Chapter 3

Constraints on the biological source(s) of the orphan branched tetraether membrane lipids

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Abstract

Branched glycerol dialkyl glycerol tetraethers (GDGTs) are membrane lipids ubiquitous in soils and peat bogs. Based on their chemical structure and their abundant occurrence in the anoxic part of peat bogs, they were suggested to be derived from anaerobic bacteria (Weijers et al., 2006a). The exact biological origin, however, remains as yet unclear and is, due to the high microbiological diversity in soils and peat bogs, difficult to establish. In order to determine which phylogenetic group(s) of bacteria might encompass branched GDGT-synthesising species we conducted both core membrane lipid and 16S rDNA analyses of a Swedish peat bog. The results show a high abundance of branched GDGT lipids relative to methanogen-derived isoprenoid archaeol and archaeal GDGT-0 lipids. The molecular ecological results show that members of Rice Clusters IV and VI of the *Crenarchaeota* are the most dominant Archaea in the peat bog and that *Acidobacteria* comprise the dominant group of Bacteria present. This suggests that the phylum of *Acidobacteria* might include the biological source of the as yet orphan branched GDGT membrane lipids.

3.1. Introduction

Based on their distinct difference in 16S ribosomal RNA content, Bacteria and Archaea are identified as two separate Domains of Life (Woese et al., 1990). This distinction is supported by the different membrane lipid composition of Bacteria and Archaea. Where bacterial cell membranes generally comprise lipids with straight or branched alkyl chains ester-linked to a glycerol backbone and arranged in a bi-layer configuration, archaeal cell membranes generally comprise lipids with isoprenoidal alkyl chains connected by ether bonds to the glycerol backbones forming dialkyl glycerol diethers (DGDs) and membrane spanning glycerol dialkyl glycerol tetraethers (GDGTs). Additionally, the enantiomeric configuration of the glycerol backbone, 1,2- vs. 2,3-di-*O*-alkyl-*sn*-glycerol for Bacteria and Archaea, respectively, is a strong distinctive characteristic (Kates, 1978).

With the development of a high performance liquid chromatography / mass spectrometry (HPLC/MS) method for analysing intact core GDGT lipids (Hopmans et al., 2000), it appeared that GDGT membrane lipids occur widespread in the environment (Schouten et al., 2000). In the terrestrial realm, isoprenoid GDGTs (e.g., GDGT-0 in Fig. 3.1) were, together with DGDs (e.g., archaeol in Fig. 3.1) detected in peat bogs (Pancost et al., 2000; Schouten et al., 2000; Weijers et al., 2004). Based on their carbon isotopic composition and their occurrence in cultured methanogenic Archaea, both isoprenoid DGDs and GDGT-0 are used in peat bogs as tracers of methanogenic Archaea (Pauly and van Vleet, 1986; Pancost et al., 2000). A newly discovered group of tetraether membrane lipids, the branched GDGTs (Fig. 3.1), has been identified over the last years and found to be ubiquitous in peat bogs as well as in soils (Schouten et al., 2000; Sinninghe Damsté et al., 2000; Weijers et al., 2006b). Despite their membrane-spanning tetraether structure, which is thought to be a typical characteristic of the Archaea, they have been shown to have a bacterial origin. This is not only based on their branched instead of isoprenoid carbon skeleton but also on the bacterial 1,2-di-*O*-alkyl-*sn*-glycerol configuration of the glycerol backbone (Weijers et al., 2006a). Because of the high abundance of branched GDGT membrane lipids in the water saturated part of peat bogs relative to the non-saturated upper part, the bacteria producing them are assumed to be anaerobes or perhaps facultative aerobes (Weijers et al., 2006a). Based on these branched GDGTs, new proxies for the fluvial input of terrestrial organic matter in marine sediments (Hopmans et al., 2004) and for the reconstruction of annual mean air temperatures on land (Weijers et al., 2007b) have been developed. Although these membrane lipids have been found in every single soil and peat bog sample analysed so far (Weijers et al., 2006b, 2007a), the bacteria producing them are as yet unknown.

Microbial community structures in soils and peat bogs are complex and difficult to disentangle. The application of different culture-independent 16S rRNA sequencing techniques, however, enables the detection of many new bacterial and archaeal sequences in soils, wetlands and peat bogs. These culture-independent techniques have been applied in

several studies of bacterial community structures and diversity in soils (e.g., Bintrim et al., 1997; Dunbar et al., 2000; Hackl et al., 2004; Chan et al., 2006), in wetlands (e.g., Høj et al., 2005; Kraigher et al., 2006) and in peat bogs (e.g., McDonald et al., 1999; Sizova et al., 2003; Pankratov et al., 2005; Dedysh et al., 2006; Morales et al., 2006). Despite this, the physiology and function of the majority of bacterial and archaeal life in soils, wetlands and peat bogs are unknown as the majority of species seem hard to isolate in pure culture and many of them are inherently slow growers (Pace, 1997; Janssen, 2006 and references therein).

With this in mind and the fact that branched GDGTs are not easily, if at all, detected with standard techniques for lipid characterisation used in species description, it is not a surprise that the organism(s) synthesising branched GDGTs is (are) yet to be discovered. In order to constrain the biological source(s), we extracted prokaryotic 16S rDNA from samples at different depths in a peat core from the Saxnäs Mosse peat bog in Sweden. Subsequently, a qualitative comparison was made between the obtained bacterial and archaeal sequences and depth profiles of the concentrations of branched and isoprenoid GDGT membrane lipids and of the isoprenoid DGD lipid archaeol in this core.

3.2. Material and Methods

3.2.1. Study site and sampling

The Saxnäs Mosse is a raised bog composed of *Sphagnum* species near the village of Lidhult in south Sweden. The top 25 cm of the bog comprises the acrotelm layer, i.e., the layer experiencing alternating oxic and anoxic conditions due to the varying water table. The zone below 25 cm depth comprises the catotelm, the part of the bog which is continuously water saturated and consequently anoxic. Using a Wardenaar-corer, 50 cm long monoliths were obtained from the peat bog for GDGT and molecular ecological analyses. The top 14 cm of the bog consists of non-decomposed Sphagnum moss twigs with leaves (*Sphagnum magellanicum*) and is followed by a 13 cm thick interval of more decomposed *Sphagnum* twigs. The lower part of the bog consists of decomposed peat containing some *Ericaceae* and *Cyperaceae* remains between 30 and 40 cm depth and decomposed *Sphagnum papillosum* below 40 cm depth. Peat samples for organic geochemical analysis were taken at a 2 cm interval from the monolith back in the laboratory. The peat samples for microbiological analysis were taken with clean spatula at a 2 cm interval from the monolith directly in the field and put in sterile 16 ml containers (Greiner), transported in a mobile fridge at ~ 4°C within 24 h to the laboratory and stored frozen at -40°C until analysis.

3.2.2. Organic geochemistry

Lipid extraction. Peat samples for lipid analysis were freeze dried and powdered with mortar and pestle prior to extraction. The soluble organic matter was extracted from the peat samples

three times for 5 min by means of an accelerated solvent extractor at a pressure of ca. 7.6 × 10^6 Pa and a temperature of 100°C using a solvent mixture of dichloromethane (DCM):methanol 9:1 (v/v). The obtained extracts were rotary evaporated and further purified by separation into a relative apolar and polar fraction over an activated Al_2O_3 column using DCM:methanol 199:1 (v/v) and DCM:methanol 1:1 (v/v) solvent mixtures, respectively. After evaporation under a pure N_2 flow, the polar fraction, containing the GDGTs and archaeol, was ultrasonically dissolved in an hexane:propanol 99:1 (v/v) solution to a concentration of ca. 2 mg ml^{-1} and filtered over an 0.45 μm PTFE filter (ø 4 mm) prior to analysis with HPLC.

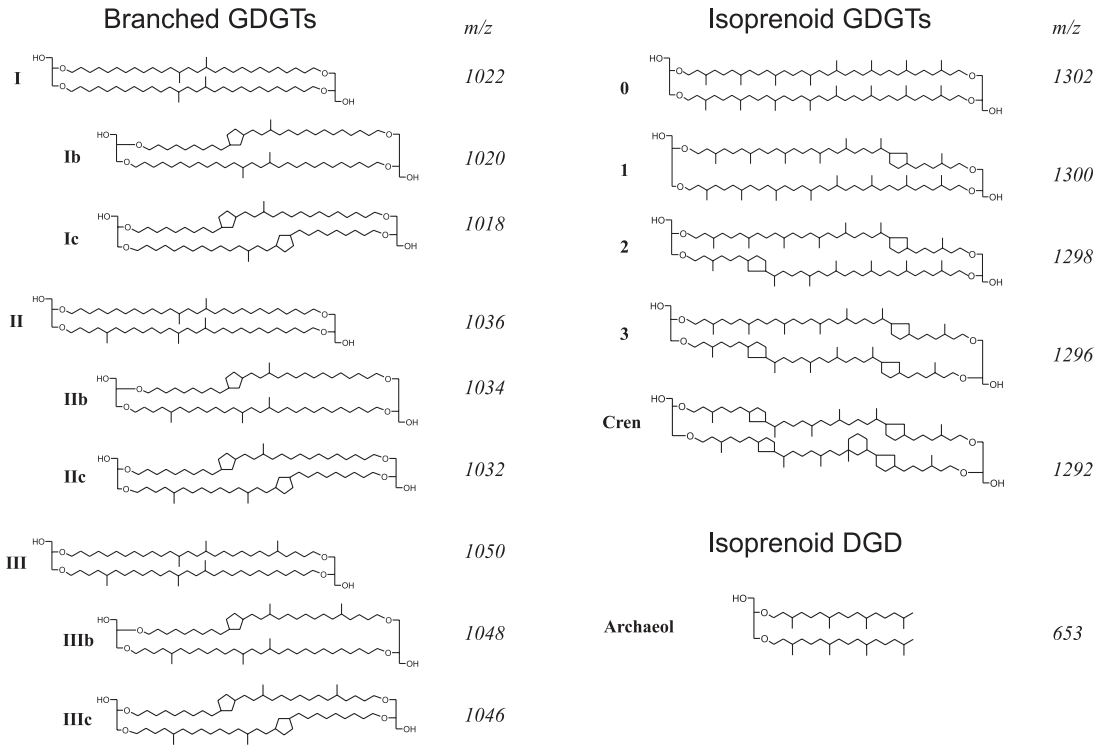

Figure 3.1: Chemical structures of the branched and isoprenoid GDGTs and the DGD archaeol. Cren = crenarchaeol

Lipid analysis. GDGTs and archaeol were analysed by high performance liquid chromatography/atmospheric pressure chemical ionisation – mass spectrometry (HPLC/APCI-MS) according to Hopmans et al. (2000) with minor modifications. Analyses were performed on an Agilent 1100 / Hewlett Packard 1100 MSD series machine equipped with automatic injector and HP-Chemstation software. Separation was achieved in normal phase on a Prevail Cyano column (150 mm × 2.1 mm; 3 μm) with a flow rate of the hexane:propanol 99:1 (v/v) eluent of 0.2 ml min^{-1}, isocratically for the first 5 min and thereafter with a linear gradient to 1.8% propanol after 45 min. Injection volume of the samples was 10 μl and quantification of the GDGTs was achieved by integrating the peak

areas in the $[M+H]^+$ and $[M+H]^+ + 1$ [i.e., protonated molecular ion (*m/z* values in Fig. 3.1) and first isotope peak] traces and comparing those with a standard curve prepared with known amounts of the isoprenoid GDGT-0. A correction was applied for the differences in mass between the branched GDGTs and GDGT-0. The DGD archaeol was analysed in a separate run, together with GDGT-0 in a single ion monitoring mode to increase sensitivity. Archaeol could not be quantified absolutely due to the absence of a DGD standard curve, but a relative quantification has been obtained by determining the ratio between archaeol and GDGT-0.

3.2.3. Molecular biology

Total DNA extraction. For molecular biological analysis 8 depth intervals were selected along the peat core (Figs. 3.4 and 3.6) covering the transition from the partly oxygenated acrotelm to the anoxic catotelm. About 2 g of peat material per sample was defrosted, centrifuged for 1 min at 1000 G to get rid of the excess liquid and subjected 3 times to a freeze-thaw cycle. DNA was extracted using the UltraClean Soil DNA Kit (Mobio laboratories Inc, Carlsbad, CA, USA) following the descriptions of the manufacturer.

PCR Amplification of archaeal and bacterial 16S rDNA. Partial archaeal 16S rDNA was amplified from the total DNA extract by polymerase chain reaction (PCR) using the forward primer Parch519f (Øvreås et al., 1997) and the reverse primer Arch-GC-915r (Stahl and Amann, 1991) including a 40-bp long GC-clamp (Muyzer et al., 1993) at the 5'-end. For the partial bacterial 16S rRNA amplification the forward eubacterial primer 341f including a 40-bp long GC-clamp (Muyzer et al., 1993) and the reverse primer 907R(a/c) (Amann et al., 1992) were used. All PCR amplifications were performed with a Geneamp PCR System 2400 (Perkin-Elmer, Connecticut, USA) using a mixture of the following components: 5 μl of 10X PCR-buffer (Pharmacia Biotechnology, Upsalla, Sweden), 10 mM of dNTP's, 20 μg of bovine serum albumine, 1 unit of Taq DNA polymerase (Pharmacia) and 0.5 μM of the respective archaeal and bacterial primers. PCR conditions included an initial denaturation step of 4 min at 96°C followed by 30 cycles including a denaturation step for 30 s at 94°C, a primer annealing step for 40 s at 57°C and a primer extension step for 40 s at 72°C. A final extension was performed for 10 min at 72°C.

DGGE analysis of 16S rDNA gene fragments. All PCR products were quantified by gel electrophoresis using a mass molecular DNA marker (Smartladder, Eurogentec) and subsequently separated by denaturing gradient gel electrophoresis (DGGE) (Schafer and Muyzer, 2001) carried out in a Bio-Rad D Gene system (Bio-Rad, München, Germany). About 100 ng PCR-product was loaded onto 6% (wt/vol) polyacrylamide gels [acrylamide/N,N'-methylene bisacrylamide ratio 37:1 (w/w)] in 1 × TAE buffer (pH 7.4). The archaeal gel contained a linear gradient from 30 to 60% denaturant [100% denaturant is 7 M urea plus 40% (v/v) formamide] and the bacterial gel contained a linear gradient from 20 to

70%. Electrophoresis of both gels proceeded for 5 h at 200 V and 60°C. Afterwards, gels were stained for 30 min in sterile double-distilled water containing ethidium bromide, de-stained for 60 min in sterile double-distilled water and photographed. DGGE-fragments were excised from the gel with sterile pipette points and the DNA of each fragment was eluted from the gel in sterile 10 mM Tris-HCL (pH 8.0) by incubation for 48 h at 4°C. The eluted DNA served as template DNA for re-amplification. Primers without GC-clamps were used for re-amplification.

Sequencing of DGGE bands. The amplified PCR products were purified using the QIAquick PCR Purification Spin Kit (Qiagen, Hilden, Germany) and quantified. Cycle sequencing reactions were performed with the ABI Prism Big Dye Terminator V3.0 kit (Applied Biosystems, CA, USA) using the forward or reverse primer (without GC clamp) at a final concentration of 0.2 µM and 10 ng of template DNA. The reaction volume was adjusted to a volume of 20 µl with molecular grade water (Sigma). The following reaction conditions were employed: 1 min initial denaturing at 96ºC followed by 25 cycles of 10 s at 96ºC, 5 s at 45ºC and 4 min at 60ºC. After an isopropanol mediated purification the pellets were dissolved in 15µl HiDi-formamide. Nucleotide positions were determined using an automated ABI-310 capillary sequencer (Applied Biosystems). Complementary sequences were aligned and manually edited using the AutoAssembler software package (Version 2.1.1; Applied Biosystems).

Phylogenetic analysis. Comparative analysis of the sequences was performed using ARB software (Ludwig et al., 2004). The partial sequences were aligned with sequences present in the database and with related full length 16S rDNA sequences obtained from NCBI using the BLAST-tool (http://www.ncbi.nlm.nih.gov/BLAST) (Benson et al., 2000). Phylogenetic trees were generated based on sequences of 1 kb and more using the maximum likelihood method. After applying a 50% variability filter, about 1400 columns of data were used for calculation of the tree. The specific sequences obtained by DGGE were added using the maximum parsimony option in ARB.

3.3. Results

3.3.1. Membrane lipids

In the upper part of the peat bog, which comprises non-decomposed peat and *Sphagnum* type vegetation, concentrations of the archaeal derived isoprenoid GDGT lipids were near the detection limit and the DGD lipid archaeol was not detected (Fig. 3.2). In this acrotelm layer, the average concentration of the summed isoprenoid GDGTs was 1 µg g^{-1} dry weight peat. In the water saturated part, the catotelm, this concentration increased considerably, with an

average value of 18 μg g^{-1} dry weight peat (Fig. 3.3). The acyclic isoprenoid GDGT-0 represented the majority of the detected isoprenoid GDGTs, i.e. ~60% in the acrotelm and ~80% in the catotelm. In the catotelm, archaeol was also detected and showed a constant abundance relative to the acyclic GDGT-0 (Fig. 3.3). A similar depth distribution was evident for the branched GDGTs although they were clearly more abundant in the peat core, by about a factor 5, than the isoprenoid GDGTs (Fig. 3.3). The average concentration of the summed branched GDGTs was only 5 μg g^{-1} dry weight peat in the upper part of the profile, but in the catotelm this concentration increased substantially to 115 μg g^{-1} dry weight peat on average (Fig. 3.3).

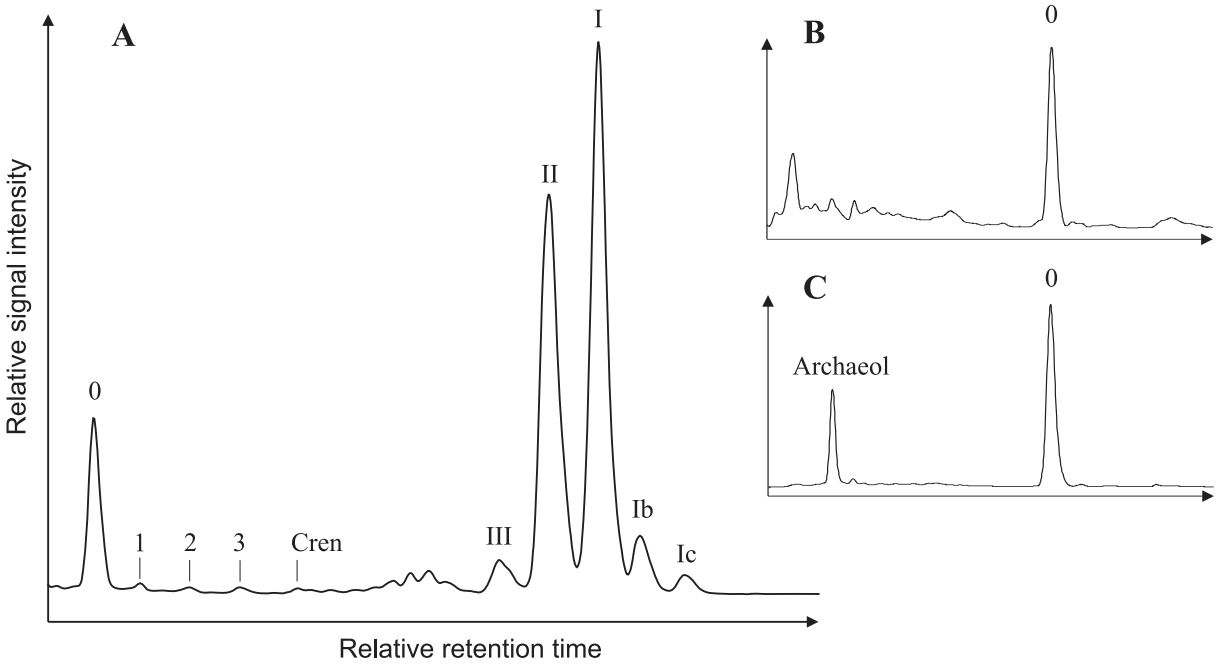

Figure 3.2: HPLC/MS base peak chromatograms of the Saxnäs Mosse peat bog. Panel A shows a typical base peak chromatogram with the relative abundances of isoprenoid and branched GDGTs. Panels B and C show the abundance of the DGD archaeol relative to GDGT-0 in the acrotelm (archaeol absent) and the catotelm of the peat bog, respectively. Numbers refer to the structures drawn in Fig. 3.1. Cren = crenarchaeol

3.3.2. Archaeal DNA sequences

Using the universal archaeal primer pair many different bands appeared on the DGGE gel containing the 8 selected depth intervals from the Saxnäs Mosse peat bog (Fig. 3.4). Of these, 31 bands could be successfully excised from the gel and sequenced (Fig. 3.4). One of the bands, band 22, appeared to be a bacterial sequence. A BLAST search revealed that the closest relative of this sequence (92% similarity) is an acidobacterial sequence from a Taiwanese forest soil. Phylogenetic analysis reveals that the majority of the obtained archaeal sequences fall within Rice Clusters (RC) IV and VI (Fig. 3.5), which are deep branching

clades in the Kingdom *Crenarchaeota* (Großkopf et al., 1998; Chin et al., 1999). Five sequences, band 10, bands 16 and 18 and bands 19 and 26, do not fall within these Rice Clusters and occupy rather isolated positions within the *Crenarchaeota*. Band 10 is only distantly related (<90%) to sequences obtained from a gold mine and a petroleum contaminated soil. The other four bands appear to be closest related (95-99%) to sequences derived from hot springs and a sulphurous lake. In general, there does not seem to be a trend towards a higher diversity of archaeal species in the deeper layers. Sequences obtained from the two deepest intervals in the peat core (38 and 40 cm) fall generally closely together within RC-VI. However, the DGGE method used does not exclude their presence in the shallower depth intervals.

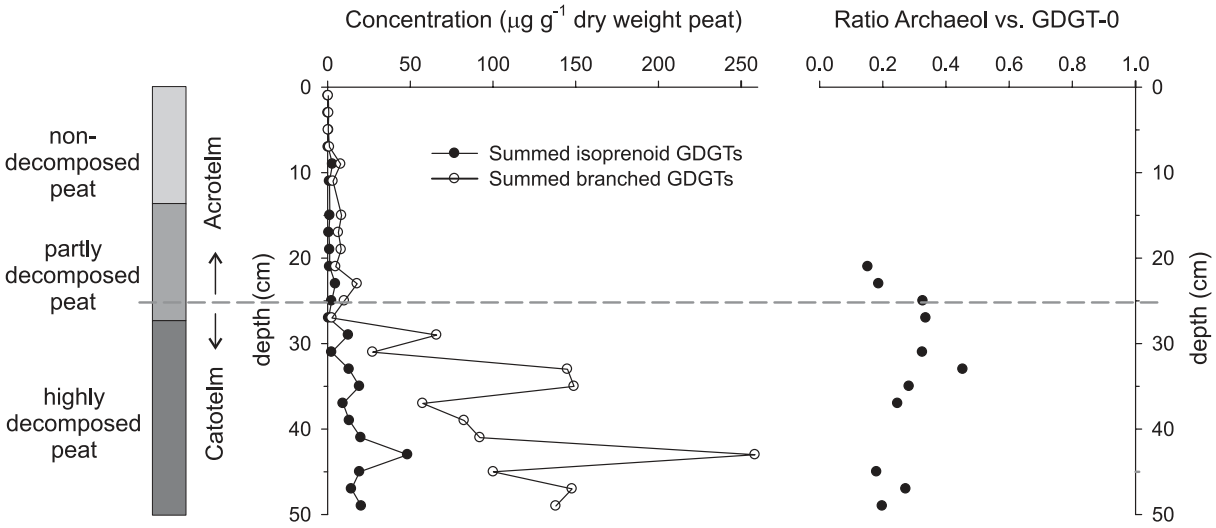

Figure 3.3: Depth profile of the Saxnäs Mosse peat core showing, on the left hand panel, the concentrations of the summed branched GDGT and summed isoprenoid GDGT (excluding crenarchaeol) membrane lipids and, on the right hand panel, the ratio between archaeol and GDGT-0. Also given are a schematic decomposition profile of the peat core and the boundary (grey striped line) between the acrotelm (zone in which the water table fluctuates) and catotelm (continuously water saturated and thus anoxic zone).

3.3.3. Bacterial DNA sequences

Using the universal bacterial primer pair several different bands were apparent on the DGGE gel. The number of bands in the gel increases from 2 in the two uppermost intervals to 9 in the deeper intervals, suggesting increased bacterial diversity although this might also be due to differences in sample concentrations (Fig. 3.6). A total of 14 different bands was successfully excised and sequenced (Fig. 3.6). Even though a universal bacterial primer was used, phylogenetic analysis revealed that 13 out of the 14 sequences belong to the phylum of *Acidobacteria* and only one sequence (band 9), detected in the 26 cm depth interval, falls within the *Syntrophobacteria* which is part of the large phylum of *δ-Proteobacteria* (Fig. 3.7).

The acidobacterial sequences are distributed along the subdivisions 1 (4 sequences), 3 (1 sequence) and 4 (3 sequences), following the classification proposed by Hugenholtz (1998), and one unknown subdivision (5 sequences).

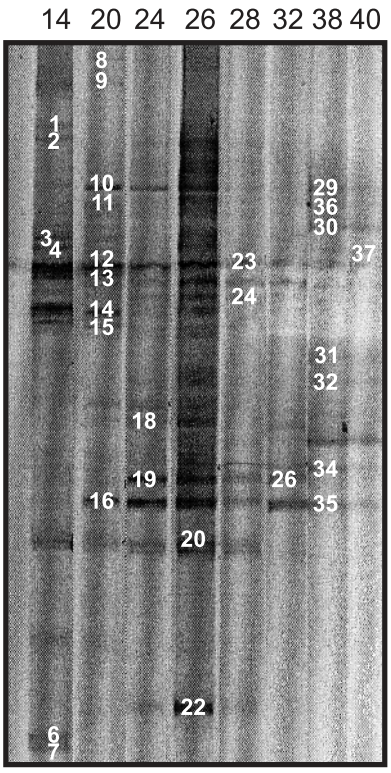

Figure 3.4: DGGE gel showing the separation of the archaeal 16S rDNA genes obtained from different depth intervals in the peat core. Numbers represent bands that were successfully excised and sequenced and correspond to the numbers in Fig. 3.5.

3.4. Discussion

3.4.1. Archaeal diversity

Although peat lands play an important role in global biogeochemical cycles via the microbe-mediated emission of CH_4 and CO_2 greenhouse gasses (Bartlett and Harriss, 1993), until recently, relatively little was known about the microbial communities present in these ecosystems. Only over the last years studies have been published aiming at the characterisation of microbial diversity and structures in peat bogs (e.g., Sizova et al., 2003; Kotsyurbenko et al., 2004; Pankratov et al., 2005; Juottonen et al., 2005; Dedysh et al., 2006; Morales et al., 2006). Microbial methanogenesis is an important process in the anaerobic degradation of organic matter in peat lands, carried out by methanogens comprising different species of *Euryarchaeota*. *Methanomicrobiales*, *Methanosarcinales*, *Methanobacteriales* and sequences belonging to Rice Cluster I (RC-I) are amongst the most regularly detected methanogen archaea in boreal peat lands (e.g., McDonald et al., 1999; Sizova et al., 2003; Horn et al., 2003; Kotsyurbenko et al., 2004; Høj et al., 2005; Juottonen et al., 2005; Brauer et al., 2006).

Figure 3.5: Maximum likelihood tree showing the phylogenetic position of the archaeal genes obtained from the Saxnäs Mosse peat core (SNM_A in bold). Numbers correspond to those in Fig. 3.4. RC = Rice Cluster

Strikingly, however, the archaeal sequences obtained from the Saxnäs Mosse bog belong to Rice Cluster IV (RC-IV) (Großkopf et al., 1998) and a cluster which most likely represents Rice Cluster VI (RC-VI) (Chin et al., 1999). These are no *Euryarchaeota* but deep branching members of the *Crenarchaeota*. Members of RC-IV have been detected on the roots of rice plants and were closely related to two earlier detected environmental sequences from a marsh environment and a freshwater sediment (Großkopf et al., 1998). RC-VI members have first

been described as such by Chin et al. (1999) in an anoxic rice field soil, but were earlier detected in an agricultural soil by Bintrim et al. (1997). Furthermore, Kemnitz et al. (2004) found, besides known methanogenic archaea, a high diversity of members of RC-IV and VI in a riparian flood plain in the Netherlands and Kotsyurbenko et al. (2004) detected these groups in an acidic west Siberian peat bog. The ecological niches and metabolic functions of members of RC-IV and VI are still unknown due to a lack of cultured relatives. It is unlikely that members of RC IV and VI are methanogens as this physiology has not been found yet in any crenarchaeotal species. The fact that we have not detected sequences closely related to methanogenic archaea might be due to the low specificity of the DGGE method, which only reveals the most abundant sequences. The diversity of methanogens in our peat could be high, but the abundance of individual methanogen species might be lower than that of the members belonging to RC IV and VI. Since with cloning and isolation studies RC IV and VI are usually detected together with methanogen communities, it might be assumed that methanogens are also present, though not detected with our methods, in the Saxnäs Mosse bog.

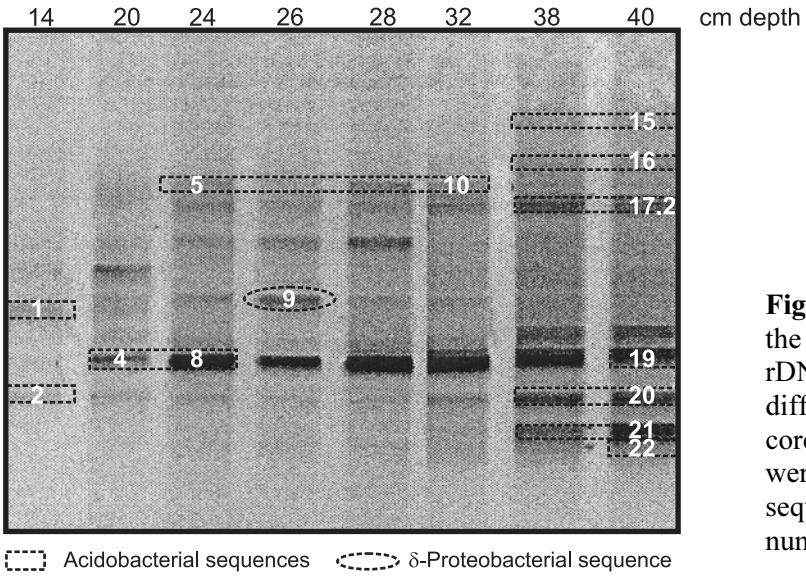

Figure 3.6: DGGE gel showing the separation of the bacterial 16S rDNA genes obtained from different depth intervals in the peat core. Numbers represent bands that were successfully excised and sequenced and correspond to the numbers in Fig. 3.7.

3.4.2. Bacterial diversity

Regarding the bacterial diversity, virtually all sequences obtained from our Swedish peat core fall within the phylogenetic cluster of *Acidobacteria*. Similar as with the Archaea, this seemingly low diversity is partially a result of the DGGE fingerprinting method used and creating a clone library may likely result in a somewhat higher diversity. However, the DGGE method screens for the most abundant sequences which in this case, clearly, belong to the *Acidobacteria*. The *Acidobacteria* are a relatively recently recognised phylogenetic cluster of

bacteria subdivided into at least eight groups (Kuske et al., 1997; Hugenholtz et al., 1998) together comprising a highly diverse phylum (Quaiser et al., 2003). Although they are found to be ubiquitous in environmental samples (Barns et al., 1999), this phylum is poorly represented by cultured organisms. Only 3 species are yet brought into culture; *Acidobacterium capsulatum*, an acidic, aerobic, mesophilic, gram-negative, non-spore forming bacterium (Kishimoto et al., 1991; Hiraishi et al., 1995) [belonging to subdivision 1; following the classification by Hugenholtz et al. (1998)]; *Holophaga foetida*, a strictly anaerobic, gram negative, homoacetogenic bacterium degrading methoxylated aromatic compounds (Liesack et al., 1994); and *Geothrix fermentans*, a strictly anaerobic, gram negative, Fe(III)-reducing bacterium (Lonergan et al., 1996; Coates et al., 1999) (both belonging to subdivision 8).

Our acidobacterial sequences are distributed over subdivisions 1, 3 and 4 and an unknown subdivision. The identity of this latter subdivision, containing 5 of the Saxnäs Mosse sequences, is rather difficult to establish as the German peat bog clone 'TM1', a closely related environmental sequence, is not classified in the original classification of Hugenholtz et al. (1998). Moreover, the soil clone 'DA052' that was originally classified in subdivision 2 (Hugenholtz et al., 1998), seems to cluster within subdivision 4, according to our phylogenetic analysis. It has to be mentioned, however, that the classification of the *Acidobacteria* by Hugenholtz et al. (1998) in some instances is only based on a few sequences combined with low bootstrap values.

Amongst the four subdivisions of *Acidobacteria* found in this study are different environmental sequences obtained from soils (e.g., Felske et al., 1998; Sait et al., 2002), a forested wetland (Brofft et al., 2002) and boreal peat bogs (e.g., Sizova et al., 2003; Juottonen et al., 2005; Dedysh et al., 2006). From the three cultured representatives of *Acidobacteria* only *Acidobacterium capsulatum* belongs to one of these subdivisions, i.e., subdivision 1, but differs by >5% sequence identity and is thus not closely related to the sequences obtained in this study (Fig. 3.7).

Molecular ecological studies of peat bog systems often reveal high abundances of *Acidobacteria*. A study of the bacterial community composition in 24 different peat bogs in New England, U.S.A., demonstrated a marked similarity in composition amongst the different bogs (Morales et al., 2006). Furthermore, 16S rDNA sequence analysis of one of these bogs showed that the *Acidobacteria* are, after the *Proteobacteria*, likely the most abundant bacteria in these bogs (11% of total number of clones) (Morales et al., 2006). Similar reports of *Acidobacteria* in peat bogs and fen soils have been made for a marsh in Slovenia (23% of total number of clones, second most abundant after *Proteobacteria*) (Kraigher et al., 2006), a Siberian peat bog (29% of total clone number, most abundant) (Dedysh et al., 2006) and four other peat bogs in Russia (*Acidobacteria* second or third most abundant after *Proteobacteria*) (Pankratov et al., 2005).

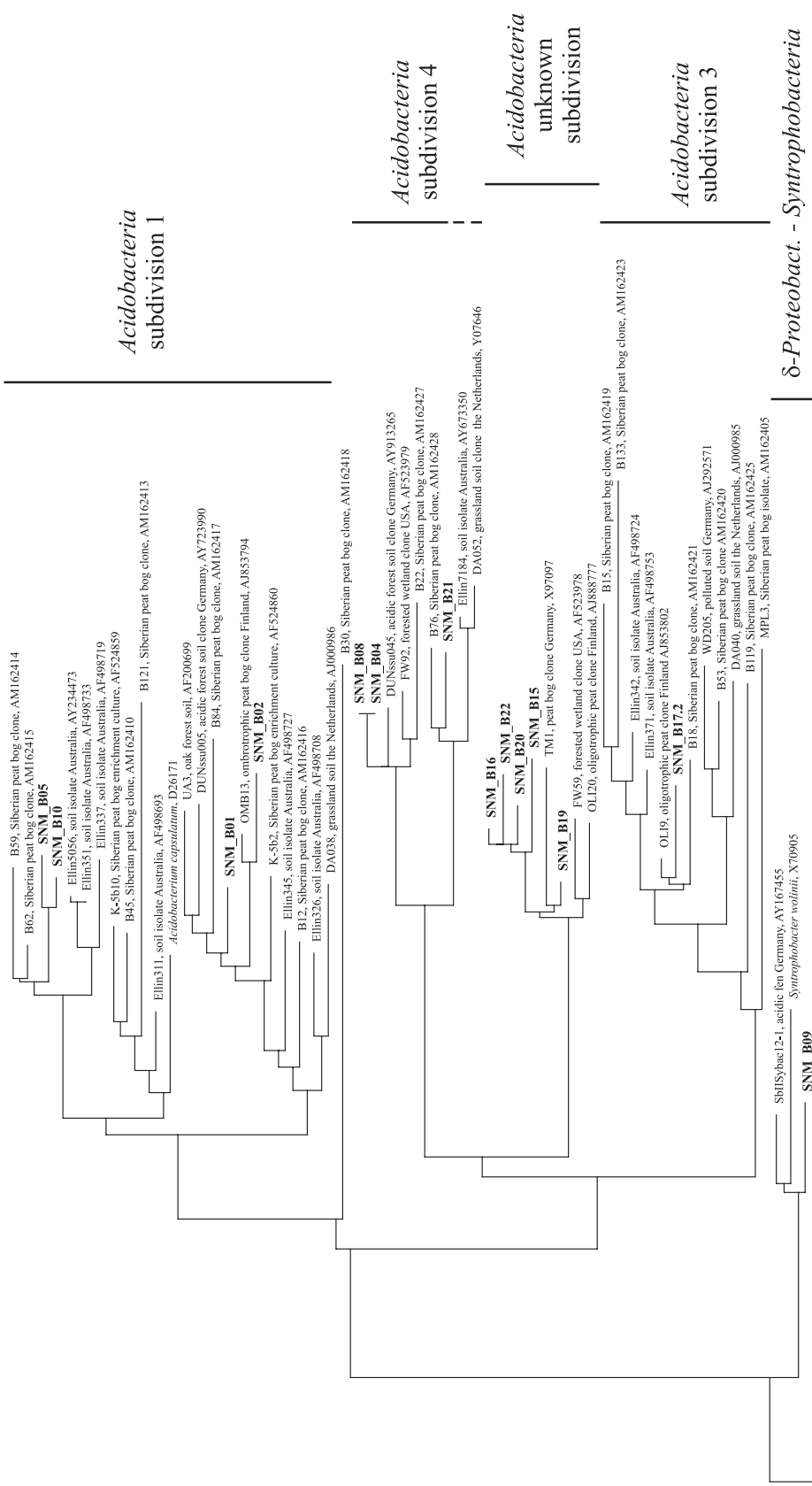

Figure 3.7: Maximum likelihood tree showing the phylogenetic position of the bacterial genes obtained from the Saxnäs Mosse peat core (SNM_B in bold). Numbers correspond to those in Fig. 3.6. Subdivisions of the *Acidobacteria* are according to the classification by Hugenholtz et al. (1998).

3.4.3. Membrane lipids

Considering non-thermophilic environments, GDGT-0 could be derived from either methanogenic *Euryarchaeota* (Kates et al., 1993; Pancost et al., 2000) or from mesophilic Group-1 *Crenarchaeota* (Schouten et al., 2000). These Group-1 *Crenarchaeota*, however, do also synthesise considerable amounts of crenarchaeol (Fig. 3.1) (Sinninghe Damsté et al., 2002a). It has been shown previously that crenarchaeol is only present in minor amounts relative to GDGT-0 in this Saxnäs Mosse peat bog (Weijers et al., 2004), which implies that Group-1 *Crenarchaeota* are not a likely source for the isoprenoid GDGTs found in this study. The Rice Cluster IV and VI *Crenarchaeota* may, however, also produce isoprenoid GDGTs. Yet, it is unlikely that they produce archaeol, which is typically found in methanogenic *Euryarchaeota* (Kates et al., 1993) and only in traces, if at all, in *Crenarchaeota*. The presence of archaeol, therefore, points to the presence of methanogens, and the relative constant ratio between archaeol and GDGT-0 in this core (Fig. 3.3) might suggest a same source for GDGT-0. The fact that no methanogen sequences were detected might be a methodological bias; crenarchaeol is present as well but no Group-1 crenarchaeotal sequences were detected either.

Strikingly, the abundance of branched GDGT membrane lipids in this core is clearly higher than that of the isoprenoid GDGTs by about a factor 5. Also in other peat bogs the average ratio of branched GDGTs versus isoprenoid GDGTs is high, i.e., the Netherlands (~2), England (~3) and Switzerland (~4) (J.W. et al., unpublished results). This suggests that the bacteria that synthesise branched GDGT lipids likely represent quite an abundant (group of) species. Based on the DGGE and subsequent 16S rDNA sequence analysis, the *Acidobacteria* are the most dominant species present in our peat bog and might represent a candidate phylum that could biosynthesise branched GDGTs. Additional evidence for this assumption is obtained from soils. In earlier studies, so far, we have detected branched GDGTs in all (>150) soils analysed, ranging from a tropical rainforest soil in Gabon to almost barren soils on Arctic Spitsbergen and at 4000 m altitude in the Andes (Weijers et al., 2006b, 2007a and J. Bendle, R. Pancost, J. Weijers and J. Sinninghe Damsté, unpublished results). Thus, branched GDGT-producing species must be very ubiquitous although we cannot fully exclude that these lipids are produced by many different species.

The *Acidobacteria* are, indeed, widespread occurring in a variety of environments like soils, swamps, fresh water lakes, hot springs and contaminated aquifers (Hugenholtz et al., 1998; Barns et al., 1999). This widespread occurrence suggests that *Acidobacteria* are significant constituents of many ecosystems (Hugenholtz et al., 1998). In soils, *Acidobacteria* are virtually always detected, ranging from Antarctic soils (Aislabie et al., 2006) to a hot desert soil in Tunisia (Chanal et al., 2006) and a tropical rainforest soil in China (Chan et al., 2006). In this latter soil, the relative abundance of *Acidobacteria*-affiliated sequences was as much as 80% (Chan et al., 2006). Based on comprehensive analysis of 32 libraries of 16S rRNA and 16S rRNA genes of members of the bacterial domain, prepared from a variety of

soils, Janssen (2006) estimated the average abundance of *Acidobacteria* in soils at 20%, which is second most abundant after the highly diverse phylum of *Proteobacteria*.

Based on the results of the microbiological analyses of the Saxnäs Mosse peat bog and the literature data, the *Acidobacterium capsulatum* (DSM 11244) and *Holophaga foetida* (DSM 6591) strains, two of the three strains available in culture from the phylum *Acidobacteria*, were analysed for the presence of branched GDGTs. Unfortunately, in both strains branched GDGTs were not detected. However, *Holophaga foetida* belongs to subdivision 8 and is rather distantly related to other environmental sequences. Secondly, although *Acidobacterium capsulatum* belongs to subdivision 1, in which more peat bog and soil clones are present, sequences obtained from our peat bog did not reveal very close relationships with this cultured strain (<95% sequence similarity). Nevertheless, the phylum of *Acidobacteria* is a highly diverse one with many subdivisions and clusters within these divisions, and the presence of branched GDGT-producing species can therefore not be excluded based on these results.

In addition, we analysed *Syntrophobacter fumaroxidans* (DSM 10017) for branched GDGT lipid content, as biomass of this species was readily available in our laboratory and the only bacterial sequences (SNM_B09) that did not fall in the *Acidobacteria* clustered within the *Syntrophobacteria*. *S. fumaroxidans* did not contain branched GDGTs either, but is also only distantly related to sequence SNM_B09, i.e., an insignificant relation based on a BLAST sequence alignment.

3.4.4. Alternative sources for branched GDGTs

An alternative approach to that described above is to examine phylogenetic relatives of bacterial species known to produce membrane lipids structurally related to that of branched GDGTs. The extreme thermophilic and strictly anaerobic bacterium *Ammonifex* is known to produce diether lipids (Huber et al., 1996) and the thermophilic *Thermoanaerobacter ethanolicus* is reported to produce membrane-spanning tetraester lipids containing α,ω-13,16-dimethyloctacosane carbon chains (Jung et al., 1994; Lee et al., 2002), which are alkyl chains identical to those found in some branched GDGTs. Possibly, low temperature relatives of these bacteria, which fall in the candidate phylum of the low G+C gram positive bacteria, might be biological sources for branched GDGTs. Therefore, from this phylum of low G+C gram positive bacteria, we analysed *Clostridium acidisoli* (DSM 12555) for the presence of branched GDGTs. This strain was chosen as it is an anaerobic acid tolerant species isolated from a German peat bog and because *Thermoanaerobacter* species show a 98-99% 16S rRNA similarity with the thermophilic and acid tolerant *Clostridium thermoamylolyticum* (Kuhner et al., 2000). Despite this seemingly close phylogenetic relation with species synthesising related types of alkyl chains, *Clostridium acidisoli* did not contain branched GDGT membrane lipids. It has to be mentioned, in addition, that these species (or relatives thereof) seem not to be ubiquitous among soils and peat bogs and that more ether bound and membrane-spanning

lipids have been reported in species belonging to other phyla, like the *Planctomycetes* (Sinninghe Damsté et al., 2002b) and the early branching *Aquificales* (Huber et al., 1992) and *Thermotogales* (Carballeira et al., 1997).

3.5. Conclusion

Branched GDGTs are present in high amounts relative to the ostensibly methanogen-derived isoprenoid GDGTs and archaeol in the Saxnäs Mosse peat bog. *Acidobacteria* seem to be the most dominant type of Bacteria present in this peat bog. As, in addition, both branched GDGTs and *Acidobacteria* are ubiquitous in globally distributed soils, we suggest that the phylum of *Acidobacteria* might encompass the organisms synthesising branched GDGT membrane lipids.

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