Supporting Information

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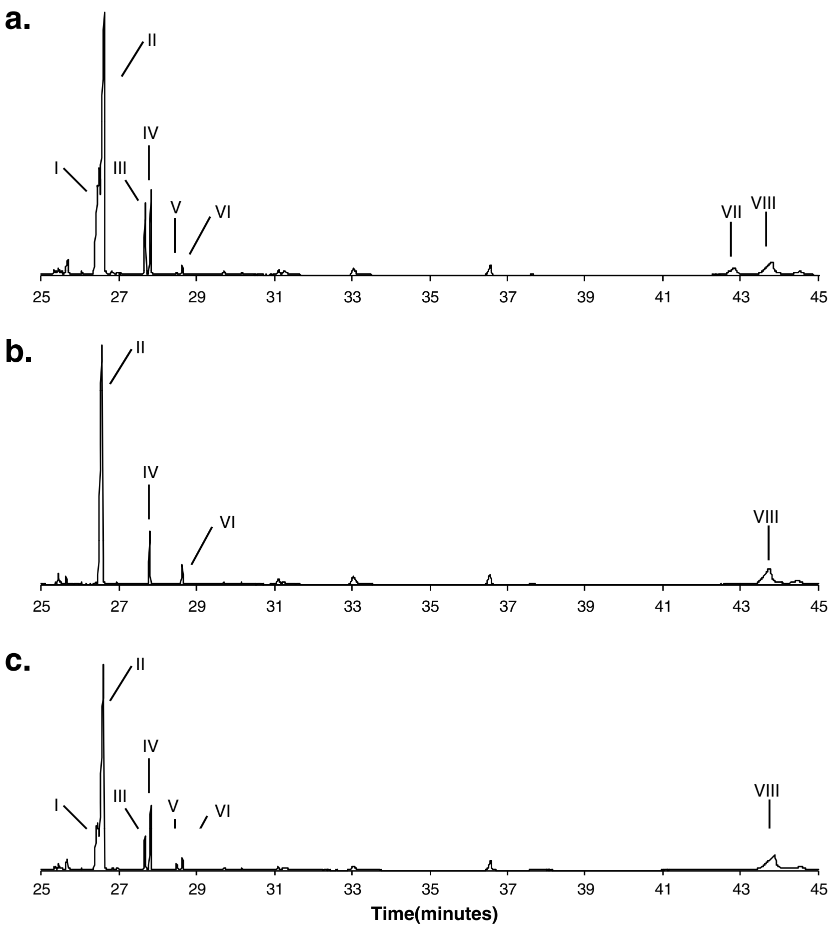

Fig. S1. Chromatograms of total lipid extract separated by GC-MS from the following strains: *a*, *Rhodopseudomonas palustris* TIE-1. *b*, *R. palustris* ΔhpnP. *c*, *R. palustris* ΔhpnP complemented with the ΔhpnP gene on a self-replicating plasmid. Numbered compounds: I, 2- methylhopenes; II, hopenes; III, 2-methyldiplopterol; IV, diplopterol; V, 2-methyltetrahymanol; VI, tetrahymanol; VII, 2-methylbacteriohopanetetrol; and VIII, bacteriohopanetetrol.

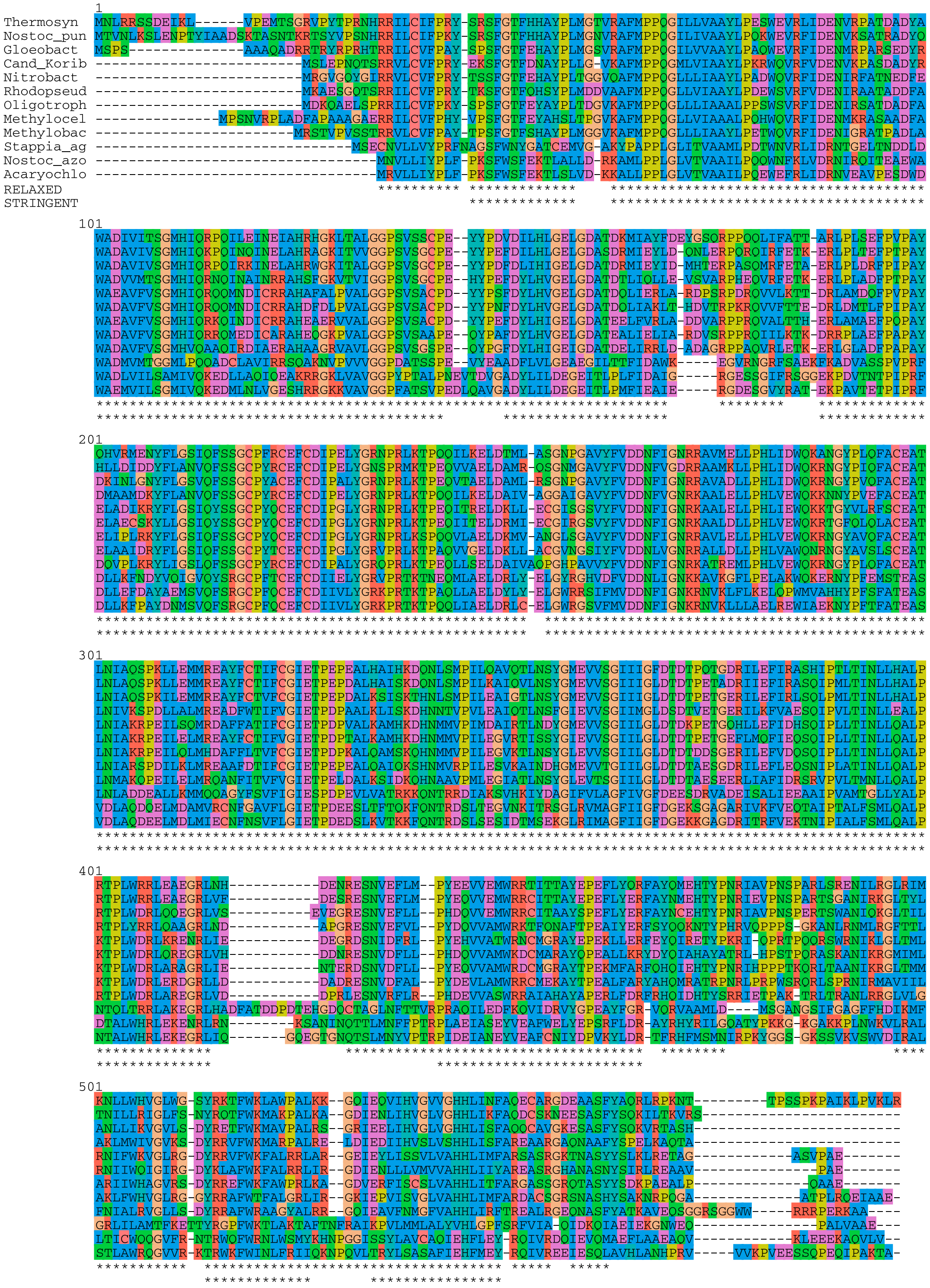

Fig. S2. Alignment of select HpnP and outgroup sequences showing filtered and unfiltered positions. The twelve representatives are as follows: *Thermosynechococcus elongatus*, *Nostoc punctiforme* PCC73102, *Gloeobacter violaceus* PCC7421, Candidatus *Koribacter versatilis* Ellin 345, *Nitrobacter* sp. Nb-311*a*, *R. palustris* TIE-1, *Oligotropha carboxidovorans* OM5, *Methylocella silvestris* BL2, *Methylobacterium nodulans* ORS2060, *Stappia aggregata* IAM12614 (outgroup), *Nostoc azollae* 0708 (outgroup), and *Acaryochloris marina* MBIC11017 (outgroup). This alignment is from trials 7–9 (Table S3) and was generated by Muscle with one outgroup family. The sites included in the filtered alignments under relaxed and stringent scenarios are marked with *.

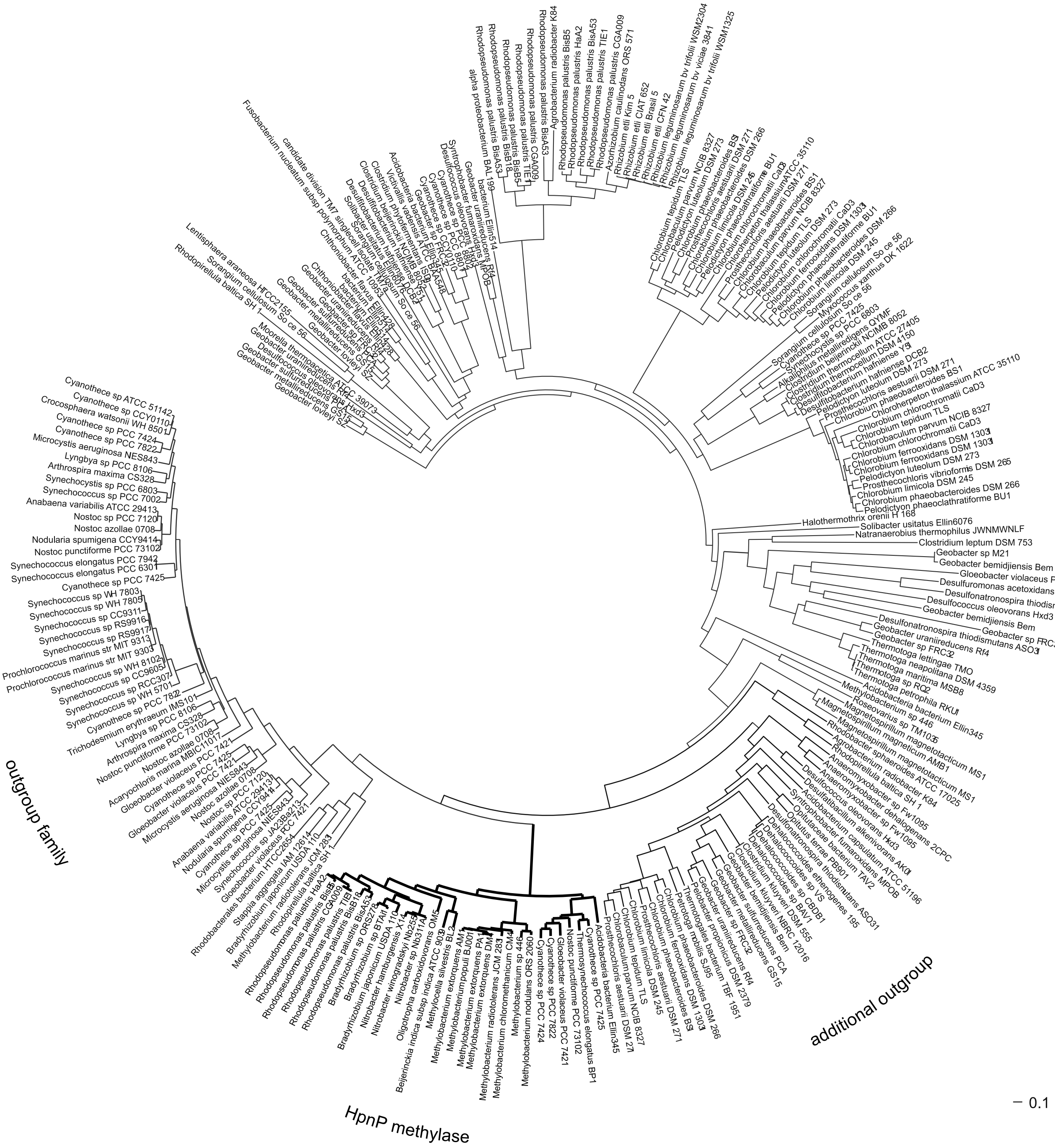

Fig. S3. Major families of putative bacterial radical SAM proteins. Maximum likelihood tree was inferred from an alignment of 247 sequences and 679 positions. The clade labeled "outgroup family" was used in all trials, and the additional outgroup was used in trials 4–6, 10–12, and 16–18 (Table S3).

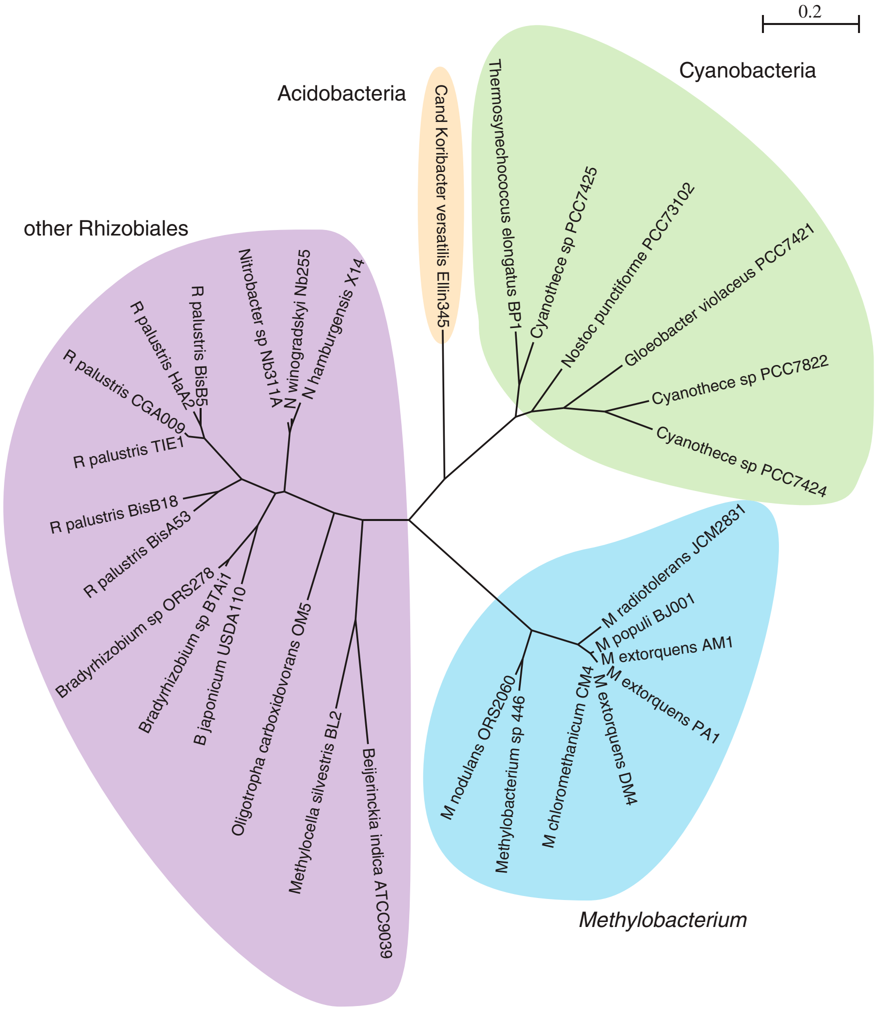

Fig. S4. Unrooted tree showing the major clusters of HpnP from different bacterial phyla. The alignment was generated by Muscle and maximum likelihood tree inferred using PhyML as described in *Methods*.

Table S1. Presence of 2-methylhopanoids in bacteria with an HpnP homolog

Bacterial genome	2-Me C30*	2-Me C35[†]	Ref.	Isolation habitat
α-Proteobacteria				
R. palustris TIE-1	+	+	(1)	Iron mat
R. palustris CGA009	+	+	(1)	Freshwater sediment
R. palustris BisB18	+	+	This study	Freshwater sediment
R. palustris BisA53	+	+	This study	Freshwater sediment
R. palustris BisB5	+	+	This study	Freshwater sediment
R. palustris HaA2	+	+	This study	Freshwater sediment
Bradyrhizobium sp. ORS278	nt[‡]	nt		Stem nodules
Bradyrhizobium sp. BTAi1	+	–	(2)	Stem nodules
Bradyrhizobium japonicum USDA110	+	–	(2)	Root nodules
Nitrobacter hamburgensis X14	nt	nt		Soil
Nitrobacter winogradskyi Nb-255	nt	nt		Soil
Nitrobacter sp. Nb-311A	nt	nt		Coastal marine waters
O. carboxidovorans OM5	nt	nt		Wastewater
Beijerinckia indica ATCC 9039	+	–	(3)	Acidic soil
M. silvestris BL2	nt	nt		Acidic forest soil
Methylobacterium extorquens AM1[§]	nt	nt		Airborne contaminant
M. extorquens PA1[§]	nt	nt		
M. extorquens DM4[§]	nt	nt		Industrial wastewater
Methylobacterium chloromethanicum CM4[§]	nt	nt		Contaminated soil
M. nodulans ORS 2060[§]	nt	nt		Root nodules
Methylobacterium populi BJ001[§]	nt	nt		Poplar tree
Methylobacterium sp. 4–46[§]	nt	nt		Root nodules
Methylobacterium radiotolerans JCM 2831[§]	nt	nt		
Cyanobacteria				
G. violaceus PCC 7421	+	+	(4)	Calcareous rocks
T. elongatus BP-1	nt	nt		Hot spring
Cyanothece sp. 7424[§]	nt	nt		Rice field
Cyanothece sp. 7425[§]	nt	nt		Rice field
Cyanothece sp. PCC7822[§]	nt	nt		Rice field
Nostoc puncitforme PCC73102	+	+	(4)	Gymnosperm symbiont
Acidobacteria				
Candidatus Koribacter versatilis Ellin345	nt	nt		Soil

*2-MeC30 indicates 2-methylhopenes.

[†] 2-MeC35 indicates 2-methylbacteriohopanetetrol.

[‡] Indicates not tested.

[§] 2-methylhopanoid production has been shown in other *Methylobacterium* and *Cyanothece* strains (4, 5).

1. Rashby SE, et al. (2007) Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proc Natl Acad Sci USA* 104:15099–15104.
2. Bravo JM, et al. (2001) Novel methylated triterpenoids of the gammacerane series from the nitrogen-fixing bacterium *Bradyrhizobium japonicum*. *Eur J Biochem* 268:1323–1331.
3. Vilcheze C, et al. (1994) Prokaryotic triterpenoids: new hopanoids from the nitrogen-fixing bacteria *Azotobacter vinelandii*, *Beijerinckia indica* and *Beijerinckia mobilis*. *Microbiology* 140:2749–2753.
4. Talbot HM, et al. (2008) Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. *Org Geochem* 39:232–263.
5. Knani M, Corpe WA, Rohmer M (1994) Bacterial hopanoids from pink pigmented facultative methylotrophs (PPFMs) and from green plant surfaces. *Microbiology* 140:2755–2759.

Table S2. Correlation of 2-methylhopanoid production with the presence of HpnP in bacteria tested for hopanoid production

Bacterial genome	2-methyls?	HpnP ortholog?	Ref.
α-Proteobacteria			
R. palustris TIE-1	Yes	Yes	(1)
R. palustris CGA009	Yes	Yes	(1)
R. palustris BisB18	Yes	Yes	This study
R. palustris BisA53	Yes	Yes	This study
R. palustris BisB5	Yes	Yes	This study
R. palustris HaA2	Yes	Yes	This study
Bradyrhizobium sp. BTAi1	Yes	Yes	(2)
Bradyrhizobium japonicum USDA110	Yes	Yes	(2)
Beijerinckia indica ATCC 9039	Yes	Yes	(3)
Zymomonas mobilis	No	No	(4)
Rhodospirillum rubrum	No	No	(5)
Gluconobacter oxydnas	No	No	(5)
Acetobacter pasteurianus	No	No	(6)
δ-Proteobacteria			
Geobacter metallireducens	No	No	(7)
Geobacter sulfurreducens	No	No	(7)
γ-Proteobacteria			
Methylococcus capsulatus	No	No	(5)
Azotobacter vinelandii	No	No	(5)
β-Proteobacteria			
Nitrosomonas europa	No	No	(5)
Burkholderia pseudomallei	No	No	(8)
Cyanobacteria			
G. violaceus PCC 7421	Yes	Yes	(9)
N. puncitforme PCC73102	Yes	Yes	(9)
Trichodesmium erythraeum	No	No	(9)
Anabaena variabilis	No	No	(9)
Microcystis aeruginosa	No	No	(9)
Crocosphaera watsonii	No	No	(9)
Synechocystis sp. PCC 6803	No	No	(9)
Actinobacteria			
Streptomyces coelicolor	No	No	(10)
Frankia alni	No	No	(11)
Firmicutes			
Alicyclobacillus acidocaldarius	No	No	(12)

1. Rashby SE, et al. (2007) Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proc Natl Acad Sci USA* 104:15099–15104.
2. Bravo JM, et al. (2001) Novel methylated triterpenoids of the gammacerane series from the nitrogen-fixing bacterium *Bradyrhizobium japonicum*. *Eur J Biochem* 268:1323–1331.
3. Vilcheze C, et al. (1994) Prokaryotic triterpenoids: new hopanoids from the nitrogen-fixing bacteria *Azotobacter vinelandii*, *Beijerinckia indica* and *Beijerinckia mobilis*. *Microbiology* 140:2749–2753.
4. Moreau RA, et al. (1995) Analysis of intact hopanoids and other lipids from the bacterium *Zymomonas mobilis* by high-performance liquid chromatography. *Anal Biochem*224:293–301.
5. Rohmer M, et al. (1984) Distribution of hopanoid triterpenes in prokaryotes. *J Gen Microbiol* 130:1137–1150.
6. Zundel M, et al. (1985) Prokaryotic triterpenoids. 1. 3 beta-Methylhopanoids from *Acetobacter* species and *Methylococcus capsulatus*. *Eur J Biochem* 150:23–27.
7. Hartner T, et al. (2005) Occurrence of hopanoid lipids in anaerobic *Geobacter* species. *FEMS Microbiol Lett* 243:59–64.
8. Cvejic JH, et al. (2000) Bacterial triterpenoids of the hopane series as biomarkers for the chemotaxonomy of *Burkholderia, Pseudomonas* and *Ralstonia* spp. *FEMS Microbiol Lett* 183:295–299.
9. Talbot HM, et al, (2008) Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. *Org Geochem* 39:232–263.
10. Poralla K, et al. (2000) Hopanoids are formed during transition from substrate to aerial hyphae in *Streptomyces coelicolor* A3(2). *FEMS Microbiol Lett* 189:93–95.
11. Nalin R, et al. (2000) High hopanoid/total lipids ratio in *Frankia* mycelia is not related to the nitrogen status. *Microbiology* 146:3013–3019.
12. Poralla K, et al. (1980) A glycolipid containing hopane isolated from the acidophilic, thermophilic *Bacillus acidocaldarius*, has a cholesterol like function in membranes. *FEBS Lett* 113:107–110.

Table S3. Sensitivity of rooted HpnP topology to alignment parameters

Trial	Total taxa incl. outgroup(s)	Alignment program	Filtering by Gblocks	No. of sites	-lnL	Best topology*
1	83	ClustalW	None	622	35,976.33	C
2	83	ClustalW	Stringent	399	21,615.26	C
3	83	ClustalW	Relaxed	469	27,187.91	A
4	118	ClustalW	None	678	57,342.19	A
5	118	ClustalW	Stringent	280	22,884.90	A
6	118	ClustalW	Relaxed	422	38,833.93	A
7	83	Muscle	None	626	36,221.90	A
8	83	Muscle	Stringent	392	20,958.59	A
9	83	Muscle	Relaxed	468	26,874.87	B
10	118	Muscle	None	704	58,004.70	B
11	118	Muscle	Stringent	306	25,363.81	A
12	118	Muscle	Relaxed	411	36,969.22	A
13	83	T-Coffee	None	689	35,924.79	A
14	83	T-Coffee	Stringent	382	20,832.01	A
15	83	T-Coffee	Relaxed	452	25,723.67	A
16	118	T-Coffee	None	812	57,199.16	B
17	118	T-Coffee	Stringent	312	25,942.82	A
18	118	T-Coffee	Relaxed	394	34,708.95	A

***A** indicates a topology similar to Fig. 4*A*, with early branching cyanobacteria. **B**, topology similar to Fig. 4*B*, with monophyletic cyanobacteria sister to the α-proteobacteria. **C**, topology similar to Fig. 4*C*, with cyanobacteria branching within the α-proteobacteria. We did not consider the position of the lone acidobacterium sequence as it was not well-supported in most trials.

Table S4. Comparing alternative tree topologies using Shimodaira's Approximately Unbiased test

		-lnL for topology*			p-value for topology†		
Alignment	No. of sites	A	B	C	A	B	C
ClustalWno filtering	622	36,001.91	35,997.57	35,976.33	0.20	0.26	0.81
ClustalW relaxed	469	27,187.91	27,199.24	27,199.77	0.69	0.32	0.38
ClustalW stringent	399	21,621.43	21,634.54	21,615.26	0.46	0.18	0.68
Muscleno filtering	626	36,221.90	36,244.39	36,259.22	0.84	0.24	0.10
Musclerelaxed	468	26,884.17	26,874.87	26,899.22	0.41	0.73	0.16
Musclestringent	392	20,958.59	20,982.64	20,964.40	0.72	0.14	0.42

*For each alignment, the best tree was found by PhyML and its likelihood is shown in bold. The two alternative topologies were fixed for each alignment to topology A, B, or C from Fig. 4 and then branch lengths and substitution parameters were optimized on that topology, giving the likelihood values shown. These three trees were then compared using the AU test.

†p-values <0.05 allow us to reject that tree as the most likely. If no p-value is <0.05, then all three trees belong to the confidence set and the true tree could be any one of them.

Table S5. Strains and plasmids used in this study

Strains or plasmids	Genotype, markers, or characteristics
Strains	
E. coli S17-1	*thi pro hdsR* hdsM^{+} *recA*; chromosomal insertion of RP4-2 (Tc::Mu Km::Tn7)
R. palustris TIE-1	Isolated from Woods Hole, MA
R. palustris ΔhpnP	*R. palustris* TIE-1 ΔhpnP
R. palustris ΔhpnP + pPVW24	*R. palustris* TIE-1 ΔhpnP transformed to Gm^{r} with pPVW24
Plasmids	
pJQ200SK	Mobilizable suicide vector; *sacB* Gm^{r}
pBBR1MCS-5	5.1-kb broad hoast range plasmid; Gm^{r} *lacZ*
pPVW9	1-kb *hpnP* upstream region PCR amplified with primers PW39 and PW40 and cloned into pJQ200SK at *NotI* and *XbaI*
pPVW10	1-kb *hpnP* downstream region PCR amplified with primers PW41 and PW42 and cloned into pJQ200SK at *XbaI* and *SpeI*
pPVW17	*XbaI* and *SpeI* cut *hpnP* downstream fragment from pPVW10 subcloned into pPVW9
pPVW24	3.5-kb *hpnP* complementation fragment PCR amplified with primers PW85 and PW86 and cloned into pBBR1MCS-5 at *HindIII* and *SpeI*

Gm, gentamicin

Table S6. Data for hopanoid analysis of *R. palustris* strains by high-temperature GC-MS

Analyte	Rt*	M^+ (RI)	Base peak	Characteristic fragments (RI)
2-methylhopene	26.45	424(18)	189	205(73), 355(23), 381(25)
hopene	26.59	410(18)	189	191(93), 341(27), 367(23), 410(19)
2-methyldiplopterol acetate	27.68	484	149	189(92), 205(80), 381(5), 424(4)
diplopterol acetate	27.82	470	191	149 (93), 189(95), 367(5), 410(4)
2-methyltetrahymanol acetate	28.49	484	205	189(32), 263(5), 355(4), 424(4)
tetrahymanol acetate	28.64	470(6)	191	189(46), 249(6), 395(5), 410(4)
2-methylBHtetrol acetate	42.84	728(1)	205/95	253(10), 383(19), 433(5), 493(38), 713(1)
BHtetrol acetate	43.81	714(.5)	191	253(9), 369(18), 433(4), 493(34), 699(1)

*Retention time (min) on 30 m DB-XLB column and using an Agilent 6890GC/5975MSD.