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Bacteriohopanepolyols preserved in silica sinters from Champagne Pool (New Zealand) indicate a declining temperature gradient over the lifetime of the vent

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ABSTRACT

Siliceous sinters from Champagne Pool (CP), a geothermal vent in the Taupo Volcanic Zone, North Island, New Zealand contained exceptionally well-preserved bacteriohopanepolyol (BHP) distributions. BHPs were present in a range of sinters deposited over the lifetime of CP (900 yr before present). The distributions correlated well with the presence of members of BHP-producing bacterial orders known to inhabit CP. For example, *Acidithiobacillales*, *Burkholderiales* and *Pseudomonadales* are known sources of composite BHPs (with a more complex functional group at C-35), such as bacteriohopanetetrol cyclitol ether, found in high relative abundance in each CP sinter analysed. The BHP distributions preserved in CP sinters were different from those described previously for any other environmental setting, with pentafunctionalised BHPs accounting for up to 55% of the total assemblage. Furthermore, composite BHPs were the most abundant compound type in each sample and accounted for up to 80% of the total BHP distribution. As the age of sinter increased a general increase in BHP abundance was observed. Similarly, in older sinters the complexity of the BHP compounds increased. The observations are consistent with a higher temperature of vent water in the past. The excellent preservation of BHPs in this setting, over the lifetime of the vent, highlights the usefulness of BHPs as biomarkers for reconstructing modern and ancient geothermal vent bacterial communities.

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1. Introduction

Terrestrial geothermal vents provide attractive localities for studying thermophilic microbial populations. Such ecosystems are commonly used as analogues for the early Earth (Walter, 1996) and as model settings for studying the preservation of bio-signatures relevant to astrobiological investigations (e.g. Summons et al., 2011). It has been shown that the mineral deposits associated with silica-depositing geothermal vents in the Taupo Volcanic Zone (TVZ; New Zealand) contain distributions of well preserved lipid biomarkers (Pancost et al., 2005; Talbot et al., 2005), which detail the presence of certain groups of microorganisms (e.g. Pancost et al., 2005; Gibson et al., 2008) and record changes in vent biogeochemistry over time (Kaur et al., 2008, 2011a).

One such family of lipid biomarkers, the bacteriohopanepolyols (BHPs), are membrane components produced by a wide range of bacteria (e.g. Rohmer et al., 1984; Talbot et al., 2008 and references therein). The structures consist of a pentacyclic hydrocarbon

skeleton with an extended, polyfunctionalised side chain (see Appendix for examples of structures). Typical functionalisation includes OH and NH₂ groups but may also comprise more complex moieties such as amino sugars at the terminal C-35 position, referred to as “composite BHPs”. These ubiquitous compounds are widespread throughout modern environments, including lacustrine and marine sediments (e.g. Talbot and Farrimond, 2007; Pearson et al., 2007; Zhu et al., 2011), soils (e.g. Cooke et al., 2008), peats (van Winden et al., 2012), water columns (Blumenberg et al., 2007; Wakeham et al., 2007; Sáenz et al., 2011a, 2011b; Berndmeyer et al., 2013) and hot spring microbial mats (Zhang et al., 2007).

Previous investigations have identified BHPs in silica sinters of the TVZ (e.g. Talbot et al., 2005; Pancost et al., 2005, 2006; Gibson et al., 2008) and have shown that incorporation into the sinter matrix of such deposits facilitates preservation of the functionality of BHP compounds (Gibson et al., 2008). However, these studies did not provide any information regarding differences in BHP distributions imposed by spatial and temporal variation in depositional settings. Therefore, in this study we have analysed BHP distributions from a variety of depositional settings within CP, including

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a sub-aqueous sulfidic precipitate, an actively precipitating sinter from the present day air–water interface and a series of older inactive sinters collected at increasing distance from the pool edge. This sampling regime allowed us to investigate BHP preservation over time and provided insight into how BHPs relate to changing bacterial populations and evolving environmental conditions.

2. Material and methods

2.1. Samples and location

Silica sinter samples and a sulfur precipitate were collected from CP, located at the Wai-o-tapu geothermal area in the TVZ (water temperature 75 °C, pH 5.5 at time of sampling). Samples were identical to those reported by Kaur et al. (2008, 2011a,b; see also Kaur, 2009) and the same sample numbers are employed here (Table 1). Maps of the individual sampling locations can be found in Kaur et al. (2011a, b). Sample CPa19 was an orange sulfur precipitate suspended in the vent waters at CP. The precipitate covers sub-aqueous sinters and domal stromatolites and is thought to be composed of As-Sb silicified filaments of thermophilic anaerobic bacteria or archaea (Jones et al., 2001). Silica sinters form when amorphous silica precipitates from geothermal fluids. The deposition of silica sinter results generally in a ‘radial contraction’ (Kaur et al., 2008) of the surface area of the pool and dictates that sinters collected from the current pool edge are the youngest and that those away from the pool edge were deposited further back in time, thereby providing a putative time sequence of silica deposition. For this investigation, a sinter sample collected from the pool edge was assumed to be the youngest (CPa1) and a range of four additional sinters (CPa4, CPa10, CPa13 and CPa16; Table 1; Kaur

et al., 2011b) taken from increasing distance from the pool edge were used to represent the depositional history at CP. The oldest sample (CPa16) was collected ca. 20 m from the pool edge (Kaur et al., 2011b) and likely dates to the time of formation of CP around 700–900 yr before present (Mountain et al., 2003).

2.2. Extraction and analysis

Prior to extraction sinters were rinsed with dichloromethane (DCM)/MeOH (1:1 v/v) to remove organic matter not incorporated into the silica matrix. Samples were freeze dried and powdered prior to extraction using a modified Bligh and Dyer method as described by Cooke et al. (2008). Ca. 3 g of powdered sinter was extracted in each case. The extract was acetylated as described by Talbot et al. (2008) and analysed using atmospheric pressure chemical ionization–high performance liquid chromatography–multistage mass spectrometry (HPLC–APCI–MSⁿ) with a Thermo-Finnigan LCQ ion trap mass spectrometer (e.g. Talbot et al., 2003a,b, 2007a,b). A semi-quantitative estimate of BHP abundance was achieved by comparison of the area of each base peak ion with the base peak area of acetylated 5 α -pregnane-3 β ,20 β -diol. Averaged response factors for BHPs were calculated by comparison with an internal standard and the response of a suite of five acetylated authentic BHP standards. This showed that BHPs containing one or more N atoms give an average response 12 x that of the standard and BHP compounds with no N atoms give a response approximately 8 x that of the standard. Peak areas of BHPs were adjusted relative to these response factors (van Winden et al., 2012). Reproducibility for the approach is \pm 20% (Cooke, 2010).

Table 1
Concentration (μ g/g TOC) and relative abundance (%) of BHPs in precipitate and sinter samples at CP.

BHP	Structure	Precipitate	Active	Inactive ^a			
		CPa19 ^b	CPa1	CPa4	CPa10	CPa13	CPa16
BHpentol cyclitol ether	I	70 ^c	420	61	140	1880	7140
		25.2 ^d	38.8	40.1	31.1	32.6	39.7
BHT cyclitol ether	II	43	180	61	140	2350	5230
		15.5	16.6	40.1	31.1	40.8	29.1
Aminotriol	III	130	320	10	34	230	1860
		46.7	29.5	6.6	7.5	4.0	10.3
BHT	IV	35	110	10	tr ^e	180	1810
		12.6	10.2	6.6	tr ^e	3.1	10.1
BHT glucosamine	V		53	tr ^e	tr ^e	59	240
			4.9			1.0	1.3
BHpentol	VI			10	69	710	1140
				6.6	15.3	12.3	6.3
AnhydroBHT	VII			tr ^e	34	180	190
AnhydroBHP ^f	VIII				7.5	3.1	1.1
				tr ^e	34	180	140
BHT pseudopentose	IX				7.5	3.1	0.8
				tr ^e		tr ^e	95
2-methyl BHT pseudopentose	X						0.5
							48
Guanidine BHT cyclitol ether	XI						0.3
							95
Total BHPs (μ g/g TOC)		278	1083	152	451	5769	17,988
Composite BHPs (%)		41	60	80	62	74	71
TOC (%) ^g		2.6	0.38	0.98	0.29	0.17	0.21

^a Samples are listed in order of increasing distance from the pool edge, i.e. according to age.

^b Sample numbers of silica sinters from CP, samples are identical to those in Kaur et al. (2008, 2011).

^c Concentration (μ g/g TOC).

^d Italic font indicates % of total BHPs.

^e Trace, compound present but at level too low for quantification.

^f Sum total of a number of isomeric forms of anhydroBHP (see Fig. 1).

^g Values are from Kaur et al. (2011a) except CPa19 from Kaur et al. (2011b).

3. Results

3.1. BHPs in an actively precipitating sinter and sub-aqueous sulfur precipitate of CP

Four commonly occurring BHPs were present in the sulfur precipitate (sample CPa19; Fig. 1a; Table 1) from the pool water of CP including: bacteriohopanepentol cyclitol ether (I, BHpentol cyclitol ether), bacteriohopanetetrol cyclitol ether (II; BHT cyclitol ether), aminobacteriohopanetriol (III; aminotriol) and bacteriohopanetetrol (IV; BHT). The active sinter (CPa1; Fig. 1b; Table 1) contained BHTglucosamine (V) as well as the same 4 compounds in the sulfur precipitate (CPa19). Total BHP concentration was 278 and 1083 $\mu\text{g/g}$ TOC (CPa19 and CPa1 respectively); however, the relative distributions varied, with aminotriol the most abundant compound in the sulfur precipitate (CPa19), whilst the composite structure BHpentol cyclitol ether had the highest concentration in the active sinter (CPa1; Table 1).

3.2. BHPs in inactive sinters

Concentrations of BHPs were higher in the oldest sinters (CPa13 and CPa16; Table 1; Fig. 2), with a maximum of 18 mg/g TOC (CPa16; Table 1; BHP distribution shown in Fig. 1c). BHpentol cyclitol ether (I) and BHT cyclitol ether (II) occurred in each of the inactive sinters (CPa4, CPa10, CPa13 and CPa16; Table 1) and were typically the most abundant BHPs, with concentrations increasing with distance from the pool edge (Table 1). BHT (IV), aminotriol (III), BHT glucosamine (V), bacteriohopanepentol (VI, BHpentol), BHT pseudopentose (IX; Talbot et al., 2008) was present in CPa4, CPa13 and CPa16 and 2-methyl BHT pseudopentose (X) in CPa16 only, which also contained guanidine-substituted bacteriohopanetetrol cyclitol ether (XI; guanidine BHT cyclitol ether; Renoux and Rohmer, 1985; Eickhoff et al., 2013).

All samples contained both tetra- and pentafunctionalised BHPs (Table 1; Fig. 2) with increasing contributions from composite structures in the older inactive samples (Fig. 2), as well as a more diverse range of structures, up to 11 in total, in the oldest sample (CPa16) compared with only 4 or 5 in the active samples (CPa19 and CPa1 respectively; Fig. 2c). Furthermore, in CPa13 and CPa16, pentafunctionalised BHPs accounted for ca. 45% of the total BHP assemblage, whereas in younger samples (CPa19 and CPa1), they accounted for only 25% and 39%, respectively (Fig. 2).

The BHP degradation products, anhydroBHT and anhydroBHP (VII and VIII; Bednarczyk et al., 2005; Talbot et al., 2005), were present only in the inactive sinters at trace level in the youngest sample (CPa4; Table 1), increasing up to 190 and 140 $\mu\text{g/g}$ TOC (for anhydroBHT and anhydroBHP respectively) in the oldest inactive sinter (CPa16). However, although total concentration increased, the relative contribution to the total BHP assemblage decreased with age (Fig. 2).

4. Discussion

4.1. Potential sources of BHPs

The four BHPs in the sulfur precipitate (BHT, aminotriol, BHT cyclitol ether and BHpentol cyclitol ether; Table 1) are all common and with a variety of sources (e.g. Talbot et al., 2008). However, culture independent investigation of the bacterial community at CP (Childs et al., 2008), in combination with reports of BHP production by different species belonging to the different phylogenetic orders identified (see Table 2 for summary of species, BHP structures and associated references), allowed us to narrow down the likely sources. Microorganisms from the orders *Aquificales*, *Thermodesulfobacteriales* and *Bacillales* were identified from 16S rRNA sequencing in the sulfidic precipitate that forms in the vent water at CP (CPa19; referred to as P zone in Childs et al., 2008). However,

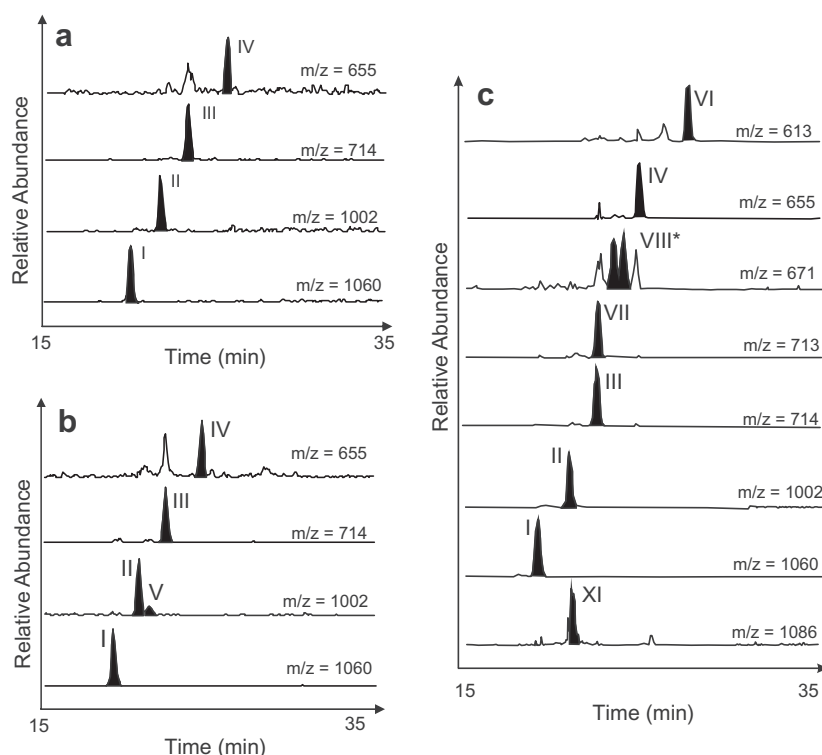


Fig. 1. Partial APCI mass chromatograms (15–35 min) showing BHPs in (a) sulfidic precipitate material (sample CPa19), (b) actively precipitating sinter (CPa1) and (c) the oldest sinter collected away from the modern day pool edge (CPa16; * indicates multiple isomers observed).

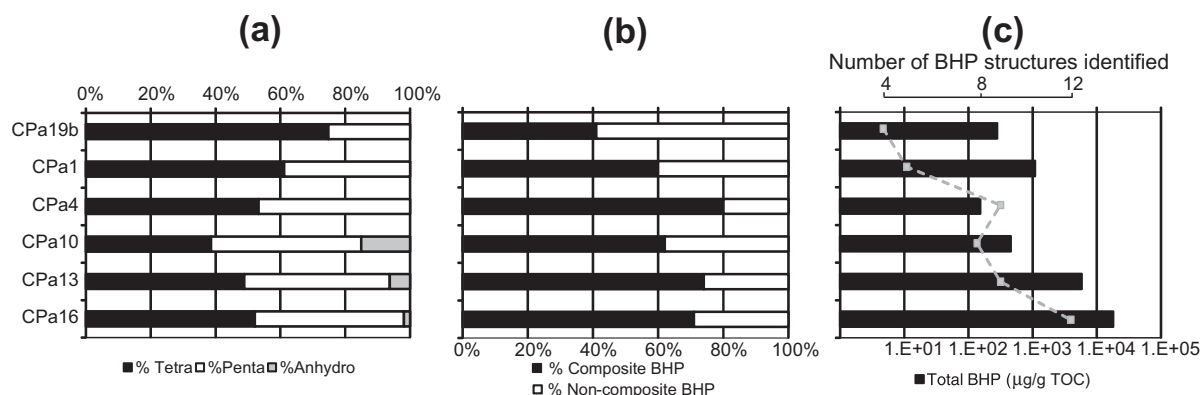


Fig. 2. Composition of BHP distributions. (a) Relative contributions of tetra-, pentafunctionalized and anhydro BHPs; (b) proportion (%) of composite BHPs and non-composite BHPs; (c) total concentration of BHPs ($\mu\text{g/g}$ TOC) and number of BHP structures in each sample.

Table 2
Bacterial orders with known BHP-producing genera identified at CP using culture-independent methods (Childs et al., 2008).

Order	Known BHP producers from same order ^a	BHPs observed
<i>Acidithiobacillales</i>	<i>Acidithiobacillus ferrooxidans</i> ³	II, III, IV, XIII
<i>Actinomycetales</i>	<i>Frankia</i> sp. ¹	IV, XIV (35-O-phenylacetyl-BHT), XV (35-O-propionyl-BHT)
	<i>Acidothermus</i> ⁴	15% of total lipid extract
	<i>Geodermatophilus</i> sp. ⁴	3% of total lipid
	<i>Streptomyces coelicolor</i> ⁶	III, IV
	<i>Streptomyces griseus</i> ⁶	III, IV
	<i>Streptomyces scabies</i> ¹⁰	III, IV
<i>Bacillales</i>	<i>Alicyclobacillus acidocaldarius</i> ^{5,12}	II, V, XVI (N-acylated BHT glucosamines)
	<i>Alicyclobacillus acidoterrestris</i> ⁹	IV, VII, XII, XVI, XVII (BHhexol)
<i>Burkholderiales</i>	<i>Burkholderia caryophylli</i> ²	II
	<i>Burkholderia gladioli</i> ²	II
	<i>Burkholderia cepacia</i> ²	I, II, V
	<i>Burkholderia pseudomallei</i> ²	II
<i>Planctomycetales</i>	<i>Candidatus "Brocadia anammoxidans"</i> ¹¹	IV
	<i>Candidatus "Scalindua" spp.</i> ¹¹	IV
	<i>Pirellula marina</i> ¹¹	IV
<i>Pseudomonadales</i>	<i>Azotobacter vinelandii</i> ¹³	I, II
	<i>Pseudomonas acidovorans</i> ⁷	b
	<i>Pseudomonas amygdali</i> ²	II
	<i>Pseudomonas syringae</i> ⁷	b

^a References to BHP production indicated by superscript number: ¹Berry et al. (1993); ²Cvejić et al. (2000); ³Jones et al. (2011); ⁴Maréchal et al. (2000); ⁵Poralla et al. (1984); ⁶Poralla et al. (2000); ⁷Rohmer et al. (1984); ⁸Rosa-Putra et al. (2001); ⁹Řezanka et al. (2011); ¹⁰Seipke and Loria (2009); ¹¹Sinninghe Damsté et al. (2004); ¹²Talbot et al. (2007a); ¹³Vilcheze et al. (1994).

^b Presence of BHPs determined via HIO₄ cleavage, whereby structure of side chain is lost.

Thermodesulfobacteriales and *Aquificales* are not known to produce BHPs or indeed to have the *sqhC* gene (squalene-hopene cyclase) which is required for BHP synthesis (e.g. Wendt, 1997; Tippelt et al., 1998).

In CPa19, composite structures (I and II; Table 1) comprised 41% of BHPs. This fits well with the presence of members of the *Bacillales* at this location. For example, *Alicyclobacillus acidocaldarius* (formerly *Bacillus acidocaldarius*) produces more composite BHPs when subjected to higher growth temperatures or under increasingly acidic culture conditions (Poralla et al., 1984; Table 2). Similarly, *A. acidoterrestris* was also recently shown to produce a high amount of composite BHPs (ca. 70% of the total BHP assemblage; Řezanka et al., 2011), including tetra and pentafunctionalised structures (Table 2); however, it also produced additional hexafunctionalised BHPs and N-acylated mannosamide BHPs (e.g. XII; Table 2) which were not found here. Furthermore, both *A. acidocaldarius* and *A. acidoterrestris* are aerobic, so the BHP distribution in CPa19, which formed under anoxic conditions, likely derived from an anaerobic member of the *Bacillales*. For example, *Bacillus anthracis* and *Bacillus subtilis* both grow under facultative anaerobic conditions with NO₃⁻ as electron acceptor (Ramos et al., 2000), *Anaerobacillus* sp. is a strictly anaerobic diazotrophic bacillus isolated from soda lake (Zavarzina et al., 2009) and *Anoxybacillus* spp. can also grow anaerobically (Pikuta et al., 2000).

Aminotriol (III), the most abundant compound in CPa19, has not been described in cultures of *Alicyclobacillus* spp. (Talbot et al., 2007a; Řezanka et al., 2011). This suggests that other BHP-producing organisms were present or that other members of the *Bacillales* produce BHPs, including aminotriol (III), when in their native environment but not under controlled culture conditions in the laboratory. The source of the aminotriol in CPa19 remains elusive.

Conditions around the perimeter of the vent are different from those in the centre of the pool. Evaporation and cooling of the vent waters provide a series of dynamic ecological niches around the pool edge where sinter formation proceeds more rapidly (Moun-tain et al., 2003). Childs et al. (2008) identified the microbial community associated with three distinct sample zones, including a sulfur deposit that forms at the air–water interface, micro-stromatolites and an acid-organic layer (represented by S, M and A zones in Childs et al. 2008). As the sample from the pool edge was homogenized before lipid extraction in this study, we assume that all three of the sample zones (air–water interface, micro-stromatolites and acid-organic layer) reported by Childs et al. (2008) were incorporated into one sample (CPa1). Although we analysed the three zones as one sample we can infer information regarding the sources of the BHPs in CPa1. For instance, the bacterial population found to exist at the air–water interface was quite similar to that in the sub-aqueous precipitate (S Zone) and consisted of *Aquificales*, *Thermodesulfobacteriales* and *Bacillales*. As mentioned above, of these orders, only members of the *Bacillales* (Table 2) are known to produce BHPs. The micro-stromatolites (M Zone)

are known to harbour a slightly different bacterial population that includes phylogenetic orders related to *Aquificales*, *Thermodesulfobacteriales*, *Bacillales*, *Burkholderiales* and *Desulfurellales*. Of these phylogenetic orders only *Bacillales* and *Burkholderiales* contain BHP producing genera (Table 2). The acid-organic layer (A zone) was shown to contain a more diverse assemblage of BHP-producing bacteria. These include the phylotypes *Acidithiobacillales*, *Actinomycetales*, *Planctomycetales* and *Pseudomonadales*.

Certain observations correlate well with the above potential source microorganisms (Table 2). For example, composite BHPs (I and II) were the most abundant compounds in each sinter and these, or related structures, are produced by members of the *Acidithiobacillales*, *Bacillales*, *Burkholderiales* and *Pseudomonadales* (Table 2). BHT (IV) and Aminotriol (III) were also identified in sample CPa1 and, based on previous studies, were most likely biosynthesised by members of the order *Actinomycetales*, such as *Frankia* spp. and *Streptomyces* spp. (Table 2). *Frankia* is closely related to *Acidotherrmus*, which also produces hopanoids in significant amount (Table 2; Maréchal et al., 2000) and is conceivably present in geothermal areas (Stetter et al., 1986). BHT has also been reported from some members of the order *Planctomycetales* (Sinnighe Damsté et al., 2004), also reported by Childs et al. 2008 (Table 2). However, members of the *Acidithiobacillales*, such as *Acidithiobacillus thioxidans* (Table 2), are particularly likely sources of BHPs in this setting as they are adapted to high temperature and low pH and have been shown to produce several of the BHPs found here, including BHT (IV), aminotriol (III) and BHT cyclitol ether (II) (Jones et al., 2011). The BHP distributions in the active sinters and sub aqueous precipitate material were therefore consistent with reports of the bacterial community at CP and demonstrate the utility of BHPs to profile bacterial communities in modern geothermal settings.

BHPentol (VII) was present in all inactive sinters at increasing concentration in the older samples (Table 1), consistent with its occurrence in a member of the *Bacillales* (*A. acidoterrestris*; Řezanka et al., 2011). However, BHPentol, together with BHT pseudopentose (IX), which was also identified at low to trace levels in three of the inactive sinters CPa4, CPa13 and CPa16 (Table 1), are most commonly associated with cyanobacteria (Bisseret et al., 1985; Zhao et al., 1996; Talbot et al., 2008). Cyanobacteria were not found at CP by Childs et al. (2008) but are known to colonise geothermal sinter formations (e.g. Walker et al., 2005; Phoenix et al., 2006), so the source of these compounds in this environment is uncertain. It should be noted that BHPentol (VII) could also be formed as a degradation product during loss of the C-35 ether linked moiety from BHPentol cyclitol ether (I; see potential sources indicated above) as recently shown for the formation of BHT from BHT glucosamine (Schaeffer et al., 2010). The C-2 methylated homologue of BHT pseudopentose (X) was also found in the oldest sample. This was the only methylated BHP observed in this study, in stark contrast to a previous report on a sinter sample from the Opaheke Pool (TVZ) that contained several C-2 and C-3 methylated BHPs but no composite BHP structures (Gibson et al., 2008). However, C-2 methylated composite structures were found in a microbial mat from a geothermal setting in North America (Zhang et al., 2007).

Guanidine-substituted cyclitol ether (XI) was present in the oldest sample (CPa16). It was first reported from methylotrophic bacteria (e.g. Renoux and Rohmer, 1985; Knani et al., 1994) and more recently in a *Geobacter* sp. (Eickhoff et al., 2013). Neither of these genera are members of the bacterial orders identified at CP in the study by Childs et al. (2008), suggesting an unknown source of guanidine-substituted cyclitol ether, or that the source of this particular compound is no longer present in the vent waters.

The BHP degradation products anhydroBHT and anhydroBHP (VII and VIII) were found exclusively in inactive sinters (Table 1). These compounds were also detected in previous studies of silica

sinters (e.g. Pancost et al., 2005, 2006; Talbot et al., 2005) and are both likely to be formed during diagenesis of tetra- and penta-functionalised BHPs, respectively (Schaeffer et al., 2008, 2010; Eickhoff et al., 2014). Where anhydroBHT and anhydroBHP were present, they represented minor components of the total BHP distribution. This suggests that BHP degradation in silica sinters is limited and it is difficult to see any trend in their distribution. Since anhydroBHT is known to be formed from tetrafunctionalised BHPs (e.g. Schaeffer et al., 2008, 2010), and anhydroBHP is assumed to follow the same pathway (Talbot et al., 2005), it would seem reasonable to expect an increase in the relative concentration of anhydroBHT and anhydroBHP (VI and VIII) with age of sample. However, as this was not the case here (Fig. 2), we suggest that BHPs are not significantly affected by early degradation processes and possibly other diagenetic pathways affect BHP distributions in silica sinters. This highlights the idea that BHPs preserved in silica sinters are excellent archives of palaeobiological information. A more detailed investigation of geohopanoid products in these systems is required to elucidate early stage degradation processes affecting BHP composition in sinters and would benefit the use of BHPs as biomarkers for ancient geothermal bacterial populations.

4.2. Environmental conditions inferred from BHP composition in silica sinters over the lifetime of CP

The BHP compositions in silica sinters appeared to agree with previous studies of the bacterial community at CP, but further important observations could be made from the data. First, BHPs were more abundant in the oldest inactive sinters than in the youngest active sinter [18 mg/g TOC and 1.1 and mg/g TOC respectively; Table 1]. Second, BHP distributions appeared to be composed predominantly of more complex compounds (i.e. composite BHPs) in older inactive sinters (62–80%; Table 1; Fig. 2).

An increase in the concentration of BHPs may indicate either a change in the bacterial community or a physiological response of a community to changing environmental conditions, such as the production of a higher concentration of BHPs at elevated temperature (e.g. Poralla et al., 1984). Indeed, work by Kaur et al. (2008, 2011a) suggests on the basis of several lines of biomarker evidence that the temperature of the vent waters at CP was higher in the past. These include an increase in the abundance of archaeol, increased cyclisation of the phytanyl moieties in glycerol dibiphytanyl glycerol tetraether lipids and a prevalence of fatty acids composed of longer chain lengths as sinter age increased (Kaur et al., 2011a).

The degree of functionalisation of the side chain of the BHPs also increased as the age of the sinters increase (Fig. 2), with pentafunctionalised BHPs accounting for 45–46% of the total BHP assemblage in the two oldest samples. If older samples were formed at higher temperature, these observations are in agreement with culture studies of the acetic acid bacterium *Frauteria aurantiaca*, where the ratio of total penta- to tetrafunctionalised BHPs increased by a factor of 5.5 when growth temperature was increased from 26 to 36 °C (Joyeux et al., 2004). Although the evidence here strongly suggests that the abundance and composition of the BHP distributions reflect higher temperature of vent waters in the past, the exact physiological role of how BHPs reinforce bacterial cell membranes remains to be determined.

The high proportion of pentafunctionalised BHPs vs. tetrafunctionalised BHPs, together with the corresponding total absence of hexafunctionalised BHPs, is in general contrast to investigations of non-geothermal settings. We have compiled a sub-set of representative literature data from our laboratory, showing the relative proportions of tetra-, penta- and hexafunctionalised BHPs (Fig. 3; note this excludes BHPs related to adenosylhopane, XIII; e.g. Talbot et al., 2007a). Many samples cluster close to the 100% Tetra-BHP apex. This signature is typical for soil/peat and also

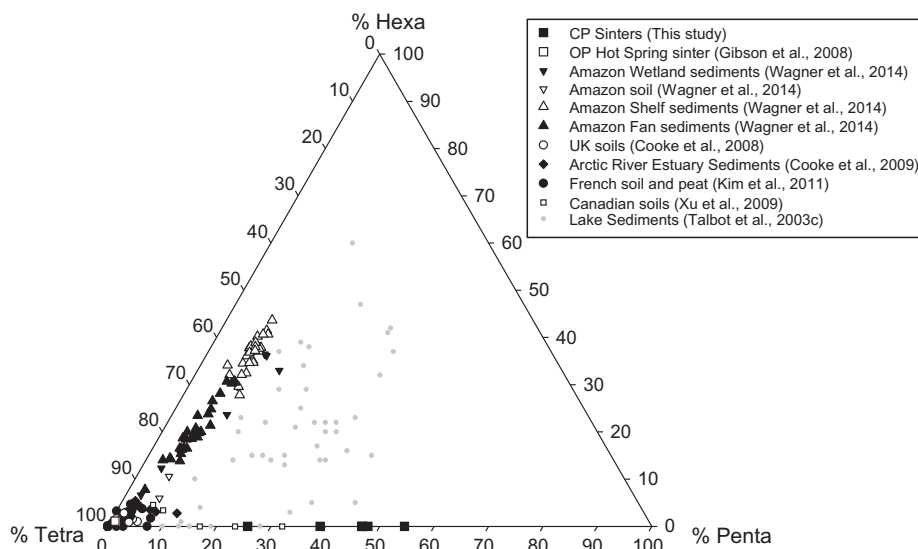


Fig. 3. Proportion (%) of tetra-, penta- and hexafunctionalised BHPs in samples from this study and selected literature sources, highlighting unusual BHP composition in CP sinters which have an unusually high relative proportion of pentafunctionalised BHPs and no hexafunctionalised BHPs. (See above-mentioned references for further information.)

estuarine/marine settings. Other workers employing LC–MS analysis have also found a low relative contribution from pentafunctionalised BHPs in a variety of settings (not shown on Fig. 3 as this region of the plot is already populated). For example, pentafunctionalised BHPs accounted for only up to 6% of total BHPs and no hexafunctionalised BHPs were found in Black Sea sediment (Blumenberg et al., 2009). Hexafunctionalised BHPs were also absent from sediments from the Benguela system and, where present, pentafunctionalised compounds accounted for no more than 2.2% of total BHPs (Blumenberg et al., 2010). Sáenz et al. (2011b) also found a low level of pentafunctionalised BHPs (< 7%) in a water column transect off Panama, with hexafunctionalised compounds present in only 1 sample nearest to the coast, whilst tetra- and hexafunctionalised BHPs dominated water column distributions in the Black Sea (up to 62% and 42% respectively and a maximum of 3% penta-BHP; samples F2 and F3, estimated from Fig. 2 in Blumenberg et al., 2007). In an earlier study Talbot et al. (2003c) did find a high level of pentafunctionalised BHPs – but also in most cases hexafunctionalised BHPs – in a number of lake sediments, which was ascribed to a methanotrophic bacterial source (Fig. 3). However, differences in the analytical methodology used in this early study means that any comparison of these data with later LC–MS based studies is not directly quantitative. First, the Talbot et al. (2003c) study employed Soxhlet extraction, which has been shown to result in a different distribution of BHPs from Bligh and Dyer extraction, the latter releasing more of the more complex (composite) and highly functionalized BHPs (Cooke, 2010) and also more BHPs with an amine at C-35, including penta- and hexafunctionalised BHPs, such as 35-aminobacteriohopanetetrol and 35-aminobacteriohopanepentol (Berndmeyer et al., 2014). Furthermore, the earlier lakes study used a different analytical method, $\text{HIO}_4/\text{NaBH}_4$ reduction of vic diols within polyfunctionalised BHPs and analysis via gas chromatography-mass spectrometry. Excluding the Talbot et al. (2003c) study from further discussion leaves only a few soil samples from Canada (Xu et al., 2009) with a relatively high proportion of pentafunctionalised BHPs (Fig. 3); however, these soils also contained adenosylhopane (XIII) and related compounds, which were not observed in the CP sinters.

Other differences in comparison with previously studied environmental settings include the observation that composite BHPs, such as BHPentol cyclitol ether (I) and BHT cyclitol ether (II),

accounted for a high proportion of total BHP abundance in CP sinters (up to 80%; Fig. 2). A higher relative abundance of composite BHPs is thought to be a response of BHP-producing bacteria to the high temperature of the vent waters, consistent with reports of the effect of temperature and reduced pH on BHP composition in cultures of BHP-producing bacteria (e.g. Poralla et al., 1984; Joyeux et al., 2004). Therefore, the prevalence of composite BHPs and composite pentafunctionalised BHPs, in particular in older sinters (CPa13 and CPa16), provides supporting evidence for the previous indications of higher temperatures of vent waters in the past (Kaur et al., 2008, 2011a).

5. Conclusions

BHPs have been detected in a number of silica sinters from the Champagne Pool (CP) geothermal vent on the North Island of New Zealand, as well as a sulfidic mineral deposit that formed in the anoxic vent waters. The BHPs most likely derive from members of the *Acidithiobacillales*, *Actinomycetales*, *Bacillales*, *Burkholderiales*, *Planctomycetales* and *Pseudomonadales*, which are known to exist at CP on the basis of previous microbiological studies. Although the exact species of source organisms of the compounds remain elusive, the BHP distributions in geothermal sinters from CP appear to be strikingly different from those observed in other environmental settings containing higher levels of pentafunctionalised relative to tetrafunctionalised compounds, as well as a higher amount of composite BHPs. This is consistent with previous work suggesting higher temperatures of vent waters in the past. Finally, BHPs appeared to retain functionalisation throughout the age range of sinters analysed, and the increased abundance of BHPs in the oldest sinters implies that BHPs would be useful tools for reconstructing geothermal vent temperature profiles and bacterial communities over time.

Acknowledgements

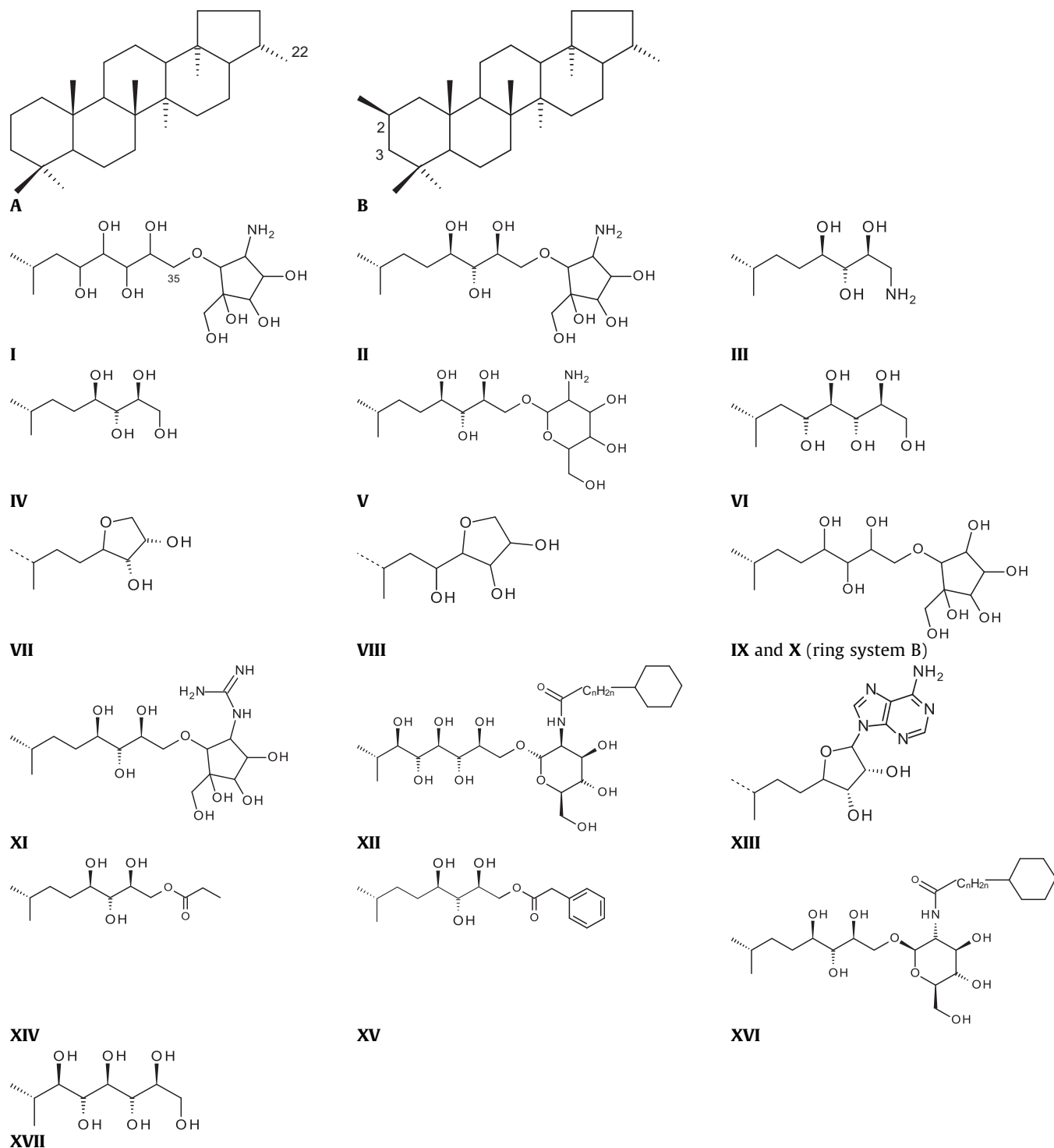
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Appendix A

Ring system and side chain structures of BHPs in silica sinters. BHPs were analysed as peracetate derivatives, but only the parent

BHP structures are shown for ease of comparison with other studies. All BHP compounds were found to contain the non-methylated ring system (A), with the exception of **X** which occurred as a C-2 methylated homologue (ring system B). Where stereochemistry is shown, all side chain structures have been unambiguously identified from NMR. When assigned using LC–MS only, where stereochemistry cannot be confirmed, we have assumed the structure to be the same as that previously



characterised although the occurrence of additional/alternative isomers cannot be excluded.

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