## Supplementary Material 16S rRNA clone library

To investigate the total bacterial community a clone library based on the 16S rRNA gene was performed of the pool *Sphagnum* mosses from Andorra peat, next to *S. magellanicum* some *S. falcatulum* was present in this pool and both these species were analysed. Both 16S clone libraries showed the presence of *Alphaproteobacteria* (17%), *Verrucomicrobia* (13%) and *Gammaproteobacteria* (2%) and since the distribution of bacterial genera among the two species was comparable an average was made. In total a 180 clones were sequenced and analyzed for the phylogenetic trees see Fig. A1 and A2

The 16S clone libraries showed a very diverse set of bacteria to be present inside or on Sphagnum mosses. Compared to other studies the microbial community in Sphagnum peat soils (Dedysh et al., 2006; Kulichevskaya et al., 2007a; Opelt and Berg, 2004) is comparable to the microbial community found here, inside and attached on the Sphagnum mosses of the Patagonian peatlands. Most of the clones showed sequence similarity to isolates or environmental samples originating from peat ecosystems, of which most of them originate from Siberian acidic peat bogs. This indicated that similar bacterial communities can be found in peatlands in the Northern and Southern hemisphere implying there is no big geographical difference in microbial diversity in peat bogs. Four out of five classes of Proteobacteria were present in the 16S rRNA clone library; Alfa-, Beta-, Gamma and Deltaproteobacteria. 42 % of the clones belonging to the Alphaproteobacteria showed a 96-97% to Acidophaera rubrifaciens, a member of the Rhodospirullales an acidophilic bacteriochlorophyll-producing bacterium isolated from acidic hotsprings and mine drainage (Hiraishi et al., 2000). This bacterium is closely related to Acidosoma sibirica, an acidophilic bacterium isolated from a Sphagnum peat bog (Belova et al., 2009). Other clones also belonged to the Rhodospirullales family, of which several showed high homology to isolates from peat bogs, like Telmatospirillum siberiense (Sizova et al., 2007). Another 42% of the clones of the Alfaproteobacteria belonged to the order of Rhizobiales, to which also all isolated methanotrophs belong of which four clones show high homology (98-99%) to the methanotrophic symbiont of Sphagnum found in the Mariapeel (Raghoebarsing et al., 2005). Unfortunately this methanotrophic symbiont has not been cultivated yet. Only a few methanotrophs have been isolated from peat ecosystems, all belonging to the Alphaproteobacteria but no close relatives to those were found in the clone library. There is a possibility more unknown methanotrophs belong to this order, but so far these 16S rRNA sequences cannot reveal a metabolic importance of the obtained clones. From other environments methanotrophs have been isolated belonging to the Gammaproteobacteria and Verrucomicrobia, but so far none from peat ecosystems and none of the clones showed high homology to other isolates methanotrophs of these classes. The clones belonging to the Verrucomicrobia were almost all highest related to Opititus spp. of which some were isolated from rice fields, they have never been demonstrated to oxidize methane (Janssen et al., 1997). Only three verrucomicrobial methanotrophs are known and all are thermophilic and originating from volcanic regions, none of the clones were closely related to these methanotrophs. As shown with the microarray more often relatives to thermophilic methanotrophs can be detected in peat ecosystems, which might indicate the presence of new methanotrophic species. Verrucomicrobial methanotrophs are recently discovered and opened new possibilities to find new and unknown methanotrophs in all methane rich ecosystems. Two clones belonged to the Deltaproteobacteria being closest relative to Bdelvibrio bacteriovorus and Spirobacillus cienkowskii, both pathogens. Pathogens have been shown to occur abundantly in Sphagnum (Opelt et al., 2007), but so far their presence in these ecosystems has not been understood. Besides these classes an abundance of Acidobacteria (28%) and Betaproteobacteria (22%) was found, both classes have been found to occur in peat ecosystems, but their role has not been discovered yet. Belova and coworkers (Belova et described the presence of Burkholderia species, belonging to al., 2006) the Betaproteobacteria in Western Siberian, Canadian and Estonian peat bogs and Opelt and coworkers showed the abundance of the genus Burkholderia of endo and ectophytic bacterial populations in bryophytes (Opelt et al., 2007; Opelt and Berg, 2004). These Burkholderia species are antagonistic bacteria with antimicrobial activity, which is thought to be used by the Sphagnum mosses as a defense strategy against fungi (Opelt et al., 2007). Acidobacteria have been detected and isolated from peat (Kulichevskaya et al., 2010; Pankratov et al., 2008), where they most likely grow on various heteropolysaccharides and galacturonic acid, which are released during decomposition of Sphagnum moss and vascular plant debris. Three percent of the clones belonged to the *Planctomycetes*, which are often detected in *Sphagnum* dominated peatlands (Dedysh et al., 2006; Kulichevskaya et al., 2006) and several have been isolated (Kulichevskaya et al., 2009; Kulichevskaya et al., 2008; Kulichevskaya et al., 2007b) and several clones show homology to these isolates. A few clones belonged to the Bacteriodetes, showing sequence similarity to the Sphingobacteria genus containing Mucilaginibacter spp. which were isolated from a Sphagnum peat bog. This species was shown to degrade pectin, xylan and laminarin in peat. The 16S rRNA clone library was able to detect a broad variety of bacteria, which is comparable to other studies. However, no clones were found to be related to isolated methanotrophs and only a few clones were related to the symbiotic methanotroph from *Sphagnum* moss. The used primer set is a general primer set, that however does not always anneal to DNA isolated from methanotrophs (Kip, personal communication), which could explain why methanotrophs were not represented in this clone library. It could be that the used primer set does not detect methanotrophs in environmental samples or is biased against methanotrophs. Described 16S rRNA clone libraries performed on peat also showed only a few clones with homology to known methanotrophs (Dedysh et al., 2006; Raghoebarsing, 2006). Another explanation for the lack of methanotrophs present that are not detected by the primer set or the DNA isolation technique is not able to extract methanotrophic DNA as easily as DNA of other bacteria. The primer set does anneal to Verrucomicrobial methanotrophs, which were neither found within these clone libraries.

## **Materials and Methods**

## 16S rRNA gene and pmoA gene clonelibraries

PCR amplification of the 16S rRNA gene was performed with two general bacterial 16S rRNA gene primers: 616F and 630R (Juretschko et al., 1998). All PCRs were performed in a gradient from 50 to 60 degrees and PCR products were combined. All PCR products were purified using the QIAquick PCR Purifications Kit (Qiagen). DNA sequencing was performed with the primers used in the PCR and for the 16S rRNA gene also the universal bacterial primer 612R (Ehrmann et al., 2003) was used. The pGEM-T Easy Vector System Kit (Promega) was used for ligation of the pmoA and 16S rRNA gene amplifications. Ligation was performed as prescribed by the manufacturer. 16S rRNA gene ligation mix was transformed to Top10 competent E. coli cells (Invitrogen<sup>TM</sup>) by heat shock exposure (30-45 seconds at 42°C). Plasmid DNA with ligated 16S rRNA gene was isolated with the FastPlasmid Mini Kit (Eppendorf) following the instructions of the manufacturer. pDNA with ligated pmoA gene was isolated with the E.Z.N.A.<sup>TM</sup> Plasmid Miniprep Kit (EZNA<sup>TM</sup>). Partial 16S rRNA gene fragments were sequenced with M13 forward and reverse primers (Invitrogen<sup>TM</sup>), targeting vector sequences adjacent to the multiple cloning site. pDNA sequencing was performed by the sequencing facility of the UMC Sint Radboud, Nijmegen. Clone library sequences and their closes relatives were analyzed using MEGA version 4 (Tamura et al., 2007). All sequences were aligned automatically using the alignment tool of MEGA4 (ClustalW).

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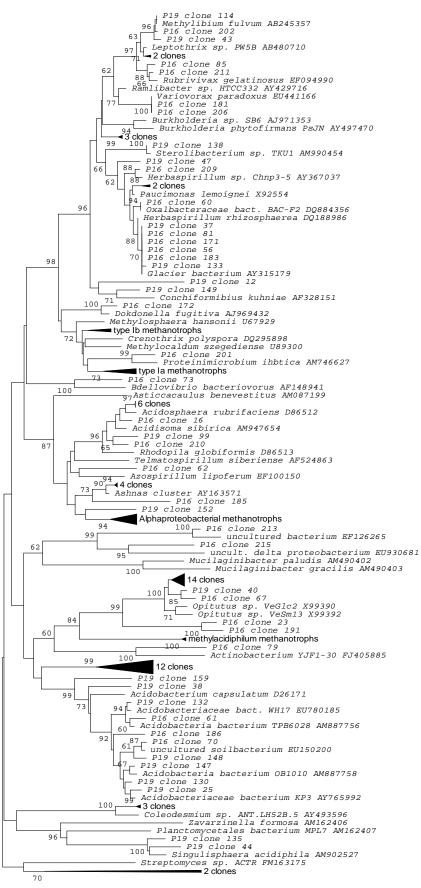
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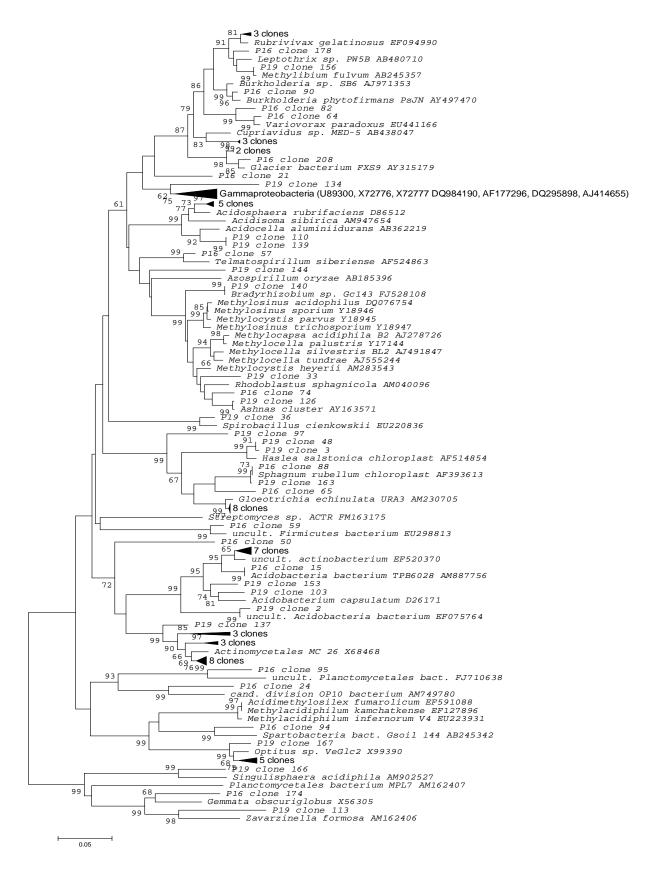
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**Fig. A1.** 16S rRNA phylogenetic tree showing relationship of the partial 16S rRNA gene sequences (position 934 to 1408 of the 16S rRNA gene, *E. coli* numbering) to representatives and closest relatives of different bacterial classes. Bootstrap values > 60 are indicated.

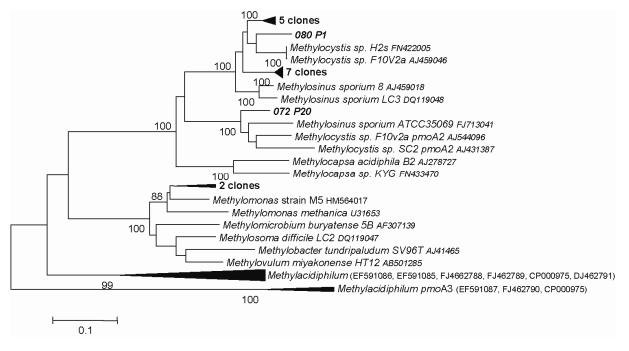
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**Fig. A2.** 16S rRNA phylogenetic tree showing relationship of the partial 16S rRNA gene sequences (position 104 to 495 of the 16S rRNA gene, *E. coli* numbering) to representatives and closest relatives of different bacterial classes. Bootstrap values > 60 are indicated.

**Fig. S1.** *pmo*A gene phylogenetic tree based on DNA showing the relationship of the clones to selected methanotrophs. The tree was based on Neighbour-Joining analysis using a bootstrap test of 500 replicates. Bootstrap values of >60 are indicated at the node of the branch.



**Supplementary Material Table S1**. Probes used for microarray analysis. The order corresponds to the order in which the probes are arranged on the microarray analysis shown in Figure 1.

Order	Name	Intended specificity
1	MbA557	Methylobacter
2	MbA486	Methylobacter
3	Mb460	Methylobacter
4	Mb_LW12-211	Methylobacter
5	Mb_SL#3-300	Methylobacter
6	Mb_SL299	soda lake Methylobacter isolates and clones
7	Mb_SL#1-418	soda lake Methylobacter isolates and clones
8	MmbB284	Mmb. Buryatense - same region as Jpn284, but 3 MM vs. that one Methylobacter and Japanese strain related
9	Jpn284	clone Jpn 07061
10	BB51-302	Methylobacter
11	Mb267	Methylobacter
12	Mb292	Methylobacter
13	Mb282	Methylobacter
14	Mb_URC278	Methylobacter
15	511-436	Methylobacter
16	LP10-424	Methylobacter LP 10 group
17	LF1a-456	Methylobacter LF 1a group
18	Mb_C11-403	Methylobacter
19	Mb380	M.bacter broad group A universal?
20	Mb271	Methylobacter
21	S14m2-270	Marine type Ia cluster, S14m#2
22	S14m2-406	Marine type Ia cluster, S14m#2
23	PS80-291	clone PS-80
24	MS1-440	Marine type Ia cluster, Marine sediment #1
25	Mm_pel467	Methylomicrobium pelagicum
26	Kuro18-205	Marine type Ia cluster, Kuro18
27	DS1-401	Deep sea cluster #1
28	Mm531	Methylomonas
29	Mm_M430	Methylomonas
30	Mm_RS311	Mm.methanica, RS clade(10-286)
31	Mm_ES294	Methylomonas
32	Mm_ES543	Methylomonas
33	Mm_ES546	Methylomonas
34	Mm_MV421	Methylomonas
35	Mm451	Methylomonas
36	Mm275	Methylomonas

37	Alp7-441	Alpine soil Methylomonas, Alp#7 (10-282)
38	peat_1_3-287	Mehtylomonas-related peat clones
39	Est514	Methylomicrobium-related clones
40	Mmb259	Methylomicrobium album + Landfill M.microbia
41	Mmb303	Methylomicrobium album
42	Mmb304	Methylomicrobium album + Landfill M.microbia and related
43	LW14-639	Methylomicrobium LW14 group
44	Mmb_RS2-443	Methylomicrobium, Mmb_RS2
45	Mmb562	Mmb. album and Methylosarcina
46	Mm229	Deep-branching M.monas (?) group (WHmb3 related group)
47	MsQ290	M.sarcina quisquilliarum related
48	MsQ295	M.sarcina quisquilliarum
49	LP20-644	Methylomicrobium-related clones
50	LP20-607	LP20 group (Type Ia, deep branching-Mmb?)
51	Ia193	Type I a (M.bacter-M.monas-M.microbium)
52	Ia575	Type I a (M.bacter-M.monas-M.microbium-M.sarcina)
53	Bsed516	Marine sediment #2, Bsed
54	SWI1-375	Marine sediment #2, SW#1
55	SWI1-377	Marine sediment #2, SW#1
56	Nc_oce426	Nitrosococcus oceani
57	DS2-287	Deep sea #2, subgroup (N.coccus and Deep sea Type Ia 10-298)
58	AIMS1-442	Deep sea #2, AIMS#1
59	DS2-220	Deep sea #2, subgroup
60	DS2-626	Deep sea #2, subgroup
61	USCG-225	Upland soil cluster Gamma
62	USCG-225b	Upland soil cluster Gamma
63	JR2-409	JR cluster #2 (California upland grassland soil)
64	JR2-468	JR cluster #2 (California upland grassland soil)
65	JR3-505	JR cluster #3 (California upland grassland soil)
66	JR3-593	JR cluster #3 (California upland grassland soil)
67	501-375	Methylococcus- related marine and freshwater sediment clones
68	501-286	Methylococcus- related marine and freshwater sediment clones
69	USC3-305	Upland soil cluster #3
70	Mc396	Methylococcus
71	MclT272	Methylocaldum tepidum
72	MclG281	Methylocaldum gracile
73	MclS402	Methylocaldum szegediense
74	MclS394	Methylocaldum szegediense and related
75	MclS400	Methylocaldum szegediense and related
76	MclE302	Methylocaldum E10
77	Mcl404	Mc.capsulatus-Mcl.tepidum-Mcl. Gracile-Mcl.Szeg and related

78	Mc1408	Methylocaldum
	fw1-286	fw-1 group: <i>M.coccus-M.caldum</i> related marine and freshwater
79	fw1-639	sediment clones fw-1 group: <i>M.coccus-M.caldum</i> related marine and freshwater
80	1W1-039	sediment clones
	fw1-641	fw-1 group: <i>M.coccus-M.caldum</i> related marine and freshwater
81		sediment clones
82	JHTY1-267	JH-TY#1
83	JRC4-432	Japanese rice cluster #4
84	OSC220	Finnish organic soil clones and related
85	OSC300	Finnish organic soil clones and related
86	JRC3-535	Japanese Rice Cluster #3
87	LK580	fw-1 group + Lake Konstanz sediment cluster
88	RSM1-419	RSM#1
89	JHTY2-562	<i>JH-TY#2</i>
90	JHTY2-578	JH-TY#2
91	JRC2-447	Japanese Rice Cluster #2
92	LW21-374	LW21 group
93	LW21-391	LW21 group
94	M90-574	<i>M.coccus-M.caldum</i> related marine and freshwater sediment clones
95	M90-253	<i>M.coccus-M.caldum</i> related marine and freshwater sediment clones
96	Mth413	Methylothermus
97	Mha-500	Methylohalobius - M.thermus and related ?
98	DS3-446	Deep sea cluster #3
99	PmoC640	PmoC
100	PmoC308	PmoC
101	Ib453	Type I b ( <i>M.thermus-M.coccus-M.caldum</i> and related)
102	Ib559	Type I b ( <i>M.thermus-M.coccus-M.caldum</i> and related)
103	McyB304	M.cystis B (parvus/echinoides/strain M)
100	Mcy255	M.cystis B (parvus/echinoides/strain M)
105	Mcy459	Methylocystis
106	Mcy264	Methylocystis
100	Mcy270	Methylocystis
107	Mcy413	Methylocystis
109	Mcy522	Methlocystis A + peat clones
110	Mcy233	Methylocystis
111	McyM309	M.cystis strain M and related
112	Peat264	peat clones
112	MsS314	Methylosinus sporium
113	MsS475	Methylosinus sporium
114	Msi263	Methylosinus sporium + 1 Msi.trichosporium subclaster
115	Msi423	Methylosinus
110	MsT214	Methylosinus trichosporium OB3b and rel.
117		

118	Msi520	Methylosinus trichosporium
119	Msi269	Methylosinus trichosporium
120	Msi294	Methylosinus
121	ARC2-518	Deep branching type II clade ARC2 - Methylosinus trichosporium 15- 084 group?
122	Msi232	<i>M.sinus</i> + most <i>M.cystis</i> -considered as additional <u>type II</u> probe
123	II509	Type II
124	II630	Type II
125	Alp8-468	Type II novel pmoA, Alpine cluster Alp#8
126	xb6-539	Novel pmoA copy of type II and related environmental clones
127	LP21-190	Novel <i>pmoA</i> copy of type II and related environmental clones
128	LP21-260	Novel <i>pmoA</i> copy of type II and related environmental clones
129	NMcy1-247	Novel pmoA copy of M.cystis #1 (?)
130	NMsi1-469	Novel pmoA copy of M.sinus
131	NMcy2-262	Novel pmoA copy of M.cystis #2 (?)
132	LP21-436	Mcy + Msi novel pmoA #1 groups
133	NMsiT-271	Novel pmoA copy of M.sinus trichpsporium (?)
134	LP21-232	Novel <i>pmoA</i> copy of type II and related environmental clones
135	RA14-299	RA14 related clones
136	RA14-594	RA14 related clones
137	RA14-591	RA14 related clones
138	Wsh1-566	Watershed + flodded upland cluster 1
139	Wsh2-491	Watershed + flodded upland cluster 2
140	Wsh2-450	Watershed + flodded upland cluster 2
141	B2rel251	Methylocapsa-related clones
142	B2-400	Methylocapsa
143	B2-261	Methylocapsa
144	B2all343	Methylocapsa and related clones
145	B2all341	Methylocapsa and related clones
146	pmoAMO3-400	clone pmoA-MO3
147	pmoAMO3-486	MO3 group
148	pmoAMO3-511	MO3 group
149	ESR-579	ESR (Eastern Snake River) cluster
150	M84P22-514	environmental clones of uncertain identity
151	TUSC409	Tropical Upland Soil Cluster #2
152	TUSC502	Tropical Upland Soil Cluster #2
153	mtrof173	Universal
154	mtrof362-I	Methanotrophs
155	mtrof661	Methanotrophs
156	mtrof662-I	Methanotrophs
157	mtrof656	Methanotrophs