

# Analysis of Bacteriohopanoids from Thermophilic Bacteria by Liquid Chromatography-Mass Spectrometry

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**Table S1.** Selected microorganisms analysed for the presence of bacteriohopanepolyols.

Current Name of Microorganism	Alternative Approved Name	Number of SHC Records	Example of GenBank Accession Number	References
<b>BACTERIA</b>				
<i>Alicyclobacillus acidocaldarius</i>	–	53	WP_014465455.1	[1–5]
strain 104-1A	ATCC 27009	–	–	[3,4]
<i>Bacillus subtilis</i> (spores)	–	>100	AAB84441.1	e.g. [6]
strain TB10	–	1	AHA77980.1	[6]
<i>Blastochloris viridis</i>	<i>Rhodopseudomonas viridis</i>	0	CUU42009.1 squalene synthase	[7]
<i>Bradyrhizobium japonicum</i>	<i>Rhizobium japonicum</i>	19	CAA60250.1	[8,9]
<i>Ca. Brocadia</i> sp.	–	18	QQR66062.1	[10]
<i>Burkholderia cepacia</i>	<i>Pseudomonas cepacia</i>	>100	KML22412.1	[11]
strain WH 8501	–	1	EAM53094.1	[12]
<i>Desulfovibrio bastinii</i>	<i>Maridesulfovibrio bastinii</i>	1	WP_034633519.1	[13]
strain DSM 16055	–	1	WP_034633519.1	[13]
<i>Frankia</i> sp.	–	–	ABW14125.1	[10]
Ea1-12	DSM 107422	3	WP_112105706.1	[10]
<i>Frateuria aurantia</i>	–	2	AFC87535.1 AFC85227.1	[14]
strain DSM 6220	–	2	AFC87535.1 AFC85227.1	[14]
<i>Geobacter metallireducens</i>	–	6	EHP88049.1	[15]
strain DSMZ 7210	GS-15	2	ABB33038.1 ABB30662.1	[15]
<i>Geobacter sulfurreducens</i>	–	11	WP_045667657.1	[15]
<i>Komagataeibacter xylinus</i>	<i>Acetobacter aceti</i> subsp. <i>xylinus</i>	15	WP_159262470.1	[10,16]
strain R-2277	–	0	–	[10]
<i>Methylococcoides burtonii</i>	–	5	WP_009061034.1	[17]
strain Sol V	–	1	CCG92847.1	[17]
<i>Methylobacterium fujisawaense</i>	–	0	WP_182591194.1 squalene synthase	[18]
<i>Methylobacterium organophilum</i>	–	5	PVY93823.1	[19]
<i>Methylocella palustris</i>	–	0	–	[10,17]
<i>Methylococcus capsulatus</i>	–	5	WP_010960137.1	[20–24]
strain NCBI 11132	–	0	–	[20]
strain ATCC 33009	–	0	–	[21]
<i>Methylomirabilis oxyfera</i>	–	2	CBE67168.1 KAB2961492.1	[25]
<i>Methylomonas methanica</i>	–	5	OAI06767.1	[20,21]
strain NCBI 11130	–	1	OAI06767.1	[20]
strain ATCC 35067	–	0	–	[21]
<i>Methylophilus methylotrophus</i>	–	0	WP_018985602.1 squalene synthase	[21]
<i>Methylosinus trichosporium</i>	–	1	ATQ69413.1	[21,23]
OB3b	–	1	ATQ69413.1	[21]
<i>Methylovulum psychrotolerans</i>	–	9	WP_103975581.1	[26]
strain Sph1 <sup>T</sup>	LMG 29227	2	POZ51441.1 POZ50074.1	[26]
strain OZ2	–	0	–	[26]
strain Sph56	–	0	MH701868.1	[26]

			partial sequence	
<i>Nitrosomonas europaea</i>	–	1	WP_011111759.1	[27]
<i>Paenibacillus arachidis</i>	–	0	–	[28]
<i>Rhodobacter capsulatus</i>	<i>Rhodopseudomonas capsulata</i>	0	P17056.1 phytoene synthase	[7]
<i>Rhodoblastus acidophilus</i>	<i>Rhodopseudomonas acidophila</i>	5	WP_155446705.1	[29,30]
strain DSM 145	–	0	–	[29,30]
<i>Rhodomicrobium vannielii</i>	–	3	WP_210336511.1	[31]
strain ATCC 17100	–	1	ADP72221.1	[31]
<i>Rhodopseudomonas palustris</i>	–	36	WP_047307546.1	[16,29,32–35]
strain DSM 139	–	0	–	[29]
strain TIE1	–	1	ACF02757.1	[32–35]
strain DSM-123	–	0	–	[34]
strain CGA009	–	1	WP_011159278.1	[34]
strain BisA53	–	1	ABJ08391.1	[34]
strain BisB5	–	1	ABE40796.1	[34]
strain BisB18	–	1	ABD87279.1	[34]
strain HaA2	–	1	ABD06434.1	[34]
<i>Rubrivivax gelatinosus</i>	<i>Rhodopseudomonas gelatinosa</i>	0	WP_200222390.1 squalene synthase	[7]
<i>Salinicoccus halitifaciens</i>	–	0	–	[36]
<i>Ca. Scalindua brodeae</i>	<i>Ca. Scalindua sorokinii</i>	1	KHE93133.1	[10]
<i>Streptomyces coelicolor</i>	–	3	QFI46551.1	[37]
strain A3(2)	–	2	QFI46551.1 CAB39697.1	[37]
<i>Streptomyces peucetius</i>	–	1	ATW51727.1	[38]
strain <i>S. peucetius</i> subsp. <i>caesi</i>	–	2	ACA52082.1 ATW51727.1	[38]
strain ATCC 27952	–	3	WP_059080096.1	[39]
<i>Streptomyces scabiei</i>	–	1	CBG68454.1 (putative)	[39]
strain 87-22	–	1	–	–
<i>Streptomyces venezuelae</i>	<i>Streptomyces venezuelensis</i>	21	WP_150501443	–
<i>Thiocapsa rosea</i>	<i>Amoebobacter roseus</i>	0	–	[11]
<i>Zymomonas mobilis</i>	–	46	WP_038259183.1	[22]
<i>Zymomonas mobilis</i> subsp. <i>mobilis</i>	–	21	ACV76194.1	[40]
strain ATCC 29191	–	2	AFN57413.1 AFN56323.1	[40]
CYANOBACTERIA - marine				
<i>Crocospaera watsonii</i>	–	9	WP_021835865.1	[12,41]
WH8501	–	1	EAM53094.1	[41]
<i>Synechococcus</i> sp.	–	10	AFY75324.1	[41]
strains PCC 6907, PCC 6714, WH8102	–	0	–	[41]
<i>Trichodesmium erythraeum</i>	–	2	WP_011610552.1	[41]
strain IMS-101	–	1	ABG50159.1	[41]
CYANOBACTERIA – non-marine				
<i>Anabena cylindrica</i> *	–	0	–	[41]
<i>Anacystis montana</i> *	–	0	–	[41]
<i>Calothrix</i> sp.	–	23	WP_096687654.1	[41]
<i>Chlorogloeopsis</i> sp.	–	0	–	[41,42]
<i>Chlorogloeopsis fritschii</i>	–	2	WP_016878379.1 RUR72374.1	[41]

<i>Chroococcidiopsis</i> sp.	–	1	WP_169244124.1	[41]
<i>Cyanothece</i> sp.	–	2	WP_107670655.1	[41]
<i>Gleocapsa</i> sp.	–	3	ELS00147.1	[41]
<i>Microcystis</i> sp.	–	> 100	WP_002732984.1	[41]
strains CCAP 1405/3, CCAP 1450/13, 110	–	0	–	[41]
<i>Microcystis aeruginosa</i>	–	100	WP_052276441.1	[41]
strain PCC 7808	–	0	–	[41]
<i>Nostoc</i> sp.	<i>Amorphonostoc</i>	96	AFY41090.1	[41]
strain PCC 6720	–	0	–	[41]
<i>Nostoc muscorum</i>	<i>Desmonostoc muscorum</i>	2	WP_193917519.1	[41]
strains B 1452-12b, CCAP 1453/12	–	0	–	[41]
<i>Oscillatoria amphigranulata</i>	<i>Pseudanabaena amphigranulata</i>	0	–	[41]
<i>Phormidium</i> sp.	–		WP_190625411.1	[41]
strain OSS4	OLV, CYX	0	–	[41]
strain RCG	PR11	0	–	[41]
strain RCO	–	0	–	[41]
<i>Phormidium luridum</i>	<i>Drouetiella lurida</i>	0	–	[41]
<i>Prochlorothrix hollandica</i>	–	1	WP_017714382.1	[41,42]
strain CCAP 1490/1	–	0	–	[41]
<i>Synechocystis</i> sp.	–	10	WP_028946973.1	[41]
PCC 6714	–	1	AIE72536.1	[41]
PCC 6803	–	8	QWO81910.1	[41]

SHC – squalene-hopene cyclase

\* no record at all in the NCBI database

**Table S2.** List of the cultivated bacterial isolates from four springs of Carlsbad (Karlovy Vary), Czech Republic and ten strains obtained from the Czech Collection of Microorganisms, Brno, Czech Republic.

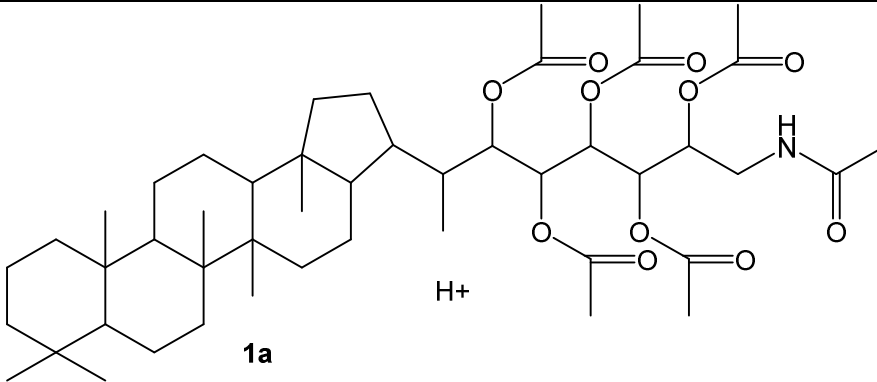
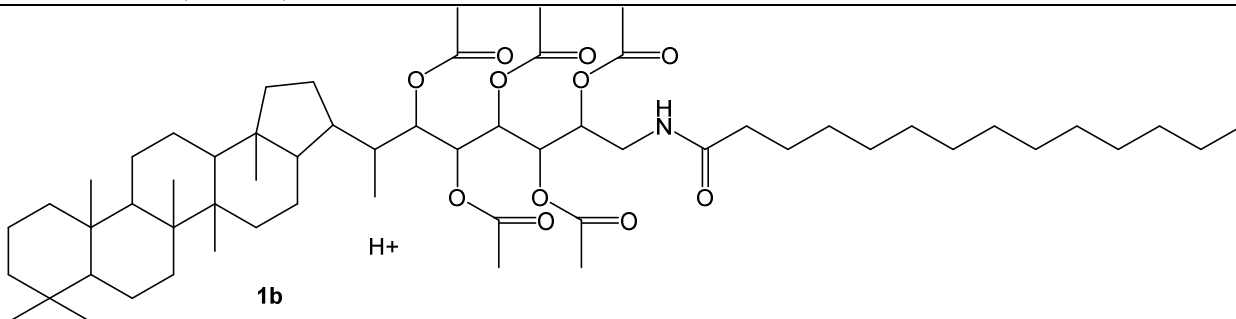
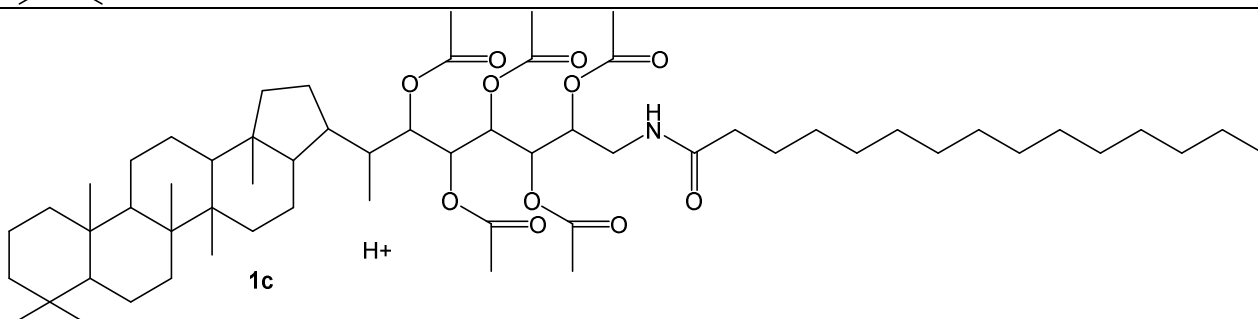
Bacterium	Cultiv. Temp.	Reference
<i>Geobacillus stearothermophilus</i> CCM 2062 (no information about source)	70	[43]
<i>Geobacillus stearothermophilus</i> CCM 5965 (Evaporated milk)	70	[43]
<b><i>Geobacillus stearothermophilus</i> ST-YPD (spring Štěpánka) <sup>a</sup></b>	<b>58</b>	[44]
<b><i>Geobacillus stearothermophilus</i> VR-1 (spring Vřídlo) <sup>a</sup></b>	<b>58</b>	[44]
<i>Geobacillus stearothermophilus</i> CCM 2062	55	[45]
<i>Meiothermus ruber</i> CCM 4212 (Thermal pools, Hveragherti, Iceland)	70	[43]
<i>Meiothermus ruber</i> CCM 4212	65	[46]
<i>Meiothermus ruber</i> CCM 4212	55	[45]
<i>Alicyclobacillus acidoterrestris</i> CCM 4660 (Apple-grape-raspberry juice)	70	[43]
<i>Alicyclobacillus acidoterrestris</i> CCM 4660	45	[47]
<i>Thermus aquaticus</i> CCM 3488 (Thermally polluted river near Brussels, Belgium)	70	[43]
<i>Thermus aquaticus</i> CCM 3488,	65	[46]
<b><i>Geobacillus kaustophilus</i> ML-1 (spring Mlýnský) <sup>a</sup></b>	<b>58</b>	[44]
<b><i>Brevibacillus agri</i> SA-1 (spring Sadový) <sup>a</sup></b>	<b>42</b>	[44]

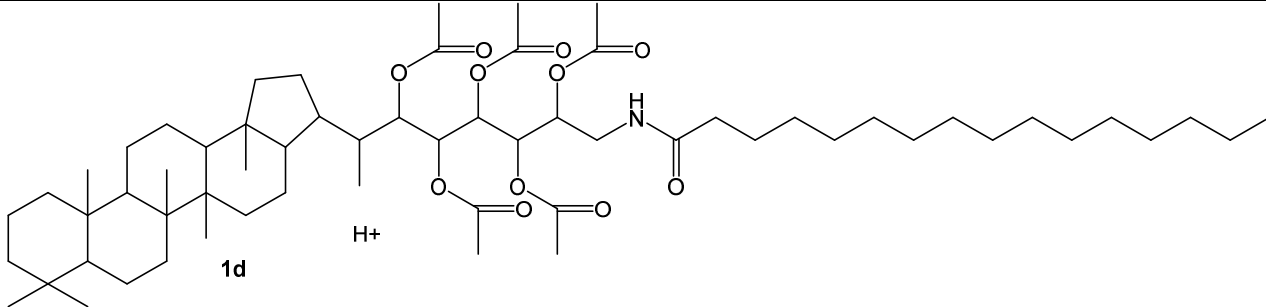
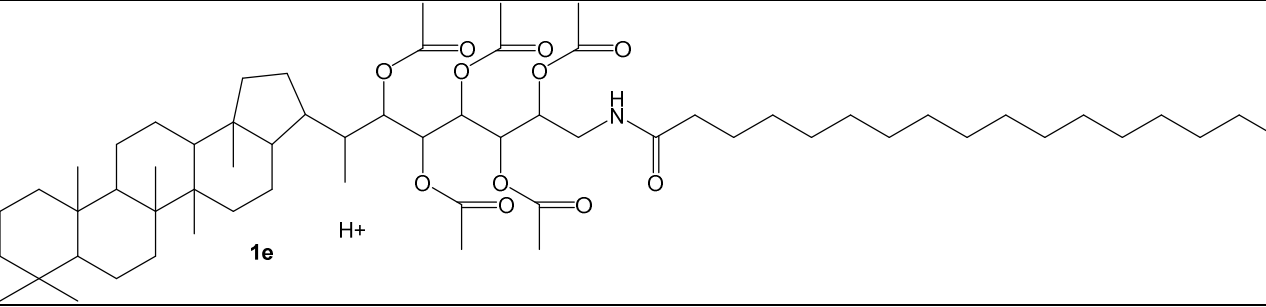
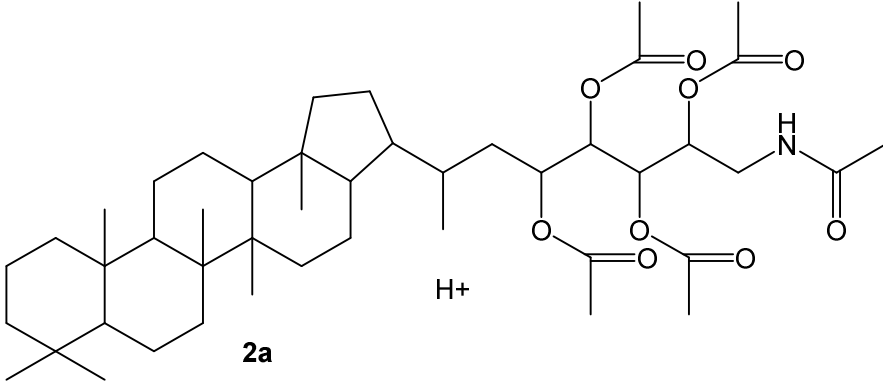
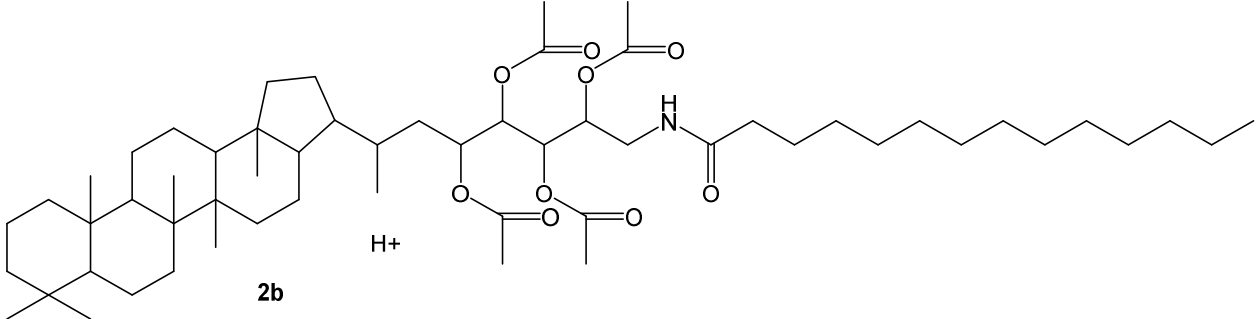
<sup>a</sup> The strains obtained from the thermal springs in Carlsbad, Czech Republic.

**Table S3.** List of the cultivated bacterial isolates from four springs of Carlsbad (Karlovy Vary), Czech Republic.

<b>Isolate</b>	<b>Hot spring</b>	<b>Cultivation</b>	<b>GenBank Accession</b>
<i>Brevibacillus agri</i> SA-1	Sadový	42	MT251434
<i>Geobacillus kaustophilus</i> ML-1	Mlýnský	58	MT251494
<i>Geobacillus stearothermophilus</i> ST-YPD	Štěpánka	58	MT251887
<i>Geobacillus stearothermophilus</i> VR-1	Vřídlo	58	MT251886

**Table S4.** Separation and identification of hopanoids from thermophilic bacteria by RP-LC/MS-ESI.

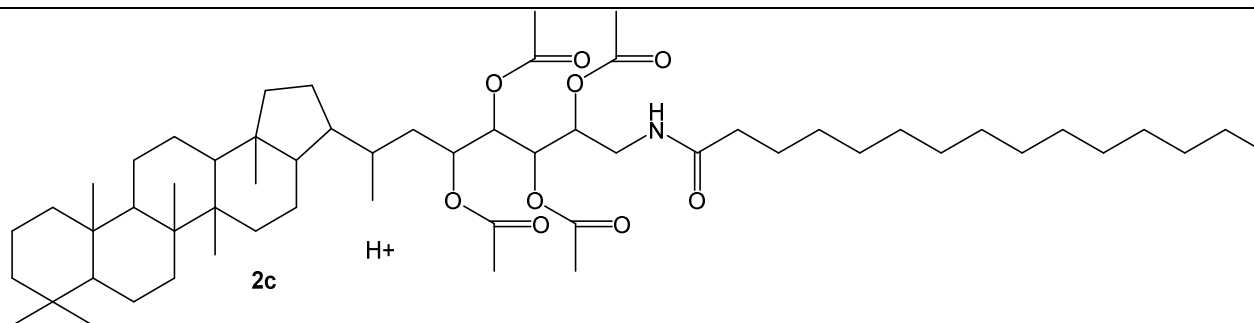
Abbreviation	Chemical Formula	<i>m/z</i>	Abundance (%)	tR (min)	Structure
<b>1a</b>	[C <sub>47</sub> H <sub>76</sub> NO <sub>11</sub> ] <sup>+</sup>	830.5413	42	23.10	 <p><b>1a</b></p>
<b>1b</b>	[C <sub>59</sub> H <sub>100</sub> NO <sub>11</sub> ] <sup>+</sup>	998.7294	8	29.38	 <p><b>1b</b></p>
<b>1c</b>	[C <sub>60</sub> H <sub>102</sub> NO <sub>11</sub> ] <sup>+</sup>	1012.7447	34	35.06+ 35.45	 <p><b>1c</b></p>

<b>1d</b>	$[\text{C}_{61}\text{H}_{104}\text{NO}_{11}]^+$	1026.7604	19	39.28	
<b>1e</b>	$[\text{C}_{62}\text{H}_{106}\text{NO}_{11}]^+$	1040.7760	22	42.67+ 43.03	
<b>2a</b>	$[\text{C}_{45}\text{H}_{74}\text{NO}_9]^+$	772.5358	21	26.17	
<b>2b</b>	$[\text{C}_{57}\text{H}_{98}\text{NO}_9]^+$	940.7236	5	33.32	

**2c** $[\text{C}_{58}\text{H}_{100}\text{NO}_9]^+$ 

954.7393

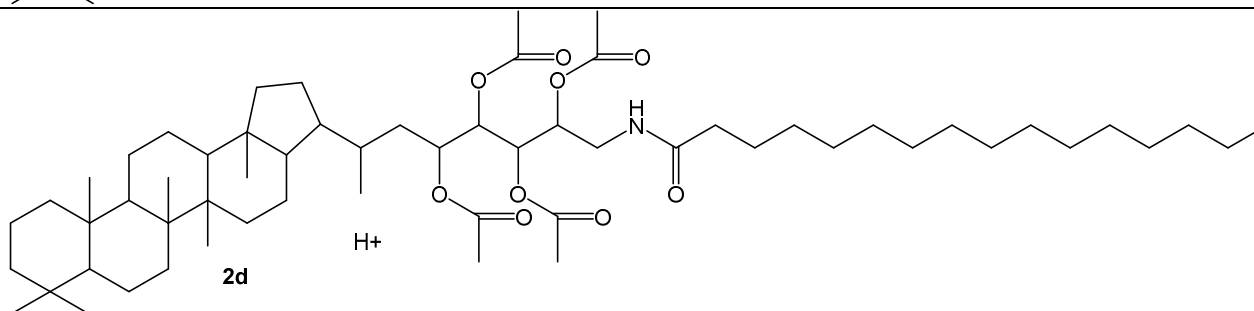
17

38.11+  
38.63**2d** $[\text{C}_{59}\text{H}_{102}\text{NO}_9]^+$ 

968.7549

11

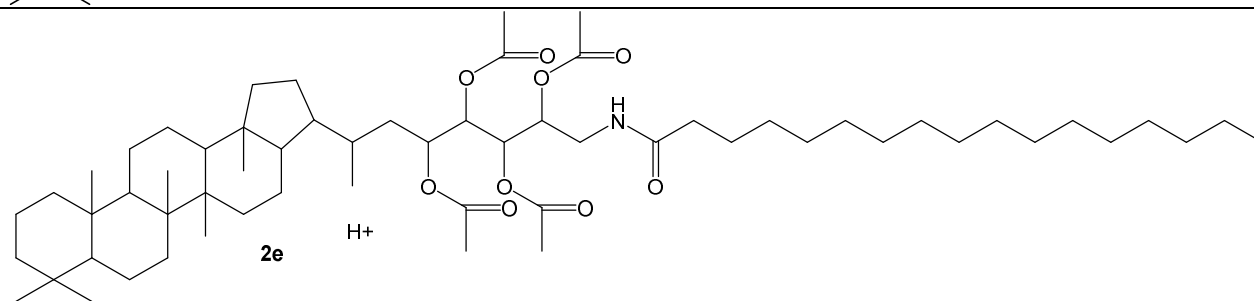
41.45

**2e** $[\text{C}_{60}\text{H}_{104}\text{NO}_9]^+$ 

982.7706

14

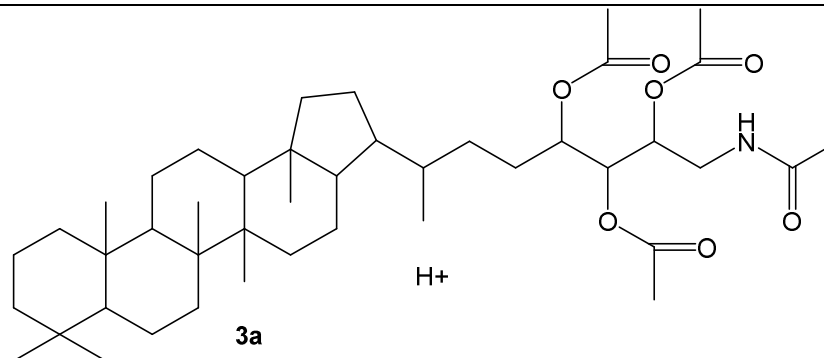
45.16

**3a** $[\text{C}_{43}\text{H}_{72}\text{NO}_7]^+$ 

714.5303

23

34.24



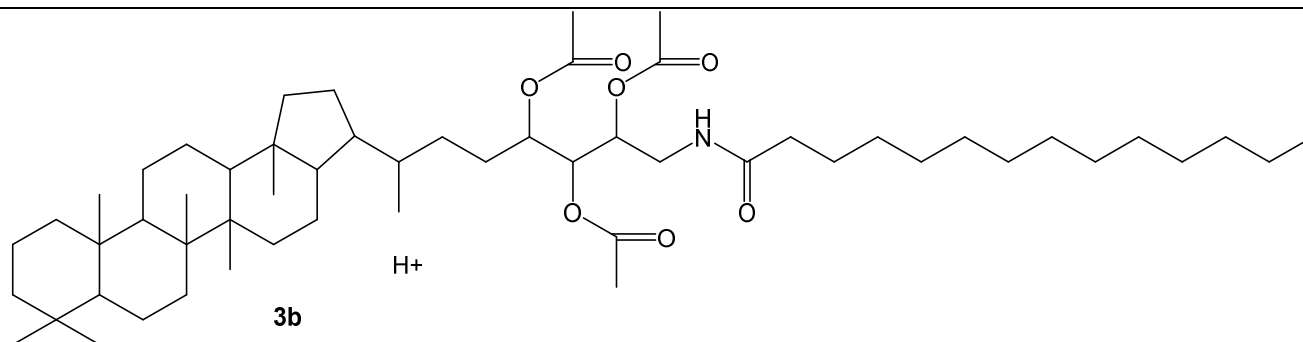


**3b** $[\text{C}_{55}\text{H}_{96}\text{NO}_7]^+$ 

882.7181

5

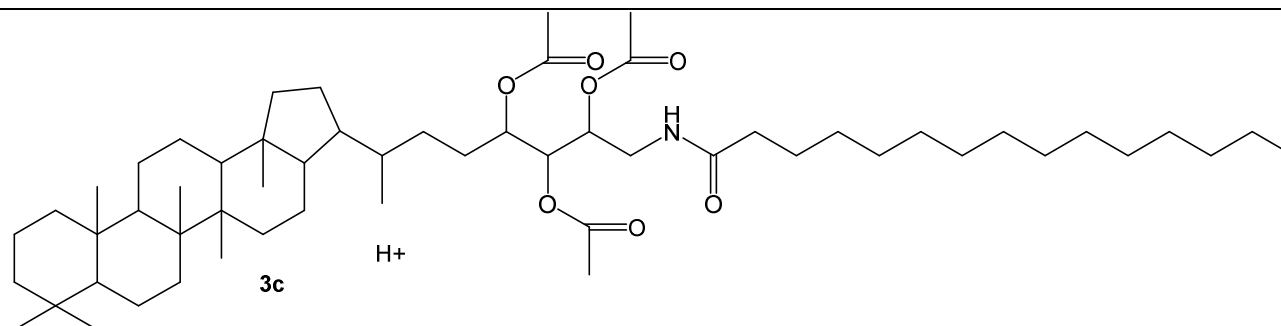
36.89

**3c** $[\text{C}_{56}\text{H}_{98}\text{NO}_7]^+$ 

896.7338

45

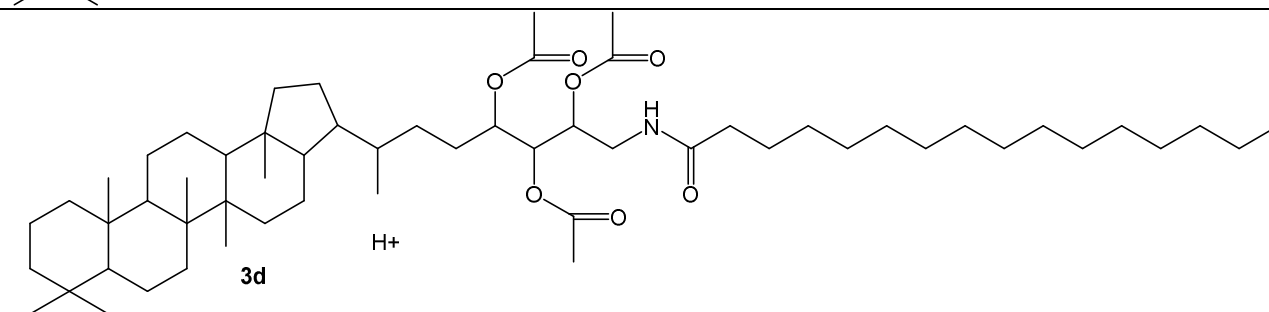
42.12

**3d** $[\text{C}_{57}\text{H}_{100}\text{NO}_7]^+$ 

910.7494

33

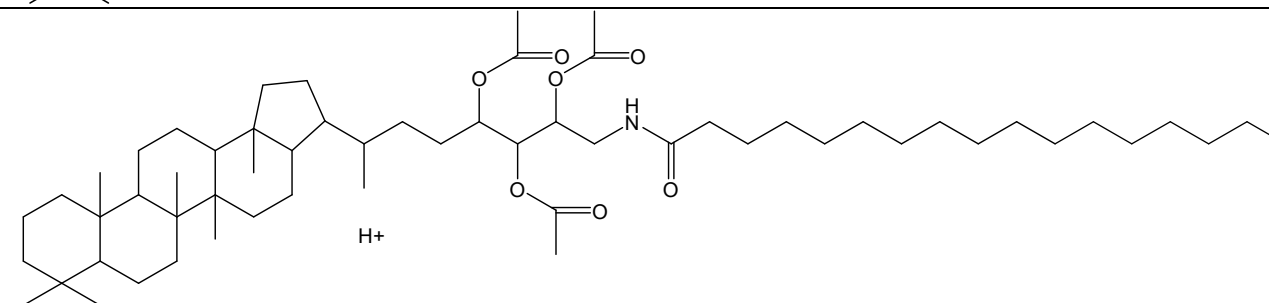
46.65

**3e** $[\text{C}_{58}\text{H}_{102}\text{NO}_7]^+$ 

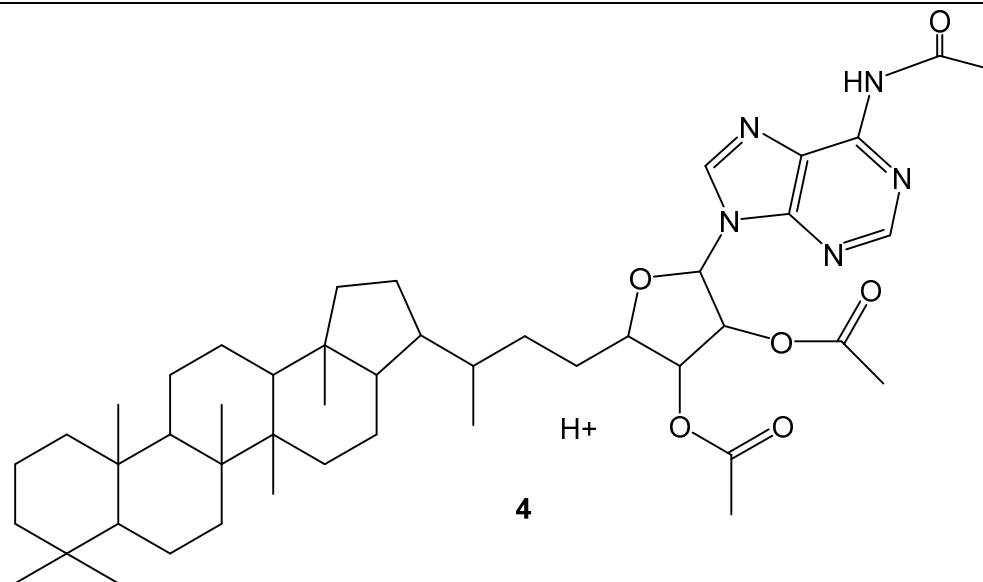
924.7651

24

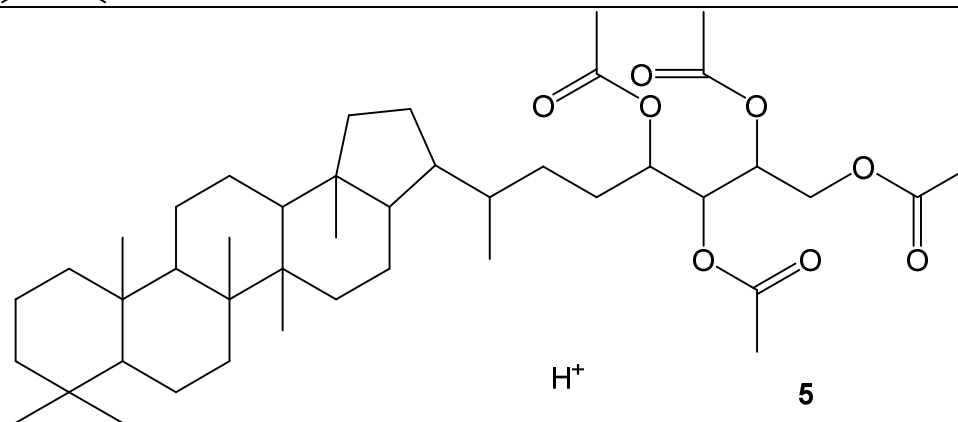
49.74



4 [C<sub>46</sub>H<sub>70</sub>N<sub>5</sub>O<sub>6</sub>]<sup>+</sup> 788.5321 18 32.13



5 [C<sub>43</sub>H<sub>71</sub>O<sub>8</sub>]<sup>+</sup> 715.5143 100 30.14



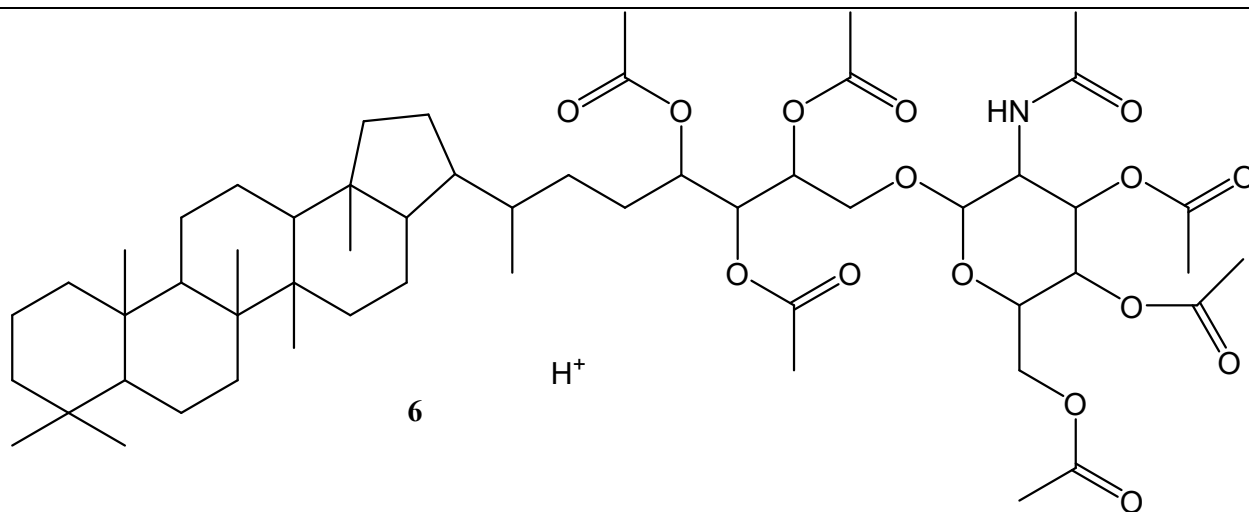
6

 $[\text{C}_{55}\text{H}_{88}\text{NO}_{15}]^+$ 

1002.6151

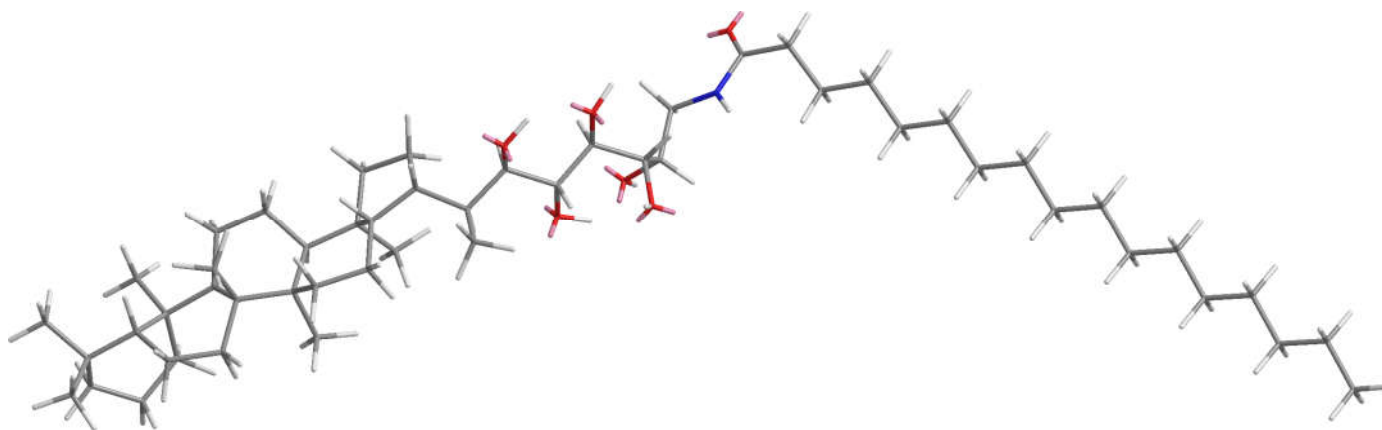
9

28.54



**Table S5.** Occurrence of hopanoid C-2 methylase and/or hopanoid C-3 methylase in analyzed thermophilic bacteria. In the table *hpnP* stands for hopanoid C-2 methylase gene and *hpnR* for hopanoid C-3 methylase. Sequencies used for analysis were as follows: B3QHD1 Hopanoid C-2 methylase from *Rhodopseudomonas palustris* (strain TIE-1) and Q60AV6 Hopanoid C-3 methylase from *Methylococcus capsulatus* (strain ATCC 33009).

	<i>Alicyclobacillus acidoterrestris</i>		<i>Brevibacillus agri</i>		<i>Geobacillus kaustophilus</i>		<i>Geobacillus stearothermophilus</i>		<i>Geobacillus thermoglucosidasius</i>	
Enzyme	<i>hpnP</i>	<i>hpnR</i>	<i>hpnP</i>	<i>hpnR</i>	<i>hpnP</i>	<i>hpnR</i>	<i>hpnP</i>	<i>hpnR</i>	<i>hpnP</i>	<i>hpnR</i>
<b>Detected protein with the highest similarity</b>	B12-binding domain-containing radical SAM protein	B12-binding domain-containing radical SAM protein	B12-binding domain-containing radical SAM protein	B12-binding domain-containing radical SAM protein	Mg-protoporphyrin IX monomethyl ester oxidative cyclase	Mg-protoporphyrin IX monomethyl ester oxidative cyclase	B12-binding domain-containing radical SAM protein	B12-binding domain-containing radical SAM protein	B12-binding domain-containing radical SAM protein	B12-binding domain-containing radical SAM protein
<b>Similarity percentage</b>	61.2 %	84.3 %	48.5 %	63.5 %	48.9 %	65.9 %	55.5 %	92.0 %	50.4 %	64.7 %
<b>ID</b>	EPZ45238.1	EPZ45238.1	ELK42704.1	QAV14985.1 RNB47569.1 GED27563.1	BAD75227.1	BAD75227.1	KZM55362.1 QOR85095.1	ALA71377.1	RDE27903.1 WP_0032521 70.1 GCD83585.1	GCD83585.1 RDE27903.1 ALF09928.1



**Figure S1.** Model of *N*-acyl derivative of bacteriohopanepentol.

## References

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