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ORIGINAL PAPER

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Alkane-1,2-diol-based glycosides and fatty glycosides and wax esters in *Roseiflexus castenholzii* and hot spring microbial mats

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Abstract The lipid composition of *Roseiflexus castenholzii*, a thermophilic filamentous phototrophic bacterium related to uncultivated filamentous phototrophic bacteria that predominate in hot spring microbial mats, is reported. *R. castenholzii* lipid extracts were dominated by components characterized by alkane-1-ol-2-alkanoate moieties glycosidically bonded to a C_6 sugar. Similar fatty glycosides, with an additional fatty acid esterified, were detected by HPLC-MS. *R. castenholzii* also produces a suite of wax esters ranging from 37 to 40 carbon atoms in length. In lipid extracts from two nonsulfidic hot spring microbial mats, similar alkane-1,2-diol-based lipids were detected in minor amounts. *R. castenholzii* lipids are compared to lipids of mats and other thermophilic mat isolates.

Keywords *Roseiflexus castenholzii* · *Chloroflexus* · Hot spring microbial mat · Alkane-1,2-diol · Lipid

Abbreviations *APCI* Atmospheric pressure chemical ionization · *GC* Gas chromatography · *HP* Hewlett Packard · *HPLC* High-performance liquid chromatography · *MS* Mass spectrometry · *NMR* Nuclear magnetic resonance

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Introduction

The most common microbial mats of hot springs in Yellowstone National Park, Wyoming, USA, are those in neutral to alkaline springs without primary sulfide, such as Octopus Spring and Mushroom Spring (Ward et al. 1989). These well-laminated, centimeters-thick mats consist predominantly of unicellular cyanobacteria and filamentous *Chloroflexus* relatives (e.g. Bauld and Brock 1973; Castenholz 1973; Ward et al. 1989; Weller et al. 1991; Ruff-Roberts et al. 1994; Ferris and Ward 1997). The most abundant *Chloroflexus* relatives in such hot springs, termed type-C organisms, have been recognized by their 16S rRNA sequences (Weller et al. 1991; Ruff-Roberts et al. 1994; Ferris and Ward 1997; Boomer et al. 2002). It has recently been shown by fluorescent in situ hybridization that type-C organisms are predominantly filaments in these mats (U. Nübel, M.M. Bateson, V. Vandieken, A. Wieland, M. Kühl, D.M. Ward, submitted). The type-C 16S rRNA sequences are only distantly related (approximately 83% similar) to sequences of cultivated *Chloroflexus aurantiacus* strains (Weller et al. 1991; U. Nübel et al. submitted). However, they are 95.7% similar to the 16S rRNA sequence of a bacteriochlorophyll-a-containing filamentous phototroph recently isolated from Japanese hot springs, *Roseiflexus castenholzii* (Hanada et al. 2002), with which they form a phylogenetic clade that excludes *Chloroflexus* spp. (U. Nübel et al. submitted; Boomer et al. 2002). In contrast to the mat systems described above, molecular analysis of mats in sulfidic hot springs revealed 16S rRNA sequences of *Chloroflexus* relatives that are only approximately 5% different from the cultivated *C. aurantiacus* strains (Ward et al. 1997).

We have studied mats in both nonsulfidic and sulfidic springs as modern analogs of stromatolitic communities, attempting to correlate lipid biomarkers and their isotopic signatures with possible source organisms (Dobson et al. 1988; Shiea et al. 1991; Zeng et al. 1992a, b; Summons et al. 1996; van der Meer et al. 2000;) and their metabolisms (van der Meer et al. 2000; M.T.J. van der Meer, et al., un-

published results). To date, our interpretation of mat lipids that might be contributed by *Chloroflexus* relatives has been based on the lipid compositions of several cultivated *C. aurantiacus* strains (Knudsen et al. 1982; Shiea et al. 1991; van der Meer et al. 2001), which produce two classes of lipids often found in hot spring mats, long-chain wax esters and alkenes. The wax esters of *C. aurantiacus* isolates range from 30 to 37 carbon atoms in length and are either saturated or mono-unsaturated (Shiea et al. 1991; van der Meer et al. 2001). Both sulfidic and nonsulfidic mats contain normal and *iso*-branched wax esters (Shiea et al. 1991; M.T.J. van der Meer et al., unpublished results) of comparable chain length (Shiea et al. 1991; van der Meer et al. 2001), but not unsaturated wax esters (Table 1). All *C. aurantiacus* cultures analyzed, grown both autotrophically and heterotrophically, also contain long-chain polyunsaturated alkenes dominated by hentriacontatriene ($C_{31:3}$ alkene) (Shiea et al. 1991; van der Meer et al. 2001). Mats in sulfidic springs contain abundant long-chain alkenes typical of *C. aurantiacus* (van der Meer et al. 2000; M.T.J. van der Meer et al., unpublished results), but these compounds were found only in trace amounts in nonsulfidic hot spring microbial mats (Dobson et al. 1988; Shiea et al. 1991; M.T.J. van der Meer et al. in preparation). The differences in lipid composition of mats and *Chloroflexus* isolates could be due to the occurrence of different *Chloroflexus* relatives (i.e., different species) in different types of mats, to differences in lipid synthesis between culture and natural environments, or both. Several observations support the first possibility. For instance, both 16S rRNA and lipid biomarker distributions suggest that *C. aurantiacus* might be more prevalent in sulfidic hot spring mats, and 16S rRNA sequence data suggest that the type-C *Chloroflexus* relatives are more prevalent in nonsulfidic mats. We studied the lipid composition of the closest cultivated relative of type-C organisms, *R. castenholzii*, to evaluate whether organisms of this phylogenetic lineage might be sources for lipids found in these mats.

Table 1 $C_{31:3}$ long-chain alkene and wax ester distribution in hot spring microbial mats and photosynthetic bacterial isolates. *Blank* Not present, *trace* present in very low amounts, + *present*, ++ dominant compounds

Compound type	Octopus Spring[a]	Mushroom Spring[b]	*Roseiflexus castenholzii*	*Chloroflexus aurantiacus*[c]
Alkene				
$C_{31:3}$	Trace	Trace		++
Wax ester				
n-C_{31}	+	+		
iso-C_{31}	+	+		
n-C_{32}	++	++		+
iso-C_{32}	+	+		
$C_{32:1}$				+
n-C_{33}	++	++		+
iso-C_{33}	+	+		
n-C_{34}	++	++		++
iso-C_{34}	+	+		
$C_{34:1}$				+
n-C_{35}	+	+		++
n-C_{36}	+	+		++
$C_{36:1}$				+
n-C_{37}	+	+	+	+
n-C_{38}			++	+
n-C_{39}			+	
n-C_{40}			++	

[a]Shiea et al. (1991); Summons et al. (1996); M.T.J. van der Meer et al., unpublished results
[b]M.T.J. van der Meer et al., unpublished results
[c]Shiea et al. (1991); van der Meer et al. (2001)

Material and methods

Culture

Roseiflexus castenholzii strain HLO8[T](DSMZ 13941[T]; JCM 11240[T]) was grown photoheterotrophically under anoxic conditions on 02YE medium (pH 7.5, 50 °C) containing 0.2% yeast extract [see Hanada et al. (1995, 2002) for details].

Environmental samples

The Octopus Spring mat was sampled on August 27, 1997 at a temperature of 58–64 °C, pH 8.3, and with sulfide concentrations below detection. The Mushroom Spring mat was sampled on August 22, 1999 at a temperature of 54 °C, pH 8.1, and with sulfide concentrations below detection. Mat samples were directly frozen on dry ice.

Lipid extraction and derivatization

Harvested cells were freeze-dried and ultrasonically extracted with methanol (3×), dichloromethane/methanol (1/1, v/v) (3×) and dichloromethane (3×). The different extracts were pooled and solvents were evaporated. The resulting total lipid extract was dissolved in a small amount of dichloromethane for storage. An aliquot of this total lipid extract was dried under a stream of N_2 followed by methylation of the fatty acids with diazomethane in diethyl ether. The methylated extract was then dissolved in ethyl acetate and passed over a SiO_2-column using ethyl acetate as eluent. This eluent was dried under a stream of N_2 followed by silylation of the alcohols in the dried eluent by adding 25 µl of bis(trimethylsilyl)trifluoroacetamide and pyridine and heating the mixture at 60 °C for 20 min. This derivatized fraction, termed the total lipid fraction, was dissolved in ethyl acetate with a final concentration of 1 mg ml^{-1}.

Acid and base hydrolysis of complex polar lipids

An aliquot of the total lipid extract was separated by elution over a silica-gel column with hexane/ dichloromethane (1/1, v/v) and ethyl acetate. The fraction eluted with ethyl acetate was concentrated and subsequently hydrolyzed in 2 N HCl in methanol by heating it at 85 °C for 24 h. After hydrolysis, the solution was neutralized with $BaCO_3$ and subsequently centrifuged, after which the supernatant was removed. The $BaCO_3$ was repeatedly washed with methanol followed by centrifugation. The supernatants were pooled and concentrated to yield the acid methanolysis fraction, which was passed over a SiO_2-column using ethyl acetate as eluent, concentrated and silylated, and finally dissolved in ethyl acetate to a final concentration of 1 mg ml^{-1}. Another aliquot of the total lipid extract was hydrolyzed by refluxing in a 1 N KOH solution in 96% methanol and 4% water for 1 h. After hydrolysis the solution was neutralized by adding 2 N HCl in methanol, and the

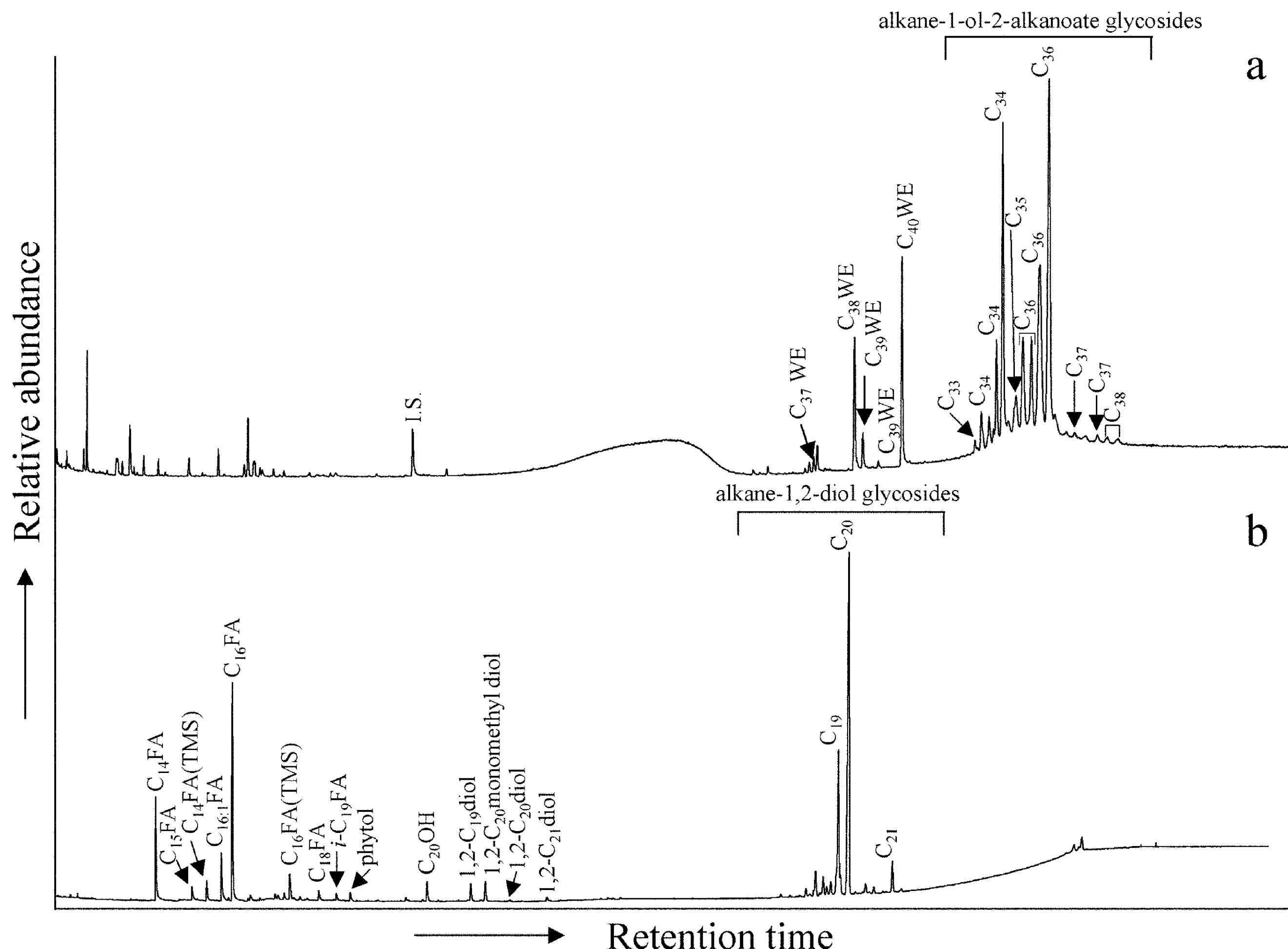

Fig. 1 **a** Partial total ion current chromatogram of the total lipid fraction from *Roseiflexus castenholzii*. The compounds indicated by "alkane-1-ol-2-alkanoate glycosides" consist of different combinations of alkane-1,2-diols, fatty acids, and C_6 sugars. The carbon numbers represent the total chain lengths of the alkane-1-ol-2-alkanoate side chains from the glycosides. **b** Partial gas chromatogram of the total lipid extract from *R. castenholzii* after basic hydrolysis. The compounds indicated by "alkane-1,2-diol glycosides" consist of different combinations of alkane-1,2-diols and C_6 sugars. The carbon numbers represent the straight-chain C_{19} diol, the C_{20} monomethyl diol, and straight-chain C_{21} diol

methanol was evaporated under a stream of N_2. The hydrolyzed material was dissolved in ethyl acetate, and traces of water were removed with a small sodium sulfate column. Subsequently, the ethyl acetate was removed by evaporation under a stream of N_2, and the basic hydrolysis fraction was methylated and silylated as described above, then dissolved in ethyl actetate to a final concentration of approximately 1 mg ml^{-1}.

The environmental samples were freeze-dried and extracted, methylated and silylated to give a total lipid fraction as described above.

Lipid analysis

The total lipid fractions of both culture and environmental samples and the hydrolyzed fractions from the culture were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) [see Schouten et al. (1998) for details].

An aliquot of the total lipid extract was analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) using an HP (Palo-Alto, Calif., USA) 1100 series LC/MS instrument equipped with an auto-injector and Chemstation chromatography manager software. Separation was achieved on an HP Eclipse XDB-C_{18} column (2.1×150 mm, 5 µm particle size), maintained at 40 °C. Injection volumes varied from 5 to 20 µl. Fatty glycosides were eluted with a combination of two solvents, A (80% methanol and 20% water) and B (100% methanol) = using the following program: a linear gradient from 75% A and 25% B to 50% B in 10 min was followed by a linear gradient from 50% B to 80% B in 5 min and by a linear gradient from 80% B to 100% B in 15 min. Flow rate was 0.6 ml min^{-1}. After each analysis the column was regenerated by back-flushing 100% methanol at 0.6 ml min^{-1} for 10 min. Lipids were detected using positive ion atmospheric pressure chemical ionization mass spectrometry (APCI-MS). Conditions for APCI-MS were as follows: nebulizer pressure at 345 kPa, vaporizer temperature was set at 400 °C, drying gas (N_2) flow 6 l min^{-1} and temperature 200 °C, capillary voltage –3 kV, corona 5 µA (~3.2 kV), fragmentor ramp 50–150 V for *m/z* 100–1,800, respectively. Positive ion spectra were generated by scanning *m/z* 100–1,000 in 1.9 s. Mass spectra presented typically represent the peak-apex spectrum and are corrected for background.

Results

Lipids of *R. castenholzii*

The total lipid fraction was dominated by unknown compounds of long retention times (Fig. 1a), the mass spectra

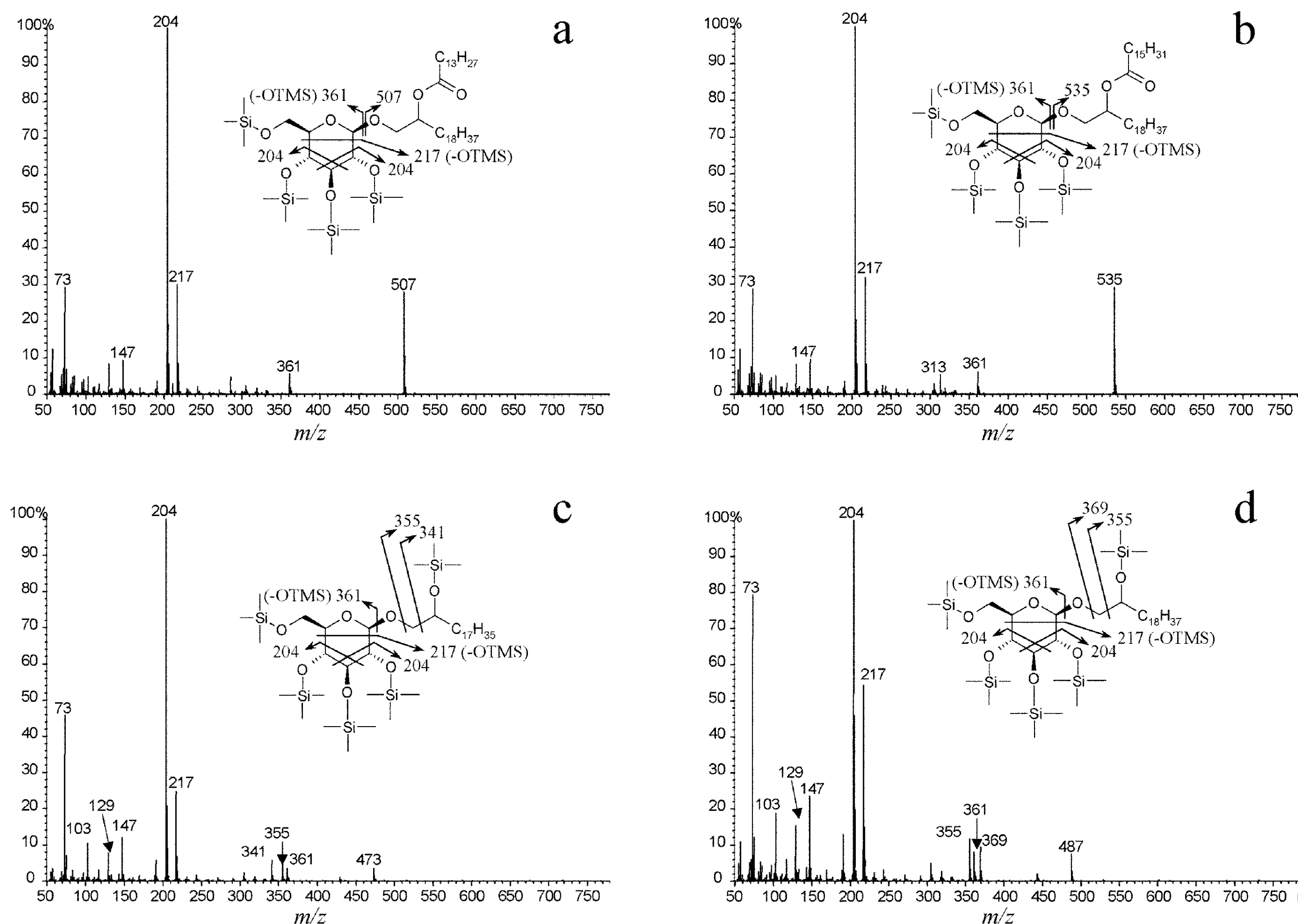

Fig. 2 Mass spectra of the dominant glycosides of *R. castenholzii* in the total lipid fraction (**a, b**), and in the total lipid extract after basic hydrolysis (**c, d**)

of which were dominated by *m/z* 204 and 217, typical fragment ions for silylated sugars (Kamerling and Vliegenthart 1989) and the *m/z* 361 fragment ion, which indicates a glycosidically bound hexose moiety (Sinninghe Damsté et al. 2001) (Fig. 2a, b). The major fragment ion at *m/z* 507, 521 or 535 suggests a homologous series of alkyl chains attached to the sugar moiety, likely at the glycosidic bond (Fig. 2a, b). No molecular ions could, however, be discerned.

To obtain more information about the structure of these unknown compounds, acid methanolysis was carried out to transform alkyl glycosides into methyl glycosides and at the same time separate the attached alkyl chain from the sugar moiety (Kamerling and Vliegenthart 1989; Sinninghe Damsté et al. 2001). The acid methanolysis fraction contained α and β isomers of two C_6 sugars in both the furanose and pyranose form, dominated by α glucose in the pyranose form, which were identified based on mass spectra and relative retention times (Kamerling and Vliegenthart 1989). This established the structure of the sugar moieties of the unknown compounds. The fraction also contained straight-chain C_{19} to C_{21} alkane-1,2-diols (Table 2), identified based on their mass spectra, relative retention times, and co-injection with a hydrolyzed red knot diester preen-gland wax fraction containing straight-chain alkane-1,2-diols ranging from C_{18} to C_{22} (Sinninghe Damsté et al. 2000). The alkane-1,2-diols were, however, dominated by the C_{20} monomethyl alkane-1,2-diol. Relative retention times showed that the position of the methyl group was internal (Kissin et al. 1986).

To gain further structural information, basic hydrolysis was also carried out on an aliquot of the total lipid extract. This treatment leaves the glycosidic bond intact but hydrolyzes ester bonds. Basic hydrolysis resulted in the release of C_{14} to C_{20} fatty acids, dominated by the C_{14} and C_{16} fatty acids, a C_{20} alkanol probably derived from the hydrolysis of the wax esters, and a relatively small amount of phytol, possibly derived from bacteriochlorophyll *a* (Hanada et al. 2002) (Fig. 1b). The fraction was, however, dominated by alkanediol glycosides (Fig. 1b). Their identification is based on the fragment ions of *m/z* 204 and 217 typical for silylated sugars (Kamerling and Vliegenthart 1989) and a fragment ion of *m/z* 361 indicating a glycosidically bound hexose moiety (Sinninghe Damsté et al. 2001) (Fig. 2c, d). The fragment ions at *m/z* 341 and 355 or *m/z* 355 and 369 indicate that the side chain consists of a C_{19} or C_{20} alkane-1,2-diol, respectively (Fig. 2c, d), as expected based on the acid methanolysis results. This also shows that the sugar moiety is attached

Table 2 Alkane-1,2-diols detected as hydrolysis products of complex polar lipids of the Octopus Spring mat and thermophilic bacteria. *Chloroflexus aurantiacus* does not contain glycosidically bound alkane-1ol-2-alkanoates. *Blank* Not present, + present, ++ present in relatively high abundance, +++ dominant alkane-1,2-diol

Diols	Carbon number	Octopus Spring[a]	*R. castenholzii*	*T. roseum*[b]	*Thermus* spp.[c]
iso-1,2-Heptadecanediol	17				+
iso-1,2-Octadecanediol	18				+++
anteiso-1,2-Octadecanediol	18				+
11-Methyl-1,2-heptadecanediol	18			+	
iso-1,2-Nonadecanediol	19				+
1,2-Nonadecanediol	19	++	++	+	
Branched-1,2-nonadecanediol[d]	20	+			
13-Methyl-1,2-nonadecanediol	20			++	
1,2-Icosanediol	20	+	+	+	
13-Methyl-1,2-icosanediol	21			+	
15-Methyl-1,2-icosanediol	21			+	
Branched-1,2-icosanediol[d]	21	+++	+++		
1,2-henicosanediol	21	+	+	+++	
15-Methyl-1,2-henicosanediol	22			+	
Branched-1,2-henicosanediol[d]	22	+			
1,2-Docasanediol	22			+	
1,2-Tricosanediol	23			+	
1,2-Tetracosanediol	24			+	

[a] Zeng et al. (1992a, b)
[b] Pond et al. (1986); Pond and Langworthy (1987); Perry (1992)
[c] Wait et al. (1997)
[d] Mid-chain mono-methyl branched, the position of the methyl branch is not exactly known and could be the same as reported for mono-methyl branched alkane-1,2-diols of *T. roseum*

to the alkane-1,2-diols via the hydroxy group at C-1. The presence of fatty acids released with basic hydrolysis indicates that they were esterified to a large molecule like the glycosides described above, either to a free hydroxyl group of the sugar moiety or the second hydroxyl group of the alkane-1,2-diol moiety. The mass spectra of the original unknown compounds in the total lipid extract shed more light on this question (Fig. 2a, b). The abundant *m/z* 507 or 535 fragment ions can be explained by fragmentation next to an alkane-1,2-diol moiety esterified to a fatty acid (Fig. 2a, b). Thus, the combined evidence indicates that the dominant compounds in the total lipid extract are glycosides consisting of an alkane-1-ol-2-alkanoate glycosidically bonded to a C_6 sugar (Fig. 2a, b). The glycoside isomers were dominated by the branched C_{20} alkane-1,2-diol/C_{14} fatty acid (*m/z* 507) and the branched C_{20} alkane-1,2-diol/C_{16} fatty acid (*m/z* 535) combinations (Figs. 1a, 2b). However, other combinations of C_{19}, C_{20} monomethyl, or C_{21} alkane-1,2-diols ester-linked to fatty acids ranging from C_{14} to C_{19}, and possibly different

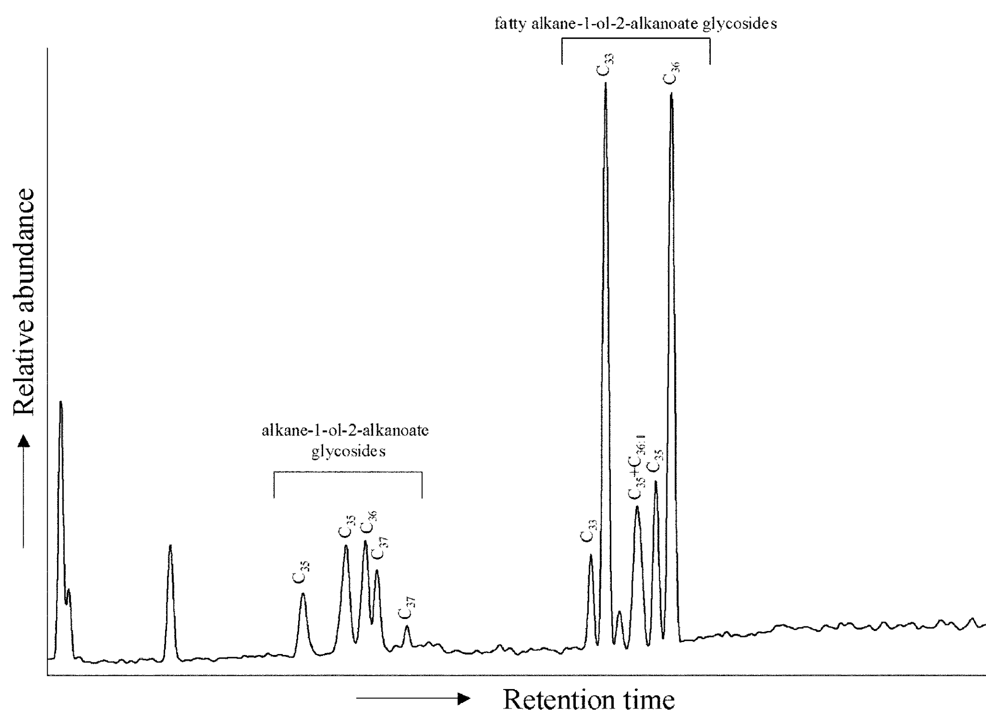

Fig. 3 Partial total ion current chromatogram of the total lipid extract from *R. castenholzii* as measured by HPLC-MS. The carbon numbers represent the total chain lengths of the alkane-1-ol-2-alkanoate side chains

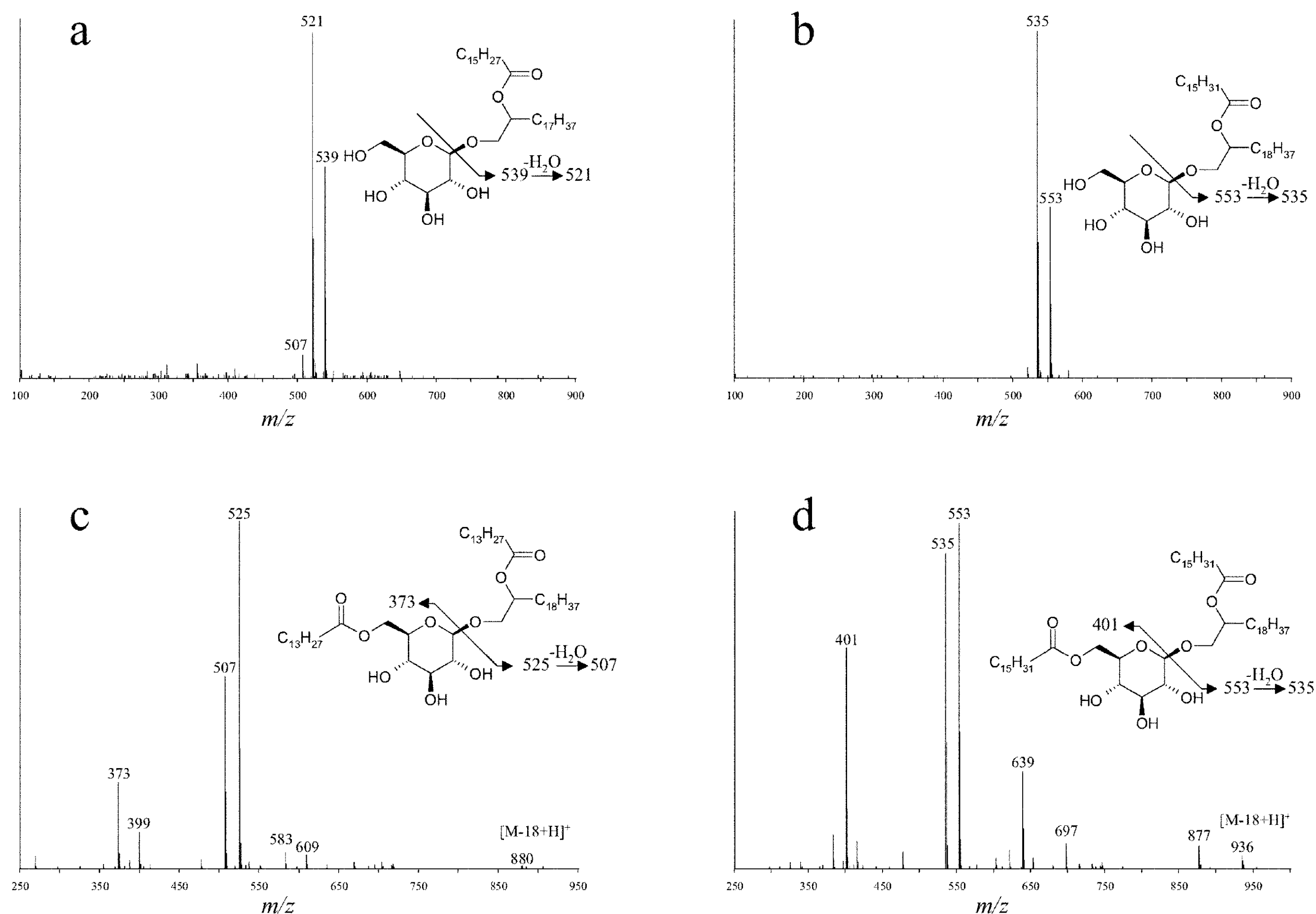

Fig. 4 Positive ion atmospheric pressure chemical ionization mass spectra of (**a, b**) glycosides and (**c, d**) fatty glycosides from the total lipid extract of *R. castenholzii,* measured by HPLC-MS

forms of C_6 sugars, are also present, resulting in the complex cluster of compounds visible in the chromatogram (Fig. 1a).

In order to extend the analysis of the total lipid extract from *R. castenholzii* to compounds with a molecular mass outside the analytical window of the GC-MS (molecular mass>800 Da) the total lipid extract was analyzed by HPLC-MS. This showed the presence of the same glycosides as determined by GC-MS (Figs. 3 and 4a, b), but the total ion current chromatogram was dominated by compounds with an even higher molecular mass. Their APCI mass spectra suggest that they are fatty glycosides, consisting of a sugar moiety glucosidically linked to an alkane-1-ol-2-alkanoate and ester-linked to a fatty acid (Fig. 4 c, d). The carbon position to which the fatty acids are linked has not been determined. However, assuming a similar configuration as reported for the monocyclic carotenoid glucoside ester isolated from *C. aurantiacus* based on ^{1}H-NMR (Takaichi et al. 1995), the alkane-1-ol-2-alkanoate would be linked to the C-1 position of the sugar, while the fatty acid would be linked to the C-6 position. Additional analyses are necessary to elucidate the exact structure of these compounds.

The total lipid fraction obtained from the *R. castenholzii* culture also contained C_{37} to C_{40} wax esters, dominated by the normal C_{38} and C_{40} wax esters, and hardly any free fatty acids and alkanols (Fig. 1a).

Occurrence of novel glycosidic lipids in hot spring microbial mats

GC-MS analyses of total lipid extracts from hot spring microbial mats containing type-C *Chloroflexus* relatives (e.g. Mushroom Spring and Octopus Spring microbial mats) (Ruff-Roberts et al. 1994; U. Nübel et al. submitted) indicate that these mats also contain long-chain glycosides similar, though not identical, to the alkanediol glycosides found in the *R. castenholzii* culture, although in relatively small amounts (Fig. 5a, b). Dominant mass fragments (see Fig. 2a, b for comparison) of the long-chain glycosides detected in the microbial mats indicate that the Mushroom Spring 54 °C mat contains glycosides with alkane-1-ol-2-alkanoate side chains ranging from C_{34} to C_{39}, dominated by C_{35} and C_{39} and including mono-unsturated C_{35} and C_{36} side chains (Fig. 5). The alkane-1-ol-2-alkanoate side chains of the alkanediol glycosides from the 58 to 64 °C Octopus Spring mat show a slightly differ-

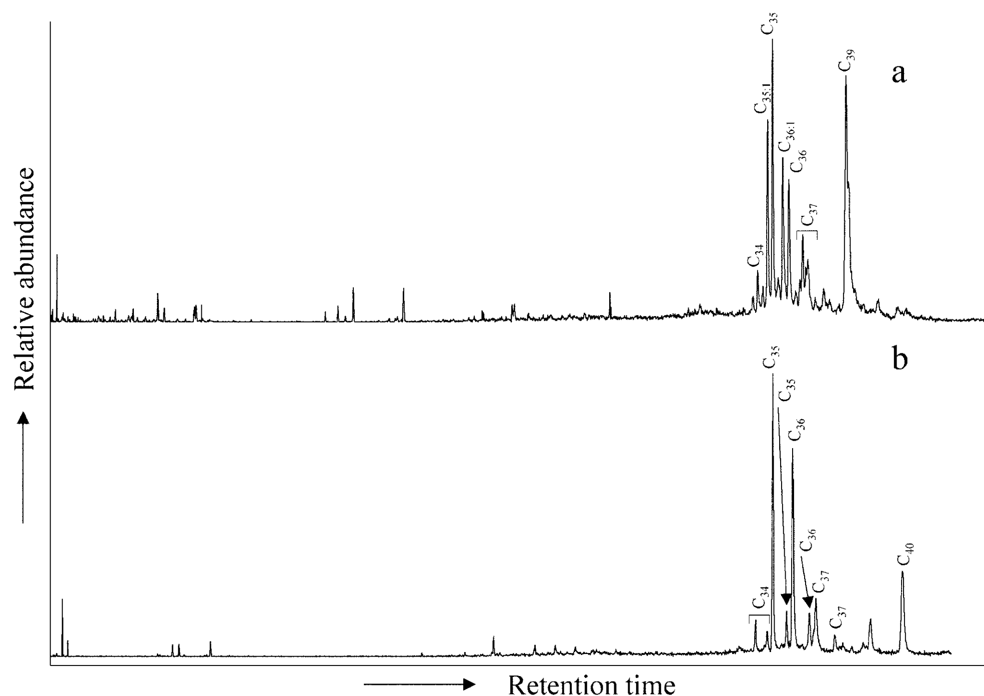

Fig. 5 Partial mass chromatogram of **a** *m/z* 204 and 217 of the total lipid extract from the Mushroom Spring 54 °C microbial mat and **b** the total lipid extract from the Octopus Spring 58–64 °C microbial mat. The carbon numbers represent total chain lengths of the alkane-1-ol-2-alkanoate side chains from the different glycosides

ent distribution from C_{34} to C_{40}, dominated by the C_{35} and C_{36} side chains (Fig. 5). There are no alkanediol glycosides in the Octopus Spring mat sample with mass fragments indicating mono-unsaturated side chains.

Discussion

The dominant lipids of *R. castenholzii* were glycosides and fatty glycosides consisting of an alkane-1-ol-2-alkanoate (mainly branched C_{20} alkane-1,2-diol/C_{14} fatty acid and branched C_{20} alkane-1,2-diol/C_{16} fatty acid) glycosidically bonded to a C_6 sugar (Fig. 2a, b). Alkane-1,2-diol-based glycosides have also been reported for other hot spring isolates (e.g., *Thermus* spp., based on fast atom bombardment/mass spectrometry analysis and acid hydrolysis of purified glycolipids (Wait et al. 1997) and *Thermomicrobium roseum*, based on acid methanolysis of purified glycolipids (Pond et al. 1986; Pond and Langworthy 1987; Perry 1992)). There are, however, differences in carbon number distributions of the alkane-1,2-diol (Table 2) and fatty acid components between these organisms and *R. castenholzii*. The long-chain diols produced by *T. roseum* include *n*-C_{19} to *n*-C_{24} and branched C_{18}, C_{20}, C_{21} and C_{22} alkane-1,2-diols, and are dominated by the *n*-C_{21} diol. The fatty acids range from C_{16} to C_{20}, including mono- and dimethyl fatty acids, and are dominated by normal and monomethyl C_{18} fatty acids (Pond et al. 1986; Pond and Langworthy 1987; Perry 1992). Several *Thermus* spp. produce mainly *iso*-C_{18}, lower amounts of *anteiso*-C_{18}, and trace amounts of *iso*-C_{17} and *iso*-C_{19} alkane-1,2-diols, while the fatty acids range from C_{13} to C_{20}, including normal, *iso*- and *anteiso*-fatty acids, and are dominated by iso-C_{15} and C_{17} fatty acids (Wait et al. 1997). The authors of these reports suggested that the studied organisms produce alkane-1,2-diol-based membrane lipids instead of or together with glycerol-based membrane lipids (Pond et al. 1986; Pond and Langworthy 1987; Perry 1992; Wait et al. 1997). Glycerol-based membrane lipids were not detected in *R. castenholzii*, suggesting that the alkane-1,2-diol-based lipids may also fulfill this role in *R. castenholzii*. It is interesting to note the greater similarity in glycosides and their alkane-1,2-diol components of *R. castenholzii* and *Thermomicrobium roseum*, since these two organisms represent relatively early diverging branches of the green nonsulfur bacterial kingdom (U. Nübel et al., submitted) and thus share a common ancestry. Interestingly, neither glycosides nor their alkane-1,2-diol components have been detected in an autotrophically grown *C. aurantiacus* OK-70 fl strain (van der Meer et al. 2000) or a heterotrophically grown *C. aurantiacus* Y-400 strain (van der Meer, unpublished results). *Thermus* spp. belong to another kingdom in Domain Bacteria (Weisburg et al. 1989).

Glycosides and fatty glycosides of *R. castenholzii* generally resembled those detected in the two nonsulfidic mat samples studied in terms of size range (compare Figs. 1a and 5). Differences in lipid compositions found in the two mat samples could be due to differences in species composition at different temperatures in the mat. For example, in the Mushroom Spring mat at 63 °C the filament population is dominated by type-C organisms while the relative contribution of *C. aurantiacus* relatives increases at higher temperature (U. Nübel et al., submitted). Alternatively, a single organism producing these alkanediol glycosides in the microbial mats might acclimate to different temperatures by adjusting the membrane lipids it produces. There is a major difference in the abundance of

glycosides and fatty glycosides produced by *R. castenholzii* and the abundance of these lipids found in the mats, suggesting either that other organisms might also be sources of these lipids in the mats, and/or that lipids synthesized in culture differ from those synthesized in nature.

Wax esters were more minor components of *R. castenholzii*; the chain lengths were longer than those detected in *C. aurantiacus* (Table 1). The dominant lipids in the total lipid fraction from nonsulfidic hot spring microbial mats (e.g. Mushroom Spring and Octopus Spring) sampled around 60 °C are usually wax esters ranging from C_{30} to C_{37}, including *iso*-branched C_{30} to C_{36} wax esters (Shiea et al. 1991). *C. aurantiacus* in culture produces wax esters ranging from C_{31} to C_{38}, including C_{32}, C_{34} and C_{36} mono-unsaturated wax esters (Shiea et al. 1991; van der Meer et al. 2001). Although the wax ester distributions in the mats are more comparable with those in *C. aurantiacus* (Table 1), our results do suggest the possibility that *R. castenholzii* or closely related organisms could be a source of the wax esters found in the mats. However, if true, there would again have to be a major difference in lipid expression between the *R. castenholzii* culture we studied and the predominant natural species (e.g., type-C organisms) to account for the differences in chain length and abundance.

In contrast to *C. aurantiacus*, *R. castenholzii* does not produce long-chain poly-unsaturated alkenes (Shiea et al. 1991; van der Meer et al. 2001). The nonsulfidic hot spring microbial mats contain only trace amounts of these long-chain alkenes (Dobson et al. 1988; Shiea et al. 1991; M.T.J. van der Meer et al., unpublished results), which could be related to the relatively low abundance of *C. aurantiacus* relatives at these temperatures (U. Nübel et al., submitted). The presence of *R. castenholzii* or closely related organisms, such as the type-C organisms, is consistent with the presence of alkanediol glycosides as well as the presence of wax esters and the low abundance of long-chain alkenes in these mats. If *C. aurantiacus* were an important source organism, a high abundance of hentriacontatriene would be expected, as observed in mats of sulfidic springs where closer phylogenetic relatives of *C. aurantiacus* are observed.

Our research points to the possibility that organisms resembling *R. castenholzii* might be the source of several mat lipids, such as alkane-1,2-diol glycosides and wax esters. However, it will be necessary to obtain pure cultures of the type-C organisms, and to understand their lipid expression under in situ conditions, before a complete explanation is obtained.

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References

Bauld J, Brock TD (1973) Ecological studies of *Chloroflexis*, a gliding photosynthetic bacterium. Arch Microbiol 92:267–284

Boomer SM, Lodge DP, Dutton BE, Pierson B (2002) Molecular characterization of novel red-green nonsulfur bacteria from five distinct hot spring communities in Yellowstone National Park. Appl Env Microbiol 68:346–355

Castenholz RW (1973) The possible photosynthetic use of sulfide by the filamentous phototrophic bacteria of hot springs. Limnol Oceanogr 18:863–876

Dobson G, Ward DM, Robinson N, Eglinton G (1988) Biogeochemistry of hot spring environments: extractable lipids of a cyanobacterial mat. Chem Geol 68:155–179

Ferris MJ, Ward DM (1997) Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl Environ Microbiol 63:1375–1381

Hanada S, Hiraishi A, Shimada K, Matsuura K (1995) *Chloroflexus aggregans* sp. nov., a filamentous phototrophic bacterium which froms dense cell agregates by active gliding movement. Int J Syst Bacteriol 45:676–681

Hanada S, Takaichi S, Matsuura K, Nakamura K (2002) *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium which lacks chlorosomes. Int J Syst Evol Microbiol 52:187–193

Kamerling JP, Vliegenthart JFG (1989) Carbohydrates. In: Lawson AM (ed) Mass spectrometry. De Gruyter, Berlin, pp 175–263

Kissin YV, Feulmer GP, Payne WB (1986) Gas chromatographic analysis of polymethyl-substituted alkanes. J Chrom Sci 24: 164–169

Knudsen E, Jantzen E, Bryn K, Ormerod JG, Sirevåg R (1982) Quantative and structural characteristics of lipids in *Chlorobium* and *Chloroflexus*. Arch Microbiol 132:149–154

Perry JJ (1992) The genus *Thermomicrobium*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes, 2nd edn. Springer, Berlin Heidelberg New York, pp 3775–3779

Pond JL, Langworthy TA (1987) Effect of growth temperature on the long-chain diols and fatty acids of *Thermomicrobium roseum*. J Bacteriol 169:1328–1330

Pond JL, Langworthy TA, Holzer G (1986) Long-chain diols: A new class of membrane lipids from a thermophilic bacterium. Science 231:1134–1136

Ruff-Roberts AL, Kuenen JG, Ward DM (1994) Distribution of cultivated and uncultivated cyanobacteria and *Chloroflexus*-like bacteria in hot spring microbial mats. Appl Environ Microbiol 60:697–704

Schouten S, Klein Breteler WCM, Blokker P, Schogt N, Rijpstra WIC, Grice K, Baas M, Sinninghe Damsté JS (1998) Biosynthetic effects on the stable carbon isotopic composition of algal lipids: implications for deciphering the carbon isotopic biomarker record. Geochim Cosmochim Acta 62:1397–1406

Shiea J, Brassell SC, Ward DM (1991) Comparative analysis of extractable lipids in hot spring microbial mats and their component photosynthetic bacteria. Org Geochem 17:309–319

Sinninghe Damsté JS, Dekker M, van Dongen BE, Schouten S, Piersma T (2000) Structural identification of diester preengland waxes of the Red Knot (*Calidris canutus*). J Nat Prod 63:381–384

Sinninghe Damsté JS, van Dongen BE, Rijpstra WIC, Schouten S, Volkman JK, Geenevasen JAJ (2001) Novel intact glycolipids in sediments from an Antarctic lake (Ace Lake). Org Geochem 32:321–332

Summons RE, Jahnke LL, Simoneit BRT (1996) Lipid biomarkers for bacterial ecosystems: studies of cultured organisms, hydrothermal environments and ancient sediments. In: Block GR, Goode JA (eds) Evolution of hydrothermal ecosystems on Earth (and Mars?). Ciba Foundation Symposium 202, Wiley, Chichester, pp 174–194

Takaichi S, Tsuji K, Matsuura K, Shimada K (1995) A monocyclic carotenoid glucoside ester is a major carotenoid in the green filamentous bacterium *Chloroflexus aurantiacus*. Plant Cell Physiol 36:773–778

Van der Meer MTJ, Schouten S, de Leeuw JW, Ward DM (2000) Autotrophy of green non-sulphur bacteria in hot spring microbial mats: biological explanations for isotopically heavy organic carbon in the geological record. Env Microbiol 2:428–435

Van der Meer MTJ, Schouten S, van Dongen BE, Rijpstra WIC, Fuchs G, Sinninghe Damsté JS, de Leeuw JW, Ward DM (2001) Biosynthetic controls on the ^{13}C-contents of organic components in the photoautotrophic bacterium *Chloroflexus aurantiacus*. J Biol Chem 276:10971–10976

Wait R, Carreto L, Nobre MF, Ferreira AM, Da Costa MS (1997) Characterization of novel long-chain 1,2-diols in *Thermus* species and demonstration that *Thermus* strains contain both glycerol-linked and diol-linked glycolipids. J Bacteriol 179:6154–6162

Ward DM, Weller R, Shiea J, Castenholz RW, Cohen Y (1989) Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionaty significance. In: Cohen Y, Rosenberg E (eds) Microbial mats, physiology ecology of benthic microbial communities. American Society for Microbiology, Washington, pp 3–15

Ward DM, Santegoeds CM, Nold SC, Ramsing NB, Ferris MJ, Bateson MM (1997) Biodiversity within hot spring microbial mat communities: molecular monitoring of enrichment cultures. Antonie van Leeuwenhoek 71:143–150

Weisburg WG, Giovannoni SJ, Woese CR (1989) 16S rRNA sequences for *Deinococcus radiodurans* and *Thermus thermophilus*. Caution about thermophilic convergence effects due to G-C enrichment. System Appl Microbiol 11:128–134

Weller R, Weller FW, Ward DM (1991) 16S rRNA sequences retrieved as randomly primed cDNA from a hot spring cyanobacterial mat community. Appl Environ Microbiol 57:1146–1151

Zeng YB, Ward DM, Brassell SC, Eglinton G (1992a) Biogeochemistry of hot spring environments 2. Lipid composition of Yellowstone (Wyoming, U.S.A.) cyanobacterial and *Chloroflexus* mats. Chem Geol 95:327–345

Zeng YB, Ward DM, Brassell SC, Eglinton G (1992b) Biogeochemistry of hot spring environments 3. Apolar and polar lipids in the biologically active layers of a cyanobacterial mat. Chem Geol 95:347–360