

1 **Distribution of PAHs and the PAH-degrading bacteria in the deep-sea**
2 **sediments of the high-latitude Arctic Ocean**

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19 **Abstract**

20 Polycyclic aromatic hydrocarbons (PAHs) are **common** organic pollutants that can be transferred
21 long distances and tend to **accumulate** in marine sediments. However, less is known regarding
22 the distribution of PAHs and their natural bioattenuation in the open sea, especially the Arctic
23 Ocean. In this report, sediment samples were collected at four sites from the Chukchi Plateau to
24 the Makarov Basin in the summer of 2010. PAH compositions and total concentrations were
25 examined with GC-MS. The concentrations of 16 EPA-priority PAHs varied from 2.0 to 41.6 ng
26 g⁻¹ dry weight and decreased with sediment depth and movement from the southern to the
27 northern sites. Among the targeted PAHs, phenanthrene was relatively abundant in all sediments.
28 The 16S rRNA gene of the total environmental DNA was analyzed with Illumina high
29 throughput sequencing (IHTS) to determine the diversity of bacteria involved in PAH
30 degradation *in situ*. **The potential degraders including *Cycloclasticus*, *Pseudomonas*, *Halomonas*,**
31 ***Pseudoalteromonas*, *Marinomonas*, *Bacillus*, *Dietzia*, *Colwellia*, *Acinetobacter*, *Alcanivorax*,**
32 ***Salinisphaera* and *Shewanella*, with *Dietzia* as the most abundant, occurred in all sediment**
33 **samples.** Meanwhile, enrichment with PAHs was initiated on board and transferred to the
34 laboratory for further enrichment and obtaining the degrading consortia. Most of the above
35 mentioned bacteria in addition to *Hahella*, *Oleispira*, *Oceanobacter* and *Hyphomonas* occurred
36 alternately as predominant members in the enrichment cultures from different sediments based
37 on IHTS and PCR-DGGE analysis. To reconfirm their role in PAH degradation, 40 different
38 bacteria were isolated and characterized, among which *Cycloclasticus* and *Pseudomonas* showed
39 the best degradation capability under low temperatures. Taken together, PAHs and
40 PAH-degrading bacteria were widespread in the deep-sea sediments of the Arctic Ocean. We
41 propose that bacteria of *Cycloclasticus*, *Pseudomonas*, *Pseudoalteromonas*, *Halomonas*,

42 *Marinomonas* and *Dietzia* may play the most important role in PAHs mineralization *in situ*.

43 **Keywords:** polycyclic aromatic hydrocarbons (PAHs), biodegradation, bacterial diversity,

44 deep-sea sediments, Arctic Ocean.

45

46 **1 Introduction**

47 The Arctic Ocean is the smallest major ocean and is almost completely surrounded by land. It
48 also has the most extensive shelves of any ocean basin. The loading of terrigenous organic carbon
49 via rivers flowing into the Arctic Ocean (Opsahl et al., 1999;Lobbes et al., 2000;Benner et al.,
50 2004) and the influence of terrigenous organic matter on bacterial diversity in coastal waters have
51 drawn attention (Ortega-Retuerta et al., 2012;Boeuf et al., 2014).

52 Polycyclic aromatic hydrocarbons (PAHs) are a type of aromatic hydrocarbons with two or
53 more fused benzene rings. PAHs tend to accumulate in marine sediments, and are a source of
54 significant environmental concern due to their toxicity, mutagenicity and carcinogenicity
55 (Haritash and Kaushik, 2009). Because PAHs are one of the main components of crude oil (in
56 addition to aliphatic hydrocarbons), the presence of PAHs in marine environments is mainly
57 attributed to oil spills, discharge and natural seepage, river import, or even air current transfer
58 (Latimer and Zheng, 2003). Therefore, the increase in human activities globally has increased the
59 risks to marine environments.

60 The Arctic Ocean remains less exploited due to its remoteness and ice cover. However, worries
61 concerning the Arctic ecosystem have increased in recent years. According to an assessment by
62 the U.S. Geological Survey, approximately 30% and 13% of the world's undiscovered gas and oil
63 reserves, respectively, may be found in the Arctic region (Gautier et al., 2009). Oil-drilling
64 platforms have been set up on some offshore shelves (McClintock, 2011;Schmidt, 2012). With
65 the oil exploitation and the future opening of the Northeast and Northwest passages, the
66 increased input of PAHs into this area is unavoidable.

67 PAHs are widespread in marine coastal sediments (Baumard et al., 1998;Witt, 1995). They
68 have also been found in surface sediments of the Arctic Ocean, with variable concentrations from

69 the shelf to basin (Yunker and Macdonald, 1995;Zaborska et al., 2011;Yunker et al., 2011). On
70 the Beaufort Sea shelf, the total concentrations of PAHs with a molecular weight of 178-278
71 reached 850 ± 230 ng g⁻¹ **dry weight (dw)**, with phenanthrene, benzo(*ghi*)perylene and
72 benzo[*b+k*]fluoranthene as the dominant constituents (Yunker and Macdonald, 1995). In the
73 western Barents Sea, the values of Σ PAHs ranged from 35 to 132 ng g⁻¹ dw.
74 Benzo[*b+k*]fluoranthene and phenanthrene dominated in the southern and northern areas,
75 respectively (Zaborska et al., 2011). In contrast, the total concentration of PAHs was much lower
76 in the high-latitude deep-sea basins, such as the Makarov Basin (35 ng g⁻¹ dw) (Yunker et al.,
77 2011).

78 As to the origin of PAHs in deep-sea sediments, **long-range atmospheric transport and**
79 **abiogenic production in deep-sea hydrothermal vents are believed to contribute to the**
80 **accumulation of PAHs (Friedman and Selin, 2012;Proskurowski et al., 2008;Simoneit et al.,**
81 **2004;Konn et al., 2009)**. In the case of the Arctic Ocean, it receives a large input of terrigenous
82 and fossil particulate organic matters delivered by fluvial transport and coastal erosion in
83 addition to combustion particulates contributed by atmospheric transport (Yunker et al., 2011).
84 For example, industry in the former Soviet Union provided a widespread source of atmospheric
85 PAHs to the Canadian High Arctic, which substantially decreased in the 1990s (Halsall et al.,
86 1997;Becker et al., 2006). **Therefore, these allochthonous PAHs could serve as carbon sources**
87 **for various PAH-degrading bacteria in the Arctic sediments.**

88 It is well known that bacterial degradation plays an important role in PAH removal from
89 marine environments. Many PAH-degrading bacteria have been found in coastal sediments,
90 including bacteria of *Cycloclasticus* (Dyksterhouse et al., 1995), *Marinobacter* (Hedlund et al.,
91 2001), *Pseudoalteromonas* (Melcher et al., 2002), *Marinomonas* (Melcher et al., 2002),

92 *Halomonas* (Melcher et al., 2002), *Sphingomonas* (Demaneche et al., 2004), and *Vibrio*
93 (Hedlund and Staley, 2001). However, less is known regarding deep-sea environments. In
94 previous studies on the deep-sea sediments of the Atlantic Ocean and Pacific Ocean, we found
95 that *Cycloclasticus* was the most important bacterium, in addition to *Alteromonas* and
96 *Novosphingobium* (Cui et al., 2008;Shao et al., 2010;Wang et al., 2008). In the Arctic Ocean,
97 *Pseudoalteromonas*, *Pseudomonas*, *Psychrobacter*, *Marinobacter* and *Shewanella* have been
98 frequently reported as crude oil degraders in coastal seawater and sea ice (Deppe et al.,
99 2005;Gerdes et al., 2005;Brakstad and Bonaunet, 2006;Giudice et al., 2010). *Pseudomonas* was
100 found to be the predominant PAH degrader in terrestrial soils (Whyte et al., 1997;Sorensen et al.,
101 2010;Eriksson et al., 2003). [To the best of our knowledge](#), the diversity of PAH-degrading
102 bacteria remains unknown in the deep-sea sediments of the high-latitude Arctic Ocean.

103 During the ecological survey of the “*Xuelong*” icebreaker in 2010, we sampled deep-sea
104 sediments across the ocean and chose four sites at the Chukchi Plateau, Canada Basin, Alpha
105 Ridge, and Makarov Basin to examine the distribution of PAHs and PAH-degrading bacteria
106 therein. Bacterial diversity in both sediments and PAHs enrichment cultures were analyzed. The
107 role of bacteria involved in PAH degradation was evaluated. The results will contribute to the
108 depiction of the distribution pattern of PAHs and PAH-utilizing bacteria in this extreme
109 environment, and help to evaluate the fate of PAHs following the contamination of such
110 environments.

111

112 **2 Material and Methods**

113 **2.1 Sediment collection**

114 A total of 19 sediment cores were collected from the Chukchi Plateau, Canada Basin, Alpha

115 Ridge, and Makarov Basin during the fourth Arctic Research Expedition of the “*Xuelong*”
116 icebreaker in the summer of 2010. PAHs and PAH-degrading bacteria are supposed to be varied
117 in the regions with large distances. Based on this hypothesis, four sites representing the four
118 typical geographical regions were chosen, including sites BN03, BN06, BN09 and BN12 (Fig. 1,
119 Table S1). The sediment cores were first sampled using a box sampler (50×50×65 cm), then
120 subsampled using a push core sampler (Φ10×60 cm) prior to releasing the box corers on deck.
121 The length of the BN03, BN06, BN09, and BN12 cores were 20, 30, 24 and 38 centimeters
122 below the surface, respectively. Subsequently, the cores were sliced into layers at depth intervals
123 of 4 cm with the exception of the surface layer, which was sliced at a depth of 2 or 4 cm
124 depending on the water content. Finally, three layers from each core, including the surface, the
125 bottom and the middle (Table S1), were selected for analysis in this report. Approximately 5 g of
126 sediment from each selected layer was used for PAH enrichment on board. The remains of the
127 sediments were frozen immediately at -20 °C on board, transported to the home laboratory on dry
128 ice, and stored at -80 °C until further analyses of PAH content and microbial diversity.

129 **2.2 Chemicals and media**

130 Naphthalene (>99.8%) was purchased from Sinopharm Chemical Reagent (Shanghai, China),
131 and phenanthrene (>97%) and pyrene (>98%) were purchased from Sigma-Aldrich (St. Louis,
132 USA). The Sixteen EPA priority pollutant PAH standards, six types of surrogate standards
133 (1,4-dichlorobenzene-d4, naphthane-d8, acenaphthene-d10, chrysene-d12, phenanthrene-d10,
134 and perylene-d12) and an internal standard (m-terphenyl) were purchased from AccuStandard
135 (New Haven, USA). Surrogate and internal standards were used for quantifying procedural
136 recovery and target PAH quantification, respectively. Crude oil was obtained from Iraq and
137 imported by the SinoChem Quanzhou Petrochemical Corporation (Quanzhou, China). Mineral

138 medium, used for enrichment of PAH-degrading bacteria, contained 1 g of NH_4NO_3 , 0.8 g of
139 KH_2PO_4 , 0.2 g of K_2HPO_4 , 2.8×10^{-3} g of FeSO_4 and 1 liter of *in situ* deep-sea water from the
140 Arctic Ocean. ONR7a medium, used for the cultivation of bacteria from the enriched cultures,
141 contained 22.8 g of NaCl , 11.2 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 3.9 g of Na_2SO_4 , 1.5 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.3 g
142 of TAPSO, 0.7 g of KCl , 0.3 g of NH_4Cl , 89 mg of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 83 mg of NaBr , 31 mg of
143 NaHCO_3 , 27 mg of H_3BO_3 , 24 mg of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, 2.6 mg of NaF , 2.0 mg of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 1
144 liter of deionized water (Dyksterhouse et al., 1995).

145 **2.3 PAHs quantification of deep-sea sediments**

146 Extraction, purification, and gas chromatography-mass spectrometry (GC-MS) quantification of
147 the PAHs in the deep-sea sediment samples were performed according to EPA method
148 8270D-2007 and previous reports (Zheng et al., 2002), with some modifications. Briefly, 0.05
149 mL of the 1 mg L^{-1} surrogate standard mixture solution was spiked into 20 g of freeze-dried
150 sediment prior to extraction. Then, the sediment was placed into Extraction System B-811 (Buchi)
151 and extracted with 250 ml of solvent consisting of a mixture of *n*-hexane and dichloromethane
152 (1:1 v/v) under the hot extraction mode for 4 h. The extract was concentrated using a vacuum
153 rotary evaporator and cleaned using column chromatography. The cleaned-up extract was further
154 concentrated to 1 mL under a gentle N_2 stream. Finally, 0.05 mL of 1 mg L^{-1} m-terphenyl was
155 added to the extract as an internal standard immediately before analysis.

156 All of the samples were analyzed using GC-MS run in the selected ion monitoring (SIM)
157 mode. The molecular ion of each PAH was used for SIM. Sixteen target PAHs were identified
158 based on both retention time relative to known standards and the mass of the molecular ion. Five
159 calibration standard solutions ($0.01\text{-}500 \text{ mg L}^{-1}$) containing the PAH standard, internal standard
160 and surrogate compounds were carefully prepared, and a calibration curve was generated. The

161 mean of the relative response factors (RRFs) for each target PAH and the surrogate compounds
162 was calculated. The quantifications were performed using the internal standard method, and the
163 concentrations of target PAHs were corrected for the recoveries. The recoveries for surrogate
164 standards were 81.6-105.2%. The lowest detection limit for each type of target PAH ranged from
165 0.13 to 0.97 ng g⁻¹ dw.

166 **2.4 PAH-degrading bacteria enrichment**

167 Approximately 5 g of sediment from each selected layer was added to 250 mL of mineral
168 medium in a 500-mL sterile polypropylene bottle. The sediments were supplied with 1 mL of
169 crude oil spiked with a PAHs mixture of naphthalene, phenanthrene, and pyrene at final
170 concentrations of 0.02, 0.01, and 0.005 g l⁻¹, respectively. Enrichment was performed on board at
171 4 °C and kept in the dark without agitation for two months. Only slight bacterial growth was
172 observed after the first round of enrichment at 4 °C. Once back to the home laboratory, the
173 enriched cultures were transferred with an inoculum of 5% to 100 ml of fresh mineral medium in
174 a 250-mL flask with the PAH mixture (without crude oil) as the sole carbon and energy source;
175 this process was repeated twice in the rotary shakers (150 rpm) at 25 °C and 15 °C every one and
176 two months, respectively. Finally, 36 PAH-degrading enriched cultures were obtained from the
177 three temperature treatments.

178 **2.5 PAH-removal rate quantification by GC-MS**

179 To determine the PAH-removal rates of each consortium, all of the consortia and uninoculated
180 controls were incubated in a 250-mL flask, which was loaded with 100 ml of fresh mineral
181 medium containing 5% inoculum and the above-mentioned PAH mixtures as the carbon source.
182 After a 45-day incubation at 15 °C and 25 °C, the residual PAHs were extracted with 100 mL of
183 dichloromethane separated into three parts. The purification and concentration of these combined

184 extracts was accomplished according to the description in “2.3 PAHs quantification of deep-sea
185 sediments”. The residual PAHs were quantified using an external standard method. The recovery
186 rate for each of PAH was calculated based on the quantity difference before and after enrichment,
187 extraction and purification in the uninoculated controls. The PAH-removal rate was calculated
188 according to the formula:

$$\text{Removal rate (\%)} = \frac{tPAH - (rPAH/R)}{tPAH} \times 100\%$$

189 $tPAH$: total quantity of each type of PAH before enrichment; $rPAH$: residual quantity of each
190 type of PAH after enrichment; R : recovery rate of each type of PAH.

191 **2.6 IHTS and data analysis**

192 Community DNA of three selected layers from each core and the enriched cultures was extracted
193 using the PowerSoil DNA Isolation Kit (MoBio) according to the manufacturer’s instructions.
194 Amplification of the 16S rRNA gene V6 region was performed using the universal bacterial
195 primers 967F (5'-CNACGCGAAGAACCTTANC-3') and 1046R
196 (5'-CGACAGCCATGCANACCT-3') as previously described (Wu et al., 2010). A set of 10
197 nucleotide (nt) barcodes was designed and added to the 5' end of 967F for multiplexing of the
198 samples in the Solexa paired-end (PE) sequencing runs. Each 25- μ L PCR mixture consisted of
199 approximately 10 ng of community DNA, 0.2 μ M of each primer, 0.2 mM of each dNTP, 1.5
200 mM $MgCl_2$, 1 \times TaKaRa *Ex Taq* Buffer (Mg^{2+} free), and 2.5 units of TaKaRa *Ex Taq* DNA
201 polymerase. PCR amplification was conducted using the following thermocycles: initial
202 denaturation at 94 $^{\circ}C$ for 2 min; 25 cycles at 94 $^{\circ}C$ for 30 s, 57 $^{\circ}C$ for 30 s, and 72 $^{\circ}C$ for 30 s; and
203 a final extension at 72 $^{\circ}C$ for 5 min. Equimolar amplicon suspensions were combined and
204 subjected to paired-end sequencing using the PE75 strategy on the Illumina HiSeq2000
205 sequencing platform at the Beijing Berrygenomics Company.

206 **2.7 PCR-DGGE**

207 PCR amplification and DGGE analysis of the 16S rRNA gene V3 fragments of the enriched
208 consortia were performed as previously reported (Cui et al., 2008).

209 **2.8 Bacterial isolation, identification and phylogenetic analysis**

210 Cultures enriched at 15 °C were chosen as representatives for the isolation of degrading bacteria.
211 Approximately 10^{-4} , 10^{-5} , and 10^{-6} dilutions of these cultures were spread onto ONR7a agar
212 plates whose lids were supplemented with naphthalene crystals and incubated at 15 °C in the dark.
213 Colonies with different morphologies were streaked onto fresh ONR7a plates twice to obtain
214 pure cultures. The PAH utilization of these isolates was tested in 100 mL of ONR7a liquid
215 medium supplemented with 0.2 g ultraviolet-sterilized naphthalene crystals at 15 °C, reflected by
216 culture color changes and an increase in the cell optical density at 600 nm.

217 **2.9 Nucleotide sequence accession numbers**

218 The Illumina high-throughput sequencing data that resulted from the present study were
219 deposited into the NCBI Sequence Read Archive under run accession numbers
220 SRR975490-SRR975525 and SRR768499-SRR768507. The partial sequences of the 16S rRNA
221 gene obtained in this study were deposited into GenBank under accession numbers
222 KF470969-KF471008 (strains) and KC581800-KC581881 (DGGE bands).

223

224 **3 Results**

225 **3.1 PAHs in sediments**

226 GC-MS quantification indicated that the total concentration of the sixteen targeted PAHs
227 ($\Sigma_{16}\text{PAH}$) ranged from 2.02 to 41.63 ng g⁻¹ dw (Table S2) and was decreased in the sediments
228 from the southern to northern sites (Fig. 2, Table S2). Among the sites, the southernmost site at

229 Chukchi Plateau (BN03) ranked the highest, whereas the northernmost site at Makarov Basin
230 (BN12) ranked the lowest. At each site, the Σ_{16} PAH decreased with sediment depth, with the
231 exception of the site at the Chukchi Plateau (BN03). The concentration of phenanthrene ranked
232 the highest among the detected PAHs, followed by naphthalene; the only exception was the
233 sediments of the Chukchi Plateau (Fig. 2, Table S2). PAHs with four to six rings, such as the
234 four-ringed pyrene, five-ringed benzo[*b*]fluoranthene, and six-ringed benzo(*ghi*)perylene and
235 indenopyrene, were significantly higher at the Chukchi Plateau site (BN03) compared to the
236 other samples. At the Alpha Ridge site (BN09), phenanthrene ranked the highest (14.61 ng g⁻¹ dw)
237 among the PAHs of all samples.

238 **3.2 Community structures of the *in situ* sediments revealed by IHTS**

239 To obtain the bacterial composition and increased insights into the PAH degraders present in the
240 *in situ* sediments, all 12 samples were subjected to Illumina high-throughput sequencing. The
241 sequencing efforts and bacterial diversity indices are presented in the Supplemental Material in
242 Table S3. Finally, a total of 1,152,388 raw reads were obtained, of which 1,051,978 clean reads
243 were used for further analyses using QIIME (v 1.7.0).

244 At the phylum level, Proteobacteria were the most abundant bacteria and occupied 44.7%-57.3%
245 of the total tags of these sediments, followed by Acidobacteria, Actinobacteria,
246 Gemmatimonadetes, and Planctomycetes (Fig. S1A). The abundant orders are presented in the
247 Supplemental Material (Fig. S1B). The top ten dominant bacteria belonged to *Oceanospirillales*,
248 *Actinomycetales*, *Rhodospirillales*, *Planctomycetales*, *Gemmatimonadales*, *Acidobacteriales*,
249 *Chromatiales*, *Alteromonadales*, *Pseudomonadales*, and *Bacillales*. Among them,
250 *Oceanospirillales*, *Alteromonadales*, and *Pseudomonadales* contained most of known oil and
251 PAH-degrading bacteria by far, such as *Pseudomonas*, *Cycloclasticus*, *Alcanivorax*,

252 *Pseudoalteromonas* and *Marinomonas*. More details are documented below.

253 The bacteria at the genus level are shown in Fig. 3A. The abundant bacteria that occupied
254 more than 1% of the total tags in at least one sample are presented, including 20 known genera
255 and 3 uncultured bacterial groups (Table S4). Among them, *Dietzia*, *Salinisphaera*,
256 *Pseudomonas*, *Acinetobacter*, *Pseudoalteromonas*, *Colwellia*, *Bacillus*, *Rhodovibrio*,
257 *Marinomonas*, and *Halomonas* have been reported as hydrocarbon-degrading bacteria in marine
258 environments. In addition, *Cycloclasticus* and *Alcanivorax* are noteworthy because they have
259 been recognized as obligate marine hydrocarbon degraders (Yakimov et al., 2007), and they were
260 widespread in all of the sediments tested in this report (Table S4). Specially, *Cycloclasticus*
261 occupied 0.2-0.5% of the 16S rRNA gene tags in each sample.

262 **3.3 PAH degradation of the PAH enrichment consortia**

263 All treatments displayed obvious bacterial growth following incubation under different
264 temperatures (4 °C, 15 °C, and 25 °C), reflected by color changes and changes in the turbidity of
265 the cultures compared to the uninoculated controls. The PAH-removal rates of the consortia after
266 45 days of incubation at 15 °C and 25 °C were calculated based on the PAH concentrations
267 determined by GC-MS. In general, the removal rates of phenanthrene and pyrene at 25 °C were
268 higher than those at 15 °C (Fig. 4). Notably, the consortia of the northernmost site (BN12)
269 generally displayed high rates at 15 °C and 25 °C. In contrast, the removal rates of the consortia
270 from sites BN03, BN06, and BN09 were relatively low.

271 **3.4 Community structures of the consortia enriched with PAHs**

272 Thirty-six enrichment cultures corresponding to the *in situ* sediments were also analyzed using
273 Illumina high-throughput sequencing (Table S3). Nearly all of the dominant bacteria in these
274 consortia were previously described as hydrocarbon degraders. For the 4 °C treatments enriched

275 with crude oil-containing PAHs, the predominant bacteria included *Pseudomonas*,
276 *Pseudoalteromonas*, *Marinomonas*, *Hahella*, *Marinobacter*, *Hyphomonas*, *Cycloclasticus*,
277 *Colwellia*, *Halomonas*, *Oceanobacter*, *Salinisphaera*, *Oleispira*, *Alteromonas*, and *Alcanivorax*
278 (Fig. 3B, Table S5). In the treatments that were enriched with PAHs at 15 °C and 25 °C,
279 *Pseudoalteromonas*, *Cycloclasticus*, *Pseudomonas*, and *Halomonas* were selected as the most
280 abundant bacteria. In the 15 °C consortia (Fig. 3C), *Pseudoalteromonas* was the most abundant
281 bacteria in the consortia of site BN03 and from the upper layer of BN06. In contrast,
282 *Cycloclasticus* dominated the three consortia of site BN12 (55.1-64.5%), whereas *Pseudomonas*
283 was clearly dominant in the consortia of the middle layer of site BN06 and upper layer of site
284 BN09, and was relatively dominant in all three consortia from site BN12 (Fig. 3C, Table S5).

285 *Halomonas* was the most dominant bacterium in the 25 °C consortia (Fig. 3D), occupying
286 33.4-71.0% of the tags of the communities from sites BN03 and BN06, with the exception of the
287 upper layer of BN03. *Pseudomonas* was dominant in the consortia of the middle layer of site
288 BN09 (named 25BN09M, 54.1% of all tags), the lower layer of BN03 (25BN03L, 32.3%), the
289 lower layer of BN06 (25BN06L, 31.6%), and the upper layer of BN12 (25BN12U, 21.6%).
290 Similar to the 15 °C consortia, *Cycloclasticus* dominated the northernmost consortia at 25 °C,
291 such as the middle-layer consortium 25BN12M (46.0%), the lower-layer consortium 25BN12L
292 (30.5%), and the upper-layer consortium 25BN12U (29.3%). Additionally, they were also
293 relatively abundant in the consortium of the upper layer of the BN09 site (25BN09U, 9.1%).

294 **3.5 Bacterial diversity comparisons based on IHTS data statistical analysis and** 295 **environmental parameters**

296 To compare the diversity indices, the tags were normalized to 7,047 (the fewest of the samples),
297 and the observed OTUs, Chao1, and Shannon indices were obtained using the software package

298 QIIME (Table S3). Overall, all of the diversity indices indicated that the sediments had the
299 highest bacterial richness and evenness (Fig. S2A-2C). Among the enriched cultures, the
300 bacterial diversity was increased with rising temperatures (Fig. S2A-2B). The Chao1 values of
301 the 25 °C-enriched cultures were significantly higher compared to the other cultures ($P < 0.01$);
302 correspondingly, the observed OTU numbers of the 25 °C-enriched cultures were also
303 significantly higher than the 4 °C treatments ($P = 0.029$). Principal coordinate analysis (PCoA)
304 showed that the communities of the *in situ* sediments and all enriched cultures could be separated
305 using the abundant OTU dataset (Fig. S2D), indicating that they had significantly different
306 bacterial community structures; this finding was supported by the results of the nonparametric
307 statistical Adonis method ($R^2 = 0.28$, $P = 0.001$). Additionally, the Mantel test results showed no
308 correlations between the community structures of the four types of samples and the individual or
309 total PAH concentrations of the *in situ* sediments.

310 **3.6 Community composition revealed by PCR-DGGE**

311 To reconfirm the bacterial composition, the PAH-degrading consortia were subjected to
312 PCR-DGGE analysis in parallel. Figure 5 presents the DGGE profiles of 12 consortia that were
313 enriched with PAHs at 25 °C. The bacteria, represented by bands, are listed in the Supplemental
314 Material Table S6, and some are noted in the pattern profiles. In general, the community
315 structures corresponded well to the IHTS results, even though the two methods targeted different
316 regions of the 16S gene. In these consortia, the three genera *Cycloclasticus*, *Pseudomonas*, and
317 *Halomonas* alternatively dominated the communities. Specifically, *Cycloclasticus* dominated all
318 three consortia from site BN12 and the consortium (25BN09U) from the upper layer of site
319 BN09; interestingly, it was accompanied by *Alcanivorax* (Fig. 5, lanes 7 and 10-12). This finding
320 was in agreement with the results of the IHTS data (Fig. 3D). *Pseudomonas* dominated or shared

321 dominance with *Cycloclasticus* or *Halomonas* in four consortia (Fig. 5, lanes 3, 6, 8 and 10) that
322 were derived from each layer of the four sites, whereas they were relatively less abundant in
323 three consortia (Fig. 5, lanes 2, 4 and 11) that were generated from three sites. *Halomonas*
324 appeared as very strong bands in five consortia (Fig. 5, lanes 2-6) from sites BN03 and BN06.
325 *Pseudoalteromonas* mainly dominated in four consortia (Fig. 5, lanes 1, 3, 5 and 6) from sites
326 BN03 and BN06. Other bacteria, such as *Marinobacter*, *Alcanivorax*, *Marinobacterium*,
327 *Colwellia*, *Thalassospira*, *Celeribacter*, and *Vibrio*, were occasionally found to be strongly or
328 weakly scattered in some of the consortia.

329 At low temperatures, the PAH-degrading communities varied to some extent in comparison to
330 the 25 °C consortia. For example, in the three consortia derived from site BN12, bacteria such as
331 *Cycloclasticus* and *Thalassolituus* became the dominant members at 4 °C (Fig. 6A, lanes 11-13),
332 while bacteria such as *Pseudomonas*, *Maritalea*, and *Thalassospira* were only dominant
333 members in 4BN12U and 4BN12M (Fig. 6A, lanes 11-12). In contrast, the most dominant
334 member was the bacteria of *Cycloclasticus* at 15 °C (Fig. 6B, lane 11-13), which was consistent
335 with the composition pattern revealed in Fig. 3C.

336 **3.7 Bacterial isolation and their potential in PAH degradation**

337 Bacteria were isolated from all of the PAH-degrading consortia enriched at 15 °C using the
338 ONR7a medium plates whose lids were supplied with naphthalene crystals as the sole carbon
339 source. Forty isolates were obtained that were affiliated with 12 genera of γ - and
340 α -proteobacteria and Actinobacteria (Fig. S3). Bacteria belonging to γ -proteobacteria were the
341 predominant isolates, including *Pseudoalteromonas* (18 isolates), *Halomonas* (6 isolates),
342 *Cycloclasticus* (3 isolates), *Pseudomonas* (3 isolates), *Marinobacter* (2 isolates), and *Shewanella*
343 (2 isolates). The PAH utilization test showed that only the bacteria of *Cycloclasticus* and

344 *Pseudomonas* showed obvious growth after two weeks in ONR7a liquid medium supplemented
345 with naphthalene at 15 °C.

346 Both the IHTS and PCR-DGGE results demonstrated that *Cycloclasticus* was predominant in
347 the PAH-degrading consortia of site BN12 (Fig. 3, Fig. 5 and Fig. 6). From these consortia, three
348 strains were isolated and named after the consortium: 15BN12U-14 (simplified as U-14),
349 15BN12L-10 (L-10), and 15BN12L-11 (L-11). These organisms had identical 16S rRNA gene
350 sequences (1497 bp) and shared 99.92% sequence similarity with the *C. pugetii* PS-1^T type strain.
351 However, they varied in morphology (Fig. S4) and genome fingerprint patterns determined by
352 Rep-PCR (Fig. S5). Strains L-10 and L-11 resembled each other in morphology and Rep-PCR
353 profiles; therefore, only strain L-10 was chosen for further analyses. Growth tests were
354 conducted at 15 °C with a single PAH as the sole carbon source in ONR7a liquid medium. The
355 results showed that strains L-10 and U-14 could assimilate naphthalene and phenanthrene, but
356 neither could utilize pyrene (Fig. 7). Moreover, strain U-14 exhibited better growth than strain
357 L-10 when utilizing phenanthrene (Fig. 7A vs. 7B).

358

359 **4 Discussion**

360 This report examined PAHs and PAH-degrading bacteria in the deep-sea sediments across the
361 Arctic Ocean. Based on the data available to date, the total PAH concentrations decreased
362 moving north toward the pole and generally decreased with sediment depth. Phenanthrene
363 (0.64-14.61 ng g⁻¹) was the most dominant among the sixteen targeted EPA-priority PAHs. Based
364 on the bacterial diversity data obtained using both culture-dependent and -independent methods,
365 the general features of PAH-degrading bacteria were revealed in the sediment samples. To the
366 best of our knowledge, this report is the first to consider the diversity and abundance of

367 PAH-utilizing bacteria in the deep-sea sediments of the high-latitude Arctic Ocean.

368 Recently, Yunker et al. examined the distribution of PAHs, plant odd alkanes, hopanes, and
369 steranes in the sediments based on a large dataset to trace the origin of organic matter in the
370 deep-sea sediments of the Arctic Ocean (Yunker et al., 2011). They found that the central Arctic
371 Ocean basins were compositionally distinct from the rivers and shelves. Moreover, PAH
372 concentrations decreased from 100-755 ng g⁻¹ dw in the coastal sea to 35 ng g⁻¹ dw in the central
373 basin (Yunker et al., 2011). Our results in this report show that the PAH concentrations are
374 generally in agreement with this tendency (Fig. 2, Table S2). According to the theory of “Global
375 distillation”, it would be easier to transfer naphthalene than other PAHs of high molecular weight
376 (HMW) over long distances (Goldberg, 1975;Friedman and Selin, 2012); however, the
377 concentration of naphthalene was less than that of phenanthrene. This discrepancy might be
378 partially due to its higher bioavailability and degradability. In contrast, HMW PAHs with four or
379 more rings accumulated in larger concentrations in areas near the south of the continent, as
380 described above (Fig. 2). Additionally, the establishment of PAH compositions can likely be
381 attributed to bacterial mineralization. To investigate the relationship between PAH composition
382 and bacterial communities, we analyzed the correlations between the community structures and
383 the concentrations of individual or total PAHs using the Mantel test method. However, no
384 definite correlation was observed. Bacterial communities *in situ* may be influenced by many
385 other factors, such as nutrients and other carbon sources, in addition to the PAHs in the tested
386 samples from the Arctic Ocean.

387 Various bacteria involved in PAH-degradation were identified in all of the sediments,
388 including *Cycloclasticus*, *Pseudomonas*, *Halomonas*, *Pseudoalteromonas*, *Marinomonas*,
389 *Bacillus*, *Dietzia*, *Colwellia*, *Acinetobacter*, *Alcanivorax*, *Salinisphaera* and *Shewanella*.

390 However, most of these bacteria occupied less than 0.5% of the total tags (Fig. S6). After PAH
391 enrichment, *Pseudomonas*, *Pseudoalteromonas*, *Cycloclasticus*, *Halomonas*, and *Marinomonas*
392 became the dominant members in the enriched cultures (Fig. S6). For example, when enriched at
393 4 °C with PAHs dissolved in crude oil, *Pseudomonas*, *Pseudoalteromonas*, *Marinomonas*,
394 *Hyphomonas*, and *Cycloclasticus* were identified as the dominant members (Fig. 3B and Fig. S6).
395 These bacteria have been previously detected as the dominant members in oil-enriched consortia
396 of the coastal seawater and sea ice from the Arctic Ocean (Deppe et al., 2005;Gerdes et al.,
397 2005;Brakstad and Bonaunet, 2006;Giudice et al., 2010). These findings are in contrast to those
398 from the deep-sea oil plume that occurred during the Deepwater Horizon oil spill, which was
399 dominated by bacteria of the order *Oceanospirillales* and the genus *Colwellia* (Hazen et al.,
400 2010;Baelum et al., 2012). When enriched with PAHs as a sole carbon and energy source at 15 °C
401 and 25 °C, the obtained PAH-degrading consortia were alternately dominated by *Pseudomonas*,
402 *Pseudoalteromonas*, *Halomonas*, and *Cycloclasticus*. A big difference in the community
403 structures occurred between cultures grown at 4 °C and 15 °C (Fig. 3 and Fig. S2); while the
404 bacterial community structures grown at 25 °C also varied to some extent in comparison with
405 those grown at 15 °C, the dominant bacteria, including *Cycloclasticus*, *Pseudomonas*,
406 *Pseudoalteromonas* and *Halomonas*, remained predominant (Fig. 3). The alpha and beta
407 diversity indices (Fig. S2) both confirmed that significant differences existed among the
408 consortia enriched under different temperatures, and indicated that temperature substantially
409 influenced the bacterial community structure. This could be explained by the enhancement of
410 PAH availability and the metabolic activity of the bacteria at high temperatures.

411 Bacteria of the genus *Cycloclasticus* have been recognized as obligate marine PAH degraders
412 (Dyksterhouse et al., 1995;Yakimov et al., 2007). They usually represent one of the most

413 predominant genera detected in crude oil-polluted sediments or seawater (Kasai et al.,
414 2002;Maruyama et al., 2003;McKew et al., 2007a;McKew et al., 2007b;Coulon et al.,
415 2007;Kappell et al., 2014;Dubinsky et al., 2013). In addition to coastal environments, they have
416 also been found in the deep-sea sediments of both the Atlantic and Pacific Oceans, as described
417 in our previous reports based on culture enrichment (Cui et al., 2008;Shao et al., 2010;Wang et
418 al., 2008). This report is the first to use IHTS to confirm the wide distribution of *Cycloclasticus*
419 bacteria in deep-sea sediments. In Arctic deep-sea sediments, bacteria of this genus occupied
420 0.2%-0.5% of the total bacteria sequenced in each sediment sample *in situ* based on the detection
421 of sequence tags on the 16S rRNA gene. Therefore, it seems likely that they play an important
422 role in PAH mineralization in this environment. Interestingly, the abundance of *Cycloclasticus*
423 increased with sediment depth and movement from the southern to northern sites (Table S4); this
424 finding was in contrast to PAH concentrations that decreased with depth and movement from the
425 south to the north. This finding is most likely due to the fact that the labile carbon sources are
426 relatively abundant in the surface sediments and are reduced in the older sediments (deep layers)
427 and remote areas such as BN12, which is close to the North Pole. In the sediments where labile
428 carbon sources are scarce, the PAHs may represent a key factor in the selection of
429 PAH-degrading bacteria that adapt to the oligotrophic circumstances.

430 *Cycloclasticus* bacteria were found in all twelve of the *in situ* sediment samples, but were
431 difficult to cultivate on plate, even when a simple carbon source, such as acetate and pyruvate,
432 was used. After many attempts, three strains were finally obtained on the ONR7a medium plates
433 supplied with naphthalene crystals on the lids, showing tiny colonies after three weeks of
434 incubation at 15 °C. Growth tests indicated that these *Cycloclasticus* strains could use
435 naphthalene or phenanthrene as a sole carbon source, but failed to utilize pyrene. In the previous

436 study, we isolated a pyrene-degrading bacterium from a Pacific deep-sea sediment that
437 represented the only one strain of *Cycloclasticus* reported to date that is capable of using pyrene
438 as its sole carbon and energy source (Wang et al., 2008); in contrast, other strains can utilize
439 pyrene only in the presence of other PAHs, such as phenanthrene (Geiselbrecht et al., 1998).

440 In addition to *Cycloclasticus*, the following PAH-degrading bacteria, which were previously
441 described to reside in coastal environments, were also found as dominant members in some
442 PAH-degrading consortia in this report (see Supplemental Material Table S5): *Pseudomonas*
443 (Niepceon et al., 2010), *Marinomonas* (Melcher et al., 2002), *Pseudoalteromonas* (Hedlund and
444 Staley, 2006), *Halomonas* (Garcia et al., 2005), *Alteromonas* (Jin et al., 2012), *Marinobacter*
445 (Hedlund et al., 2001), *Vibrio* (Hedlund and Staley, 2001), and *Thalassospira* (Kodama et al.,
446 2008). In fact, we also isolated 64 strains from the consortia enriched at 25 °C using M2 media
447 plates (Wang et al., 2008), which contain more distinct carbon compounds than ONR7a media
448 plates (i.e., sodium acetate, glucose, sucrose, sodium citrate, and malic acid). Among them, only
449 three strains (*Marinomonas* sp. D104, *Sphingobium* sp. C100 and *Pseudomonas* sp. C39) showed
450 a good PAH-degradation capability at 15 °C and 25 °C. Genome sequencing revealed that strains
451 D104 and C100 possessed several genes involved in the initial hydroxylation and intermediate
452 metabolic steps of PAHs (Dong et al., 2014b, a). Particularly, strain *Marinomonas* sp. D104
453 could even degrade the PAH mixture of naphthalene, phenanthrene, and pyrene at 4 °C
454 (unpublished data). Although *Pseudoalteromonas* and *Halomonas* were the most predominant
455 members in many consortia (Fig. 3 and Fig. 5), in this study they failed to grow in the presence
456 of the tested PAHs.

457 *Pseudomonas* is a common PAH-degrader in cold environments, and is frequently found in
458 Arctic and Antarctic soils (Whyte et al., 1997; Sorensen et al., 2010; Eriksson et al., 2003; Ma et

459 al., 2006). In this study, *Pseudomonas* occurred *in situ* as one of the most dominant bacteria and
460 occupied 1.5-1.8% of the total bacteria sequenced from the three samples (Table S4 and Fig. 3A).
461 In many cultures enriched with PAHs, *Pseudomonas* was the dominant member and even
462 occupied up to 70% of the total populations in the 4BN03M consortium (Table S5 and Fig. 3B).
463 Coincidentally, three *Pseudomonas* strains were obtained from the 15 °C enrichments (Fig. S3) and
464 were able to grow with naphthalene. However, to the best of our knowledge, *Pseudomonas* is
465 less abundant in oceanic sediments (Cui et al., 2008; Wang et al., 2008; Shao et al., 2010).

466 In addition to the bacteria mentioned above, many other bacteria belonging to *Dietzia*,
467 *Alcanivorax*, *Colwellia*, *Thalassolituus*, *Oceanobacter*, *Hahella*, and *Roseovarius* were also
468 relatively dominant in some of the PAH-degrading communities. *Dietzia* (Alonso-Gutierrez et al.,
469 2011), *Alcanivorax* (Schneiker et al., 2006), *Colwellia* (Baelum et al., 2012), *Thalassolituus*
470 (Yakimov et al., 2004) and *Oceanobacter* (Teramoto et al., 2009) have been reported as
471 hydrocarbon-degrading bacteria, but not as PAH degraders. It is noteworthy that bacteria of
472 *Dietzia* were abundant *in situ*, particularly in the middle or lower layers of all sediments, and
473 occupied 1.65%-7.8% of the total tags (Fig. 3A, Table S4). These organisms might thrive on
474 alkanes in these environments, but are not likely to thrive on PAHs because they only occupied
475 11.25% of the total tags in one consortium (the upper layer of site BN03, enriched at 15 °C) (Fig.
476 3C, Table S5). Interestingly, bacteria of *Thalassolituus* occurred as the rare species (abundance
477 <0.01%) in all *in situ* sediments. They became predominant in the 4 °C cultures of site BN12
478 enriched with oil containing PAHs (Fig. 6A), but were diluted from the PAH-enriched cultures at
479 both 15 °C and 25 °C (Fig. 6B and Fig. 5). These results were quite consistent with their aliphatic
480 hydrocarbon-degrading and psychrotolerant characteristics (Yakimov et al., 2004).

481 It is worth mentioning that the once frequently reported PAH-degrading bacteria

482 *Novosphingobium* spp. were not detected in the sediments of the Arctic Ocean. These bacteria
483 are common PAH degraders in both marine and terrestrial environments (Gan et al., 2013). In our
484 previous reports, they occurred as a predominant PAH degrader in nearly all PAH enrichment
485 cultures, such as those from the deep-sea column of the Indian Ocean (Yuan et al., 2009) and
486 those from hydrothermal sediments of the Lau Basin (Dong et al., 2011). Other reports also
487 proved the widespread distribution of *Novosphingobium* bacteria and confirmed their roles as
488 effective PAH degraders (Balkwill et al., 1997; Sohn et al., 2004; Yuan et al., 2009; Notomista et
489 al., 2011). Similar to *Novosphingobium*, bacteria of *Sphingomonas* and *Neptunomonas* were not
490 found in the sediments of the Arctic Ocean, although they are also common PAH-degraders in
491 marine environments (Demaneche et al., 2004; Hedlund et al., 1999).

492 In summary, various PAHs and degrading bacteria are ubiquitous in the Arctic deep-sea
493 sediments. In general, the total PAH concentrations decreased with sediment depths and
494 movement from the south to the north, and ranged from 2.0 to 41.6 ng g⁻¹ dw. Correspondingly,
495 various bacteria involved in PAH degradation existed in the deep-sea sediments, including the
496 obligate marine hydrocarbon-degrading bacteria *Cycloclasticus*, *Alcanivorax*, and *Thalassolituus*,
497 as well as *Pseudomonas*, *Pseudoalteromonas*, *Marinobacter*, *Marinomonas*, *Acinetobacter*,
498 *Bacillus*, *Colwellia*, *Dietzia*, *Halomonas*, *Rhodovibrio*, *Salinisphaera* and *Shewanella*. Among
499 them, *Cycloclasticus*, *Pseudomonas*, *Pseudoalteromonas*, *Marinomonas*, *Halomonas* and *Dietzia*
500 may play a more important role in PAH degradation *in situ* in the Arctic Ocean. Bioattenuation
501 of PAHs occurs whilst bacteria survive in the remote deep-sea areas, which are cold, dark,
502 oligotrophic, high pressure, and perennially covered in ice.

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505 ***Supplementary material***

506 The supplement related to this article is uploaded during the manuscript submission.

507

508 *Author contribution.* Z. Z. S. and C. M. D. designed research; X. H. B. and C. M. D. performed
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519 **References**

520 Alonso-Gutierrez, J., Teramoto, M., Yamazoe, A., Harayama, S., Figueras, A., and Novoa, B.:
521 Alkane-degrading properties of *Dietzia* sp. HOB, a key player in the Prestige oil spill biodegradation (NW
522 Spain), *Journal of applied microbiology*, 111, 800-810, 10.1111/j.1365-2672.2011.05104.x, 2011.
523 Baelum, J., Borglin, S., Chakraborty, R., Fortney, J. L., Lamendella, R., Mason, O. U., Auer, M., Zemla,
524 M., Bill, M., Conrad, M. E., Malfatti, S. A., Tringe, S. G., Holman, H. Y., Hazen, T. C., and Jansson, J. K.:
525 Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill, *Environ Microbiol*,
526 14, 2405-2416, 10.1111/j.1462-2920.2012.02780.x, 2012.
527 Balkwill, D. L., Drake, G. R., Reeves, R. H., Fredrickson, J. K., White, D. C., Ringelberg, D. B., Chandler,
528 D. P., Romine, M. F., Kennedy, D. W., and Spadoni, C. M.: Taxonomic study of aromatic-degrading
529 bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp.
530 nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov, *Int J Syst Bacteriol*, 47,
531 191-201, 1997.
532 Baumard, P., Budzinski, H., and Garrigues, P.: Polycyclic aromatic hydrocarbons in sediments and
533 mussels of the western Mediterranean sea, *Environmental Toxicology and Chemistry*, 17, 765-776,
534 10.1002/etc.5620170501, 1998.
535 Becker, S., Halsall, C. J., Tych, W., Hung, H., Attewell, S., Blanchard, P., Li, H., Fellin, P., Stern, G.,
536 Billeck, B., and Friesen, S.: Resolving the Long-Term Trends of Polycyclic Aromatic Hydrocarbons in the
537 Canadian Arctic Atmosphere, *Environmental science & technology*, 40, 3217-3222, 10.1021/es052346l,

2006.

Benner, R., Benitez-Nelson, B., Kaiser, K., and Amon, R. M. W.: Export of young terrigenous dissolved organic carbon from rivers to the Arctic Ocean, *Geophysical Research Letters*, 31, L05305, 10.1029/2003GL019251, 2004.

Boeuf, D., Humily, F., and Jeanthon, C.: Diversity of Arctic pelagic Bacteria with an emphasis on photoheterotrophs: a review, *Biogeosciences*, 11, 3309-3322, 10.5194/bg-11-3309-2014, 2014.

Brakstad, O. G., and Bonaunet, K.: Biodegradation of petroleum hydrocarbons in seawater at low temperatures (0-5 degrees C) and bacterial communities associated with degradation, *Biodegradation*, 17, 71-82, 10.1007/s10532-005-3342-8, 2006.

Coulon, F., McKew, B. A., Osborn, A. M., McGenity, T. J., and Timmis, K. N.: Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters, *Environ Microbiol*, 9, 177-186, 10.1111/j.1462-2920.2006.01126.x, 2007.

Cui, Z., Lai, Q., Dong, C., and Shao, Z.: Biodiversity of polycyclic aromatic hydrocarbon-degrading bacteria from deep sea sediments of the Middle Atlantic Ridge, *Environ Microbiol*, 10, 2138-2149, 2008.

Demaneche, S., Meyer, C., Micoud, J., Louwagie, M., Willison, J. C., and Jouanneau, Y.: Identification and functional analysis of two aromatic-ring-hydroxylating dioxygenases from a *shingomonas* strain that degrades various polycyclic aromatic hydrocarbons, *Appl Environ Microbiol*, 70, 6714-6725, 10.1128/AEM.70.11.6714-6725.2004, 2004.

Deppe, U., Richnow, H. H., Michaelis, W., and Antranikian, G.: Degradation of crude oil by an arctic microbial consortium, *Extremophiles : life under extreme conditions*, 9, 461-470, 10.1007/s00792-005-0463-2, 2005.

Dong, C., Chen, L., Liao, Y., and Shao, Z.: Phylogenetic and degrading genes analysis of a PAHdegrading bacterium TVG9-VII from deep-sea hydrothermal environment, *Acta Microbiologica Sinica*, 51, 1548-1554, 2011.

Dong, C., Bai, X., Lai, Q., Xie, Y., Chen, X., and Shao, Z.: Draft Genome Sequence of *Sphingobium* sp. Strain C100, a Polycyclic Aromatic Hydrocarbon-Degrading Bacterium from the Deep-Sea Sediment of the Arctic Ocean, *Genome announcements*, 2, 10.1128/genomeA.01210-13, 2014a.

Dong, C., Bai, X., Lai, Q., Xie, Y., Chen, X., and Shao, Z.: Draft Genome Sequence of *Marinomonas* sp. Strain D104, a Polycyclic Aromatic Hydrocarbon-Degrading Bacterium from the Deep-Sea Sediment of the Arctic Ocean, *Genome announcements*, 2, 10.1128/genomeA.01211-13, 2014b.

Dubinsky, E. A., Conrad, M. E., Chakraborty, R., Bill, M., Borglin, S. E., Hollibaugh, J. T., Mason, O. U., Y., M. P., Reid, F. C., Stringfellow, W. T., Tom, L. M., Hazen, T. C., and Andersen, G. L.: Succession of hydrocarbon-degrading bacteria in the aftermath of the deepwater horizon oil spill in the gulf of Mexico, *Environmental science & technology*, 47, 10860-10867, 10.1021/es401676y, 2013.

Dyksterhouse, S. E., Gray, J. P., Herwig, R. P., Lara, J. C., and Staley, J. T.: *Cycloclasticus pugetii* gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium from marine sediments, *Int J Syst Bacteriol*, 45, 116-123, 1995.

Eriksson, M., Sodersten, E., Yu, Z., Dalhammar, G., and Mohn, W. W.: Degradation of Polycyclic Aromatic Hydrocarbons at Low Temperature under Aerobic and Nitrate-Reducing Conditions in Enrichment Cultures from Northern Soils, *Appl Environ Microbiol*, 69, 275-284, 2003.

Friedman, C. L., and Selin, N. E.: Long-range atmospheric transport of polycyclic aromatic hydrocarbons: a global 3-D model analysis including evaluation of Arctic sources, *Environmental science & technology*, 46, 9501-9510, 10.1021/es301904d, 2012.

Gan, H. M., Hudson, A. O., Rahman, A. Y., Chan, K. G., and Savka, M. A.: Comparative genomic analysis of six bacteria belonging to the genus *Novosphingobium*: insights into marine adaptation, cell-cell signaling and bioremediation, *BMC genomics*, 14, 431, 10.1186/1471-2164-14-431, 2013.

Garcia, M. T., Ventosa, A., and Mellado, E.: Catabolic versatility of aromatic compound-degrading halophilic bacteria, *FEMS Microbiol Ecol*, 54, 97-109, 10.1016/j.femsec.2005.03.009, 2005.

Gautier, D. L., Bird, K. J., Charpentier, R. R., Grantz, A., Houseknecht, D. W., Klett, T. R., Moore, T. E., Pitman, J. K., Schenk, C. J., and Schuenemeyer, J. H.: Assessment of undiscovered oil and gas in the Arctic, *Science*, 324, 1175-1179, 2009.

589 Geiselbrecht, A. D., Hedlund, B. P., Tichi, M. A., and Staley, J. T.: Isolation of Marine Polycyclic
590 Aromatic Hydrocarbon (PAH)-Degrading *Cycloclasticus* Strains from the Gulf of Mexico and
591 Comparison of Their PAH Degradation Ability with That of Puget Sound *Cycloclasticus* Strains, Applied
592 and Environmental Microbiology, 64, 4703-4710, 1998.

593 Gerdes, B., Brinkmeyer, R., Dieckmann, G., and Helmke, E.: Influence of crude oil on changes of
594 bacterial communities in Arctic sea-ice, FEMS Microbiology Ecology, 53, 129-139,
595 10.1016/j.femsec.2004.11.010, 2005.

596 Giudice, A. L., Bruni, V., Domenico, M. D., and Michaud, L.: Psychrophiles - Cold-Adapted
597 Hydrocarbon-Degrading Microorganisms, in: Handbook of Hydrocarbon and Lipid Microbiology, edited
598 by: Timmis, K. N., Springer Berlin Heidelberg, 1897-1921, 2010.

599 Goldberg, E. D.: Synthetic organohalides in the sea, Proc R Soc Lond B Biol Sci, 189, 277-289, 1975.

600 Halsall, C. J., Barrie, L. A., Fellin, P., Muir, D. C. G., Billeck, B. N., Lockhart, L., Rovinsky, F. Y.,
601 Kononov, E. Y., and Pastukhov, B.: Spatial and Temporal Variation of Polycyclic Aromatic Hydrocarbons
602 in the Arctic Atmosphere, Environmental science & technology, 31, 3593-3599, 10.1021/es970342d,
603 1997.

604 Haritash, A. K., and Kaushik, C. P.: Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs):
605 a review, J Hazard Mater, 169, 1-15, 10.1016/j.jhazmat.2009.03.137, 2009.

606 Hazen, T. C., Dubinsky, E. A., DeSantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh, N., Jansson, J. K.,
607 Probst, A., Borglin, S. E., Fortney, J. L., Stringfellow, W. T., Bill, M., Conrad, M. E., Tom, L. M.,
608 Chavarria, K. L., Alusi, T. R., Lamendella, R., Joyner, D. C., Spier, C., Baelum, J., Auer, M., Zemla, M.
609 L., Chakraborty, R., Sonnenthal, E. L., D'Haeseleer, P., Holman, H. Y., Osman, S., Lu, Z., Van Nostrand, J.
610 D., Deng, Y., Zhou, J., and Mason, O. U.: Deep-sea oil plume enriches indigenous oil-degrading bacteria,
611 Science, 330, 204-208, 10.1126/science.1195979, 2010.

612 Hedlund, B. P., Geiselbrecht, A. D., Bair, T. J., and Staley, J. T.: Polycyclic aromatic hydrocarbon
613 degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov, Appl Environ
614 Microbiol, 65, 251-259, 1999.

615 Hedlund, B. P., Geiselbrecht, A. D., and Staley, J. T.: *Marinobacter* strain NCE312 has a
616 Pseudomonas-like naphthalene dioxygenase, FEMS microbiology letters, 201, 47-51,
617 10.1111/j.1574-6968.2001.tb10731.x, 2001.

618 Hedlund, B. P., and Staley, J. T.: *Vibrio cyclotrophicus* sp. nov., a polycyclic aromatic hydrocarbon
619 (PAH)-degrading marine bacterium, International journal of systematic and evolutionary microbiology, 51,
620 61-66, 2001.

621 Hedlund, B. P., and Staley, J. T.: Isolation and characterization of *Pseudoalteromonas* strains with
622 divergent polycyclic aromatic hydrocarbon catabolic properties, Environmental Microbiology, 8, 178-182,
623 2006.

624 Jin, H. M., Kim, J. M., Lee, H. J., Madsen, E. L., and Jeon, C. O.: *Alteromonas* As a Key Agent of
625 Polycyclic Aromatic Hydrocarbon Biodegradation in Crude Oil-Contaminated Coastal Sediment,
626 Environmental science & technology, 46, 7731-7740, 10.1021/es3018545, 2012.

627 Kappell, A. D., Wei, Y., Newton, R. J., Van Nostrand, J. D., Zhou, J., McLellan, S. L., and Hristova, K. R.:
628 The polycyclic aromatic hydrocarbon degradation potential of Gulf of Mexico native coastal microbial
629 communities after the Deepwater Horizon oil spill, Front Microbiol, 5, 205, 10.3389/fmicb.2014.00205,
630 2014.

631 Kasai, Y., Kishira, H., and Harayama, S.: Bacteria belonging to the genus *cycloclasticus* play a primary
632 role in the degradation of aromatic hydrocarbons released in a marine environment, Appl Environ
633 Microbiol, 68, 5625-5633, 2002.

634 Kodama, Y., Stiknowati, L. I., Ueki, A., Ueki, K., and Watanabe, K.: *Thalassospira tepidiphila* sp. nov., a
635 polycyclic aromatic hydrocarbon-degrading bacterium isolated from seawater, International journal of
636 systematic and evolutionary microbiology, 58, 711-715, 10.1099/ijs.0.65476-0, 2008.

637 Konn, C., Charlou, J. L., Donval, J. P., Holm, N. G., Dehairs, F., and Bouillon, S.: Hydrocarbons and
638 oxidized organic compounds in hydrothermal fluids from Rainbow and Lost City ultramafic-hosted vents,
639 Chemical Geology, 258, 299-314, 2009.

640 Latimer, J. S., and Zheng, J.: The Sources, Transport, and Fate of PAHs in the Marine Environment, in:
641 PAHs: An Ecotoxicological Perspective, John Wiley & Sons, Ltd, 7-33, 2003.

642 Lobbes, J. M., Fitznar, H. P., and Kattner, G.: Biogeochemical characteristics of dissolved and particulate
643 organic matter in Russian rivers entering the Arctic Ocean, *Geochimica et Cosmochimica Acta*, 64,
644 2973-2983, 2000.

645 Ma, Y., Wang, L., and Shao, Z.: *Pseudomonas*, the dominant polycyclic aromatic hydrocarbon-degrading
646 bacteria isolated from Antarctic soils and the role of large plasmids in horizontal gene transfer, *Environ*
647 *Microbiol*, 8, 455-465, 10.1111/j.1462-2920.2005.00911.x, 2006.

648 Maruyama, A., Ishiwata, H., Kitamura, K., Sunamura, M., Fujita, T., Matsuo, M., and Higashihara, T.:
649 Dynamics of microbial populations and strong selection for *Cycloclasticus pugetii* following the
650 Nakhodka oil spill, *Microb Ecol*, 46, 442-453, 10.1007/s00248-002-3010-z, 2003.

651 McClintock, J. B.: Arctic: sea-ice loss sparks oil drilling boom, *Nature*, 479, 478, 10.1038/479478e, 2011.

652 McKew, B. A., Coulon, F., Osborn, A. M., Timmis, K. N., and McGenity, T. J.: Determining the identity
653 and roles of oil-metabolizing marine bacteria from the Thames estuary, UK, *Environ Microbiol*, 9,
654 165-176, 10.1111/j.1462-2920.2006.01125.x, 2007a.

655 McKew, B. A., Coulon, F., Yakimov, M. M., Denaro, R., Genovese, M., Smith, C. J., Osborn, A. M.,
656 Timmis, K. N., and McGenity, T. J.: Efficacy of intervention strategies for bioremediation of crude oil in
657 marine systems and effects on indigenous hydrocarbonoclastic bacteria, *Environ Microbiol*, 9, 1562-1571,
658 10.1111/j.1462-2920.2007.01277.x, 2007b.

659 Melcher, R. J., Apitz, S. E., and Hemmingsen, B. B.: Impact of irradiation and polycyclic aromatic
660 hydrocarbon spiking on microbial populations in marine sediment for future aging and biodegradability
661 studies, *Appl Environ Microbiol*, 68, 2858-2868, 2002.

662 Nieperon, M., Portet-Koltalo, F., Merlin, C., Motelay-Massei, A., Barray, S., and Bodilis, J.: Both
663 *Cycloclasticus* spp. and *Pseudomonas* spp. as PAH-degrading bacteria in the Seine estuary (France),
664 *FEMS Microbiol Ecol*, 71, 137-147, 10.1111/j.1574-6941.2009.00788.x, 2010.

665 Notomista, E., Pennacchio, F., Cafaro, V., Smaldone, G., Izzo, V., Troncone, L., Varcamonti, M., and Di
666 Donato, A.: The marine isolate *Novosphingobium* sp. PP1Y shows specific adaptation to use the aromatic
667 fraction of fuels as the sole carbon and energy source, *Microb Ecol*, 61, 582-594,
668 10.1007/s00248-010-9786-3, 2011.

669 Opsahl, S., Benner, R., and Amon, R. M. W.: Major flux of terrigenous dissolved organic matter through
670 the Arctic Ocean, 8, *American Society of Limnology and Oceanography*, Waco, TX, ETATS-UNIS, 1999.

671 Ortega-Retuerta, E., Jeffrey, W. H., Babin, M., Bédanger, S., Benner, R., Marie, D., Matsuoka, A.,
672 Raimbault, P., and Joux, F.: Carbon fluxes in the Canadian Arctic: patterns and drivers of bacterial
673 abundance, production and respiration on the Beaufort Sea margin, *Biogeosciences*, 9, 3679-3692,
674 10.5194/bg-9-3679-2012, 2012.

675 Proskurowski, G., Lilley, M. D., Seewald, J. S., Fruh-Green, G. L., Olson, E. J., Lupton, J. E., Sylva, S. P.,
676 and Kelley, D. S.: Abiogenic Hydrocarbon Production at Lost City Hydrothermal Field, *Science*, 319,
677 604-607, 2008.

678 Schmidt, C. W.: Offshore exploration to commence in the Arctic: can shell's oil-spill response plans keep
679 up?, *Environmental health perspectives*, 120, A194-199, 10.1289/ehp.120-a194, 2012.

680 Schneiker, S., Martins dos Santos, V. A., Bartels, D., Bekel, T., Brecht, M., Buhmester, J., Chernikova, T.
681 N., Denaro, R., Ferrer, M., Gertler, C., Goesmann, A., Golyshina, O. V., Kaminski, F., Khachane, A. N.,
682 Lang, S., Linke, B., McHardy, A. C., Meyer, F., Nechitaylo, T., Puhler, A., Regenhardt, D., Rupp, O.,
683 Sabirova, J. S., Selbitschka, W., Yakimov, M. M., Timmis, K. N., Vorholter, F. J., Weidner, S., Kaiser, O.,
684 and Golyshin, P. N.: Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium
685 *Alcanivorax borkumensis*, *Nature biotechnology*, 24, 997-1004, 10.1038/nbt1232, 2006.

686 Shao, Z., Cui, Z., Dong, C., Lai, Q., and Chen, L.: Analysis of a PAH-degrading bacterial population in
687 subsurface sediments on the Mid-Atlantic Ridge, *Deep Sea Research Part I: Oceanographic Research*
688 *Papers*, 57, 724-730, 2010.

689 Simoneit, B. R. T., Lein, A. Y., Peresyphkin, V. I., and Osipov, G. A.: Composition and origin of
690 hydrothermal petroleum and associated lipids in the sulfide deposits of the Rainbow field (Mid-Atlantic

691 Ridge at 36N), *Geochimica et Cosmochimica Acta*, 68, 2275-2294, 2004.

692 Sohn, J. H., Kwon, K. K., Kang, J. H., Jung, H. B., and Kim, S. J.: *Novosphingobium pentaromativorans*
693 sp. nov., a high-molecular-mass polycyclic aromatic hydrocarbon-degrading bacterium isolated from
694 estuarine sediment, *International journal of systematic and evolutionary microbiology*, 54, 1483-1487,
695 10.1099/ijs.0.02945-0, 2004.

696 Sorensen, S. R., Johnsen, A. R., Jensen, A., and Jacobsen, C. S.: Presence of psychrotolerant
697 phenanthrene-mineralizing bacterial populations in contaminated soils from the Greenland High Arctic,
698 *FEMS microbiology letters*, 305, 148-154, 10.1111/j.1574-6968.2010.01920.x, 2010.

699 Teramoto, M., Suzuki, M., Okazaki, F., Hatmanti, A., and Harayama, S.: *Oceanobacter*-related bacteria
700 are important for the degradation of petroleum aliphatic hydrocarbons in the tropical marine environment,
701 *Microbiology*, 155, 3362-3370, 10.1099/mic.0.030411-0, 2009.

702 Wang, B., Lai, Q., Cui, Z., Tan, T., and Shao, Z.: A pyrene-degrading consortium from deep-sea sediment
703 of the West Pacific and its key member *Cycloclasticus* sp. P1, *Environ Microbiol*, 10, 1948-1963,
704 10.1111/j.1462-2920.2008.01611.x, 2008.

705 Whyte, L. G., Bourbonniere, L., and Greer, C. W.: Biodegradation of petroleum hydrocarbons by
706 psychrotrophic *Pseudomonas* strains possessing both alkane (*alk*) and naphthalene (*nah*) catabolic
707 pathways, *Applied and environmental microbiology*, 63, 3719-3723, 1997.

708 Witt, G.: Polycyclic aromatic hydrocarbons in water and sediment of the Baltic Sea, *Marine pollution*
709 *bulletin*, 31, 237-248., 10.1016/0025-326X(95)00174-L, 1995.

710 Wu, J. Y., Jiang, X. T., Jiang, Y. X., Lu, S. Y., Zou, F., and Zhou, H. W.: Effects of polymerase, template
711 dilution and cycle number on PCR based 16S rRNA diversity analysis using the deep sequencing method,
712 *BMC microbiology*, 10, 255, 10.1186/1471-2180-10-255, 2010.

713 Yakimov, M. M., Giuliano, L., Denaro, R., Crisafi, E., Chernikova, T. N., Abraham, W. R., Luensdorf, H.,
714 Timmis, K. N., and Golyshin, P. N.: *Thalassolituus oleivorans* gen. nov., sp. nov., a novel marine
715 bacterium that obligately utilizes hydrocarbons, *International journal of systematic and evolutionary*
716 *microbiology*, 54, 141-148, 2004.

717 Yakimov, M. M., Timmis, K. N., and Golyshin, P. N.: Obligate oil-degrading marine bacteria, *Curr Opin*
718 *Biotechnol*, 18, 257-266, 10.1016/j.copbio.2007.04.006, 2007.

719 Yuan, J., Lai, Q., Zheng, T., and Shao, Z.: *Novosphingobium indicum* sp. nov., a polycyclic aromatic
720 hydrocarbon-degrading bacterium isolated from a deep-sea environment, *International journal of*
721 *systematic and evolutionary microbiology*, 59, 2084-2088, 2009.

722 Yunker, M. B., and Macdonald, R. W.: Composition and Origins of Polycyclic Aromatic Hydrocarbons in
723 the Mackenzie River and on the Beaufort Sea Shelf, Arctic, 48, 118 -129, 1995.

724 Yunker, M. B., Macdonald, R. W., Snowdon, L. R., and Fowler, B. R.: Alkane and PAH biomarkers as
725 tracers of terrigenous organic carbon in Arctic Ocean sediments, *Org Geochem*, 42, 1109-1146, 2011.

726 Zaborska, A., Carroll, J., Pazdro, K., and Pempkowiak, J.: Spatio-temporal patterns of PAHs, PCBs and
727 HCB in sediments of the western Barents Sea, *Oceanologia*, 53, 1005-1026., 2011.

728 Zheng, G. J., Man, B. K. W., Lam, J. C. W., Lam, M. H. W., and Lam, P. K. S.: Distribution and sources
729 of polycyclic aromatic hydrocarbons in the sediment of a sub-tropical coastal wetland, *Water Res*, 36,
730 1457-1468, 2002.

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732

733 **Figure Legends**

734 **Fig. 1.** Locations of the deep-sea sediment sampling sites in the high-latitude Arctic Ocean.

735

736 **Fig. 2.** The distribution and relative abundance of sixteen EPA-priority PAHs in the sediments of
737 the Arctic Ocean. For each PAH, its relative abundance in all twelve samples was presented
738 using different bar lengths in a sub-plot. The longest bar in a sub-plot indicates that this sample
739 has the highest concentration value of a PAH in all twelve samples. The length of the other bars
740 was proportionately shorted based on the ratios of the concentration values of other samples
741 divided by the most abundant PAH in this sample. Acenaphthylene, acenaphthene and anthracene
742 were not presented in this plot because their concentrations were below the detection limits in all
743 sediment samples. U, M, and L in the sample names refer to the upper, middle, and lower layers
744 of the sediments, respectively.

745

746 **Fig. 3.** Relative abundances of bacteria (genus level) in the sediments and enriched consortia
747 from the Arctic Ocean. A: sediment; B: 4 °C consortia; C: 15 °C consortia; D: 25 °C consortia. The
748 genera of abundance more than 1% of the total tags at least in one consortium were listed in each
749 plot. “Others” refer to the genera constituting less than 1% of the total tags of a sample for all
750 samples.

751

752 **Fig. 4.** PAH-removal rates of the consortia that were enriched from the sediments of the Arctic
753 Ocean. The consortia were grown with a mixture of PAHs (naphthalene, phenanthrene, and
754 pyrene) as the sole carbon and energy source. PAHs were quantified using GC/MS after
755 pretreatments. Naphthalene was not included due to an error caused by its high volatility.
756 Consortia names with prefixes “15” or “25” indicated they were enriched at 15°C or 25°C,

757 respectively.

758

759 **Fig. 5.** PCR-DGGE profiles of 12 PAH-degrading consortia that were enriched at 25 °C. Lanes
760 1-3, site BN03; Lanes 4-6, site BN06; Lanes 7-9, site BN09; Lanes 10-12, BN12; and Lane 13,
761 negative control.

762

763 **Fig. 6.** PCR-DGGE profiles of PAH-degrading consortia that were enriched low temperatures.
764 Lane CK, negative control; A: consortia enriched at 4 °C; and B: consortia enriched at 15 °C.

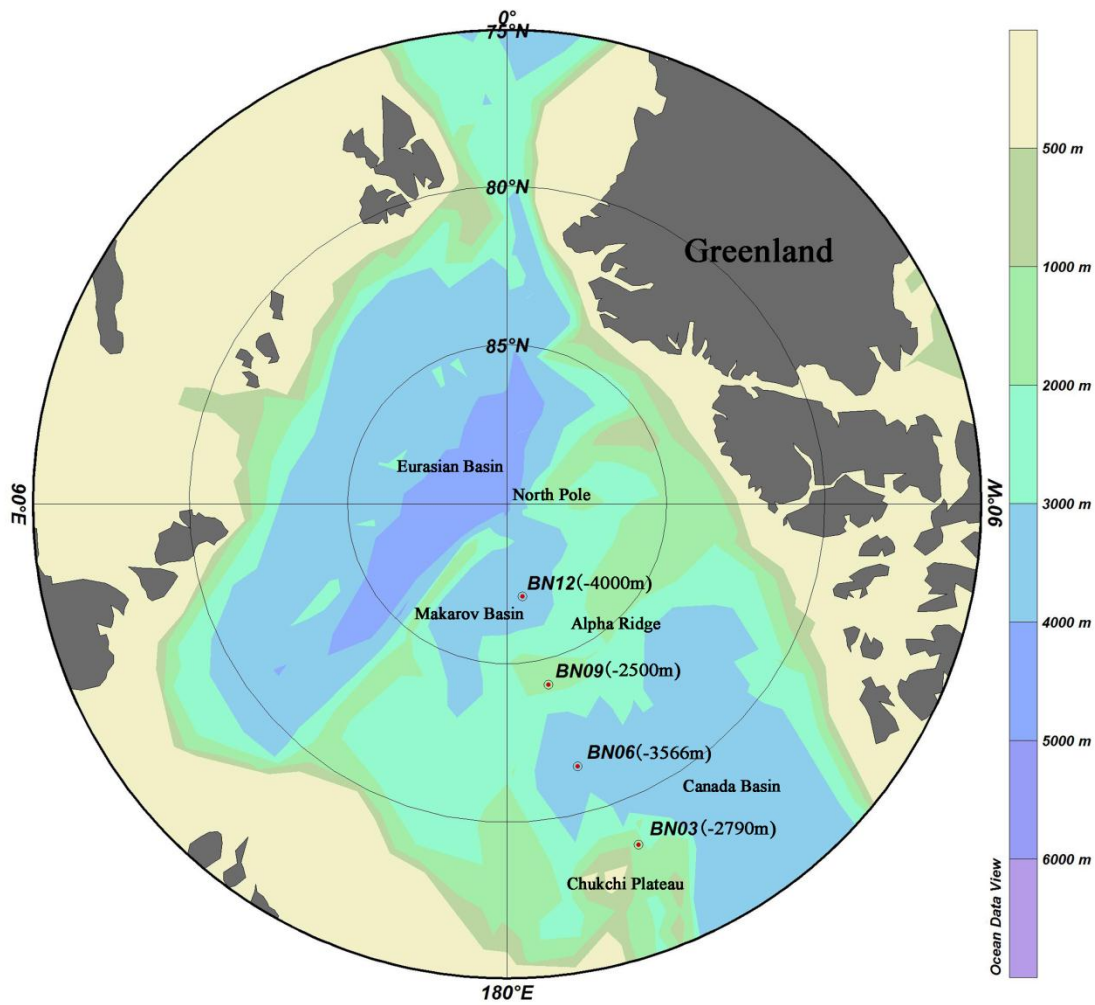
765

766 **Fig. 7.** Growth curves of strains 15BN12U-14 and 15BN12L-10 using individual PAHs as the
767 sole carbon and energy source in ONR7a medium. A and B: strain U-14 and L-10 cultivated at
768 15 °C, respectively. OD₆₀₀ was defined as meaning optical density at 600 nm.

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770

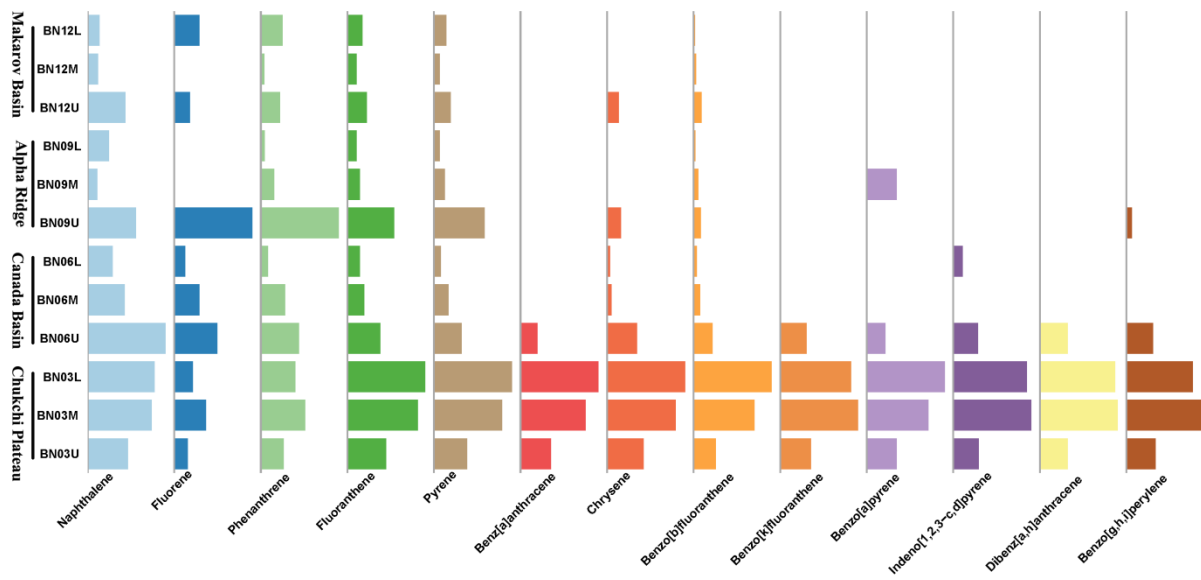
771 Fig.1



772

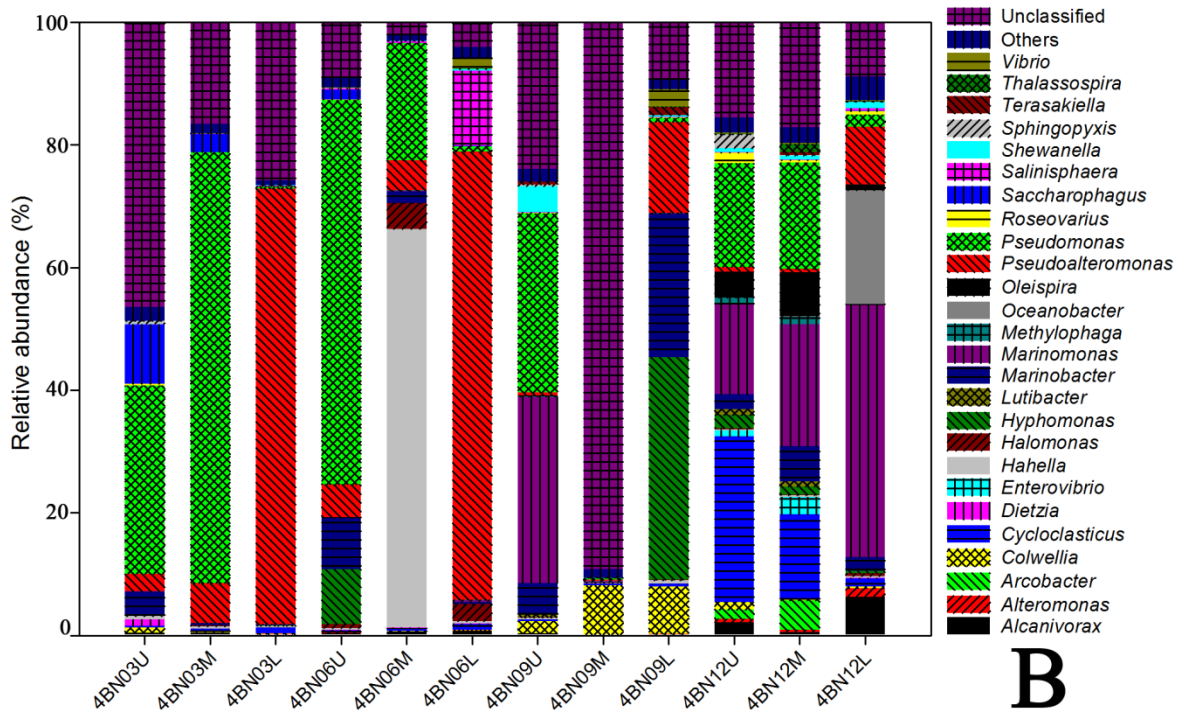
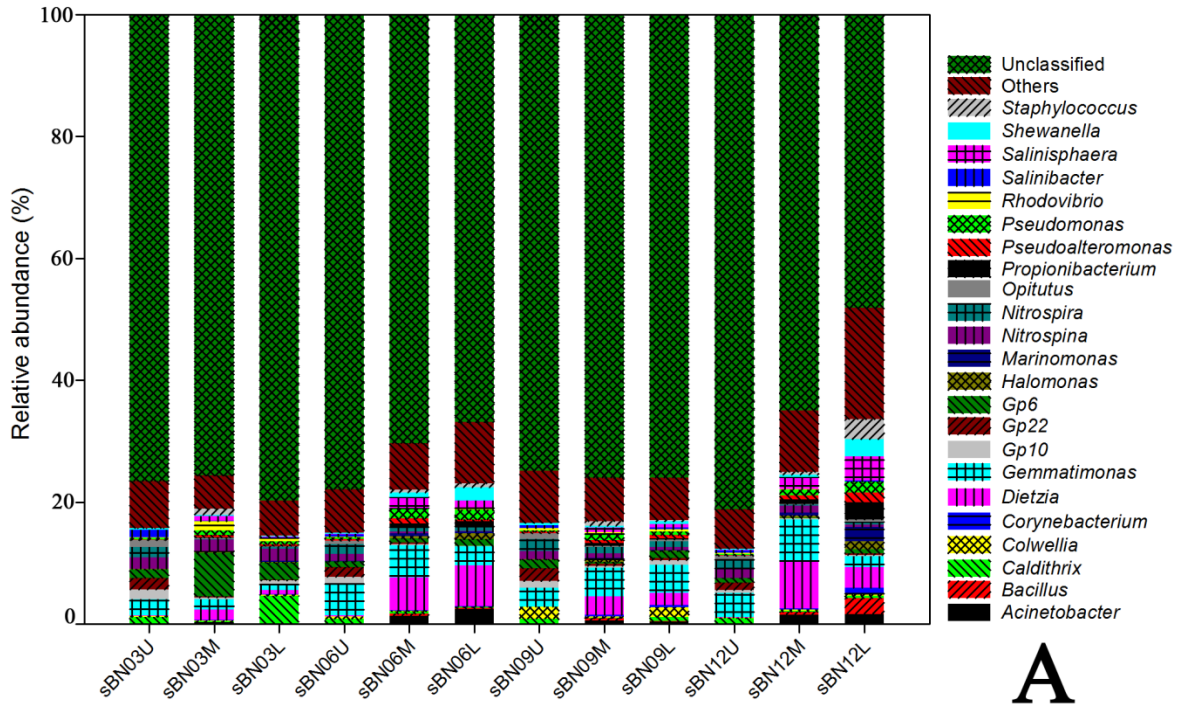
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774 Fig. 2

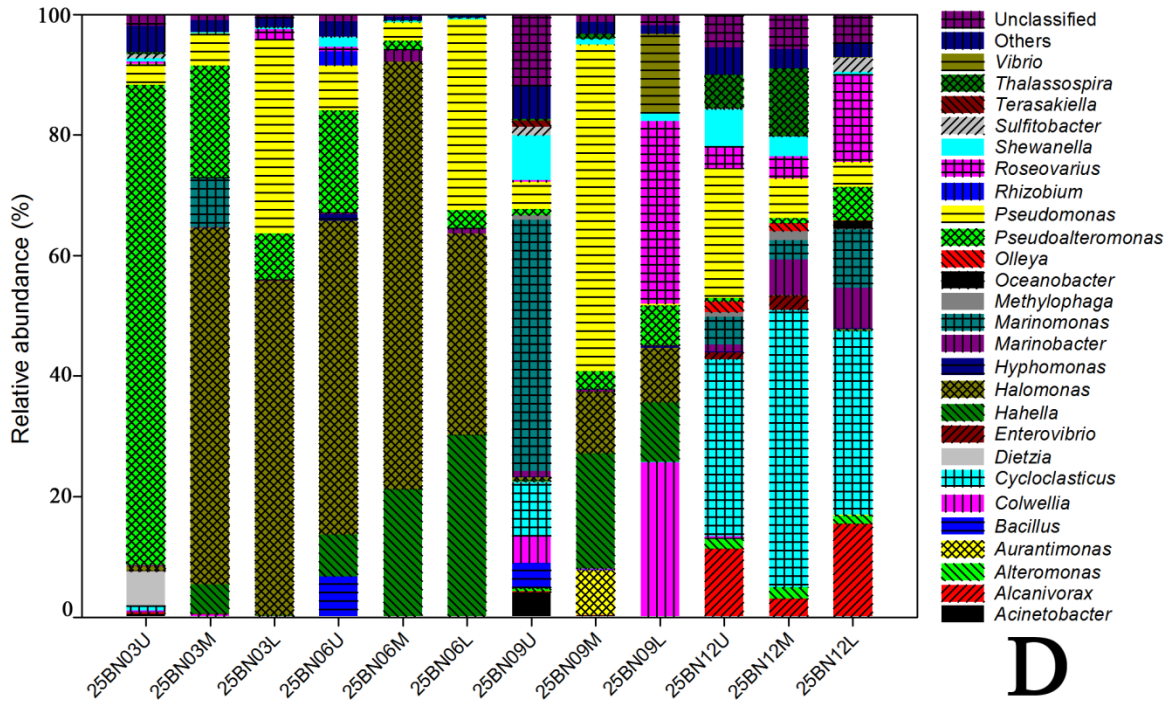
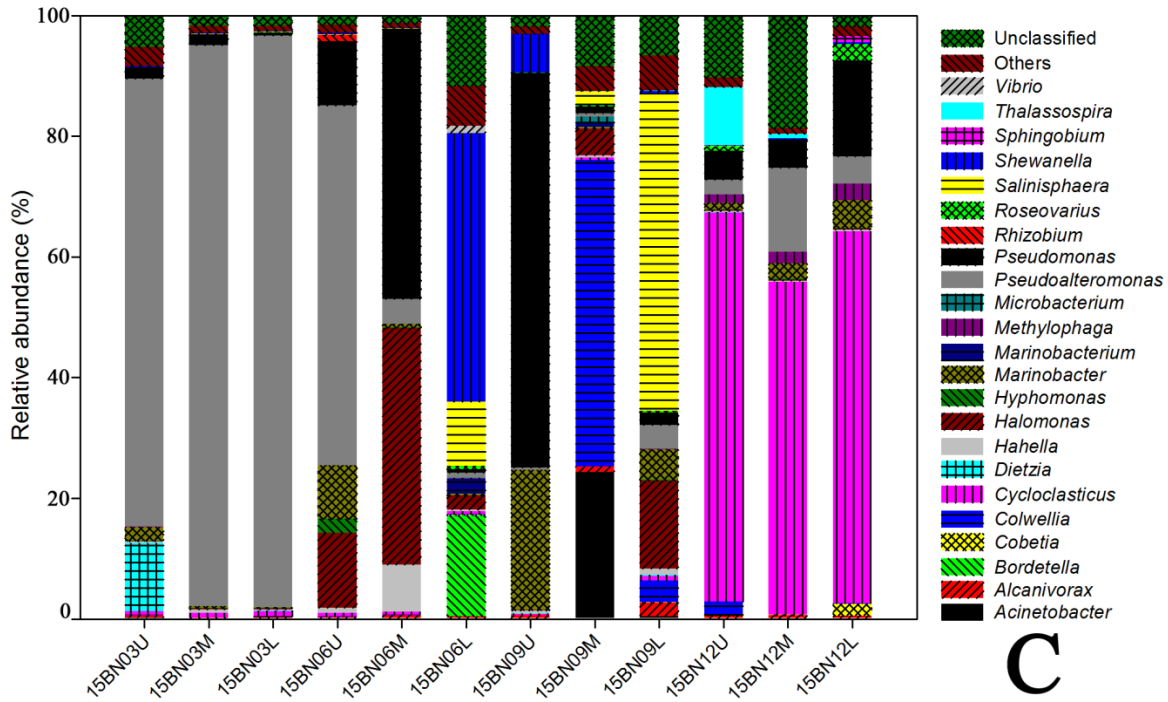


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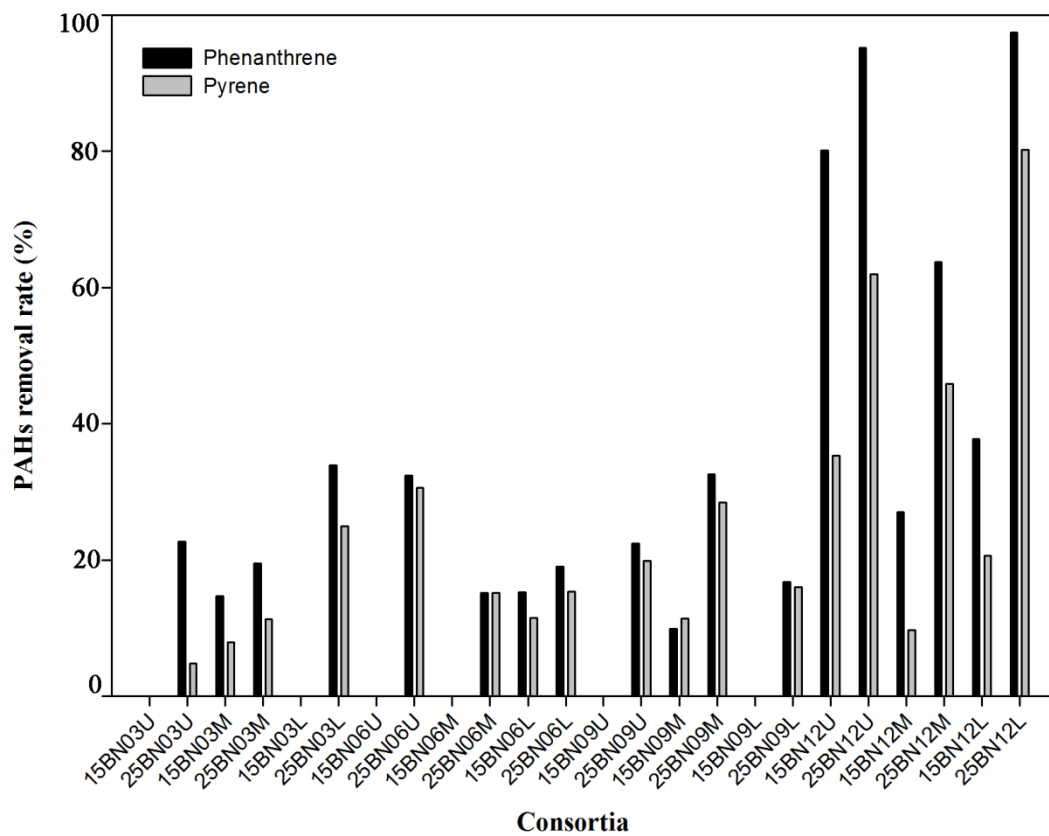
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780 Fig.3 (continue)



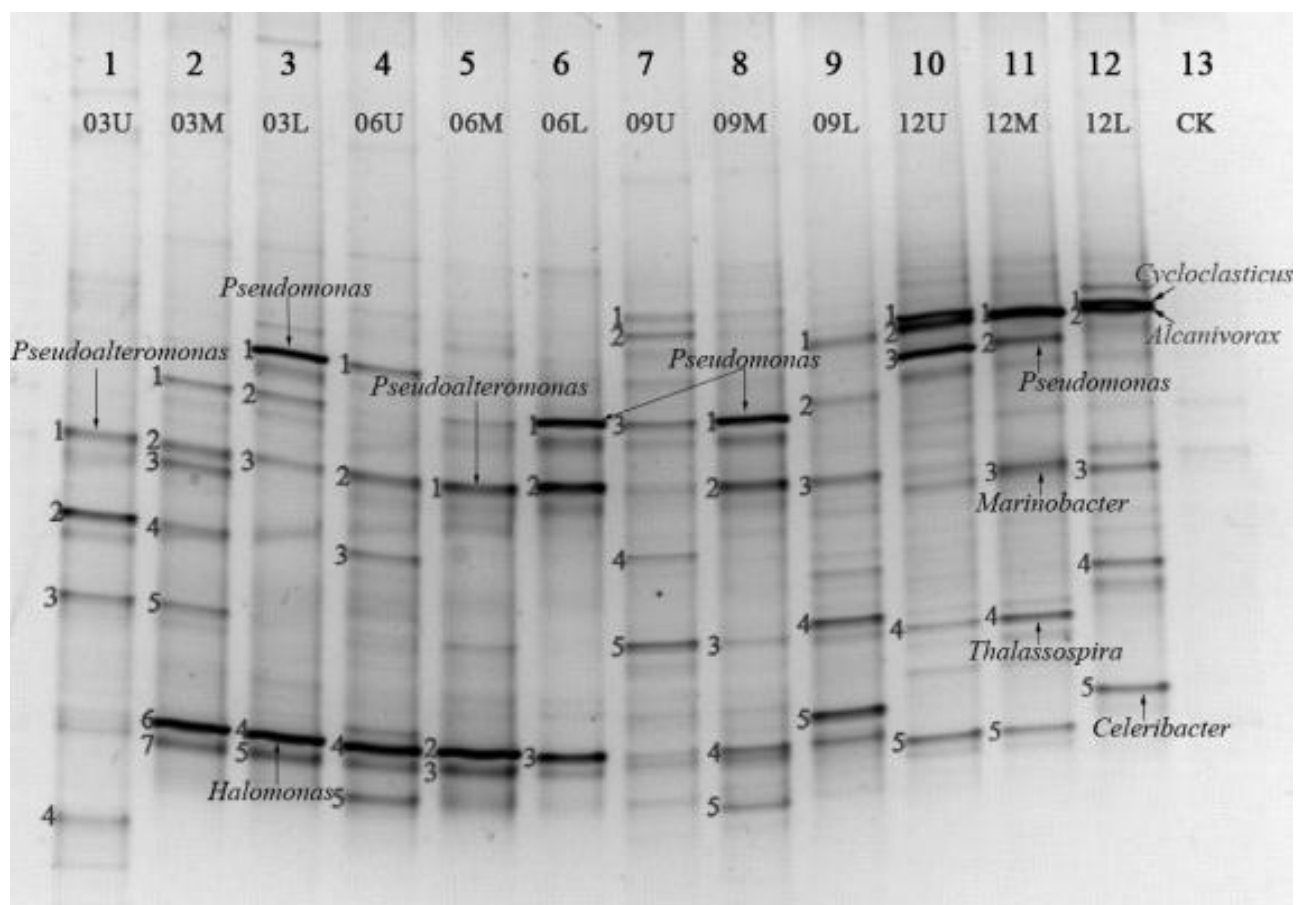
783 Fig. 4



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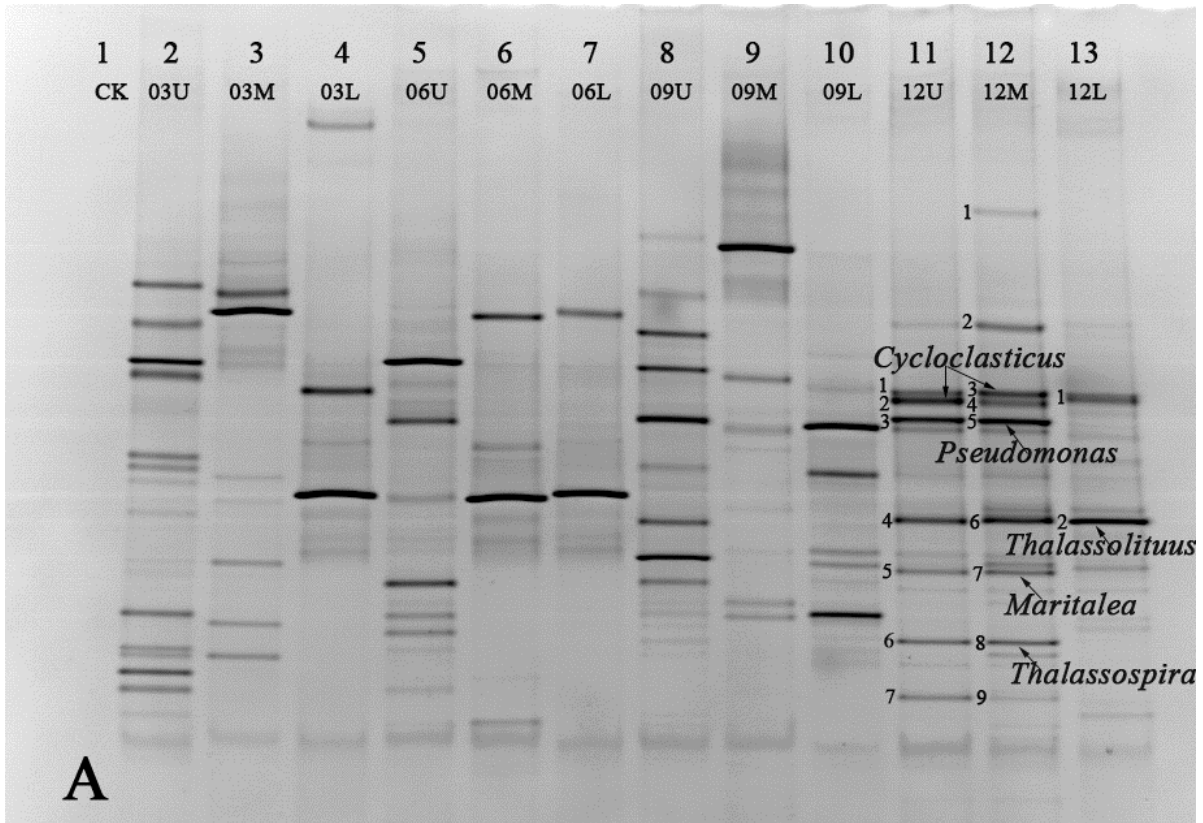
786 Fig. 5



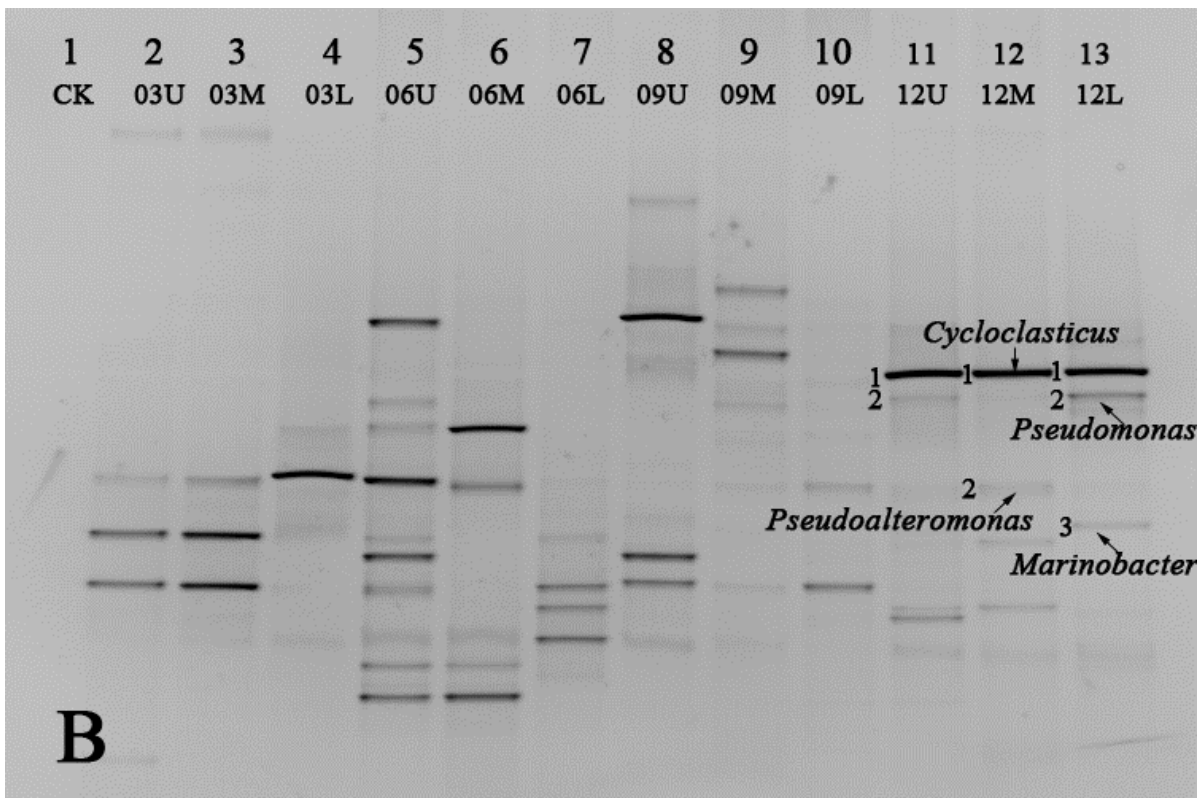
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789 Fig. 6

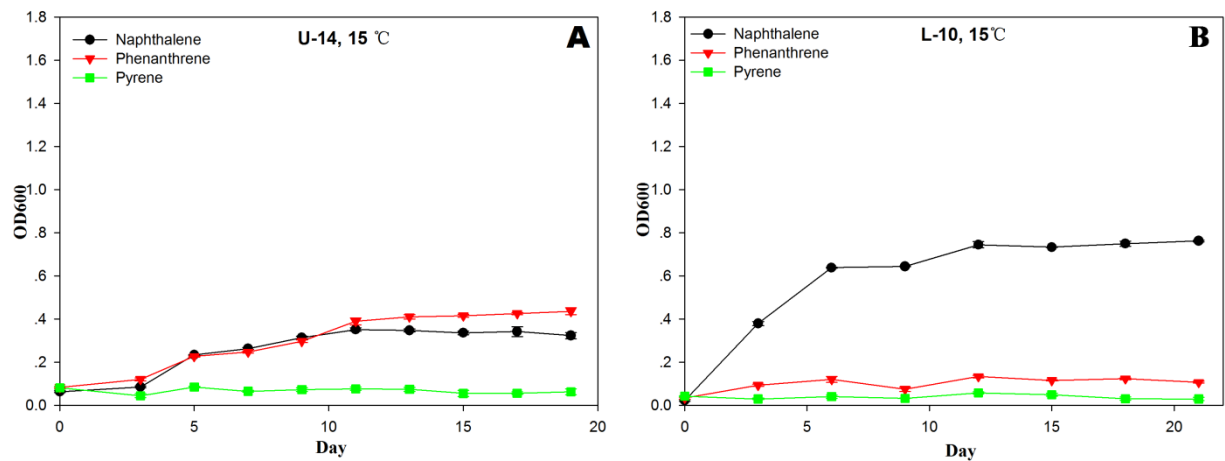


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792 Fig. 7



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