Organic Geochemistry 41 (2010) 1075–1081

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Adenosylhopane: The first intermediate in hopanoid side chain biosynthesis

Alexander S. Bradley [a,*], Ann Pearson [b], James P. Sáenz [c], Christopher J. Marx [a,**]

[a] Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA
[b] Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138, USA
[c] MIT-WHOI Joint Program in Chemical Oceanography, Woods Hole, MA 02543, USA

ARTICLE INFO

Article history:
Received 1 February 2010
Received in revised form 29 June 2010
Accepted 20 July 2010
Available online 3 August 2010

ABSTRACT

The hopanoid products synthesized by two mutant strains of *Methylobacterium* together suggest a biosynthetic pathway for the formation of the hopanoid side chain. Mutants deficient in the gene *hpnH* lack side chains entirely, while those deficient in *hpnG* accumulate adenosylhopane. These results are in accordance with adenosylhopane as a precursor to extended hopanoids and suggest that adenine is subsequently cleaved, possibly forming phosphoribohopane. We propose that the great diversity of microbial bacteriohopanepolyols and composite hopanoids reflects processes occurring downstream of this intermediate.

1. Introduction

Hopanoids are pentacyclic triterpenoids used widely as biomarkers for bacterial productivity in ancient and modern environments (Brocks and Summons, 2004). It has been proposed that hopanoid side chain structures carry taxonomic and/or physiological information (Talbot and Farrimond, 2007) and recent developments in analytical technology now allow rapid elucidation of hopanoid distributions in natural environments (Talbot et al., 2007). However, our increased understanding of hopanoid structural diversity has come without similar gains in unraveling how this diversity is linked to hopanoid sources (Talbot et al., 2008) and only limited information is available regarding hopanoid function (Welander et al., 2009). In addition, studies on pure cultures are laborious and can never be applied to the full range of hopanoid producing bacteria, some of which may not be cultivable, or which may not produce hopanoids under all conditions (Poralla et al., 2000). The factors influencing composite side chain hopanoid products therefore remain largely undiscovered.

Identifying the genes responsible for various hopanoid modifications will provide tools to predict their structures based on genomic or metagenomic sequence information. Similar approaches have been used with the squalene–hopene cyclase gene to detect putative hopanoid biosynthetic capacity in bacterial cultures (Fischer et al., 2005; Hartner et al., 2005) and the environment (Pearson and Rusch, 2008; Pearson et al., 2009).

In this study, we identify the first two steps in the formation of hopanoid side chains. We suggest the pathway involves addition of the adenosyl moiety from S-adenosyl methionine, followed by loss of adenine. This mechanism sheds light on some of the hopanoid structures observed in the environment and it suggests the possibility that some of the observed diversity of extended hopanoids could be affected by diagenesis or degradation, rather than solely by physiological synthesis.

2. Methods

2.1. Construction of plasmids and generation of strains

Mutant strains of *Methylobacterium extorquens* AM1 (hereafter referred to as *Methylobacterium*) were constructed as described in Marx (2008). The target genes were chosen because they are associated with a cluster of genes that co-occur with squalene–hopene cyclase in several genomes (Perzl et al., 1998). In *Methylobacterium*, the operon structure of *hpnH* and *hpnG* suggests that disruption of these genes is unlikely to lead to polar effects. DNA sequences flanking the upstream and downstream regions of each target gene (*hpnH* and *hpnG*) were amplified by PCR, cloned into pCR2.1-TOPO (Invitrogen, Carlsbad, CA, USA), sequenced and then introduced into the broad-host-range allelic exchange vector pCM433 (Marx, 2008). The plasmid for excision of *hpnH* was generated by amplifying the region upstream of *hpnH* using primers AB-15uf (GACGTCCGCGAAGAGCTCGATAAAGA) and AB-15ur (CATATGATCTTCG CGACGTACCGTAT), the product of which was cloned into pCR2.1-TOPO, and subsequently into pCM433 using the restriction enzymes *AatII* and *NdeI*. The downstream flank of *hpnH* was generated by PCR using primers AB-15df (GGGCCCACCGCTTACCATGAGAGACC) and AB-15dr (ACGCGTCATGACCGTTGCCGATAAG), then cloned into pCR2.1-TOPO, and subsequently, with *ApaI* and *MluI*, cloned into the vector containing the upstream flank, yielding pAB32. The

* Corresponding author. Tel.: +1 617 495 9137; fax: +1 617 495 8848.
** Corresponding author.
E-mail addresses: bradley@fas.harvard.edu (A.S. Bradley), cmarx@oeb.harvard.edu (C.J. Marx).

0146-6380/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.orggeochem.2010.07.003

plasmid for excision of *hpnG* was generated by amplifying the region upstream of *hpnG* with primers AB-16uf (GACGTCCCAGAAG CACTTCCTCAACC) and AB-16ur (CATATGACGCCCTTGAAATGAAA-AGA), which was cloned into pCR2.1-TOPO and then into pCM433 using *AatII* and *NdeI*. The downstream region was generated with AB-16df (GGGCCCGAAGCTCTGGGTCAGGAACA) and AB-16dr (ACC GGTTCACCAAACCGATCTCACAA), cloned into pCR2.1-TOPO, and then into the final plasmid with *ApaI* and *AgeI*, yielding pAB34. The sequences of pAB32 and pAB34 have been deposited in GenBank (Accession numbers GU952841–GU952842).

These constructs were conjugated from *Escherichia coli* into wild-type strain CM501 (Marx, 2008), where selection for the tetracycline resistance gene on pCM433 resulted in strains with a single-crossover homologous recombination. Subsequent plating onto succinate agar containing sucrose (5% w/v) selected against the lethal *sacB* locus, resulting in a second recombination event that removed the integrated plasmid to leave behind the deleted (or original) allele. Regions of the chromosome including the deleted sequence were amplified by PCR and sequenced to confirm generation of the desired unmarked deletion. Table 1 details the plasmid used to generate each mutant, the locus on the *Methyobacterium* chromosome of the amplified upstream and downstream flanks, and the region deleted as confirmed by PCR and sequencing.

2.2. Media, growth condition and lipid analysis

Methylobacterium strains were grown at 30 °C in a minimal medium (Lee et al., 2009) with 15 mM succinate as the carbon source. *E. coli* strains were grown at 37 °C on Luria–Bertani agar. Antibiotics added for selection were at the following concentrations: ampicillin 50 μg/ml, chloramphenicol 20 μg/ml, kanamycin 50 μg/ml, rifamycin 50 μg/ml, streptomycin 35 μg/ml and tetracycline 10 μg/ml.

Lipids were extracted from 10 ml of culture grown to a final optical density of at least 0.35 following the method of Bligh and Dyer (1959). Total lipid extracts were analyzed following methods previously established (Talbot et al., 2003a,b, 2007), using a Thermo Surveyor LC interfaced to an LTQ-MS. Hopanoids were identified by comparison of retention times and mass spectra to published information (Talbot et al., 2003a,b, 2007) and to samples with previously established hopanoid composition, including *Rhodopseudomonas palustris* CGA-009 and *Crocosphaera watsonii* WH801. For hopanoid analysis by GC-MS the total lipid extract was subjected to periodic acid oxidation and then reduced with $NaBH_4$ (Rohmer et al., 1984) and subsequently derivatized with acetic anhydride or *N*,*O*-bis(trimethylsilyl)trifluoro-acetamide (BSTFA + 1% TMCS) in the presence of pyridine. The limit of detection for individual lipids was approximately 1 ng per μg of total lipid extract.

3. Results

Wild-type *Methylobacterium* has hopanoid genes encoded mainly in three gene clusters (Table 2). When grown on succinate in liquid media it accumulates diploptene (I) diplopterol (II), methyldiplopterol (III), trace bacteriohopanetetrol (BHT, IV), BHT cyclitol ether (V) and guanidine-substituted BHT cyclitol ether (VI), similar to other *Methylobacterium* strains (Knani et al., 1994). Approximately 63% of diplopterol is methylated. The mutant strain Δ*hpnH* (CM2180) produces only I, II and III, i.e., it does not accumulate any hopanoids > C_{31} (Fig. 1). The Δ*hpnG* mutant strain (CM2181) accumulates I, II, III and it also accumulates adenosylhopane (VII). It does not, however, accumulate any bacteriohopanetetrol (IV), BHT cyclitol ether (V) or guanidine-substituted BHT cyclitol ether (VI) (Fig. 2).

The products accumulated in mutants lacking *hpnH* and *hpnG* can be interpreted by comparison of the deleted genes to homologs of known function. The genome annotation for *Methylobacterium* classifies *hpnH* as encoding a Radical SAM superfamily enzyme and its classification in the InterPro database (Hunter et al., 2009) supports this annotation. Radical SAM enzymes rely on S-adenosyl methionine (SAM) as a substrate. Similar assessment of

Table 1
Plasmid names and resulting strain numbers used in this study, indicating the target gene of each plasmid (excised from resulting strain), the target region (nucleotide numbers for each gene's coding sequence), the upstream and downstream flanks inserted into each plasmid and the excised region based on PCR amplification and sequencing of the target region in mutant strains. Numbers refer to the nucleotide numbers within the *Methylobacterium extorquens* AM1 genome (Vuilleumier et al., 2009).

Strain	Plasmid	target	Coding region	Upstream flank	Downstream flank	Deleted region
CM2180	pAB32	*hpnH*	3838139–3839302	3837610–3838171	3839315–3839835	3838172–3839315
CM2181	pAB34	*hpnG*	3839497–3840204	3838762–3839377	3840333–3840862	3839377–3840332

Table 2
Genes hypothetically involved in hopanoid biosynthesis. AM1 locus refers to the CoDing Sequence (CDS) number in the genome of *M. extorquens* AM1 (Vuilleumier et al., 2009). References indicate the publication in which each gene's function was demonstrated or hypothesized 1: Perzl et al. (1998); 2: Reipen et al. (1995); 3: This work; 4: Hypothesized function; 5: Hausmann et al. (2009) and 6: Welander et al. (2010).

Gene	Annotation	AM1 locus	Hypothesized or known function	Reference
hpnA	Hopanoid associated sugar epimerase	Meta1_1814	Involved in side chain formation	1
hpnB	Putative glycosyltransferase	No homolog	Involved in side chain formation	1
hpnC	Squalene synthase	Meta1_1815	Synthesis of squalene	1
hpnD	Putative dehydrosqualene synthase	Meta1_1816	Synthesis of squalene – alternative pathway	1
hpnE	Putative dehydrosqualene reductase	Meta1_1817	Synthesis of squalene – alternative pathway	1
hpnF	Squalene–hopene cyclase	Meta1_1818	Formation of hopanoid cyclic backbone	2
hpnG	Nucleoside phosphorylase	Meta1_3691	Cleavage of adenine from side chain	3
hpnH	Hopanoid biosynthesis associated radical SAM protein	Meta1_3690	Addition of adenosyl group to side chain	3
hpnI	Glycosyltransferase	Meta1_3685	Possibly involved in side chain formation	4
hpnJ	Hopanoid biosynthesis associated radical SAM protein	Meta1_3690	Possibly involved in side chain formation	4
hpnK	Hopanoid biosynthesis associated protein (ydjC)	Meta1_3687	Possibly involved in side chain formation	4
hpnL	Conserved hypothetical protein	Meta1_3683	Possibly involved in side chain formation	4
hpnM	Hopanoid biosynthesis associated membrane protein	No homolog	Possibly involved in side chain formation	4
–	Conserved hypothetical protein	Meta1_3688	Unknown. Occurs only in Methylobacterium spp.	4
hpnN	RND superfamily exporter	Meta1_3689	Probable hopanoid transporter, similar to eukaryotic Ptc	5
hpnP	Hopanoid A-ring methylase	Meta1_4400	Methylates A ring at C2	6

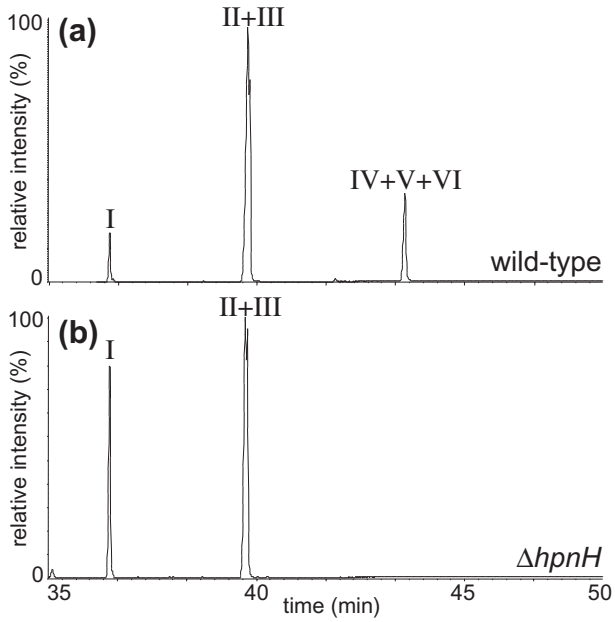


Fig. 1. GC-MS chromatogram showing hopanoid products in (a) wild-type *Methylobacterium* (peak representing structures IV + V + VI is the TMS derivative of the alcohol product after periodic acid oxidation and $NaBH_4$ reduction) and (b) the Δ*hpnH* mutant strain. The mutant strain lacks hopanoids > C_{31}.

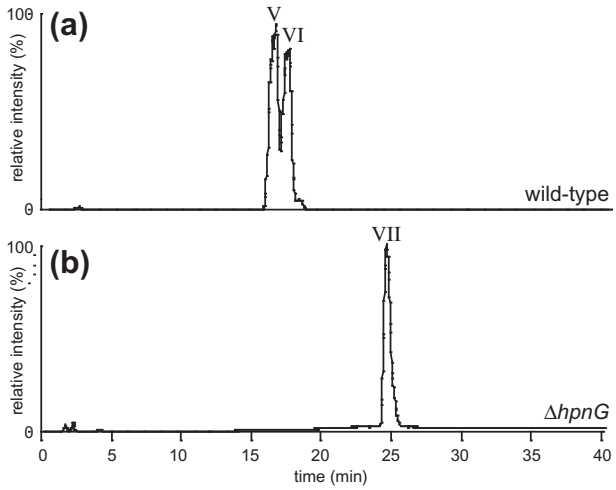


Fig. 2. LCMS partial chromatogram showing extended hopanoids in (a) wild-type *Methylobacterium* (b) Δ*hpnG* mutant strain. The chromatogram is a combination of ions *m*/*z* 655 + 746 + 1002 + 1086, the molecular ions for IV, VII, V and VI, respectively.

hpnG reveals that it belongs to a large family of genes that encode purine nucleoside phosphorylases (PNPs), which act to cleave a nucleoside (like adenosine) into a nucleobase plus a phosphorylated ribose (Mao et al., 1997; Silva et al., 2007).

4. Discussion

The pattern of hopanoid accumulation in these two strains is consistent with the functions predicted for their respective mutated genes. The prediction for the function of each gene derives from the general function of homologous genes within the same the gene family. We suggest that the enzyme encoded by *hpnH* is responsible for the transformation of I to adenosylhopane (VII). The enzyme encoded by *hpnG* then cleaves adenine from adenosylhopane.

Based on its homology to other genes, *hpnH* encodes a radical SAM enzyme. The absence of *hpnH* in *Methylobacterium* yields hopanoids with no side chains. The C_5 unit of extended hopanoid side chains is known to originate from a ribosugar (Flesch and Rohmer, 1988; Neunlist et al., 1988; Rohmer et al., 1989), although to date the origin of this ribose has remained unknown. Our pathway suggests it is derived from the adenosyl group of SAM. Many radical SAM enzymes such as *hpnH* generate a 5′-deoxyadenosyl radical (Layer et al., 2004), which could serve as the adenosyl donor to the double bond of I (Fig. 3).

The Δ*hpnG* mutant strain of *Methylobacterium* accumulates only a single extended hopanoid: adenosylhopane. This is consistent with the mechanism proposed above. The *hpnG* gene is located downstream of *hpnH* and disruption of *hpnG* leads to the accumulation of adenosylhopane, because our *hpnG* mutant still contains a functional copy of the adenosylating *hpnH*. Adenosylhopane has been previously proposed as a hopanoid intermediate based on the correlation of the stereochemistry in IV and VII (Neunlist et al., 1988). We postulate that adenosylhopane is an essential intermediate in hopanoid synthesis not only in *Methylobacterium*, but also in all hopanoid producing bacteria. This suggestion is strengthened by analyzing co-occurring proteins in all published microbial genomes (Jensen et al., 2009). We found that *hpnH* and *hpnG* homologs are present in all genomes in the STRING (http://string.embl.de/) database that encode an apparent squalene–hopene cyclase. This implies the presence of these genes in the last common ancestor of hopanoid containing organisms and significantly, it also implies that side chain addition is significant in the physiological role of hopanoids. The absence of adenosylhopane in wild-type *Methylobacterium* indicates that in this species adenosylhopane normally undergoes further modifications mediated by gene products acting downstream of *hpnG*.

The sequence of *hpnG* supports this argument, indicating it encodes for a purine nucleoside phosphorylase (PNP) homolog. The activity of PNPs is to cleave adenine (or guanine) from its corresponding nucleoside using a mechanism that universally requires phosphate addition to the ribose (Erion et al., 1997; Silva et al., 2007); it is likely that the HpnG enzyme is no exception. Therefore, it is probable that the next intermediate step in hopanoid biosynthesis involves formation of phosphoribohopane. This intermediate has yet to be observed experimentally and may be short lived.

The next steps of hopanoid synthesis in *Methylobacterium* involve opening the ribose ring (Duvold and Rohmer, 1999). It is unclear whether this occurs before or after loss of the phosphate group and the genes involved are as yet unknown. Subsequently, in *Methylobacterium* most of the compound hopanoid is converted into BHT cyclitol ether. Previous studies have determined that the polyhydroxylated cyclopentane group in the hopanoid side chain derives from *N*-acetyl-D-glucosamine (GlcNAc) (Vincent et al., 2003), very likely via UDP-GlcNAc, which is a common enzymatic cofactor in bacterial metabolism. This is consistent with the presence of the gene annotated as *hpnI* (Table 2), a homolog of other bacterial glycosyltransferases that require nucleotide diphosphate sugars as substrates. Current work is aimed at confirming this role for the *hpnI* gene and whether its substrate is phosphoribohopane or IV. The biosynthetic steps downstream of adenosylhopane are likely to differ among the various hopanoid producing organisms and may include a range of genes involved in the production of the many diverse hopanoid structures that have been observed in nature (Talbot et al., 2007; Talbot and Farrimond, 2007).

Our proposed pathway (Fig. 3) has a number of direct implications for hopanoid structures commonly found in bacterial samples. The *hpnH* results reveal that synthesis of 2-methyl hopanoids is independent of side chain formation in *Methylobacterium*. Both

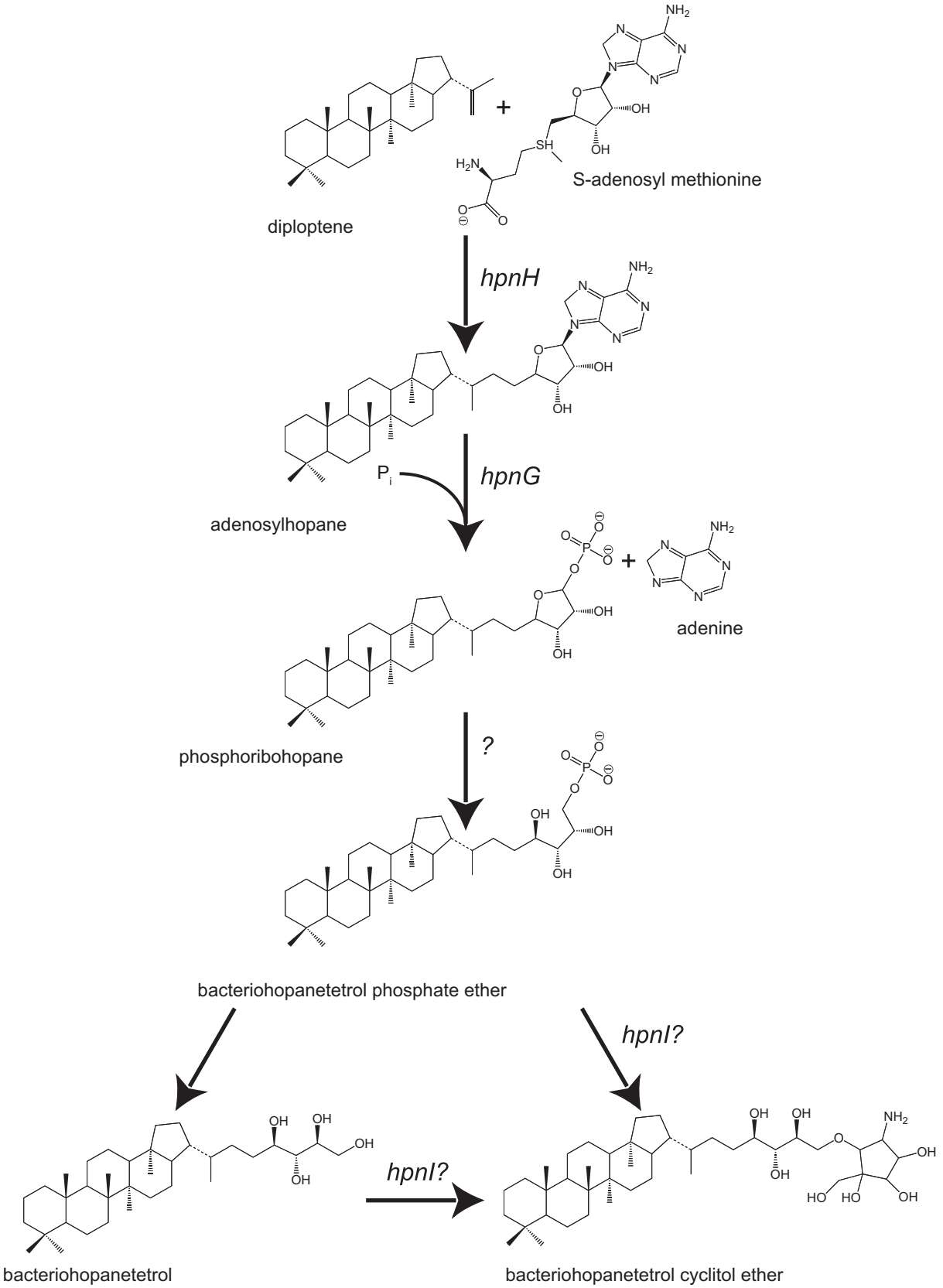


Fig. 3. Proposed first steps in the biosynthesis of the hopanoid side chain: the enzyme encoded by *hpnH* adds SAM to I, forming VII. Adenine is subsequently cleaved from VII by *hpnG*, likely forming phosphoribohopane.

the wild-type and Δ*hpnH* strains of *Methylobacterium* produce III, but our strain of *Methylobacterium* has not been observed to produce 2-methyl hopanoids with extended side chains. This suggests that the methylating enzyme (encoded by *hpnP*; Welander et al., 2010) in *Methylobacterium* may accept only diplopterol as a substrate and not diploptene or extended hopanoids. In contrast, *Rhodopseudomonas palustris* TIE-1 is reported to contain 2-methyldiploptene, 2-methyltetrahymanol and 2-methylbacteriohopanetetrol, along with their desmethyl homologs, but contains only desmethyl 35-aminobacteriohopane-32,33,34-triol (Rashby et al., 2007). Therefore, in *R. palustris* – unlike in *Methylbacterium* – the A-ring methylase HpnP is likely to act on non-extended hopanoids and tetrahymanol and also on some extended hopanoids. This is consistent with the phylogenetic analysis suggesting that *hpnP* can be divided into subfamilies and that the *hpnP* of *Methylobacterium* species are distinct from that of other proteobacteria (Welander et al., 2010). Alternatively, the lack of extended methylhopanoids could be due to the inability of HpnH to catalyze the reaction of SAM with a methylated diploptene, if such a molecule is ever present. There may be some flexibility in the substrate affinities of these enzymes, since small amounts of 2-methylbacteriohopanetetrol have been isolated from *Methylobacterium organophilum* (Renoux and Rohmer, 1985).

Adenosylhopane has been identified in the environment (Talbot and Farrimond, 2007; Blumenberg et al., 2009; Pearson et al., 2009) and in some bacterial cultures (Neunlist and Rohmer, 1985; Seemann et al., 1999; Bravo et al., 2001; Talbot et al., 2007). It accumulates preferentially in terrigenous and lacustrine settings, leading to the suggestion that it may be a good marker for soil bacteria (Talbot and Farrimond, 2007). Alternatively, the accumulation of adenosylhopane could simply reflect a lower activity of *hpnG* in organisms that accumulate it. We propose that adenosylhopane has limited potential as a phylogenetic marker but may reflect physiological or environmental conditions.

The implied formation of a phosphorylated intermediate by the PNP analogue *hpnG* also suggests a possible connection to the hopanoid lactones that have been observed in cultures and the environment (Seemann et al., 1999). Hopaneribonolactone (VIII) has an oxo group at the 1′ position of the ribose, which could result from oxidative loss of phosphate. Similarly, reductive loss of phosphate would yield 32,35-anhydrobacteriohopanetetrol (IX), which has been observed in sediments (Bednarczyk et al., 2005; Cooke et al., 2008b), soils (Cooke et al., 2008a) and hydrothermal vents (Talbot et al., 2005). These two reactions can proceed abiotically, suggesting that VIII and IX could be diagenetic or accidental products. Schaeffer et al. (2008) have also suggested that IX may form as a diagenetic product from IV. The ratio of VIII to IX may be a product of sedimentary pH and Eh, carrying little phylogenetic information. Finally, in the alternative case of catalyzed hydrolysis of phosphoribolactone or its downstream products, it is possible that some fraction of bacteriohopanetetrol also is a metabolic or environmental breakdown product, rather than enzymatically biosynthesized.

5. Conclusions

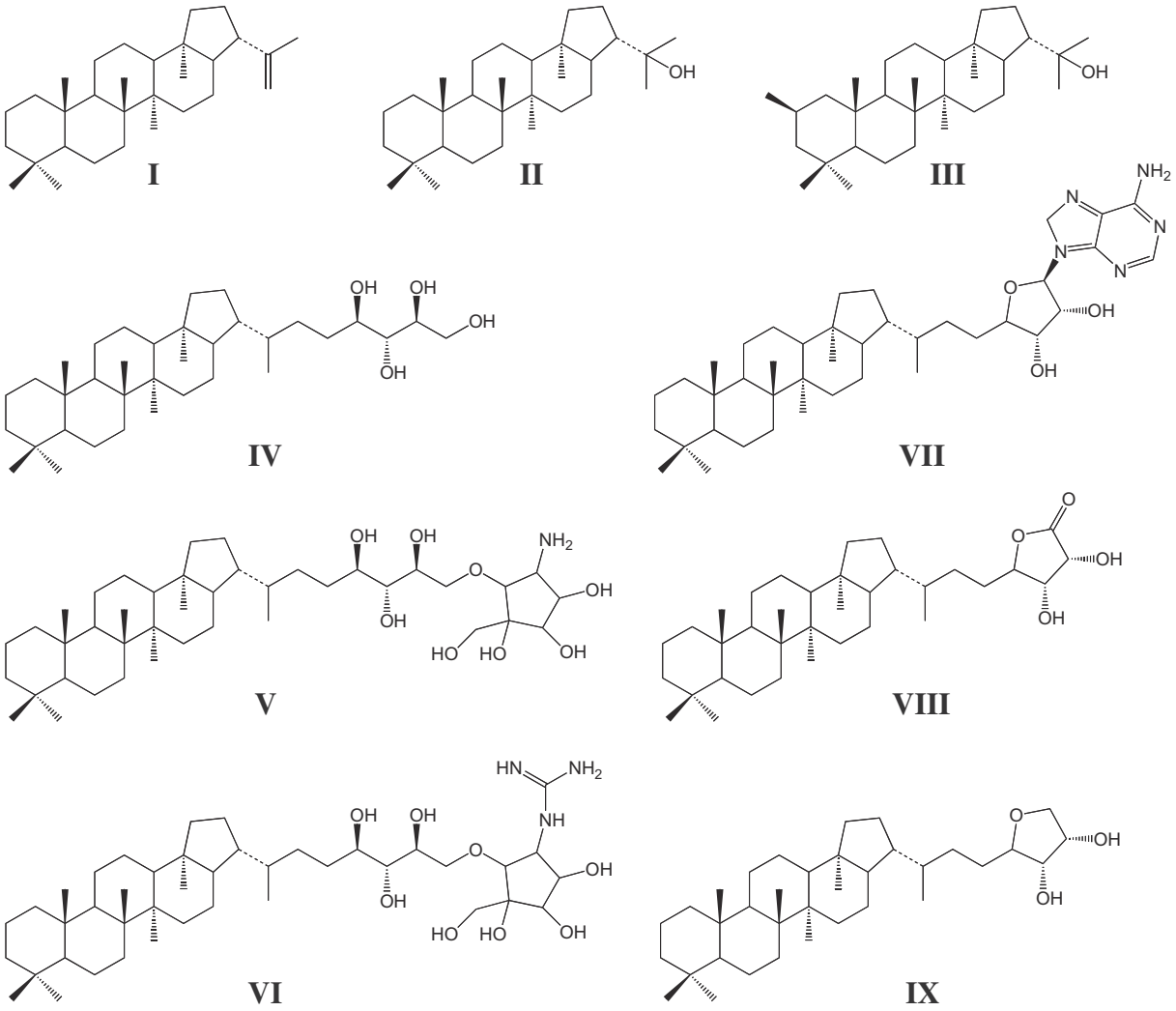
Mutations of two genes, *hpnH* and *hpnG*, produced strains of *Methylobacterium* with two distinct hopanoid profiles that differed from the profiles found in the wild-type. This work provides evidence linking the genes *hpnH* and *hpnG* to specific aspects of hopanoid side chain biosynthesis that are likely present in all hopanoid producing organisms and suggests a central role for adenosylhopane in the biosynthesis of hopanoid side chains. Examination of the nucleotide sequences of these genes in bacteria and in the environment has potential to help us further elucidate the utility of hopanoids as biomarkers.

Acknowledgements

A.S.B. thanks the Agouron Institute for a fellowship supporting this research (AI-F-GB9.08.2). J.P.S acknowledges the WHOI-MIT Joint Program and funding support from the National Science Foundation, Emerging Topics in Biogeochemical Cycles program, through an award to Roger Summons. We thank Roger Summons for comments on the manuscript, Susie Carter for laboratory assistance, and Helen Talbot for editorial handling. We gratefully acknowledge the comments of two anonymous reviewers, which helped us to improve the manuscript.

Associate Editor—**Helen Talbot**

Appendix A



References

Bednarczyk, A., Hernandez, T.C., Schaeffer, P., Adam, P., Talbot, H.M., Farrimond, P., Riboulleau, A., Largeau, C., Derenne, S., Rohmer, M., Albrecht, P., 2005. 32,35-Anhydrobacteriohopanetetrol: an unusual bacteriohopanepolyol widespread in recent and past environments. Organic Geochemistry 36, 673–677.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Blumenberg, M., Seifert, R., Kasten, S., Bahlmann, E., Michaelis, W., 2009. Euphotic zone bacterioplankton sources major sedimentary bacteriohopanepolyols in the Holocene Black Sea. Geochimica et Cosmochimica Acta 73, 750–766.

Bravo, J.-M., Perzl, M., Hartner, T., Kannenberg, E.L., Rohmer, M., 2001. Novel methylated triterpenoids of the gammacerane series from the nitrogen-fixing bacterium *Bradyrhizobium japonicum* USDA 110. European Journal of Biochemistry 268, 1323–1331.

Brocks, J.J., Summons, R.E., 2004. Sedimentary hydrocarbons, biomarkers for early life. In: Schlesinger, W.H. (Ed.), Biogeochemistry, Treatise on Geochemistry, vol. 8. Elsevier, Oxford, pp. 63–116.

Cooke, M.P., Talbot, H.M., Farrimond, P., 2008a. Bacterial populations recorded in bacteriohopanepolyol distributions in soils from northern England. Organic Geochemistry 39, 1347–1358.

Cooke, M.P., Talbot, H.M., Wagner, T., 2008b. Tracking soil organic carbon transport to continental margin sediments using soil-specific hopanoid biomarkers: a case study from the Congo fan (ODP site 1075). Organic Geochemistry 39, 965–971.

Duvold, T., Rohmer, M., 1999. Synthesis of ribosylhopane, the putative biosynthetic precursor of bacterial triterpenoids of the hopane series. Tetrahedron 55, 9847–9858.

Erion, M., Takabayashi, K., Smith, H., Kessi, J., Wagner, S., Honger, S., Shames, S., Ealick, S., 1997. Purine nucleoside phosphorylase. 1. Structure–function studies. Biochemistry 36, 11725–11734.

Fischer, W.W., Summons, R.E., Pearson, A., 2005. Targeted genomic detection of biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe. Geobiology 3, 33–40.

Flesch, G., Rohmer, M., 1988. Prokaryotic hopanoids – the biosynthesis of the bacteriohopane skeleton – formation of isoprenic units from 2 distinct acetate pools and a novel type of carbon–carbon linkage between a triterpene and D-ribose. European Journal of Biochemistry 175, 405–411.

Hartner, T., Straub, K.L., Kannenberg, E., 2005. Occurrence of hopanoid lipids in anaerobic Geobacter species. FEMS Microbiology Letters 243, 59–64.

Hausmann, G., Von Mering, C., Basler, K., Scott, M.P., 2009. The hedgehog signaling pathway: where did it come from? PLoS Biology 7, e1000146.

Hunter, S., Apweiler, R., Attwood, T.K., Bairoch, A., Bateman, A., Binns, D., Bork, P., Das, U., Daugherty, L., Duquenne, L., Finn, R.D., Gough, J., Haft, D., Hulo, N., Kahn, D., Kelly, E., Laugraud, A., Letunic, I., Lonsdale, D., Lopez, R., Madera, M., Maslen, J., Mcanulla, C., Mcdowall, J., Mistry, J., Mitchell, A., Mulder, N., Natale, D., Orengo, C., Quinn, A.F., Selengut, J.D., Sigrist, C.J.A., Thimma, M., Thomas, P.D., Valentin, F., Wilson, D., Wu, C.H., Yeats, C., 2009. InterPro: the integrative protein signature database. Nucleic Acids Research 37, D211–D215.

Jensen, L.J., Kuhn, M., Stark, M., Chaffron, S., Creevey, C., Muller, J., Doerks, T., Julien, P., Roth, A., Simonovic, M., Bork, P., Von Mering, C., 2009. STRING 8 – a global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Research 37, D412–D416.

Knani, M., Corpe, W., Rohmer, M., 1994. Bacterial hopanoids from pink-pigmented facultative methylotrophs (PPFMs) and from green plant surfaces. Microbiology 140, 2755–2759.

Layer, G., Heinz, D., Jahn, D., Schubert, W., 2004. Structure and function of radical SAM enzymes. Current Opinion in Chemical Biology 8, 468–476.

Lee, M.-C., Chou, H.-H., Marx, C.J., 2009. Asymmetric, bimodal trade-offs during adaptation of *Methylobacterium* to distinct growth substrates. Evolution 63, 2816–2830.

Mao, C., Cook, W., Zhou, M., Koszalka, G., Krenitsky, T., Ealick, S., 1997. The crystal structure of *Escherichia coli* purine nucleoside phosphorylase: a comparison with the human enzyme reveals a conserved topology. Structure 5, 1373–1383.

Marx, C.J., 2008. Development of a broad-host-range *sacB*-based vector for unmarked allelic exchange. Biomed Central Research Notes 1, 1.

Neunlist, S., Rohmer, M., 1985. A novel hopanoid, 30-(5′-adenosyl)hopane, from the purple non-sulfur bacterium *Rhodopseudomonas acidophila*, with possible DNA interactions. Biochemical Journal 228, 769–771.

Neunlist, S., Bisseret, P., Rohmer, M., 1988. The hopanoids of the purple non-sulfur bacteria *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila* and the absolute configuration of bacteriohopanetetrol. European Journal of Biochemistry / FEBS 171, 245–252.

Pearson, A., Rusch, D.B., 2008. Distribution of microbial terpenoid lipid cyclases in the global ocean metagenome. ISME Journal 3, 352–363.

Pearson, A., Leavitt, W.D., Saenz, J.P., Summons, R.E., Tam, M.C.-M., Close, H.G., 2009. Diversity of hopanoids and squalene–hopene cyclases across a tropical land–sea gradient. Environmental Microbiology 11, 1208–1223.

Perzl, M., Reipen, I.G., Schmitz, S., Poralla, K., Sahm, H., Sprenger, G.A., Kannenberg, E.L., 1998. Cloning of conserved genes from *Zymomonas mobilis* and *Bradyrhizobium japonicum* that function in the biosynthesis of hopanoid lipids. Biochimica et Biophysica Acta 1393, 108–118.

Poralla, K., Muth, G., Hartner, T., 2000. Hopanoids are formed during transition from substrate to aerial hyphae in *Streptomyces coelicolor* A3(2). FEMS Microbiology Letters 189, 93–95.

Rashby, S.E., Sessions, A.L., Summons, R.E., Newman, D.K., 2007. Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. Proceedings of the National Academy of Sciences of the United States of America 104, 15099–15104.

Reipen, I., Poralla, K., Sahm, H., Sprenger, G., 1995. *Zymomonas mobilis* squalene–hopene cyclase gene (SHC): cloning, DNA-sequence analysis, and expression in *Escherichia coli*. Microbiology 141, 155–161.

Renoux, J., Rohmer, M., 1985. Prokaryotic triterpenoids – new bacteriohopane tetrol cyclitol ethers from the methylotrophic bacterium *Methylobacterium organophilum*. European Journal of Biochemistry 151, 405–410.

Rohmer, M., Bouvier-Nave, P., Ourisson, G., 1984. Distribution of hopanoid triterpenes in prokaryotes. Journal of General Microbiology 130, 1137–1150.

Rohmer, M., Sutter, B., Sahm, H., 1989. Bacterial sterol surrogates - –biosynthesis of the side-chain of bacteriohopanetetrol and of a carbocyclic pseudopentose from C-13-labeled glucose in *Zymomonas mobilis*. Chemical Communications 1471, 1472.

Schaeffer, P., Schmitt, G., Adam, P., Rohmer, M., 2008. Acid-catalyzed formation of 32,35-anhydrobacteriohopanetetrol from bacteriohopanetetrol. Organic Geochemistry 39, 1479–1482.

Seemann, M., Bisseret, P., Tritz, J., Hooper, A., Rohmer, M., 1999. Novel bacterial triterpenoids of the hopane series from *Nitrosomonas europaea* and their significance for the formation of the C-35 bacteriohopane skeleton. Tetrahedron Letters 40, 1681–1684.

Silva, R.G., Nunes, J.E.S., Canduri, F., Borges, J.C., Gava, L.M., Moreno, F.B., Basso, L.A., Santos, D.S., 2007. Purine nucleoside phosphorylase: a potential target for the development of drugs to treat T-cell- and apicomplexan parasite-mediated diseases. Current Drug Targets 8, 413–422.

Talbot, H.M., Farrimond, P., 2007. Bacterial populations recorded in diverse sedimentary biohopanoid distributions. Organic Geochemistry 38, 1212–1225.

Talbot, H.M., Squier, A.H., Keely, B.J., Farrimond, P., 2003a. Atmospheric pressure chemical ionisation reversed-phase liquid chromatography/ion trap mass spectrometry of intact bacteriohopanepolyols. Rapid Communications in Mass Spectrometry 17, 728–737.

Talbot, H.M., Summons, R., Jahnke, L., Farrimond, P., 2003b. Characteristic fragmentation of bacteriohopanepolyols during atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 17, 2788–2796.

Talbot, H., Farrimond, P., Schaeffer, P., Pancost, R., 2005. Bacteriohopanepolyols in hydrothermal vent biogenic silicates. Organic Geochemistry 36, 663–672.

Talbot, H., Rohmer, M., Farrimond, P., 2007. Rapid structural elucidation of composite bacterial hopanoids by atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 21, 1–13.

Talbot, H., Summons, R., Jahnke, L., Cockell, C., Rohmer, M., Farrimond, P., 2008. Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. Organic Geochemistry 39, 232–263.

Vincent, S., Sinay, P., Rohmer, M., 2003. Composite hopanoid biosynthesis in Zymomonas mobilis: N-acetyl-D-glucosamine as precursor for the cyclopentane ring linked to bacteriohopanetetrol. Chemical Communications 782, 783.

Vuilleumier, S., Chistoserdova, L., Lee, M.-C., Bringel, F., Lajus, A., Zhou, Y., Gourion, B., Barbe, V., Chang, J., Cruveiller, S., Dossat, C., Gillett, W., Gruffaz, C., Haugen, E., Hourcade, E., Levy, R., Mangenot, S., Muller, E., Nadalig, T., Pagni, M., Penny, C., Peyraud, R., Robinson, D.G., Roche, D., Rouy, Z., Saenampechek, C., Salvignol, G., Vallenet, D., Wu, Z., Marx, C.J., Vorholt, J.A., Olson, M.V., Kaul, R., Weissenbach, J., Médigue, C., Lidstrom, M.E., Ahmed, N., 2009. *Methylobacterium* genome sequences: a reference blueprint to investigate microbial metabolism of C1 compounds from natural and industrial sources. PLoS ONE 4, e5584.

Welander, P.V., Hunter, R.C., Zhang, L., Sessions, A.L., Summons, R.E., Newman, D.K., 2009. Hopanoids play a role in membrane integrity and pH homeostasis in *Rhodopseudomonas palustris* TIE-1. Journal of Bacteriology 191, 6145–6156.

Welander, P.V., Coleman, M., Sessions, Alex L., Summons, R.E., Newman, D.K., 2010. Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. Proceedings of the National Academy of Sciences of the United States of America 107, 8537–8542.