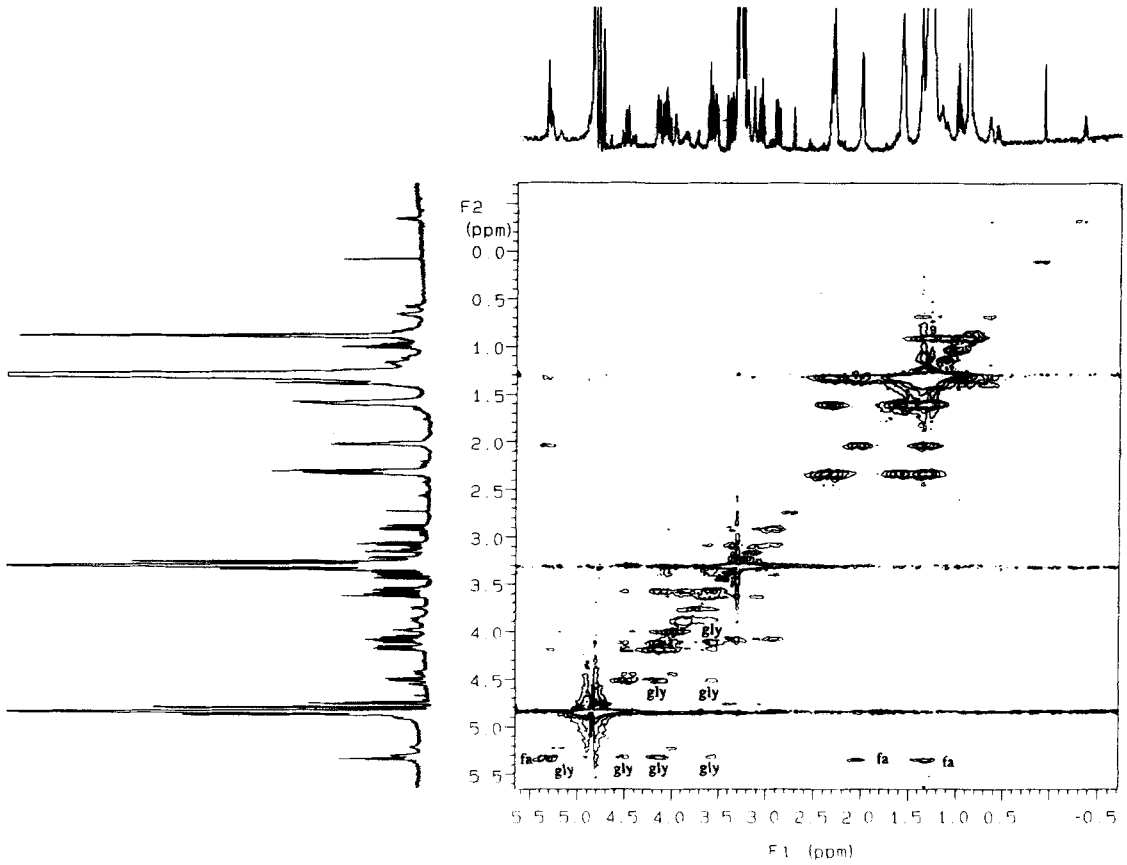


Fig. 3. ^{1}H-HOHAHA NMR spectrum. Note the connectivities for glycerol (gly) and for the fatty acid components (fa). The carbohydrate connectivities are not marked.

assigned to the glycosyl residue. The DQF-COSY spectrum (**Fig. 4**) allowed the assignments of the spin connectivities in the spectrum. Hence, the signal at 3.39 ppm was assigned to H-2 (a doublet of doublets with J = 7.3 Hz and 2.0 Hz). Based on these coupling constants, it was determined that the glycosyl H-3 signal at 3.62 ppm (triplet with J = 7.3 Hz) was axial and that the hydroxyl group at this position was therefore equatorial. The large coupling with both H-4 and H-2 indicated the *gluco* configuration. H-4 appeared further upfield (3.08 ppm) as a triplet (J = 7.3 Hz). The signal for one of the H-6 protons (dd, J = 13.5 + 7.9 Hz) appeared at 2.90 ppm, indicating that it was not attached to a carbon atom bearing oxygen. It was concluded that this carbon was attached directly to sulfur. Based on the chemical shift a sulfonic acid moiety was proposed. The proton assignments agreed well with the ^{13}C-NMR assignments. These were confirmed by an HMQC experiment (**Fig. 5**). The carbon attached to sulfur appeared at 53.5 ppm as expected. The glyceryl methylene carbons appeared between 62 and 67 ppm while the remaining glyceryl resonance, as well as those for the carbohydrate carbons, was further downfield. Assignments for the shifts in the fatty acid region also appear as expected. The complete proton assignments are given in an expanded ^{1}H-NMR carbohydrate region in **Fig. 6.** Based on the NMR analyses, the molecule was determined to be a diacyl-glyceryl derivative of a 6-deoxy-α-glucopyranose-6-sulfonic acid. These assignments agree with previously published results (25). According to integration of NMR signals in analyses of crude lipid extract, SQDG comprises approximately 5–10% of the total lipid in *Rhizobium meliloti.*

The ^{1}H-NMR spectrum of the faster migrating component (R_f 0.22) had essentially the same features as that of the slower migrating other major component (R_f 0.18). The fatty acid distribution was, however, different as signals for multiple unsaturation were more dominant. This splitting of TLC components for these lipids is quite common and has been demonstrated and studied by other workers who also attribute differences in mobility to fatty acid class (25). These workers also observed differences in the orcinol response for the two TLC components.

Fatty acid determination. The fatty acids were determined by gas chromatography–mass spectrometry (GC–MS) to

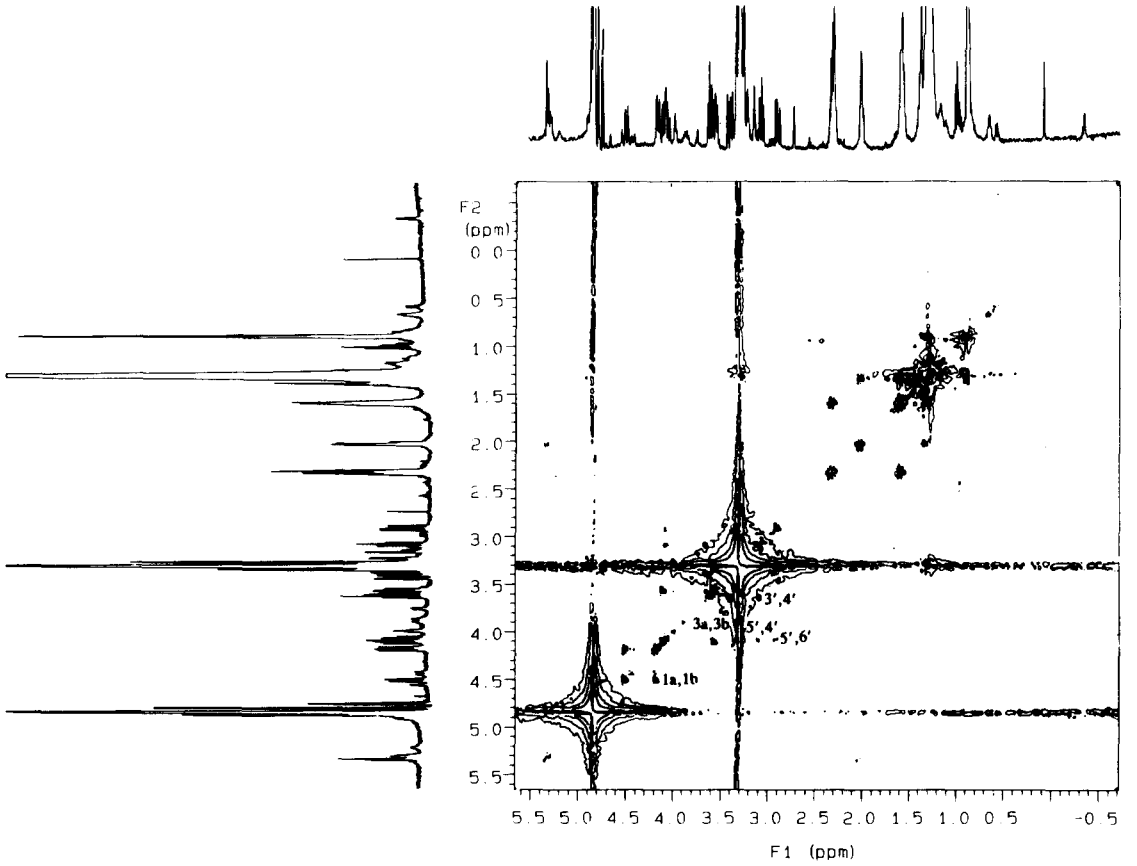


Fig. 4. ^{1}H-DQF-COSY spectrum. Note the assignments for the carbohydrate and glycerol components. Primed numbers refer to the carbohydrate component.

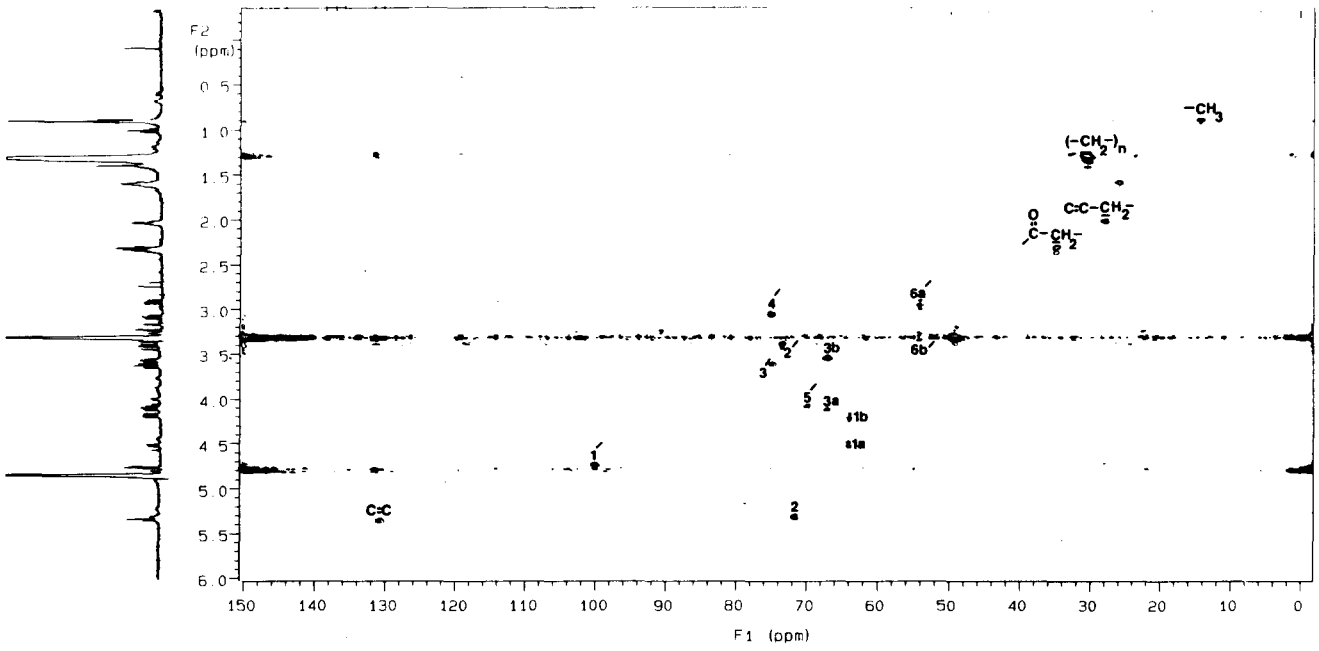


Fig. 5. ^{1}H/^{13}C-HMQC spectrum of purified ^{35}S-labeled lipid component (R_f 0.22). All cross peaks due to the carbohydrate and glycerol components are assigned. Note the proton and carbon chemical shifts at the 6-position, the site of sulfonylation. Primed numbers refer to the carbohydrate components.

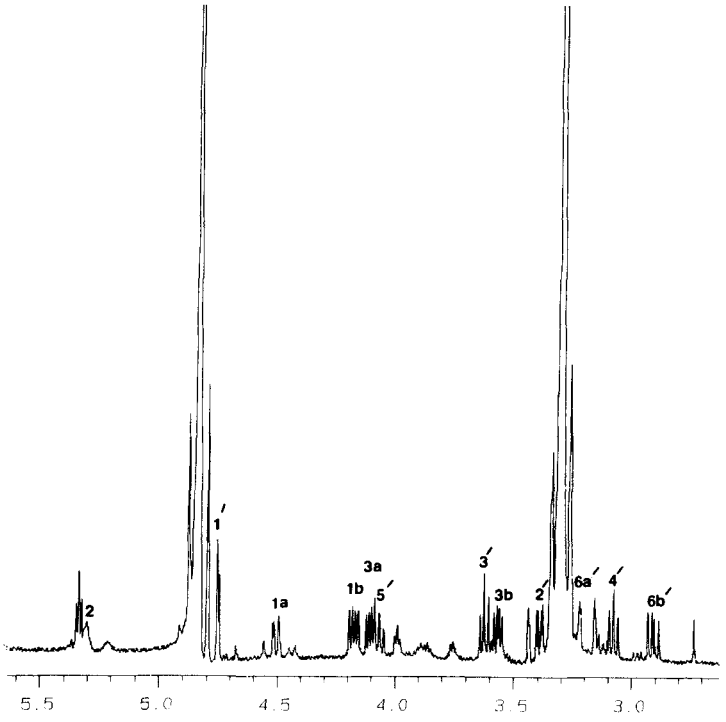


Fig. 6. ^{1}H-NMR spectrum of purified ^{35}S-labeled lipid component (R_f 0.22). Complete proton assignments for sulfoquinovosyl diacylglycerol are shown. Primed numbers refer to the carbohydrate protons.

be predominantly hexadecanoic acid, octadecenoic acid, and a methyleneoctadecanoic acid. There were other minor peaks assignable to a bis-unsaturated C-18 fatty acid, tetradecanoic acid, and tetradecenoic acid.

Mass spectrometry analysis. The proposed structure was confirmed by performing fast atom bombardment mass spectrometry on the faster migrating component (**Fig. 7**). The major ion in the pseudo-molecular ion region ($(M-H)^-$ = 793 u) was assigned to sulfoquinovosyl dihexadecanoyl glycerol (**Structure 1**). The next major ion

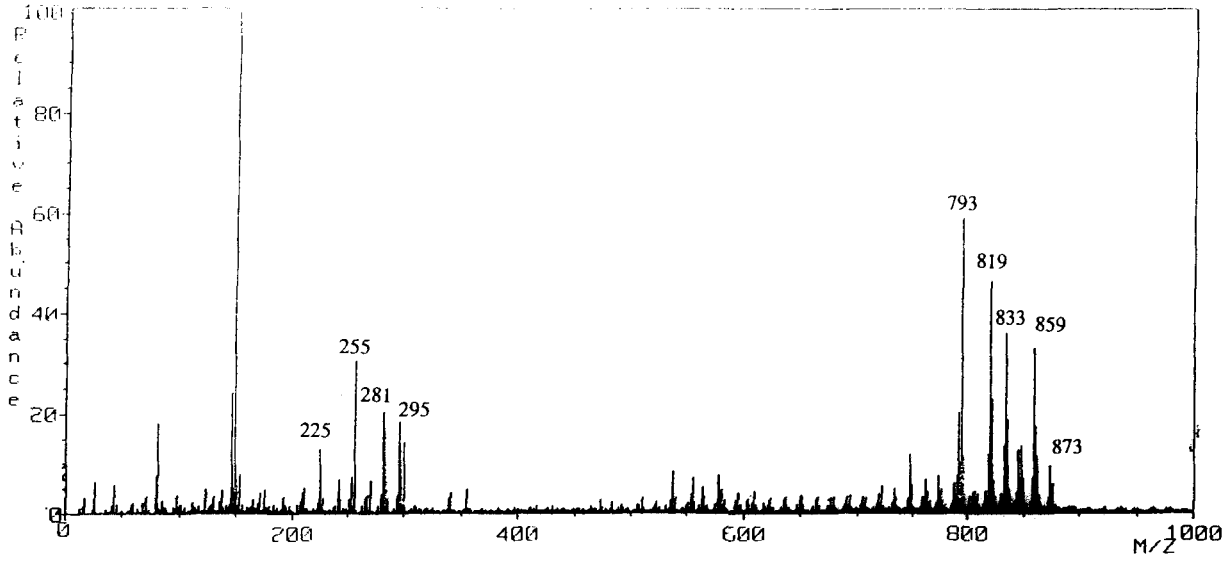
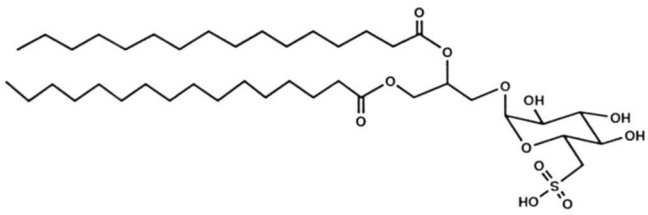


Fig. 7. Negative ion fast atom bombardment mass spectrum of purified lipid component. The cluster of ions between 700 and 900u are $(M-H)^-$ pseudo-molecular ions for the different species. The ion at *m/z* 793 is due to the molecule containing two hexadecanoyl residues (Structure 1). This is the predominant component. The other ions are due to heterogeneity in the fatty acyl components. The assignments are made in the text. Signals between 250 and 300 are due to carboxylate ions of the fatty acyl components formed by primary cleavage. The assignments are: *m/z* 255 (C_{16}) the predominant component, 281 ($C_{18:1}$), 295 (C_{18} with one cyclopropyl group) and 279 ($C_{18:2}$).



Structure 1. Structure of the major component in the family of sulfoquinovosyl diacylglycerols elaborated by *R. meliloti* 2011. The most prominent fatty acyl species are C_{16}, $C_{18:1}$, and C_{18}, cyclopropyl species. The 1- and 2-positions of glycerol are acylated.

at *m/z* 819 corresponded to a form in which the fatty acids were a hexadecanoyl component and an octadecenoyl component. The next major peak at *m/z* 833 corresponded to the presence of a methyleneoctadecenoyl and a hexadecanoyl fatty acid residue. The next most abundant peak appeared at *m/z* 859 and corresponded to the presence of octadecenoic acid and a methyleneoctadecenoic acid. Other minor peaks corresponding to different combinations of the fatty acids present were also observed. Signals for the carboxylate ions of the fatty acids appear between 250 and 300 u. The peaks corresponding to C_{16} and $C_{18:1}$ are the most abundant (*m/z* 255 and 281, respectively). The peak at *m/z* 225 is most likely due to the dehydrosulfoglycosyl anion corresponding to a minor sulfoglycosyl ion at *m/z* 243. The stereochemistry of the glycerol to carbohydrate linkage was confirmed by removing the fatty acid by base treatment and subjecting the carbohydrate component to polarimetry. Not enough material was obtained to allow an accurate measurement of the optical rotation of the glycoside but the sign could be determined. The rotation of the glyceryl glycoside in water was small and positive, consistent with the proposed D-configuration (26).

Occurrence of sulfoquinovosyl diacylglycerol (SQDG) in symbiotic species of the *Rhizobiaceae*

Organic extracts of several strains belonging to *Rhizobium, Bradyrhizobium,* and *Agrobacterium* were screened for the presence of SQDG by TLC followed by visualization with orcinol. The results are shown in **Fig. 8A.** Glycosyl-containing bands (R_f 0.22 and 0.18) with mobilities similar to the standard SQDG bands seemed to be present in all members of the rhizobia species tested. No such bands occurred in the *Escherichia coli* control. The *A. tumefaciens* sample showed what appeared to be a faint band in this region also. However, when the TLC plates were subjected to autoradiography (Fig. 8B), the *Rhizobium* bands were found to be radiolabeled while the same region of the *A. tumefaciens* lane was not. These results were verified by scraping the bands from the plate, extracting with chloroform–methanol 1:1, and checking radioactivity by scintillation counting. In addition, a third faint radioactive band with slower mobility than SQDG appeared in the autoradiogram in the lanes of the rhizobia species. This band has not yet been identified. The *Bradyrhizobium* strain tested also showed a band with a mobility similar to the standard and when extracted showed about twice

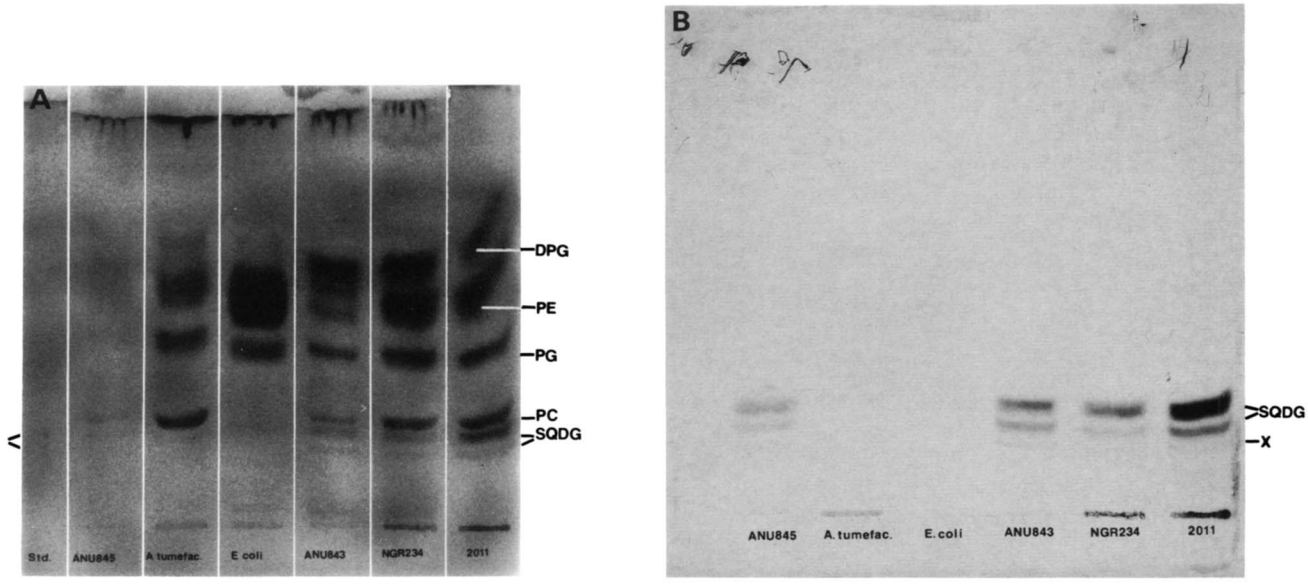


Fig. 8. Orcinol-stained TLC plate (A) and the corresponding autoradiogram (B) (not same scale) of membrane extracts from members of the *Rhizobiaceae* (strains are identified in text). Two bands can be seen with mobilities similar to the SQDG standard in all strains except the *E. coli* control and the *A. tumefaciens* sample. Some major lipids are identified as a reference in the *Rhizobium* wild-type (2011) strain. They are phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and diphosphatidylglycerol (DPG). The minor radiolabeled component (X) has yet to be identified. (The standard was not radiolabeled.)

baseline radioactivity (results not shown). In a previous study, TLC of the total lipid extract from *R. meliloti* failed to show the presence of SQDG (27). This was most likely due to the different methods of extraction. The other major bands, however, had mobilities similar to those observed in this study.

Extracted radioactive TLC bands were also analyzed by Fourier transform infrared spectroscopy. All samples as well as the standard showed broad absorption bands for the hydroxyl groups at about 3400 cm^{-1} along with strong bands for CH_2 and CH_3 (2800–2950 cm^{-1}), for acyl ester groups (1750 cm^{-1}) and overlapping bands in the region for sulfonic acid groups (1000–1400 cm^{-1}). These values agree with previously published data (25).

DISCUSSION

We have shown that sulfoquinovosyl diacylglycerol (SQDG) is a membrane lipid in certain cultured rhizobial species. The occurrence of SQDG outside of plants or photosynthetic bacteria has never been reported before. A strict exclusivity of this lipid to photosynthetic systems has always been assumed. It is, therefore, remarkable that this lipid component is found in a gram-negative bacterium. It is at once puzzling and understandable that it is found in a plant symbiont. Several questions immediately arise in connection with this finding. The first one is: Do these lipids have any functional role in symbiosis? It is tempting to propose that, perhaps, the presence of this lipid might be necessary in order to ensure some compatibility between the plant and bacterial membranes during symbiosis. It has been known for many years that another typical plant lipid, phosphatidylcholine (PC), exists in the membrane of members of the *Rhizobiaceae.* However, a role for PC in symbiosis and/or pathogenesis has not yet been elucidated. The second question is: What is the origin of the genes responsible for biosynthesis of SQDG; were they transferred from the plant or were they developed in the bacterium? Based on zero evidence in either direction, the former scenario seems much more reasonable since the *Rhizobia* and legumes have shared a long association and there has been sufficient chance for this to have occurred. Another alternative is that these bacteria were, at one stage, plant organelles.

With an aim of determining how general the occurrence of SQDG is among legume bacterial symbionts, we have included in this study a representative sampling of the family *Rhizobiaceae. R. leguminosarum* bv. *trifolii* ANU843 and *R.* sp. strain NGR234 are wild-type strains that represent a variety in the range of *Rhizobium* host specificity. The finding of SQDG in these organisms increases the consequence of the previous finding and strengthens the possibility of the widespread importance of this lipid in symbiosis. *R. leguminosarum* bv. *trifolii* ANU845, which lacks the "symbiotic plasmid," was studied in an effort to determine whether the pSym genes are involved in the biosynthesis of SQDG, thus indicating a possible role in nodulation as opposed to a later stage in infection. The presence of SQDG in this mutant suggests that this lipid may have a role (if any) during or after bacterioid formation, possibly by ensuring membrane compatibility between the bacterium and the plant thus allowing for release from the infection thread. More studies will need to be performed on other mutant strains that lack this ability. *B. japonicum* was studied because of its genetic dissimilarity to *R. meliloti* yet similarity in the function of symbiosis. The lower amounts of SQDG found in *B. japonicum* do not disallow the possibility of its importance to symbiosis in this organism. The possibility exists that SQDG is a major component only of the bacterioid form and that the levels of expression observed here are background. Further studies on the presence of this lipid in bacterioid membranes are ongoing. Finally, *A. tumefaciens* was investigated to probe the possibility that SQDG is a key determinant in the distinction of symbiosis from pathogenesis. Although there appeared to be an orcinol-positive band corresponding to the faster migrating SQDG component in the TLC analysis for this species, this band did not show any detectable levels of radioactivity either by autoradiography or scintillation counting. This species is genetically quite similar to *R. meliloti* so the lack of SQDG suggests that this lipid plays a role in symbiosis. Although this study was not exhaustive in the strains tested, it does reinforce the possibilities of a role for sulfoquinovosyl diacylglycerol in the *Rhizobium*/legume relationship.

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