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Abstract

Heterotrophic bacteria isolated from five aquatic microbial mat samples from different locations in continental Antarctica and the Antarctic Peninsula were compared to assess their biodiversity. A total of 2225 isolates obtained on different media and at different temperatures were included. After an initial grouping by whole-genome fingerprinting, partial 16S rRNA gene sequence analysis was used for further identification. These results were compared with previously published data obtained with the same methodology from terrestrial and aquatic microbial mat samples from two additional Antarctic regions. The phylotypes recovered in all these samples belonged to five major phyla, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Firmicutes* and *Deinococcus-Thermus*, and included several potentially new taxa. Ordination analyses were performed in order to explore the variance in the diversity of the samples at genus level. Habitat type (terrestrial versus aquatic) and specific conductivity in the lacustrine systems significantly explained the variation in bacterial community structure. Comparison of the phylotypes with sequences from public databases showed that a considerable proportion (36.9%) is currently known only from Antarctica. This suggests that in Antarctica both cosmopolitan taxa as well as taxa with limited dispersal and a history of long-term isolated evolution occur.

1. Introduction

Microbial mats and surface crusts that may develop in wet Antarctic habitats (Laybourn-Parry and Pearce 2007; Vincent 2000), are dense communities of vertically stratified microorganisms and are believed to be responsible for much of the primary production under the extreme polar conditions. The mats and crusts typically consist of mucilage in which cyanobacteria and other algal cells are embedded, together with other heterotrophic and chemoautotrophic microorganisms, sand grains and other inorganic materials (Fernández-Valiente et al. 2007). Particularly the lacustrine ecosystems, which range from relatively deep freshwater and hypersaline lakes, to small ponds and seepage areas (Verleyen et al. in press) act as true biodiversity and primary production hotspots in a matrix of polar desert and ice.

In recent years, Antarctic microbial mats have attracted a lot of scientific interest, with the photoautotrophic taxa such as cyanobacteria (Taton et al. 2006), green algae (De Wever et al. 2009) and diatoms (Sabbe et al. 2003) probably being the best-studied groups. Water depth (and hence light climate), liquid water availability, and conductivity or related parameters are the most important variables in structuring these communities (Hodgson et al. 2004; Verleyen et al. 2010). Surprisingly, only a small number of studies have focussed on the heterotrophic bacterial diversity in these microbial mats (Brambilla et al. 2001; Van Trappen et al. 2002). Other land-based habitats in Antarctica that have been studied for their heterotrophic bacterial diversity include soils in dry valleys (Aislabie et al. 2006b) and maritime Antarctica (Chong et al. 2010), the plankton in freshwater lakes (Pearce. 2005), and anoxic waters in meromictic lakes (Franzmann et al. 1991). The few studies focussing on the heterotrophic bacterial diversity in aquatic microbial mats comprised samples from lakes in the McMurdo Dry Valleys, the Vestfold Hills and the Larsemann Hills and included culture-dependent as well as independent approaches. They reported a large diversity with an important number of previously unknown taxa (Brambilla et al. 2001; Van Trappen et al. 2002). As a result, several new species have been described in

the phyla *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* and *Firmicutes* (Reddy et al. 2003a, b; Reddy et al. 2002a, b; Shivaji et al. 2005; Van Trappen et al. 2003, 2004a, b, c, d). The relationship between the bacterial diversity of microbial mats and environmental parameters has not yet been studied although Brambilla et al. (2001) suggested some general features expected of the organisms obtained based on their phylogenetic position.

The aims of this study were (i) to contribute to a better understanding of the diversity of heterotrophic bacteria in microbial mat communities from a range of terrestrial and aquatic habitats in coastal and inland ice-free regions in Continental and Maritime Antarctica, and (ii) to explore the relationship between the bacterial communities and a set of environmental parameters. We applied a cultivation-based approach using several media and growth conditions to access heterotrophic bacteria. A large number of isolates was obtained and identified through genotypic characterization using rep-PCR fingerprinting and phylogenetic analysis of the 16S rRNA gene sequences. Comparison of the sequences with those available in public databases allowed identification of the bacteria and an assessment of their geographic distribution.

2. Experimental Procedures

2.1. Source of samples

Five samples (PQ1, LA3, SK5, WO10 and SO6) from lacustrine habitats in different locations in Continental Antarctica and the Antarctic Peninsula (Figure 1) were analysed (Table 1). All samples were kept frozen continuously after collection (in January 2003 [PQ1] and January 2007 [LA3, SK5, WO10 and SO6]) until processing in the laboratory. Specific conductivity and pH were measured in the field using a YSI 600 meter. Details regarding the analysis of the concentration of the major ions and nutrients have been described by Hodgson et al. (2010) and Verleyen et al. (in press).

Data for the new samples was also compared with information on four further samples previously studied using the same methods, including two terrestrial mat samples from Utsteinen (Sør Rondane Mountains, East Antarctica) (Peeters et al. 2011a) and two microbial mat samples from lakes in the Pensacola Mountains and the Shackleton Range (Peeters et al. 2011b).

2.2. Enumeration and isolation of heterotrophic bacteria

One gram of sample was aseptically weighed and homogenized in 9 ml sterile cold (4°C) physiological saline (0.86% NaCl) using a vortex. Tenfold dilution series (kept at 4°C) were plated on four different media (Marine agar 2216 (MA) (BD Difco™), R2A (BD Difco™), ten times diluted R2A (R2A/10), and PYGV (Pepton-Yeast-Glucose-Vitamin) medium (DSMZ medium 621)) and incubated at 20°C, 15°C and 4°C. R2A (Difco) contains pyruvate, starch and dextrose as C sources and yeast extract, peptone and casaminoacids as N and C sources and PYGV (DSMZ medium 621) contains peptone, yeast extract and glucose as C and/or N sources and additional vitamins and minerals. Both are considered oligotrophic media because the amounts of these components are at least two to ten times lower than in more general media such as nutrient broth. In addition to regular physiological saline (PS) dilution series, sea water (SW) dilutions were used for the LA3 and WO10 samples which originated from lakes close to the ocean and had elevated conductivity values.

All plates were incubated for several weeks during which the number of colony forming units (CFU) was counted. When the number of CFU's had stabilized, the total number of CFU/g for each combination of culture conditions was calculated for the plates showing between 20 and 400 colonies. At the end of the incubation period, three colonies (or less in case of insufficient growth) of each morphological type (colony parameters used include color, margin, elevation, shape, diameter, surface appearance) were isolated and purified. Pure cultures were cryopreserved at -80°C using broth medium plus 15% glycerol or the MicroBank™ system (Pro-Lab Diagnostics, Ontario, Canada).

112

113

114 **2.3. Genotypic fingerprinting**

115 To reduce the large number of isolates, duplicates were eliminated using a whole-genome fingerprinting
116 technique, repetitive element palindromic (rep)-PCR, resulting in a smaller number of clusters and
117 unique isolates. DNA preparation was carried out as described by Baele et al. (2003). Rep-PCR
118 fingerprinting using the GTG_5 primer (5'-GTG GTG GTG GTG GTG-3') was performed according to Gevers
119 et al. (2001). Resulting fingerprints were processed using the BioNumerics (v 5.1.) software (Applied-
120 Maths). Rep-PCR profiles were compared by calculating pairwise Pearson's correlation coefficients (*r*). A
121 cluster analysis was performed on the resulting matrix using the Unweighted Pair Group Method using
122 Arithmetic averages (UPGMA). An 80% Pearson correlation coefficient threshold was used (Gevers et al.
123 2001) in combination with visual inspection of bands to delineate rep-clusters. Rep-types included both
124 rep-clusters as well as isolates grouping separately.

125

126 **2.4. 16S rRNA gene sequencing**

127 The 16S rRNA genes of the representatives of all the different rep-types were amplified and partially
128 sequenced as previously described (Vancanneyt et al. 2004). PCR products were purified using a
129 Nucleofast 96 PCR clean up membrane system (Machery-Nagel, Germany) and Tecan Workstation 200.
130 The BKL1 primer was used for sequencing (Coenye et al. 1999). The fragments obtained (approximately
131 400 bp of the first and most variable part of the gene) were cleaned with the BigDye® xTerminator™
132 Purification Kit according to the protocol of the supplier (Applied Biosystems). Sequence analysis was
133 performed using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, USA). Phylogenetic analysis
134 was performed using the BioNumerics (v 5.1.) software package (Applied-Maths). The sequences were
135 compared and pairwise similarity values were calculated to delineate phylotypes at 99.0% 16S rRNA

136 gene sequence similarity (Acinas et al. 2004; Stach et al. 2003). The classifier of the Ribosomal Database
137 Project, containing the sequences of all described species, was used to obtain a genus identification for
138 the phylotypes (Wang et al. 2007). Identifications with confidence estimates lower than 80% (Wang et al.
139 2007) were verified by phylogenetic analysis with all neighbouring taxa. A multiple alignment of the
140 sequences was made and after visual inspection, distances were calculated using the Kimura-2
141 correction. A neighbour joining dendrogram (Saitou and Nei 1987) was constructed and bootstrap
142 analysis was undertaken using 500 bootstrap replicates. When the analysis showed that a phylotype was
143 not part of an existing genus and was either equally related to multiple genera or had 16S rRNA gene
144 sequence similarities with neighbouring genera below the threshold value of 96.4% (Yarza et al. 2010),
145 the phylotype was classified as a potentially new genus.
146 The 16S rRNA gene sequences determined in this study have been deposited in the EMBL database
147 under accession numbers FR772052 - FR772080 and FR772100 - FR772289.
148

149 **2.5. Sample coverage**

150 Rarefaction curves were used to estimate how well our method covers the fraction of bacteria viable in
151 the growth conditions used. They were calculated with an online rarefaction calculator
152 (http://biome.sdsu.edu/fastgroup/cal_tools.htm). The Shannon biodiversity index was calculated as
153 described by Magurran et al. (1988).
154

155 **2.6. Multivariate analysis**

156 Direct and indirect ordinations were performed using CANOCO 4.5 for Windows (ter Braak and Smilauer
157 2002). A principal component analysis (PCA) was applied of the number of rep-types assigned to the
158 different genera for each sample. Redundancy analysis (RDA) was applied to assess whether differences
159 in bacterial community structure are underlain by differences in habitat type. Therefore, we created

three dummy variables (Table S2). The forward selection procedure and unrestricted Monte Carlo permutations tests (499 permutations, p = 0.05) was used to select the minimal number of variables explaining the variation in the distribution of the different rep-types over the genera for the different samples. The importance of limnological variability was assessed for the lacustrine samples only, because no chemical data were available for the terrestrial samples

2.7. Geographic distribution of the phylotypes

The 16S rRNA gene sequence of each phylotype was compared with sequences available in public databases (EMBL and NCBI) including cultured strains as well as environmental sequences (both from metagenomics and high throughput sequencing). Based on the origin of sequences showing ≥ 99.0% sequence similarity, the phylotypes were classified as Antarctic (when no high scoring sequences, or only high scoring sequences originating from other Antarctic environments, were found), bipolar (only high scoring sequences from polar environments), cold (only high scoring sequences from cold environments) or cosmopolitan (at least one high scoring sequence from non-Antarctic/cold/polar environment) (Table 4). Phylotypes that showed no significant similarity with any other sequences, were classified as Antarctic.

3. Results

3.1. Isolation, rep-PCR fingerprinting and 16S rRNA gene sequencing

Dilution series of the different samples (Table 1) were plated on four different media and incubated at three relatively cold temperatures compared to those used for more temperate bacteria. After three weeks incubation for plates at 20 and 15°C and eight weeks for 4°C, the number of colony forming units (CFU) was counted for the different conditions. When comparing the number of CFU/g for the five samples, there were clear differences (Table 2). Sample WO10 had the highest CFU/g of all samples. The

highest value for samples PQ1 and SK5 was low in comparison with the other samples although a large diversity in colony morphologies was observed and consequently many isolates were taken (Fig. 1). For samples PQ1, SK5 and SO6 the highest number of CFU/g was found at 15 or 20°C, while for samples LA3 and WO10 4°C gave best growth. The samples originating from saline and brackish lakes and ponds (LA3 and WO10) yielded the highest number of CFU/g on marine medium, whereas the other samples yielded the highest number of CFU/g on an oligotrophic medium.

Between 253 and 550 isolates (Fig. 1), were purified from the five new samples. This gave a total of 2225 isolates that were grouped in 810 rep-types. To compare the diversity obtained under each culture condition, the relative diversity yield was calculated as the number of rep-types recovered from a sample for each medium and temperature combination, divided by the total number of rep-types obtained for that sample. The highest values are summarized in Table 3. For all samples the highest values for the colony counts (Table 2) and the highest diversity (Table 3) were found on either oligotrophic media (R2A, R2A/10 and PYGV) or marine media (MA PS and MA SW). The highest CFU/g and diversities for each sample were in the same temperature categories (high temperature category: 15-20°C; low temperature category: 4°C) for samples PQ1, SK5 and SO6, however, for samples LA3 and WO10 the highest CFU/g was at 4°C while the highest diversity was recovered at 20°C.

Representatives of the different rep-types were subjected to 16S rRNA gene sequence analysis. Based on these sequences, phylotypes were delineated at 99% sequence similarity. The number of phylotypes recovered per sample ranged from 39 (LA3) to 89 (PQ1) (Fig. 1). Interestingly, only an intermediate number of isolates was taken in this latter sample in comparison with the other samples, suggesting that it harbours a relatively large diversity. This was confirmed by the higher Shannon diversity index based on the number of isolates per rep-type: 5.17 for PQ1, compared to 4.24, 4.62, 4.54 and 4.82 for samples LA3, SK5, WO10 and SO6, respectively. Rarefaction curves (Fig. S1) were calculated to assess the coverage of the culturable diversity under these culture conditions. The curves for most samples

approached a plateau. However, for sample PQ1, the rarefaction curve continued to rise despite a high number of isolates being recovered from this sample.

3.2. Distribution of the phylotypes over different phyla, classes, genera and samples

The different phylotypes were identified using the classifier tool of the Ribosomal Database Project and phylogenetic analysis of the 16S rRNA gene sequences. The diversity found in the different samples was considered at different taxonomic levels. At phylum level, for most samples, the phylotypes were affiliated with four major phylogenetic groups, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Firmicutes*. In addition, isolates of the *Deinococcus-Thermus* phylum were recovered from samples PQ1 and SO6 (Fig. 1). At genus level, variation between the five samples was larger: 70 genera were recovered as well as 18 potentially novel genera (Table S1). Only *Salinibacterium* and *Flavobacterium* were found in all five samples.

Previously we studied two terrestrial samples, BB50 and BB115 from the Utsteinen region (Peeters et al. 2011a), and two aquatic microbial mat samples, TM2 and TM4 from the Pensacola Mountains and the Shackleton Range, respectively (Peeters et al. 2011b), using the same isolation conditions and the same characterization methods. Below we compare our new findings with those from these four samples. To facilitate comparison and to provide an overview, bacterial genus diversity data from these two studies are also included in Table S1. No genera were recovered from all nine samples. The genera *Arthrobacter*, *Brevundimonas* and *Hymenobacter* were found in eight samples whereas *Cryobacterium*, *Rhodococcus*, *Sphingomonas*, *Flavobacterium* and *Bacillus* were found in seven of the nine samples. Some 38% (31/82) of the genera were recovered from only one sample (e.g. *Frigoribacterium*, *Saxeibacter*, *Aurantimonas*, *Caulobacter*, *Lysobacter*, *Maribacter*, *Brevibacillus*).

The genus *Arthrobacter* (Table S1) was best represented among the isolates (733 isolates, representing 20 different phylotypes), although the largest number of different phylotypes (50) was found in the

genus *Hymenobacter*, which also had a rather high number of isolates (230). Other well represented genera based on either the number of isolates or the number of phylotypes included *Brevundimonas*, *Flavobacterium*, *Polaromonas*, *Psychrobacter*, *Massilia*, *Sphingopyxis*, *Sphingomonas* and *Deinococcus*.

At the phylotype level, none of the phylotypes was found in all nine locations (Table S1). Only one phylotype (R-36741), identified as *Brevundimonas,* was found in eight samples. Phylotype R-36538, identified as *Arthrobacter,* was isolated from six samples. Furthermore, phylotypes belonging to the genera *Brevundimonas*, *Rhodococcus*, *Salinibacterium*, *Sphingomonas* and *Massilia* were found in five samples and phylotypes belonging to the genera *Arthrobacter*, *Cryobacterium*, *Rothia*, *Polaromonas*, *Bacillus*, *Paenibacillus* and a potentially new genus in the class *Betaproteobacteria* were found in four samples. Additionally, fifteen (4.2%) of the 356 phylotypes were recovered from three samples, 68 (19.1%) were found in two samples and 260 (73.0%) were restricted to a single sample. Table 4 shows the distribution of shared phylotypes over the different samples. Sample SK5 shared the highest percentage of phylotypes with other samples, especially with samples PQ1, LA3 and SO6. Also samples TM2 and WO10 and TM4 and SO6 shared an important percentage (≥ 10%) of phylotypes.

In all nine samples, only 3.4% (47) of the rep-types contained isolates from more than one sample. The majority of these mixed rep-types contained isolates from two different samples and only two comprised isolates from three different samples. All samples contained isolates that were part of these mixed rep-types, whereas the highest number was shared between samples SK5 and SO6. A large portion of the mixed rep-types was affiliated with *Actinobacteria,* while the remainder was related to all other classes and phyla obtained except for the *Deinococcus-Thermus* phylum. The mixed rep-types belonged to diverse genera, with several from the genera *Arthrobacter*, *Brevundimonas*, *Hymenobacter*, *Pedobacter* and *Rothia*.

3.3. Bacterial community structure in relation to environmental conditions

Also here, we included information from our previous studies (Peeters et al. 2011a, b) to enhance the comparison. The principal component analysis at genus level (Fig. 2) confirmed the differences observed between the nine samples. The two terrestrial samples from Utsteinen (BB50 and BB115) are located relatively close to each other in the top half of the scatter plot. The two samples from the saline lakes (LA3 and WO10) and the brackish lake (TM2) are situated on the negative side of the first ordination axis. A redundancy analysis revealed that the dummy variable denoting the difference in habitat type and grouping terrestrial and freshwater habitats significantly explained 27.3% of the differences in community composition between terrestrial and aquatic samples. This indicates that the samples from saline lakes are different to those from freshwater systems and terrestrial environments. In the subset of the samples from aquatic habitats for which limnological data are available, RDA confirmed that conductivity significantly explained 34.4% of the variation in community structure at genus level.

3.4. Geographical distribution of the phylotypes

The sequences of the different phylotypes were compared with public databases to assess their geographical distribution. For the five new samples a large number of the phylotypes (36.0-64.6%) showed a cosmopolitan distribution as was also found in the four previously studied samples (Table 5). All nine samples also contained a large number of phylotypes currently known only from Antarctica (20.6-58.4%) and many of these shared no significant similarity (≥ 99.0%) with any other sequence in public databases. In general, only small numbers of phylotypes have been classified as cold (≤ 10.4%) or bipolar (≤ 8.3%). It is clear that for most phyla/classes the phylotypes were mainly cosmopolitan (Table 5). Notable exceptions were the phyla *Bacteroidetes* and *Deinococcus-Thermus*, of which the majority of phylotypes were currently known only from Antarctica, many of them without significant sequence similarity with any other sequence.

4. Discussion

We studied the cultured diversity of the heterotrophic bacteria recovered under standardised conditions from five aquatic microbial mat samples from different locations in Maritime and Continental Antarctica and compared the results with previously published data from terrestrial and aquatic microbial mats from two additional regions. Although only a limited number of isolates was studied from each sample, and the culturable diversity represents only a fraction of the total diversity present (Amann et al. 1995), some clear differences between the samples were apparent. The most diverse sample was PQ1, with the highest Shannon diversity index and the largest number of phylotypes recovered, despite only an intermediate number of isolates obtained in comparison with the other samples (Fig. 1). This relatively high diversity may be explained by the location of the sampling site on the Antarctic Peninsula where environmental conditions are less extreme than on the Antarctic continent.

The distribution of the different phyla, classes and genera varied considerably. In most samples, the phylotypes belonged to four major phylogenetic groups (*Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Firmicutes*) that have been reported frequently from various Antarctic habitats including aquatic microbial mats, soil from continental Antarctica and the sub-Antarctic islands and from sediments (Aislabie et al. 2006b, 2008; Babalola et al. 2009; Bowman et al. 2000a; Bowman and McCuaig 2003; Brambilla et al. 2001; Cary et al. 2010; Chong et al. 2010; Selbmann et al. 2010; Van Trappen et al. 2002). The phylum *Deinococcus-Thermus* was only recovered from four samples (BB50, BB115, PQ1 and SO6), including both terrestrial and aquatic samples. The genus *Deinococcus* has been found previously in Antarctic soils and especially in the McMurdo Dry Valleys (Aislabie et al. 2006a, 2008; Cary et al. 2010; Niederberger et al. 2008) although several other studies focussing on Antarctic soils (Gesheva 2009; Shivaji et al. 2004) as well as on marine environments (Bowman et al. 2003, 2000b) and microbial mats in Antarctic lakes (Brambilla et al. 2001; Van Trappen et al. 2002) did not report the presence of this taxon. Most of the frequently occurring genera (genera that were found in more than four samples or

304 from which more than 100 isolates were recovered) have been reported previously from Antarctica (Ah
305 Tow and Cowan 2005; Busse et al. 2003; Irgens et al. 1996; Selbmann et al. 2010; Shivaji et al. 2004; Van
306 Trappen et al. 2002).
307 Besides genera found in multiple samples, also some phylotypes were found in more than one sample.
308 The observation that sample PQ1, the only sample originating from the Antarctic Peninsula, shared
309 comparable percentages of phylotypes with all samples (Table 4), irrespective of geographical distance is
310 interesting. Moreover, these percentages are in the same range as those shared between the other
311 samples. For some higher organisms such as Acari and Nematoda, a strong boundary has been observed
312 between the species present in the Antarctic Peninsula and continental Antarctica, although for
313 Tardigrada and Bryophyta no continental/maritime divide has been found (Convey et al. 2008). Our
314 results suggest that this boundary probably does not exist for bacterial taxa.
315 The abovementioned differences between the samples are related to lake water conductivity and the
316 type of habitat (terrestrial versus aquatic) as revealed by direct ordination analyses. The importance of
317 conductivity was also evident from the fact that the medium used affected the colony yield and the
318 diversity recovered for each sample. For example, the highest yield was obtained using the marine
319 medium for the samples derived from saline and brackish lakes. A number of genera were only obtained
320 from the saline lakes (e.g. *Loktanella*, *Halomonas*, *Gelidilacus* and *Algoriphagus*), whereas only small
321 numbers of the less salt tolerant class *Betaproteobacteria* (Philippot et al. 2010) were isolated in these
322 samples. Only the genera *Aeromicrobium* and *Micrococcus* were isolated both from terrestrial and saline
323 samples. Interestingly, conductivity appears to be more important than the type of habitat, as revealed
324 by the ordination analysis. Although our results may be influenced by the limited number of isolates and
325 samples studied, this observation corroborates previous studies (Philippot et al. 2010; Tamames et al.
326 2010), reporting that the diversity obtained from freshwater samples is more comparable with that of
327 terrestrial samples than with saline ones. The importance of conductivity and related variables rather

328 than extremes of temperatures, pH, or other physical and chemical factors (Tamames et al. 2010)
329 corroborates findings in other microbial organisms in Antarctic lakes, including diatoms and
330 cyanobacteria (Verleyen et al. 2010).
331
332 In the nine samples, a significant number of phylotypes were found to represent potentially novel
333 genera. From the terrestrial samples (BB50 and BB115), the saline samples (TM2, LA3 and WO10) and
334 the freshwater samples (TM4, PQ1, SK5 and SO6) respectively 4, 12 and 22 phylotypes represented
335 potentially new genera. The majority of potentially new genera were found in the classes
336 *Alphaproteobacteria* and *Betaproteobacteria* (35% each) and in samples SO6 (19%), SK5 (16%) and LA3
337 (16%). Further polyphasic studies are necessary to confirm their status and classification. The isolated
338 taxa can be investigated for antimicrobial activities or other products of biotechnological significance
339 (examples reviewed in Margesin and Feller 2010). Moreover, several phylotypes obtained here belonged
340 to genera which at present contain only one species or even one strain (e.g. *Rhodoglobus*, *Saxeibacter*,
341 *Enhydrobacter* and the recently described *Marisedimicola*). The additional cultures obtained in this work
342 may give more insight into the diversity present in these genera.
343
344 A comparison of our sequences to those available in public databases (including sequences from cultured
345 strains as well as environmental community samples and clone libraries) revealed that the majority of
346 the taxa showed a cosmopolitan distribution (Table 5). Although the geographic distribution reflects
347 current and therefore limited knowledge of bacterial diversity and ecology (Curtis and Sloan 2004), some
348 interesting observations can be made. For the BB samples, an important number of phylotypes are
349 currently restricted to Antarctica. This may be explained partly by the terrestrial, more exposed nature of
350 these samples from the pristine environment of the new Princes Elisabeth Station in Utsteinen. These
351 samples were also taken inland, whereas most previous microbial studies on terrestrial samples in

Antarctica have focussed on regions closer to the coast, and generally in close vicinity to research stations (Aislabie et al. 2006b; Chong et al. 2009; Shivaji et al. 2004). The other samples in our comparison originated from locations closer to the ocean and may have experienced inflow of non-Antarctic species, which may have contributed to the lower percentage of phylotypes with an Antarctic distribution. In addition, some strains may have been isolated previously in one of the few earlier studies in the regions of the Schirmacher and Syowa Oasis (Satoh et al. 1989; Shivaji et al. 2004). An important percentage of phylotypes currently restricted to Antarctica was also recovered from sample PQ1, although this sample was taken on the Antarctic Peninsula, closer to the ocean and to civilization.

Comparing the geographical distribution of the phylotypes in more detail, it is clear that the majority of those belonging to the *Actinobacteria*, *Proteobacteria* and *Firmicutes* have a more general distribution whereas most *Bacteroidetes* and *Deinococcus-Thermus* phylotypes are currently restricted to the Antarctic continent. This high number of Antarctic phylotypes within the *Bacteroidetes*, with several potentially new taxa, is in agreement with the increasing number of new species described from Antarctica within this phylum (Bowman et al. 1997, 1998; Bowman and Nichols 2002; Hirsch et al. 1998; McCammon et al. 1998; Shivaji et al. 1992; Van Trappen et al. 2003, 2004b, c; Yi et al. 2005; Yi and Chun, 2006). Our observations therefore appear to indicate that both cosmopolitan and specific Antarctic phylotypes, possibly with a limited dispersal capacity, are present.

5. Conclusion

Although only a limited number of microbial mat samples were studied, these revealed a large diversity of culturable heterotrophic bacteria. There were important differences between the taxa obtained from each of the samples and only limited overlap was observed between the diversity obtained. Phylotypes belonged to five major phylogenetic groups (*Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Deinococcus-Thermus*) and several represented potentially new taxa. The bacterial diversity was

found to relate to conductivity and habitat type. A comparison of our data with sequences in public databases showed that an important proportion of phylotypes (36.9%) are currently known only from the Antarctic continent, although a large proportion of cosmopolitan taxa (56.3%) was also recovered. This suggests that, in Antarctica, cosmopolitan taxa as well as taxa with limited dispersal, which potentially evolved in isolation, occur.

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Captions for Figures

Fig. 1 Division of the phylotypes over the different phylogenetic groups. The number of obtained isolates and phylotypes are mentioned for the different samples. Information for samples BB50, BB115, TM2 and TM4 was based on Peeters et al. 2011a, b

Fig. 2 Principal component analysis (PCA) of the samples showing the differences in bacterial diversity (at genus level) based on the number of rep-types. Information for samples BB50, BB115, TM2 and TM4 was based on Peeters et al. 2011a, b

Fig. S1 Rarefaction curves representing the number of phylotypes isolated from the different samples

Table 1 Overview of samples with their location, coordinates and description

Sample number	Place	Region	Latitude	Longitude	Sample description
PQ1	Narrows Lake	Pourqoui-Pas Island, AntarcticPeninsula	67°42'S	67°27'W	Littoral cyanobacterial mat with green algae and diatoms
LA3	Langhovde lake 3	Syowa Oasis	69°13'S	39° 48'E	Littoral brown crusts of cyanobacteria or diatoms from a small salt lake, sampling depth 0.2 m
SK5	Naka Tempyo	Syowa Oasis	69°28'S	39°40'E	Littoral epipsammic and interstitial microbial mat, brown or orange pigmented on top with a green surface layer, sampling depth 0.1 m
WO10	West Ongul Island, lake 10	Syowa Oasis	69°01'S	39°32'E	Littoral orange mat below a black decomposed mat. Shallow pool with evidence of higher lake level, sampling depth 0.15 m
SO6	Schirmacher Oasis, lake	Schirmacher Oasis	70°45'S	11°40'E	Littoral microbial mat sample from freshwater lake, sampling depth 0.1 m

Table 2 Plate counts (10^5 CFU/g) for the different growth conditions per sample. The maximum plate count for each sample is shown in bold and underlined; nd, not determined

Medium	Temperature	PQ1	LA3	SK5	WO10	SO6
MA-PS	4°C	0,00026	**21,6**	0,0008	**368,4211**	0,282759
	15°C	0,000341	17,78333	0,0021	177,7632	0,398276
	20°C	0,000345	16,13333	0,003	244,7368	0,614828
MA-SW	4°C	nd	9,1	nd	52,28571	nd
	15°C	nd	11	nd	55,71429	nd
	20°C	nd	14,1	nd	48	nd
R2A	4°C	0,003245	0,000167	0,187	41,31579	8,241379
	15°C	0,0128	0,0003	0,86	57,63158	**79,2069**
	20°C	**0,02195**	0,000133	1,89	114,2105	19,91379
R2A/10	4°C	0,0022	0	0,16	9,013158	7,862069
	15°C	0,0148	0,00007	0,507	63,42105	26,44828
	20°C	0,0309	17,66667	0,9	30	24,34483
PYGV	4°C	0,00127	0,00007	0,2085	15,52632	7,034483
	15°C	0,0132	0.0007	1,38	34,73684	25,7069
	20°C	0,022	0,0001	**2,1**	37,89474	26,82759

Table 3 Highest relative values for the number of rep-types and corresponding conditions

Samples	**PQ1**	**LA3**	**SK5**	**WO10**	**SO6**
Highest relative diversity yield	0.167	0.271	0.274	0.258	0.294
Medium	R2A	MA PW	PYGV	MA PW	PYGV
Temperature (°C)	15	20	15	20	20

Table 4 Number of phylotypes defined at 99% sequence similarity (lower left triangle) and percentage of phylotypes (upper right triangle) shared between the samples

Sample	PQ1	LA3	SK5	WO10	SO6	BB50 [a]	BB115 [a]	TM2 [b]	TM4 [b]
PQ1	x	5%	11%	4%	9%	5%	2%	2%	4%
LA3	7	x	11%	7%	4%	1%	1%	3%	7%
SK5	16	11	x	7%	14%	7%	4%	5%	8%
WO10	5	6	7	x	8%	0%	2%	10%	5%
SO6	15	5	20	10	x	5%	6%	4%	10%
BB50	7	1	8	0	7	x	7%	3%	4%
BB115	3	1	4	2	7	7	x	4%	7%
TM2	3	3	6	10	6	4	4	x	9%
TM4	5	5	7	4	11	4	5	8	x

[a] Data from Peeters et al. 2011a

[b] Data from Peeters et al. 2011b

Table 5 Number of phylotypes recovered with cosmopolitan, cold, bipolar or Antarctic distribution for the different classes and phyla and the different samples. Distribution types were assigned to phylotypes by evaluating the geographic origin of highly similar sequences (≥ 99.0%) present in public databases and originating from cultured strains as well as environmental samples and clone-libraries

Distribution type	PQ1	LA3	SK5	WO10	SO6	BB50 [a]	BB115 [a]	TM2 [b]	TM4 [b]
Actinobacteria									
cosmopolitan	8/14	4/5	7/12	10/16	13/20	12/20	10/13	4/5	12/13
cold	4/14	1/5	2/12	4/16	2/20	0/20	1/13	0/5	0/13
bipolar	0/14	0/5	0/12	0/16	0/20	0/20	0/13	1/5	0/13
Antarctic [c]	2/14 (1)	0/5 (0)	3/12 (3)	2/16 (2)	5/20 (5)	8/20 (7)	2/13 (2)	0/5 (0)	1/13 (1)
Alphaproteobacteria									
cosmopolitan	10/12	8/10	15/17	6/7	15/17	5/7	5/5	8/13	6/7
cold	0/12	0/10	0/17	0/7	0/17	0/7	0/5	1/13	0/7
bipolar	0/12	0/10	0/17	0/7	0/17	0/7	0/5	0/13	0/7
Antarctic [c]	2/12 (1)	2/10 (2)	2/17 (2)	1/7 (0)	2/17 (2)	2/7 (2)	0/5 (0)	4/13 (3)	1/7 (1)
Betaproteobacteria									
cosmopolitan	8/11	1/1	10/13	0/0	14/16	5/6	2/2	5/6	4/5
cold	0/11	0/1	1/13	0/0	1/16	0/6	0/2	0/6	0/5
bipolar	0/11	0/1	1/13	0/0	0/16	0/6	0/2	1/6	0/5
Antarctic [c]	3/11 (1)	0/1 (0)	1/13 (1)	0/0 (0)	1/16 (0)	1/6 (1)	0/2 (0)	0/6 (0)	1/5 (1)
Gammaproteobacteria									
cosmopolitan	4/6	2/10	1/3	7/13	2/2	0/1	0/0	2/3	1/2
cold	0/6	0/10	0/3	1/13	0/2	0/1	0/0	0/3	0/2
bipolar	0/6	1/10	0/3	3/13	0/2	0/1	0/0	0/3	0/2
Antarctic [c]	2/6 (1)	7/10 (3)	2/3 (0)	2/13 (0)	0/2 (0)	1/1 (1)	0/0 (0)	1/3 (0)	1/2 (0)
Bacteroidetes									
cosmopolitan	1/41	1/10	1/10	2/8	4/19	4/15	0/12	4/11	1/4
cold	1/41	0/10	0/10	0/8	0/19	1/15	0/12	0/11	0/4
bipolar	0/41	0/10	0/10	1/8	1/19	2/15	2/12	0/11	0/4
Antarctic [c]	39/41 (31)	9/10 (5)	9/10 (8)	5/8 (0)	14/19 (14)	8/15 (7)	10/12 (10)	7/11 (6)	3/4 (3)
Firmicutes									
cosmopolitan	0/0	3/3	4/4	3/4	3/3	6/6	1/1	15/18	3/3
cold	0/0	0/3	0/4	0/4	0/3	0/6	0/1	0/18	0/3
bipolar	0/0	0/3	0/4	0/4	0/3	0/6	0/1	1/18	0/3
Antarctic [c]	0/0 (0)	0/3 (0)	0/4 (0)	1/4 (0)	0/3 (0)	0/6 (0)	0/1 (0)	2/18 (1)	0/3 (0)
Deinococcus-Thermus									
cosmopolitan	1/5	0/0	0/0	0/0	0/2	1/8	0/4	0/0	0/0
cold	0/5	0/0	0/0	0/0	0/2	0/8	0/4	0/0	0/0
bipolar	0/5	0/0	0/0	0/0	0/2	0/8	0/4	0/0	0/0
Antarctic [c]	4/5 (2)	0/0 (0)	0/0 (0)	0/0 (0)	2/2 (2)	7/8 (5)	4/4 (3)	0/0 (0)	0/0 (0)
All isolates									
% cosmopolitan	36.0	48.7	64.4	58.3	64.6	52.4	48.6	67.9	79.4
% cold	5.6	2.6	5.1	10.4	3.8	1.6	2.7	1.8	0.0
% bipolar	0.0	2.6	1.7	8.3	1.3	3.2	5.4	5.4	0.0
% Antarctic [c]	58.4 (41.6)	46.2 (25.6)	28.8 (23.7)	22.9 (4.2)	30.4 (29.1)	42.9 (36.5)	43.2 (40.5)	25.0 (17.9)	20.6 (17.6)

[a] Data from Peeters et al. 2011a

[b] Data from Peeters et al. 2011b

[c] In brackets, the number/percentage of phylotypes that shared no significant similarity with any other sequence in the public database

Figure 1

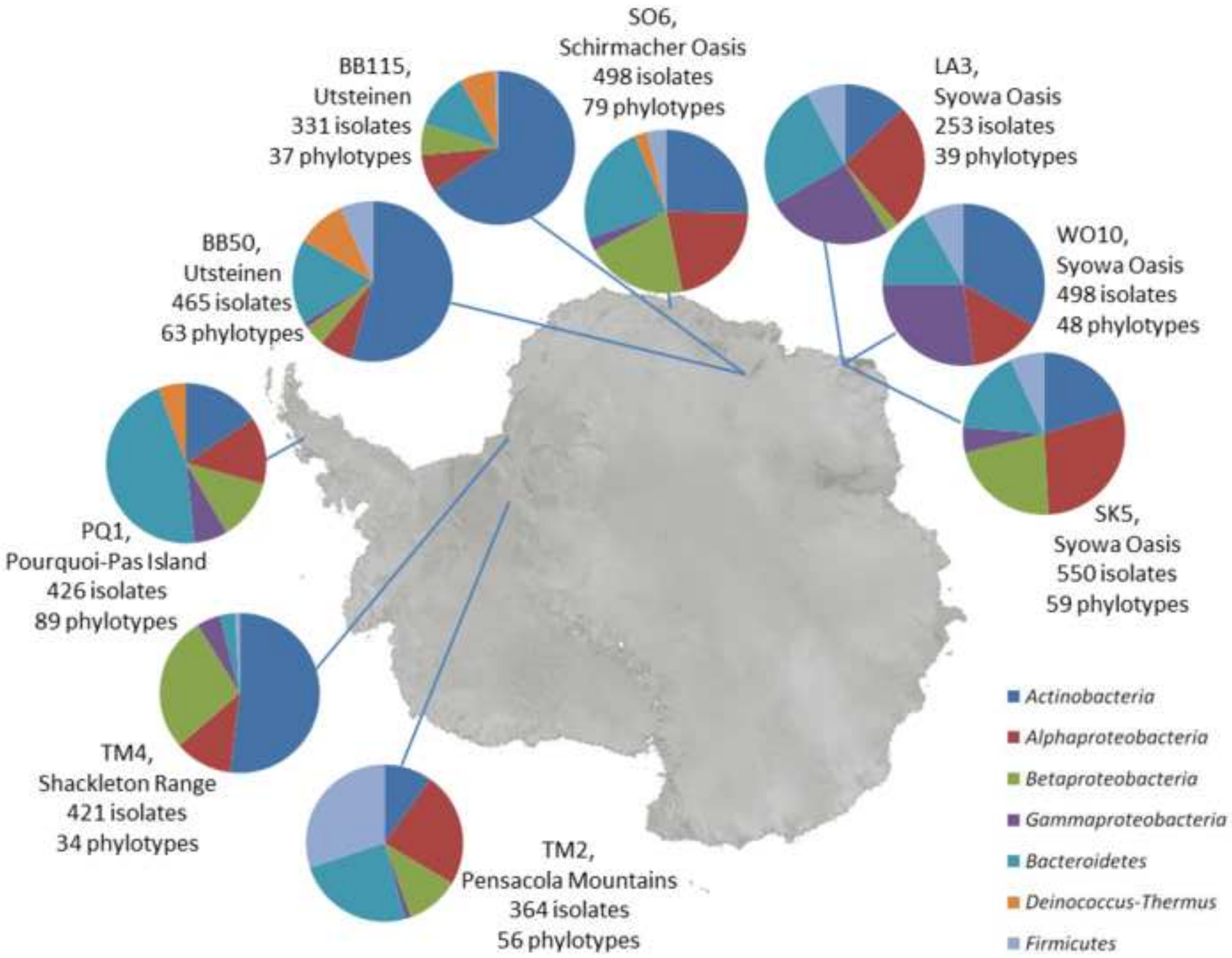
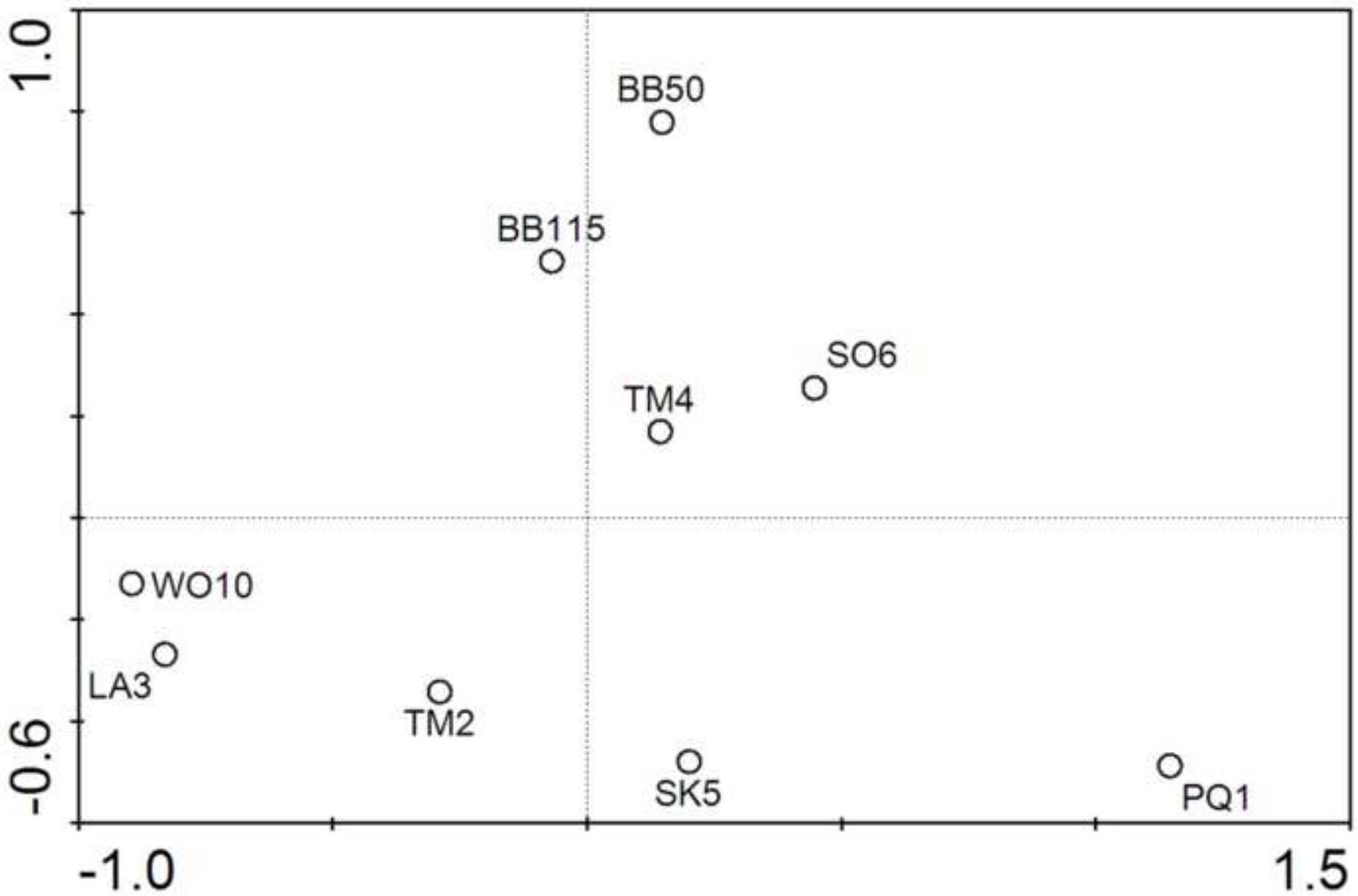

SO6,
Schirmacher Oasis
498 isolates
79 phylotypes
BB115,
Utsteinen
331 isolates
37 phylotypes
LA3,
Syowa Oasis
253 isolates
39 phylotypes
BB50,
Utsteinen
465 isolates
63 phylotypes
WO10,
Syowa Oasis
498 isolates
48 phylotypes
PQ1,
Pourquoi-Pas Island
426 isolates
89 phylotypes
SK5,
Syowa Oasis
550 isolates
59 phylotypes
TM4,
Shackleton Range
421 isolates
34 phylotypes
TM2,
Pensacola Mountains
364 isolates
56 phylotypes
Actinobacteria
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Bacteroidetes
Deinococcus-Thermus
Firmicutes

Figure 2



Electronic clean Supplementary Tables

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Supplementary Fig. S1 for:

Heterotrophic bacterial diversity in aquatic microbial mat communities from Antarctica.
in Polar Microbiology
by Karolien Peeters, Elie Verleyen, Dominic A. Hodgson, Peter Convey, Damien Ertz, Wim Vyverman, Anne Willems*

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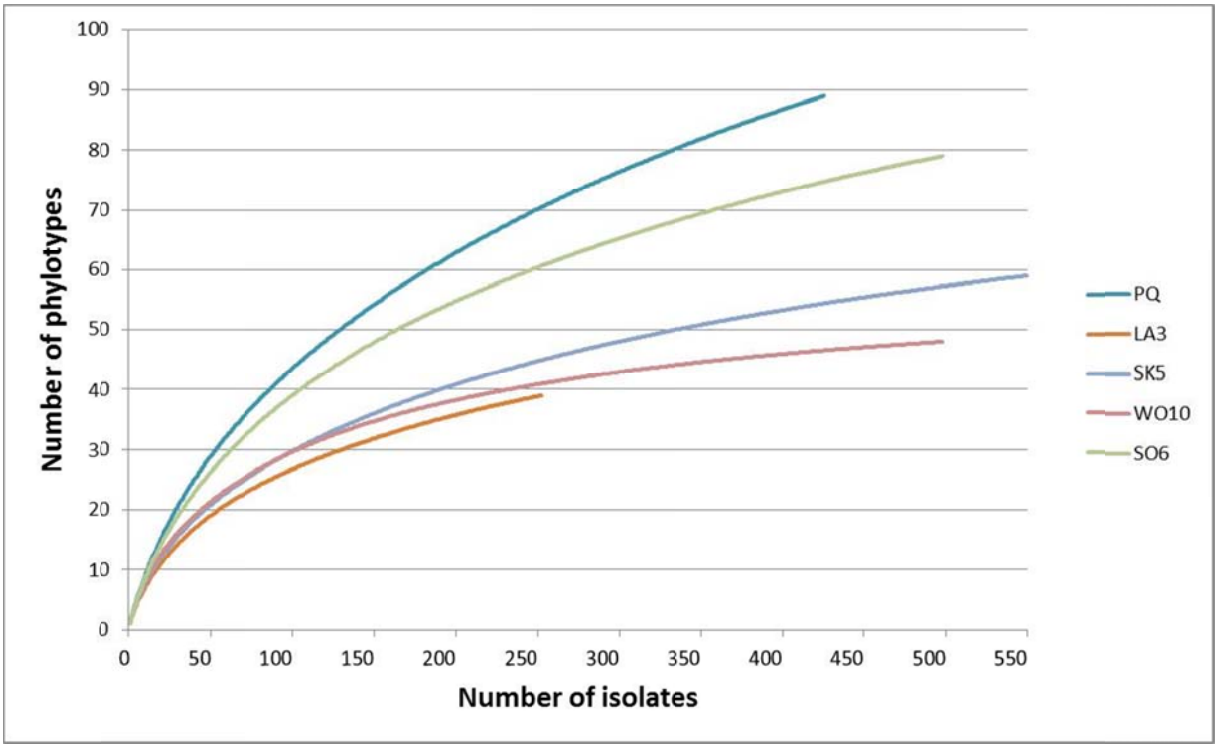
Fig. S1 Rarefaction curves representing the number of phylotypes isolated from the different samples



Supplementary Tables S1 and S2 for:

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in Polar Microbiology
by Karolien Peeters, Elie Verleyen, Dominic A. Hodgson, Peter Convey, Damien Ertz, Wim Vyverman, Anne Willems*

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Table S1: Distribution of the phylotypes over the different genera. Phylotypes were labelled with the isolate number of a representative strain that was sequenced. Per sample, phylotypes are listed as well as the number of isolates of this phylotype (#). Phylotypes shared between several samples are marked with the same number in superscript. In some cases, different isolate numbers carry the same number in superscript; these are different representatives of the same phylotype. In some phyla, novel genera were tentatively assigned for phylotypes that did not cluster inside existing genera or whose 16S rRNA gene sequence similarity was equally low with multiple neighbouring genera.

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
Actinobacteria																		
Aeromicrobium							R-42664	8			R-36485	1						
Arthrobacter	R-36707[2]	11			R-36538[5]	16	R-36538[5]	1	R-36534[4]	21	R-36535[1]	1	R-36535[1]	68	R-43110[5]	15	R-37013[4]	120
	R-36193[3]	1			R-36715[7]	32	R-36715[7]	1	R-36538[5]	32	R-36707[2]	160	R-36534[4]	25			R-43110[5]	38
	R-36715[7]	31			R-36751[8]	1	R-36751[8]	12	R-43938[6]	2	R-36193[3]	29	R-36538[5]	12			R-43938[6]	25
	R-38507	3					R-41531	2	R-36715[7]	31	R-36487	1	R-36550	14			R-39621	10
	R-44216	2							R-36751[8]	1	R-36708	5	R-36556	1			R-38429	1
									R-44261	1	R-36371	7						
Cryobacterium	R-37019[10]	1			R-42756	2	R-41532	3	R-42736	2	R-36515[9]	12	R-36515[9]	58			R-37019[10]	1
	R-38273	2			R-43143	3												
Frigoribacterium									R-43109	1								
Janibacter			R-39538	1														
Kocuria			R-39201[12]	1	R-36519[11]	1					R-36519[11]	3					R-39201[12]	2
											R-42745	1						
Knoellia					R-39574	5							R-36688	19			R-43433	3
													R-43101	1				
Marisedimicola							R-36750[13]	9	R-36750[13]	6					R-36750[13]	6	R-38315	1
	R-38376[14]	3			R-38376[14]	34			R-38376[14]	9								
Microbacterium											R-36360	1	R-36588	1			R-43968	1
Micrococcus			R-43944[15]	2													R-43944[15]	2
Modestobacter											R-36506	1						
Nocardioides			R-39112	1	R-39601	3	R-43252	3	R-42691	1	R-36473	2	R-36680	1				
							R-42721	4	R-42658	5								
Patulibacter											R-36497	2						

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
Rhodococcus	R-37022[18]	2			R-37022[18]	3	R-37022[18]	1	R-43119[17]	1	R-36475[16]	4	R-36475[16]	2			R-37022[18]	10
	R-37575[20]	2					R-37551[19]	1	R-37022[18]	1			R-43119[17]	15			R-37551[19]	4
	R-43120	1							R-37551[19]	1								
									R-37575[20]	4								
Rhodoglobus							R-36762[21]	54							R-36762[21]	4		
							R-41578	5							R-36754	6		
Rothia	R-36507[22]	5			R-36507[22]	4			R-36507[22]	1	R-36507[22]	5						
Salinibacterium	R-39128[23]	10	R-39128[23]	2	R-39128[23]	51	R-39128[23]	1	R-39128[23]	14								
	R-37573[24]	8					R-37573[24]	2										
							R-42713	2										
Saxeibacter													R-36686	1				
Subtercola											R-36477	1						
Tessaracoccus											R-36529	14						
											R-36527	5						
gen. nov. *Actinobacteria 1*											R-36375	1						
gen. nov. *Actinobacteria 2*									R-41477	1					R-36733	1		
gen. nov. *Actinobacteria 3*									R-41567	2								
total *Actinobacteria*		82		7		155		109		137		256		218		32		218
Alphaproteobacteria																		
Altererythrobacter			R-39115	1														
Aurantimonas											R-36516	8						
Bosea	R-38307[25]	1			R-39149	8									R-38307[25]	4		
					R-39584	1												
Brevundimonas	R-36554[26]	44	R-36554[26]	6	R-36554[26]	121			R-36554[26]	10	R-36244[26]	1	R-36554[26]	6	R-36741[26]	14	R-36741[26]	34
	R-37024[28]	1	R-37014[29]	3	R-37014[29]	25			R-37014[29]	2					R-37030[27]	1	R-37030[27]	11
	R-37014[29]	2	R-40155	1					R-41484[30]	12					R-36759	22	R-37024[28]	2
	R-41484[30]	2															R-37014[29]	4
Caulobacter					R-39136	4												
Devosia	R-36756[32]	4					R-36585[31]	3					R-36585[31]	5	R-43424	1	R-43964	1
															R-36756[32]	27		
															R-36938	1		
Hyphomicrobium					R-40143	1												
Loktanella			R-39046[33]	9			R-39046[33]	59										

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
							R-44293	3										
Mesorhizobium									R-41592	3								
Paracoccus							R-41610[34]	26	R-41610[34]	1							R-42686	1
Phenylobacterium					R-44236	3												
Porphyrobacter	R-38345[35]	4			R-38345[35]	6												
Rhizobium					R-39528	2												
Rhodobacter															R-36943	3		
Roseomonas									R-41594	1								
Roseovarius			R-39071	8														
Sphingomonas	R-40141[38]	4			R-40141[38]	21			R-36533[37]	1	R-36544[36]	9	R-36544[36]	10	R-36940[37]	1	R-36940[37]	1
	R-39544[39]	1			R-39544[39]	1			R-39544[39]	5	R-36533[37]	8	R-36533[37]	2				
					R-39586[40]	6			R-39586[40]	1	R-36505	1	R-36583	4				
					R-39596	1			R-41554	1								
					R-39146	9			R-43106	1								
									R-44566	2								
Sphingopyxis	R-41479[42]	12					R-36742[41]	14	R-41479[42]	57					R-36742[41]	8		
									R-41480	2								
Sphingosinicella									R-41563	3								
									R-41564	1								
Sulfitobacter	R-39094[43]	3	R-39094[43]	2	R-39094[43]	2	R-44292	1										
gen. nov. *Alphaproteobacteria* 1					R-36492[44]	2			R-36492[44]	1	R-36492[44]	2						
gen. nov. *Alphaproteobacteria* 2					R-36501[45]	5					R-36501[45]	2						
gen. nov. *Alphaproteobacteria* 3			R-36760[46]	2											R-36760[46]	4		
gen. nov. *Alphaproteobacteria* 4															R-39199	1		
gen. nov. *Alphaproteobacteria* 5															R-36935	1		
gen. nov. *Alphaproteobacteria* 6	R-38319	1																
gen. nov. *Alphaproteobacteria* 7			R-39043	1														
gen. nov. *Alphaproteobacteria* 8			R-39117	1														
gen. nov. *Alphaproteobacteria* 9							R-43079	1										
total *Alphaproteobacteria*		79		34		218		107		104		31		27		82		54
Betaproteobacteria																		
Albidiferax																	R-37567	1
Curvibacter	R-36930[47]	2													R-36930[47]	1		
Duganella					R-42680[48]	6			R-42680[48]	56								

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
									R-41603	6								
Hydrogenophaga					R-38517[49]	1									R-38517[49]	2		
Massilia	R-36558[50]	4			R-36558[50]	55			R-36558[50]	28	R-36558[50]	4	R-36558[50]	18				
					R-44262[51]	1			R-44262[51]	5								
									R-42682	5								
									R-41596	7								
									R-43135	1								
									R-41598	1								
Polaromonas	R-37596[54]	26			R-36732[52]	1			R-37550[53]	8	R-40127[53]	2			R-36732[52]	22	R-37550[53]	98
	R-38414[55]	1			R-37550[53]	3			R-37596[54]	4	R-36500	2			R-37550[53]	8		
	R-38383	1							R-38414[55]	2					R-38520	4		
	R-38293	1							R-42676	2								
	R-38390	2																
	R-38278	1																
Rhodoferax	R-43137[56]	3			R-43137[56]	2												
	R-37606	1			R-42715	1												
Variovorax					R-39150	1					R-38535[57]	5	R-38535[57]	3				
Xylophilus											R-36498	3						
gen. nov. *Betaproteobacteria 1*									R-36369[58]	8	R-36369[58]	3					R-36369[58]	1
			R-37018[59]	1	R-37018[59]	2			R-37018[59]	2							R-37018[59]	2
gen. nov. *Betaproteobacteria 2*															R-36978	1		
gen. nov. *Betaproteobacteria 3*																	R-43960	1
gen. nov. *Betaproteobacteria 4*	R-42728[60]	1			R-42728[60]	19												
					R-42750	9												
gen. nov. *Betaproteobacteria 5*					R-39153	13												
gen. nov. *Betaproteobacteria 6*									R-41601	1								
gen. nov. *Betaproteobacteria 7*									R-41500	1								
total *Betaproteobacteria*		43		1		114		0		137		19		21		38		103
Gammaproteobacteria																		
Enhydrobacter	R-37587[61]	1							R-37587[61]	1								
Halomonas			R-39097[62]	20			R-39097[62]	9										
			R-39074	5			R-43069	1										
Idiomarina			R-39100	12														

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
Luteimonas									R-37032[63]	2							R-37032[63]	1
Lysobacter											R-36483	6						
Marinobacter			R-43132[64]	4			R-43132[64]	14							R-36953	1		
			R-39083	9			R-44565	2										
			R-39119	7			R-43103	9										
			R-39065	2			R-43199	6										
Pseudomonas	R-37619	15			R-39154	1	R-43128	23										
	R-37583	1					R-44307	2										
	R-38323	2																
Pseudoxanthomonas	R-38407	1													R-37036[65]	1	R-37036[65]	18
Psychrobacter	R-39101[67]	4	R-39101[67]	7	R-39101[67]	3	R-36959[66]	51							R-36959[66]	3		
			R-39551[68]	1	R-39551[68]	24	R-42705	56										
							R-43075	3										
							R-41527	16										
							R-41516	5										
gen. nov. *Gammaproteobacteria*			R-39122	3														
total *Gammaproteobacteria*		24		70		28		197		3		6		0		5		19
Bacteroidetes																		
Aequorivita							R-41536	6							R-36724	1		
Algoriphagus							R-36749[69]	4							R-36749[69]	9		
															R-36727	4		
Arcicella	R-38331	1																
Chryseobacterium	R-38366	4									R-36526	5	R-36555	1				
											R-36517	1						
Flavobacterium	R-38322[70]	16	R-38322[70]	2	R-38367[71]	2	R-38388[73]	1	R-43115	2	R-40838	2			R-36963	32		
	R-38367[71]	18	R-38378[72]	1					R-42675	10	R-36233	15			R-36964	1		
	R-38378[72]	19							R-41499	7					R-36968	2		
	R-38388[73]	1																
	R-38349	2																
	R-37579	1																
	R-38284	2																
	R-38295	1																
	R-38274	5																
	R-38359	3																

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
	R-38392	1																
	R-38423	10																
	R-40835	2																
	R-38377	2																
	R-38373	3																
	R-38339	12																
	R-38296	21																
	R-37608	5																
	R-38358	2																
	R-41446	1																
Gelidibacter							R-36722[74]	32							R-36722[74]	24		
Gillisia			R-39057[75]	28	R-39057[75]	1	R-39057[75]	6							R-36928	6		
Hymenobacter	R-36374[76]	1	R-40152[81]	1	R-37569[80]	1			R-42743[78]	2	R-36374[76]	1	R-42743[78]	6	R-36960[79]	2	R-37569[80]	3
	R-36215[77]	4			R-40152[81]	4			R-36960[79]	1	R-36215[77]	1	R-42653	2			R-37565	2
	R-37600	1			R-39159[82]	7			R-37569[80]	2	R-36503	1	R-36552	5				
	R-38509	1			R-39177[83]	2			R-39159[82]	2	R-43420	2	R-36548	1				
	R-38267	1			R-39133	3			R-39177[83]	8	R-36490	4	R-36591	13				
	R-38290	1			R-40142	1			R-42654	1	R-36364	8	R-36557	2				
	R-40138	2			R-39126	3			R-41473	4	R-36486	8	R-36541	1				
	R-38389	1							R-43236	1	R-38500	8	R-36616	1				
	R-38365	18							R-43117	11	R-36359	8	R-36692	1				
	R-38384	1							R-41490	3	R-36499	6	R-36595	1				
	R-37603	7							R-43240	4			R-36553	5				
	R-44218	1							R-44547	2								
	R-38268	1							R-42674	9								
									R-41496	27								
Maribacter			R-39054	1														
Pedobacter	R-38348	2					R-43111[84]	2	R-43111[84]	8	R-36480	9			R-36962	1	R-38393	11
	R-43090	1																
	R-38357	2																
Pontibacter															R-36965	7		
Psychroflexus			R-39078[85]	13	R-39078[85]	1	R-39078[85]	8										
			R-39107	8														
Salegentibacter			R-39056	77														

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
Spirosoma	R-41450	1							R-43202	3							R-37560	1
Winogradskyella			R-39121	1			R-43254	5										
gen nov. *Bacteroidetes 1*	R-38326	1																
gen nov. *Bacteroidetes 2*	R-38398	9																
gen nov. *Bacteroidetes 3*			R-39049	2														
total *Bacteroidetes*		188		134		25		64		107		79		39		89		17
Firmicutes																		
Aerococcus															R-38529	1		
Alkalibacterium							R-41513	4										
Bacillus			R-44214	1	R-39577	1	R-43946[87]	8	R-38416[86]	2	R-38416[86]	9			R-37580[86]	4	R-37580[86]	2
									R-43946[87]	1	R-36702	5			R-43422	1	R-43946[87]	1
											R-43891	1			R-36721	7		
											R-36493	5						
Brevibacillus													R-36717	2				
Carnobacterium							R-36987[88]	2							R-36987[88]	9	R-36982[89]	7
															R-36982[89]	33		
Jeotgalibacillus															R-42990	2		
Ornithinibacillus															R-38538	1		
Paenibacillus			R-42742[90]	4	R-42742[90]	6			R-42742[90]	4	R-42742[90]	3			R-36731	1		
					R-44233	1									R-36746	4		
Paenisporosarcina			R-36758[91]	2											R-36744	1		
															R-36758[91]	13		
Planococcus							R-36948[92]	7							R-36948[92]	28		
															R-36970	1		
															R-36952	1		
Staphylococcus					R-36520[93]	2					R-36520[93]	4			R-38534[93]	1		
															R-36936	2		
															R-36971	2		
total *Firmicutes*		0		7		10		21		7		27		2		112		10
Deinococcus-Thermus																		
Deinococcus	R-43890[94]	1							R-36713[95]	2	R-43890[94]	1	R-36713[95]	6				
	R-36590[96]	3							R-44264	1	R-36502	5	R-36590[96]	14				
	R-38506	1									R-36711	17	R-36685	1				
	R-37627	1									R-36479	8	R-38408	3				

Genus	PQ1		LA3		SK5		WO10		SO6		BB50 [a]		BB115 [a]		TM2 [b]		TM4 [b]	
	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#	Phylotype	#
	R-38289	4									R-36366	3						
											R-36206	1						
											R-36489	1						
											R-38476	11						
total *Deinococcus -Thermus*		10		0		0		0		3		47		24		0		0

[a] Data from Peeters et al. 2011a.

[b] Data from Peeters et al. 2011b.

Table S2: Dummy variables for habitat type and water chemistry data for the different samples. Water chemistry data were not available for samples BB50 and BB115. NA = data not available. Measurement procedures are described in (Hodgson et al. (2010) and Verleyen et al. (in press) .

	BB50	BB115	TM2	TM4	PQ1	LA3	SK5	WO10	SO6
Dummy variables									
terrestrial-aquatic	0	0	1	1	1	1	1	1	1
terrestrial-freshwater-saline 1	0	0	0	1	1	0	1	0	1
terrestrial-freshwater-saline 2	0	0	1	0	0	1	0	1	0
Water chemistry parameters									
Conductivity (mS/cm)	/	/	2.220	0.22702	0.1312	26.83	0.014	26.8	0.009
Sampling depth (m)	/	/	0.1	0.1	0.1	0.1	3.5	0.1	0.1
pH	/	/	8.15	9.04	NA	7.93	8.58	7.97	7.5
Al (mg/L)	/	/	<0.002	0.005	<0.002	0.278	0.005	0.343	NA
Fe (mg/L)	/	/	0.004	<0.001	<0.001	0.205	0.015	0.309	NA
Mg (mg/L)	/	/	13.9	1.18	2.26	6280	1.04	2270	0.58
Ca (mg/L)	/	/	11.4	3.34	1.63	885	2.01	363	0.61
K (mg/L)	/	/	1.36	0.612	0.758	1560	0.248	432	0.61
Na (mg/L)	/	/	45	3.47	17.2	43800	3.08	12000	2.59
Cl (mg/L)	/	/	88.6	60.1	34	92600	4.08	25400	3.33
SO4 (mg/L)	/	/	17.5	27.9	11.8	3840	0.57	1270	3.08
TN (mg/L)	/	/	4.3	0.18	0.04	0.66	0.11	45	NA
TOC (mg/L)	/	/	0.97	0.89	0.43	5.1	0.84	270	NA
DOC (mg/L)	/	/	1.04	0.96	0.58	5.11	0.9	258	NA
NO3-N (mg/L)	/	/	4.42	<0.100	<0.100	<0.100	<0.100	<0.100	0
NH4-N (mg/L)	/	/	0.043	0.026	0.018	2.07	0.012	16.6	<0.100
PO4-P (mg/L)	/	/	<0.005	<0.005	<0.005	6	<0.005	26	<0.005
Silicate-Si (mg/L)	/	/	0.222	0.319	0.136	3.5	0.71	9.44	NA