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Isolation and characterization of aerobic anoxygenic phototrophs from exposed soils from the Sør Rondane Mountains, East Antarctica

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ABSTRACT

This study investigated the culturable aerobic phototrophic bacteria present in soil samples collected in the proximity of the Belgian Princess Elisabeth Station in the Sør Rondane Mountains, East Antarctica. Until recently, only oxygenic phototrophic bacteria (*Cyanobacteria*) were well known from Antarctic soils. However, more recent non-cultivation-based studies have demonstrated the presence of anoxygenic phototrophs and, particularly, aerobic anoxygenic phototrophic bacteria in these areas. Approximately 1000 isolates obtained after prolonged incubation under different growth conditions were studied and characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Representative strains were identified by sequence analysis of 16S rRNA genes. More than half of the isolates grouped among known aerobic anoxygenic phototrophic taxa, particularly with *Sphingomonadaceae*, *Methylobacterium* and *Brevundimonas*. In addition, a total of 330 isolates were tested for the presence of key phototrophy genes. While rhodopsin genes were not detected, multiple isolates possessed key genes of the bacteriochlorophyll synthesis pathway. The majority of these potential aerobic anoxygenic phototrophic strains grouped with *Alphaproteobacteria* (*Sphingomonas*, *Methylobacterium*, *Brevundimonas* and *Polymorphobacter*).

Introduction

The permanently ice-free regions of Antarctica constitute only 0.32–0.4% of the continent's surface area [2,28]. The largest exposed regions are situated mainly along the coastal lowlands of the Peninsula and continental Antarctica, as well as in the Transantarctic Mountains. In the higher altitude inland areas, ice-free regions are very scarce [14]. However, in Dronning Maud Land (East Antarctica), the Sør Rondane Mountains (SRM) – located ~200 km inland from the King Haakon VII Sea – contain ~900 km^2 of exposed surface area. This 220 km long wedge-shaped mountain chain (71° 30′ – 72° 40′ S, 22–28° E) mainly consists of groups of mountains and individual nunataks (i.e. isolated mountain tops projecting above the surrounding ice layer) [76,120]. Similar to many other exposed inland continental Antarctic areas, terrestrial regions in the SRM are characterized by very low levels of organic matter, low soil moisture and extremely low soil surface temperatures, which provide the conditions for selection of a specialized, highly adapted microbial community [24,43,107,120]. In this oligotrophic environment, sunlight, abundantly present during the austral summer, may be an important energy resource for phototrophic bacterial groups that can harvest sunlight and convert it into chemical energy in order to support life.

Phototrophy represents one of the oldest and most important bacterial processes on Earth for which two mechanisms have been described. The simplest mechanism involves ion-pumping rhodopsin proteins [10,106], and environmental studies in the last decade have revealed the enormous diversity of microbial rhodopsins. Although they comprise a diverse group of photoactive transmembrane proteins, the proteo- and actinorhodopsin proton pumping families, predominantly found in aquatic environments all over the planet, are by far the most abundant and widespread [5,6,15,20,59,86,87,89,93,94].

The second bacterial phototrophy mechanism, which is less widespread but more efficient, relies on (bacterio)chlorophyll-containing photochemical reaction centers, and chlorophyll-dependent species are found solely in the *Cyanobacteria*. Anoxygenic phototrophic bacteria (APB) (i.e. those relying on bacteriochlorophyll (Bchl)) are found in the *Acidobacteria*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes* and *Proteobacteria* [17,121]. Most known APB are aerobic anoxygenic phototrophs (AAP).

Abbreviations: AAP, aerobic anoxygenic phototrophs; APB, anoxygenic phototrophic bacteria; BchL, bacteriochlorophyll; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SRM, Sør Rondane Mountains.

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These AAP do not contain carbon fixation enzymes [117] and use light as an auxiliary energy source for their mostly heterotrophic metabolism [37,58].

Several genes encoding subunits of key enzymes in the (bacterio)chlorophyll synthesis pathway are well conserved among phototrophic bacteria. The dark-operative protochlorophyllide oxidoreductase enzyme complex is present in all known phototrophic bacteria using photochemical reaction centers. In *Cyanobacteria*, the complex is encoded by *chlLNB* genes, whereas APB rely on the homologous *bchLNB* genes. Additionally, APB contain a second enzyme complex involved in the Bchl synthesis pathway: chlorophyllide oxidoreductase, encoded by *bchXYZ* genes [22,41,47]. For light-harvesting, the majority of APB rely on a type 2 photochemical reaction center. These reaction centers have a heterodimeric structure, with *pufL* and *pufM* encoding the conserved proteins. Hence, these genes have proven to be excellent markers for studying APB diversity [58,60,84].

Previously, we reported the diversity of key protein encoding genes involved in (bacterio)chlorophyll- and rhodopsin-dependent phototrophy in exposed areas of the SRM, which appeared to be suitable habitats for phototrophic microorganisms, especially AAP, due to the availability of sunlight, oxygen and the minimum quantity of organic nutrients [101,102]. The results suggested the presence of a diverse AAP community, including novel representative bacteria. However, since most of these bacteria still have not yet been cultivated, their characteristics and biochemical potential remain unknown. Although amplicon sequencing of 16S rRNA genes only provides insights into what is present, such inventories of protein-encoding genes, in general, cannot be linked to specific bacteria because of possible horizontal gene transfer and gene duplication events [30,31,69]. Even though metagenome sequencing may reveal functional potential, recreating and closing genomes from such data is difficult due to genomic microheterogeneity. Furthermore, a function cannot be assigned to a considerable number of genes [72,82]. Thus, while culture-independent methods can describe the functional capacities of whole microbial communities, isolation and characterization of Antarctic bacteria, and microorganisms in general, is of great scientific relevance for investigating ecophysiology and adaptive strategies and linking function to identity. Cultured microorganisms not only permit testing for certain functions (e.g. phototrophy) or expression of genes, but also the sequencing of their genomes. In addition, they may help extend identifications obtained from metagenome data [38,72,103,108].

Previous research using deep sequencing of 16S rRNA genes revealed that oxygenic phototrophs (*Cyanobacteria*) are sometimes only present in relatively small numbers in soils of the SRM [101,Tahon et al. Submitted for publication]. In contrast, these environments seem to contain a broad diversity of anoxygenic phototrophs, although rhodopsins have not been detected [101,102]. Therefore, the present study focused on the isolation of APB, and more specifically AAP relying on a type 2 photochemical reaction center, from the same exposed samples used previously for non-cultivation based studies. Isolates were identified at the molecular level and screened for the presence of different phototrophy genes. This data, contributing to the bacterial characterization of exposed surface soils of the SRM, represents one of the first reports on cultivation of aerobic anoxygenic bacteria from continental Antarctica.

Materials and methods

Sampling site and sampling method

As previously described [101,102], top surface soil samples were collected aseptically from four exposed sites in the proximity of the Belgian Princess Elisabeth Station, Utsteinen, East Antarctica (71° 57′ S, 23° 20′ E, elevation 1382 m). Three samples (KP15, KP43 and KP53) were taken on the eastern part of the Utsteinen nunatak, ~500 m north of the research station, whereas sample KP2 was taken ~1.3 km south of the Belgian base, on the eastern part of the Utsteinen ridge. Samples were stored in sterile polypropylene containers at −20 °C on collection until they were returned to the Laboratory for Microbiology (Ghent University, Belgium), where they were stored in a cold room facility (−20 °C).

Media and isolation of bacterial strains

For isolation of aerobic phototrophic microorganisms, two defined low nutrient media were prepared, one selective for aerobic photoautotrophs (PA) and one for aerobic photoheterotrophs (PH). Media compositions were based on media previously used for the isolation of phototrophic bacteria [23,49,54,99,104,110,118]. Both media contained 3.50 mM $K_2HPO_4{\cdot}3H_2O$, 1.47 mM KH_2PO_4, 0.81 mM $MgSO_4{\cdot}7H_2O$, 3.42 mM NaCl, 0.58 mM $CaSO_4{\cdot}2H_2O$, 25 μM $Fe_2(SO_4)_3$, 69.6 nM $ZnSO_4{\cdot}7H_2O$, 0.252 μM $MnCl_2{\cdot}4H_2O$, 25.2 nM $CoCl_2{\cdot}6H_2O$, 10 nM $CuCl_2{\cdot}2H_2O$ and 25 nM $NiCl_2{\cdot}H_2O$. No carbon sources were added to the PA medium but the PH medium was enriched with a mix of six different carbon sources (glucose, sucrose, sodium succinate, sodium pyruvate, sodium acetate and malate), which are frequently used for isolating phototrophic bacteria. Concentrations of carbon sources were set at 0.5 mM each in order to mimic the oligotrophic Antarctic environment. Nitrogen traces in the aforementioned components mimicked the low *in situ* Antarctic nitrogen conditions [19,39], and no additional nitrogen source was added. To support growth of photodiazotrophs, 24.32 μM MoO_3 and 1 μM V_2O_5 were added to the media [12,13,55,83]. For solid media, 15 g L^{-1} Bacto agar (BD) was added. The final pH of both media was set to 7.0.

The isolation of phototrophic bacteria was performed as follows: a ten-fold dilution series (10^{-1}–10^{-5}) was prepared for each sample, starting from one gram of aseptically weighed soil homogenized in 9 mL sterile liquid growth medium using a vortex. Finally, 100 μL of each dilution was plated out. For liquid enrichments, sterile 120 mL glass vials containing 20 mL of dilutions 10^{-2}–10^{-5} were set up and sealed with a gas permeable seal (4titude®).

Culture plates and liquid enrichments were set up with four replicates incubated under an aerobic atmosphere at 4 and 15 °C (two replicates each). For bacteria in Antarctic surface samples, residing in a dark freezer for several years was considered similar to a long austral winter. To aid recovery of dormant bacteria, an isolation procedure was devised that would mimic the gradual light transition from winter to summer. Incubation was performed in illuminated incubators, starting at 0 h of light per day for one week, and increasing by two hours day^{-1} every week until a maximum of 18 h day^{-1} by week 10, which was designed to simulate the increasing day length during the transition from winter to summer at the sampling locations. Distinct colonies were purified from solid media from week 10 onwards. For liquid enrichments, one vial of each condition was non-continuously shaken (aeration once per day by gentle manual shaking for 10 s) and the other vial was not aerated. After 21 weeks, 1/100 dilutions were plated and incubated for up to seven months. For each condition, all colonies with a distinct morphology were purified by seeding single colonies on new plates filled with the same medium.

MALDI-TOF mass spectrometry

Due to the very slow growth of the isolates and limited amount of available biomass, a modified version of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) sample preparation protocol was used, as previously described by Wieme et al. [112], but with cell suspensions rather

than cell extracts. One loopful (1 μL yellow Looplast® loop, LP Italiana) of bacterial cells was suspended in 10 μL of sterile MilliQ water and mixed. If an isolate did not produce biomass equivalent to one loopful, all the biomass from a 4.5 cm ø petri dish was used. 1 μL of freshly prepared suspension was then spotted in duplicate onto a 384 OptiTOF stainless steel MALDI plate (AB Sciex) and dried at room temperature. Afterwards, 1 μL of 0.5% (w/v) α-cyano-4-hydroxycinnamic acid in 50:48:2 acetonitrile:water:trifluoroacetic acid solution was added to each spot and allowed to dry.

Acquisition of the bacterial fingerprints was undertaken using a 4800 Plus MALDI TOF/TOF™ Analyzer (Applied Biosystems) in linear positive ion mode, as previously described [112]. Raw mass spectra were extracted as t2d files from the analyzer, imported into the Data Explorer 4.0 software (Applied Biosystems) and then converted into text files. Subsequently, these text files were imported into BioNumerics 7.5 (Applied Maths) and transformed into fingerprints. The similarity between spectra was determined using Pearson's product moment correlation. Spectra were then clustered using the unweighted pair group with arithmetic mean method.

Identification and characterization of bacterial strains

Bacterial DNA was extracted using the alkaline lysis protocol [73] and stored at −20 °C until further processing. Gene fragments were amplified (Table 1) using a Veriti thermal cycler (Life Technologies). All amplification reactions were carried out with two replicates in 25 μL reaction mixtures containing 1× Qiagen PCR buffer (Qiagen), 0.2 mM of each deoxynucleotide triphosphate, 0.625 units of Qiagen *Taq* polymerase (Qiagen) and forward and reverse primers (Table 1). Each reaction mixture received 2.5 μL of template DNA.

PCR products were purified using a Nucleofast 96 PCR cleanup membrane system (Macherey-Nagel) and Tecan Genesis Workstation 200 (Tecan). Sequencing of the protein encoding genes was performed using the amplification primers (Table 1). For 16S rRNA genes, the sequencing primers listed by Coenye et al. [27] and Cleenwerck et al. [26] were used. Sequencing was carried out using a BigDye Xterminator™ purification kit (Applied Biosystems) and an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Analyses of sequences

Sequences were assembled using the BioNumerics 7.5 software (Applied Maths). To ensure high quality data, every position of the assembly was the consensus of a minimum of two separate sequences. Afterwards, sequences were manually curated to eliminate all low-quality sequences containing indels, stop codons and ambiguous bases. The 16S rRNA genes were initially partially sequenced (V1-V3 region). Partial sequences were grouped at a 98% similarity level using CD-HIT [40,64]. Partial sequences with ≥98% similarity were considered to represent one phylotype. Afterwards, the full 16S rRNA gene of one representative of each phylotype was sequenced and identified using the EzTaxon database (http://www.ezbiocloud.net/identify) [25].

For analysis of the overlap between the diversity picked up by cultivation and by a culture-independent approach, partial 16S rRNA gene data (V1–V3 region) from the same samples, previously obtained using Illumina MiSeq 2 × 300 bp sequencing [Tahon et al. Submitted for publication], were compared with 16S rRNA gene data from the isolates. Sequences were compared using CD-HIT. This comparison also allowed verification of the taxonomy previously inferred from the Illumina sequence data using the May 2013 GreenGenes training set [Tahon et al. Submitted for publication] Additionally, one representative of each phylotype together with one representative of each Illumina OTU (binned at 97% similarity), was used to construct a phylogenetic tree. For phylogenetic analysis of *pufLM*, an updated version of our previously described database containing publicly available sequences as at May 8th 2017 [101] was used. For 16S rRNA and *pufLM* genes, sequences were aligned using Clustal Omega [44,96] on the Stevin supercomputer at UGent. A total of 20 iterations were performed in order to optimize the alignments. The alignment was then trimmed to the size of the Illumina sequences (16S rRNA genes) or the size of the sequenced amplicons (*pufLM* genes) and visually inspected, excluding all non-overlapping reference sequences from further analysis. Remaining sequences were realigned and the resulting alignment was used to construct a maximum likelihood phylogenetic tree (1000 bootstrap replicates) using the FastTree tree building software [81] with the general time-reversible model and the discrete gamma model with 20 rate categories. For *pufLM*, the closest relatives of the newly obtained isolates were selected from the resulting phylogenetic tree in order to prepare a smaller tree following the same protocol. Sequences from uncultured bacteria were not included in the final *pufLM* tree. Trees were visualized using the iTOL software [62,63].

Accession numbers

The sequences determined in this study were deposited in the National Center for Biotechnology GenBank database under accession numbers KY386300 to KY386629 (16S rRNA) and KY437105 to KY437155 (*pufLM*).

Results

Isolation of bacteria

While all media showed a good yield, the use of oligotrophic media and incubation under low temperatures (4 and 15 °C) resulted in growth only after prolonged periods of time. From solid media, purification of distinct colonies was performed from week 10 onwards, whereas for the liquid setups – originally enriched for 21 weeks – this was after up to seven months. From the various conditions, a total of 1552 colonies were randomly picked. The numbers of isolates picked per sample and per incubation condition are listed in Table S1.

The majority of the colonies were extremely small (<0.5 mm ø after four weeks) and many showed no visual pigmentation, except after concentration of the biomass. Furthermore, the liquid enrichments resulted in a large number of cyanobacterial liquid cultures. Samples KP2 and KP15 resulted in most cyanobacterial growth and diversity. For samples KP43 and KP53, cyanobacterial growth was absent from most of the setups. These samples, however, displayed growth of green microalgae that was identified as *Stichococcus* sp. by 18S rRNA gene sequencing.

Since the focus was on AAP, the cyanobacterial enrichment cultures, which could have harbored a broad variety of potentially novel cyanobacterial strains, were donated to the *Cyanobacteria* culture collection at the University of Liège for further isolation and characterization. As determined by microcopy, they contained a large variety of *Nostoc* sp., *Stigonema* sp., as well as cyanobacterial growth that could not be identified (A. Wilmotte, personal communication).

MALDI-TOF MS

For the dereplication, identification and characterization of the isolates, an approach combining MALDI-TOF MS fingerprinting and sequence analysis of 16S rRNA and phototrophy genes was used. First, MALDI-TOF MS was performed in order to characterize all 1552 isolates. Only spectra of good quality were considered for further analysis: quality mass spectrometry profiles (minimum

Table 1
PCR primers and conditions used for screening different genes.

Gene	Target	Primer	Sequence 5′-3′	Final concentration	Region	Amplicon size	Program
16S rRNA	Universal	pA[a] pH[a]	AGA GTT TGA TCC TGG CTC AG AAG GAG GTG ATC CAG CCG CA	0.1 μM 0.1 μM	8–1541[l]	±1500 bp	95 °C (5 min); 3 × 95 °C (1 min), 55 °C (2 min 15 s), 72 °C (2 min 15 s); 30 × 95 °C (35 s), 55 °C (1 min 15 s), 72 °C (1 min 15 s); 72 °C (10 min)
pufLM	AAP	pufLF[b] pufMR[c]	CTK TTC GAC TTC TGG GTS GG CCA TSG TCC AGC GCC AGA A	0.2 μM 0.2 μM	64–1612[m]	±1500 bp	94 °C (3 min); 30 × 94 °C (1 min), 60 °C (1 min), 72 °C (2 min); 72 °C (10 min) [3]
pufM	Universal	pufM_uniF[d] pufM_WAW[d]	GGN AAY YTN TWY TAY AAY CCN TTY CA AYN GCR AAC CAC CAN GCC CA	1.0 μM 0.5 μM	584–825[n]	±240 bp	94 °C (4 min); 35 × 94 °C (40 s), 49 °C (30 s), 72 °C (30 s); 72 °C (7 min)
proteorhodopsin	Universal	PR-1aF[e] PR-1aR[e]	GAT CGA GCG NTA YRT HGA RTG G GAT CGA GCR TAD ATN GCC CAN CC	1.87 μM 1.87 μM	340–665[o]	±335 bp	94 °C (2 min), 30 × 94 °C (30 s), 52 °C (30 s), 72 °C (30 s); 72 °C (7 min) [20]
actinorhodopsin	Clade LG1 & LG2	LG-for[f] LG1A-for[f] LG2-for[f] LG-rev[f]	TAY MGN TAY GTN GAY TGG MGN TAY ATH GAY TGG YT TAY MGN TAY GCN GAY TGG ATN GGR TAN CAN CCC CA	0.4 μM 0.4 μM 0.4 μM 0.8 μM	283–614[p]	±330 bp	95 °C (7 min), 45 × 94 °C (30 s), 51.5 °C (1 min 30 s), 72 °C (30 s); 72 °C (10 min)
nifH, *bchL*, *bchX*	Universal	IGK3[g] DVV[g]	GCI WTH TAY GGI AAR GGI GGI ATH GGI AA ATI GCR AAI CCI CCR CAI ACI ACR TC	1.0 μM 1.0 μM	19–413[q][p]	395 bp	95 °C (10 min); 40 × 95 °C (45 s), 52 °C (30 s), 72 °C (40 s); 72 °C (10 min)
nifH	Universal	F2[h] R6[h]	TGY GAY CCI AAI GCI GA GCC ATC ATY TCI CCI GA	1.0 μM 1.0 μM	115–473[q]	359 bp	95 °C (5 min); 35 × 95 °C (45 s), 51 °C (45 s), 72 °C (45 s); 72 °C (7 min)
cbbL	RuBisCO IA & IB	RubIgF[i] RubIgR[i]	GAY TTC ACC AAR GAY GAY GA TCR AAC TTG ATY TCY TTC CA	0.4 μM 0.4 μM	571–1382[r]	±800 bp	95 °C (3 min); 3 × 95 °C (1 min), 49 °C (2 min 15 s), 72 °C (2 min 15 s); 30 × 95 °C (35 s), 49 °C (1 min 15 s), 72 °C (1 min 15 s); 72 °C (7 min)
cbbL	RuBisCO IA & IC	K2f[j] V2r[j]	ACC AYC AAG CCS AAG CTS GG GCC TTC SAG CTT GCC SAC CRC	0.2 μM 0.2 μM	496–990[r]	492–495 bp	95 °C (3 min); 35 × 95 °C (1 min), 62 °C (1 min), 72 °C (1 min 30 s); 72 °C (10 min) [116]
cbbM	RuBisCO II	cbbM343F[k] cbbM1126R[k]	GGY AAY AAC CAR GGY ATG GG CGY ARB GCR TTC ATR CCR CC	0.1 μM 0.1 μM	343–1126[k]	700–800 bp	95 °C (3 min); 30 × 95 °C (1 min), 50 °C (2 min), 72 °C (3 min); 72 °C (7 min) [56]

[a] From Ref. [33].
[b] From Ref. [70].
[c] From Ref. [11].
[d] From Ref. [119].
[e] From Ref. [20].
[f] From Ref. [93].
[g] From Ref. [4].
[h] From Ref. [66].
[i] From Ref. [97].
[j] From Ref. [71].
[k] From Ref. [56].
[l] Based on the 16S rRNA gene sequence of *Escherichia coli* (A14565).
[m] Based on the *pufLM* sequence of *Sphingomonas sanxanigenens* DSM 19645 (CP006644).
[n] Based on the *pufM* sequence of *Sphingomonas sanxanigenens* DSM 19645 (CP006644).
[o] Based on the *proteorhodopsin* sequence of *Vibrio campbellii* BAA-1116 (FJ985782).
[p] Based on the *actinorhodopsin* sequence of *Leifsonia rubra* CMS 76R (ATIA01000023).
[q] Based on the *nifH* sequence of *Azotobacter vinelandii* (M20568).
[r] Based on the *cbbL* IA sequence of *Bradyrhizobium* sp. ORS278 (CU234118).

highest peak intensity of 200 and <35% slope for the maximum peak intensity) could be generated for 1038 isolates. For the other isolates, even on repetition, no good quality profile could be obtained, mostly due to the very low quantity of biomass available. As a result, these isolates were not used in further analyses. Subsequently, cluster analysis (Pearson correlation) of MALDI-TOF MS profiles was performed, and visual inspection resulted in 141 clusters (data not shown). Approximately 15% of the isolates did not group in these clusters and they were characterized by unique profiles often of a somewhat lower quality. Prior to further analyses, the initial number of isolates was reduced to a subset of isolates representing all MALDI-TOF MS clusters, as well as good quality unique profiles (330 isolates total) (Table S2).

Identification based on 16S rRNA sequences

After partial 16S rRNA sequencing, the 330 representative isolates were binned in 77 phylotypes (98% sequence similarity). Based on the full 16S rRNA gene sequence, taxon assignment with the EzTaxon cultured database allocated 63 phylotypes (295 isolates) to 29 genera (95% cutoff [92]) belonging to the *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Deinococcus-Thermus* (Table 2). Of the remaining phylotypes, the 16S rRNA was less than 95% similar to that of their closest cultured neighbor. Of these, 12 grouped with the aforementioned phyla and potentially represented new genera or families (90–95% 16S rRNA similarity to the closest cultured neighbor) (Table 2). Interestingly, two isolates displayed less than 80% similarity to named species and thus may represent the first cultured isolates of novel or uncultured taxa at a less detailed phylogenetic level. The sequence of isolate R-68168 (phylotype 64) was most similar (78.70%) to that of *Streptacidiphilus rugosus* AM-16 (*Actinobacteria*), whereas the sequence of R-68213 (phylotype 76) was most similar (79.07%) to that of *Hippea maritima* DSM 10411 (*Deltaproteobacteria*). Therefore, these sequences, which were 89.99% similar to each other, were additionally compared with the GreenGenes database [32,67,91]. The results grouped the isolates with environmental sequences of the candidate phylum FBP (96.26 and 97.83% highest sequence similarity, respectively) [61]. They are currently being studied in detail and will be reported on separately.

The identifications obtained were compared to the MALDI-TOF MS dendrogram. A total of 126 of the 141 clusters and several unique profiles, accounting for 892 of the 1038 isolates enclosed in the MALDI-TOF MS dendrogram, were well defined and contained isolates belonging to the same genus or species, based on the 16S rDNA identification of the closest EzTaxon hit. Other clusters were taxonomically heterogeneous and contained isolates belonging to different genera. This may be explained by the fact that the profiles were a somewhat lower quality due to the low biomass obtained for these strains. The comparison also revealed that several phylotypes were represented by multiple MALDI-TOF MS clusters and unique spectra, indicating that several distinct strains were isolated within these groups. Most diversity was retrieved from the solid media and least from the liquid enrichments. The distribution of the recovered taxa for the different terrestrial samples and cultivation setups is shown in Table S3. Several genera, including *Sphingomonas*, *Nocardioides*, *Rhodococcus* and *Hymenobacter*, were retrieved from all samples and nearly all setups, whereas others (e.g. *Rhodopseudomonas*, *Polymorphobacter*, *Knoellia*) were retrieved only from one sample and cultivation setup (Table S3). The most abundantly retrieved genera considering all isolation conditions were *Sphingomonas* (397 isolates), *Nocardioides* (83 isolates) and *Arthrobacter* (83 isolates) (Table S3). A subset of strains representing the total diversity recovered was stored in the research collection of the Laboratory of Microbiology at Ghent University and is available for further research.

In a previous study [Tahon et al. Submitted for publication], the bacterial communities present in the samples had been investigated by sequencing partial 16S rRNA genes (V1–V3 region) using Illumina MiSeq 2 × 300 bp sequencing. Grouping at a 97% similarity resulted in a total of 703 OTUs. Comparison of these sequences with those of the isolates allowed more insight into the diversity overlap retrieved between cultivation and the culture-independent approach. Many of the isolates' 16S rRNA genes grouped together at high similarities (≥97%) with the environmental sequences (Table 2, Figs. S1–S9). However, for 30 of the 77 cultured phylotypes, no sequence sharing more than 97% similarity was present in the culture-independent dataset.

Although deep sequencing has revolutionized the current knowledge of the bacterial world, these techniques also have weaknesses. Currently, for the widely used Illumina MiSeq platform, the maximum amplicon length after merging reads is restricted to ~550 bp. For 16S rRNA genes, these partial sequences encompass only one third of the complete gene and, as a result, their identification at more detailed taxonomic levels (i.e. genus and species) may prove difficult. Therefore, the identity of the isolates based on full 16S rRNA gene sequences allowed tentative confirmation or improvement of the previous identification of the Illumina sequences obtained from the same samples. Maximum likelihood analysis clearly showed that the neighboring Illumina sequences of the 327 representative isolates (74 phylotypes) grouping with *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Deinococcus-Thermus* had previously all been assigned a correct taxonomy at the phylum, class and order level (Figs. S1 and S3–S9). For 63 phylotypes, the neighboring sequences had also been assigned a correct family. For the others, related Illumina sequences had been unclassified at the family level. For phylotypes 29, 49 and 77, the identity of the isolates made it possible to assign a tentative genus and species identity to the highly similar Illumina sequences that were previously identified only to the family level. In a few other cases, there were discrepancies in the identification of isolates and the OTUs they were grouped with. For example, the V1–V3 region of isolates grouping in phylotypes 4 and 20 (identified as *Brevundimonas variabilis* and *Noviherbaspirillum* sp.) was almost identical to the sequences recovered using Illumina (Table 2, Figs. S3 and S7) that had been identified as *Mycoplana* and *Collimonas* using the May 2013 GreenGenes training set [32,67,91]. Repeating the identification of these sequences, but with the current EZTaxon and GreenGenes databases, led to the same identification as *B. variabilis* and *Noviherbaspirillum* sp. Thus, although any differences could sometimes be due to using a short sequence for identification purposes, they might also be explained by differences in the sequence databases used, but the ongoing addition of new sequence data and novel taxa improves the identifications.

Protein coding gene analyses

For all 330 representative isolates, phototrophy potential was tested by amplification of key genes involved in rhodopsin- and (bacterio)chlorophyll-dependent light-harvesting. However, proteorhodopsin and actinorhodopsin genes could not be amplified from any of the isolates, although amplification of *pufLM* resulted in 50 positive reactions. The more universal *pufM* primers of Yutin et al. [119] did not provide any additional isolates. Positive isolates belonged to *Sphingomonas* (30 isolates), *Methylobacterium* (9 isolates), *Brevundimonas* (9 isolates), *Sphingomonadaceae* (1 isolate) and *Hymenobacter* (1 isolate) (Tables 2 and S2). Comparison of these *pufLM* sequences with *pufLM* cloned sequences from a previous study [101] revealed that only one sequence was recovered using both approaches. The *pufLM* sequence of isolate R-68361 (*Hymenobacter* sp.) was identical to two cloned sequences (accession no. KT154478) obtained from the same terrestrial sample (i.e.

Table 2
Overview of phylotypes recovered from the samples. For each phylotype, the number of representative strains (330 total) enclosed in the group are listed as well as the nearest phylogenetic neighbor and the number of isolates testing positive for different phototrophy genes. Types previously retrieved [Tahon et al. Submitted for publication] using Illumina MiSeq sequencing (≥97% similarity) are indicated with an *.

Phylotype	No. of strains	Identification	Representative strain	Accession no. strain	Nearest phylogenetic neighbor			*pufLM*	*bchL/bchX*
					Type strain	Accession no.	Sequence similarity (%)		
Proteobacteria									
Alphaproteobacteria									
3	1	*Aureimonas* sp.	R-68373	KY386318	*Aureimonas ferruginae* CC-CFT023	JQ864240	96.64	0	1
4*	10	*Brevundimonas variabilis*	R-68295	KY386469	*Brevundimonas variabilis* ATCC15255	AJ227783	99.79	9	6
1*	10	*Methylobacterium* sp.	R-68173	KY386380	*Methylobacterium iners* 5317S-33	EF174497	98.52	9	2
6	1	*Polymorphobacter* sp.	R-68699	KY386562	*Polymorphobacter multimanifer* 262-7	AB649056	95.99	0	0
2*	1	*Rhodopseudomonas* sp.	R-67878	KY386506	*Rhodopseudomonas pseudopalustris* DSM123	AB498818	98.67	0	0
18*	1	*Roseomonas aquatica*	R-68475	KY386403	*Roseomonas aquatica* TR53	AM231587	99.93	1	0
19*	1	*Roseomonas* sp.	R-68165	KY386462	*Roseomonas tokyonensis* K-20	AB297501	98.60	0	0
5	2	*Sphingomonadaceae* bacterium	R-67883	KY386496	*Polymorphobacter multimanifer* 262-7	AB649056	94.94	1	1
9*	53	*Sphingomonas* sp.	R-68274	KY386532	*Sphingomonas aerolata* NW12	AJ429240	99.09	3	7
12	12	*Sphingomonas* sp.	R-68304	KY386332	*Sphingomonas cynarae* SPC-1	HQ439186	98.24	6	3
7	18	*Sphingomonas* sp.	R-67984	KY386379	*Sphingomonas faeni* MA-olki	AJ429239	99.62	4	4
16	12	*Sphingomonas* sp.	R-68260	KY386460	*Sphingomonas hunanensis* JSM 083058	FJ527417	98.52	10	4
13	3	*Sphingomonas* sp.	R-68524	KY386357	*Sphingomonas mucosissima* CP173-2	AM229669	98.27	0	0
8	1	*Sphingomonas* sp.	R-68700	KY386453	*Sphingomonas oligophenolica* JCM 12082	AB018439	98.24	0	0
17	1	*Sphingomonas* sp.	R-67954	KY386585	*Sphingomonas pituitosa* EDIV	AJ243751	95.58	0	0
15	7	*Sphingomonas* sp.	R-68270	KY386304	*Sphingomonas yantingensis* 1007	JX566547	96.79	7	5
11*	1	*Sphingomonas* sp.	R-68222	KY386535	*Sphingomonas yunnanensis* YIM 003	AY894691	97.52	0	0
Betaproteobacteria									
20*	2	*Noviherbaspirillum* sp.	R-67997	KY386345	*Noviherbaspirillum psychrotolerans* PB1	JN390675	98.62	0	0
21*	1	*Noviherbaspirillum suwonensis*	R-68579	KY386599	*Noviherbaspirillum suwonensis* 54105-65	JX275858	98.95	0	0
22*	2	*Massilia* sp.	R-67978	KY386448	*Massilia eurypsychrophila* B528-3	KJ361504	97.70	0	0
23	5	*Variovorax* sp.	R-67932	KY386486	*Variovorax boronicumulans* BAM-48	AB300597	97.55	0	0
24	2	*Variovorax* sp.	R-67871	KY386393	*Variovorax ginsengisoli* Gsoil 3165	AB245358	99.26	0	0
Actinobacteria									
43*	2	*Actinomycetales* bacterium	R-67786	KY386350	*Jatrophihabitans endophyticus* S9650	JQ346802	93.6	0	0
44*	1	*Actinomycetales* bacterium	R-68701	KY386494	*Jatrophihabitans soli* KIS75-12	KP017569	94.37	0	0
45*	7	*Actinomycetales* bacterium	R-68183	KY386330	*Frankia alni* ACN14A	CT573213	94.11	0	0
46*	1	*Actinomycetales* bacterium	R-67810	KY386542	*Cryptosporangium minutisporangium* IFO15962	AB037007	94.41	0	0
47*	3	*Actinomycetales* bacterium	R-67836	KY386609	*Modestobacter versicolor* CP153-2	AJ871304	94.28	0	0
48*	2	*Actinomycetales* bacterium	R-68223	KY386537	*Sporichthya brevicatena* IFO 16195	AB006164	93.78	0	0
77*	1	*Angustibacter* sp.	R-68259	KY386618	*Angustibacter aerolatus* 7402J-48	JQ639056	96.64	0	0
54*	4	*Aquipuribacter* sp.	R-67807	KY386530	*Aquipuribacter hungaricus* IV-75	FM179321	97.88	0	1
57*	22	*Arthrobacter* sp.	R-67818	KY386623	*Arthrobacter agilis* DSM 20550	X80748	99.46	0	0
58*	2	*Arthrobacter flavus*	R-67793	KY386372	*Arthrobacter flavus* TB 23	ALPM01000083	99.25	0	0
75	1	*Arthrobacter* sp.	R-68384	KY386384	*Arthrobacter oxydans* DSM 20119	X83408	99.25	0	0
55*	5	*Arthrobacter pityocampae*	R-68518	KY386301	*Arthrobacter pityocampae* Tp2	EU885749	99.34	0	0

42*	1	*Auraticoccus monumenti*	R-68201	KY386505	*Auraticoccus monumenti* MON 2.2	FN552748	99.58	0	0
59*	3	*Dermacoccaceae* bacterium	R-68253	KY386608	*Calidifontibacter indicus* PC IW02	EF187228	94.71	0	0
41*	1	*Friedmanniella* sp.	R-68221	KY386527	*Friedmanniella luteola* FA1	AB445453	98.22	0	0
40	5	*Friedmanniella* sp.	R-67749	KY386408	*Friedmanniella sagamiharensis* FB2	AB445456	96.09	0	0
10	1	*Geodermatophilus* sp.	R-68085	KY386308	*Geodermatophilus terrae* PB261	JN033773	99.02	0	0
53*	1	*Knoellia* sp.	R-68061	KY386418	*Knoellia sinensis* DSM 12331	AVPJ01000034	98.70	0	0
33*	2	*Marmoricola* sp.	R-67804	KY386524	*Marmoricola aquaticus* CBMAI	JN615437	97.75	0	0
32	2	*Marmoricola* sp.	R-67781	KY386333	*Marmoricola korecus* Sco-A36	FN386723	98.67	0	0
51	7	*Modestobacter* sp.	R-68230	KY386550	*Modestobacter lapidis* MON 3.1	LN810544	97.28	0	0
49*	2	*Nakamurella* sp.	R-68216	KY386516	*Nakamurella lactae* DLS-10	AM778124	98.07	0	0
50*	3	*Nakamurella* sp.	R-68216	KY386516	*Nakamurella lactae* DLS-10	AM778124	98.57	0	0
34	9	*Nocardioides* sp.	R-67800	KY386498	*Nocardioides antarcticus* M-SA3-94	KM347967	97.76	0	0
27*	1	*Nocardioides aquaticus*	R-68162	KY386458	*Nocardioides aquaticus* EL-17K	X94145	99.38	0	0
25	2	*Nocardioides* sp.	R-68562	KY386518	*Nocardioides ginsengagri* BX5-10	GQ339904	97.38	0	0
28	3	*Nocardioides plantarum*	R-68307	KY386526	*Nocardioides plantarum* NCIMB 12834	AF005008	100.00	0	0
26*	1	*Nocardioides* sp.	R-68482	KY386444	*Nocardioides salarius* CL-Z59	DQ401092	97.00	0	0
29*	14	*Nocardioides* sp.	R-68154	KY386423	*Nocardioides terrigena* DS-17	EF363712	97.79	0	1
30*	1	*Nocardioides* sp.	R-67827	KY386592	*Nocardioides terrigena* DS-17	EF363712	96.06	0	0
31*	7	*Nocardioides* sp.	R-68145	KY386386	*Nocardioides terrigena* DS-17	EF363712	97.52	0	1
52*	9	*Phycicoccus* sp.	R-68264	KY386392	*Phycicoccus ochangensis* L1b-b9	GQ344405	98.09	0	0
35	2	*Rhodococcus* sp.	R-68019	KY386523	*Rhodococcus aerolatus* PAMC 27367	KM044053	96.34	0	0
36*	1	*Rhodococcus* sp.	R-68187	KY386338	*Rhodococcus aerolatus* PAMC 27367	KM044053	95.64	0	1
37*	1	*Rhodococcus* sp.	R-68509	KY386574	*Rhodococcus aerolatus* PAMC 27367	KM044053	95.92	0	0
38*	2	*Rhodococcus* sp.	R-67872	KY386395	*Rhodococcus aerolatus* PAMC 27367	KM044053	95.85	0	0
39	7	*Rhodococcus* sp.	R-68273	KY386321	*Rhodococcus fascians* LMG 3623	JMEN01000010	98.77	0	2
14*	1	*Solirubrobacterales* bacterium	R-68159	KY386450	*Conexibacter arvalis* KV-962	AB597950	94.02	0	0
Deinococcus-Thermus									
60*	3	*Deinococcus* sp.	R-68561	KY386437	*Deinococcus marmoris* DSM12784	JNIV01000230	100.00	0	0
61*	3	*Deinococcus saxicola*	R-68514	KY386612	*Deinococcus saxicola* AA-1444	AJ585984	99.93	0	0
Firmicutes									
56	1	*Paenibacillus* sp.	R-68670	KY386433	*Paenibacillus frigoriresistens* YIM 016	JQ314346	97.66	0	0
FBP									
64*	1	FBP bacterium	R-68168	KY386300	*Nocardioides echinoideorum* CC-CZW004	KM085325	78.70	0	0
76*	1	FBP bacterium	R-68213	KY386500	*Hippea maritima* DSM 10411	CP002606	79.07	0	0
Bacteroidetes									
70*	1	*Adhaeribacter* sp.	R-68225	KY386541	*Adhaeribacter aquaticus* DSM16391	AXBK01000007	96.37	0	0
67*	1	*Hymenobacter* sp.	R-67758	KY386449	*Hymenobacter arcticus* R2-4	KC213491	98.05	0	0
62	4	*Hymenobacter* sp.	R-68471	KY386441	*Hymenobacter roseosalivarius* AA718	Y18833	98.01	0	0
63	1	*Hymenobacter* sp.	R-68402	KY386432	*Hymenobacter roseosalivarius* AA718	Y18833	97.94	0	0
65	1	*Hymenobacter* sp.	R-68403	KY386435	*Hymenobacter roseosalivarius* AA718	Y18833	98.89	0	0
66*	2	*Hymenobacter* sp.	R-68243	KY386583	*Hymenobacter roseosalivarius* AA718	Y18833	94.75	0	0
68*	8	*Hymenobacter* sp.	R-68178	KY386311	*Hymenobacter terrae* DG7A	KF862488	93.96	1	0
69	1	*Hymenobacter* sp.	R-68030	KY386555	*Hymenobacter terrae* DG7A	KF862488	91.29	0	0
74*	10	*Spirosoma* sp.	R-68079	KY386376	*Spirosoma rigui* WPCB118	EF507900	97.94	0	1
72	1	*Pedobacter* sp.	R-67967	KY386410	*Pedobacter duraquae* WB2.1-25	AM491368	98.36	0	0
71	2	*Pedobacter* sp.	R-68289	KY386572	*Pedobacter panaciterrae* Gsoil 042	AB245368	97.59	0	0
73	2	*Pedobacter* sp.	R-68191	KY386353	*Pedobacter ruber* W1	HQ882803	95.43	0	0

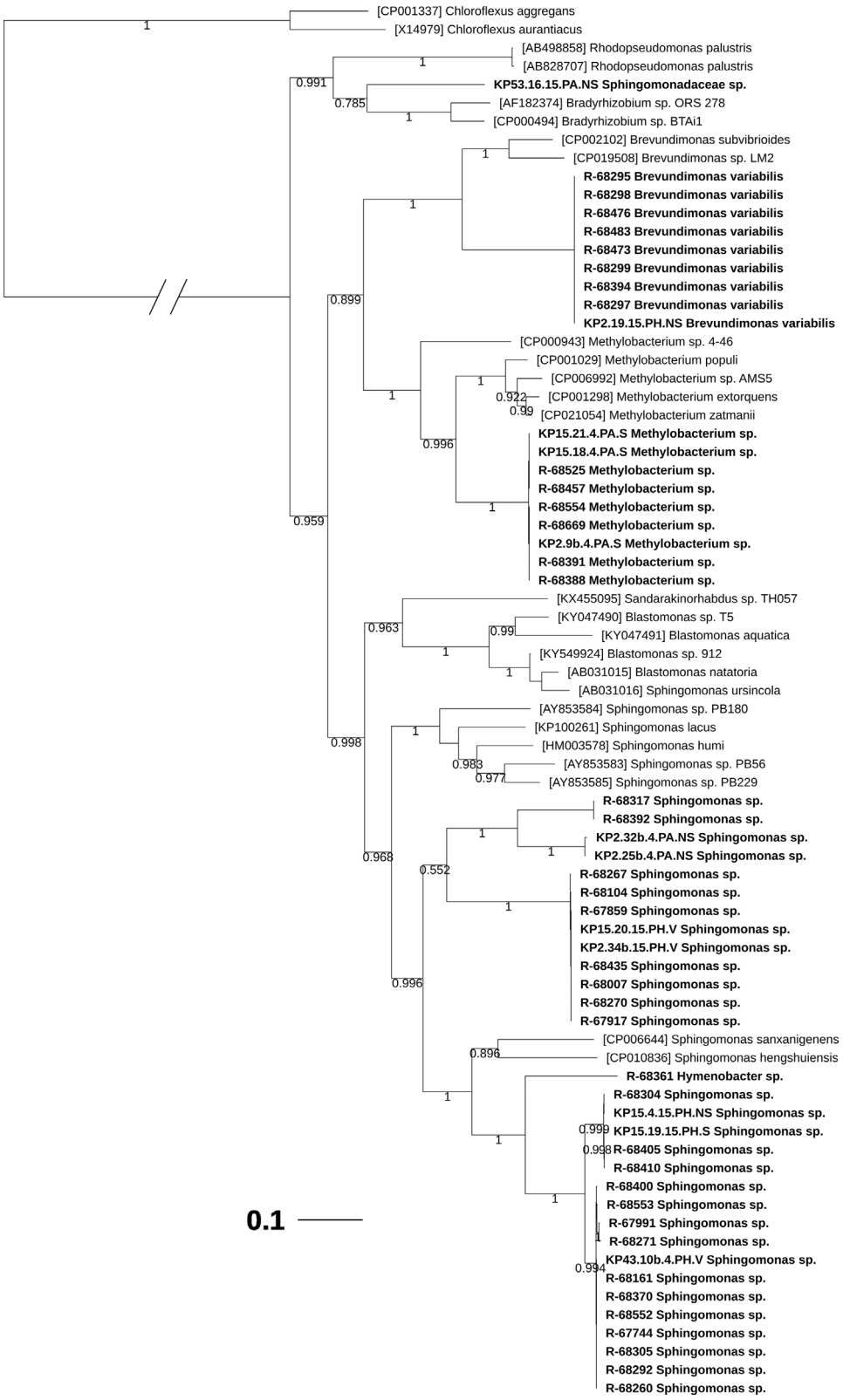

Fig. 1. Maximum likelihood phylogenetic tree (1000 bootstrap replicates) of *pufLM* sequences (1554 nucleotide positions). Scale bar indicates 10 substitutions per 100 nucleotide positions. For reference data, the accession number and taxon name are listed. *PufLM* sequences from newly obtained strains are labelled in bold. Bootstrap values of at least 50% are provided. *Chloroflexi pufLM* sequences were used as an outgroup. Identifications of the strains obtained can be found in Table S2.

KP15). All other *pufLM* isolate sequences were less than 86% similar to cloned sequences. The maximum likelihood tree in Fig. 1 clearly shows newly obtained *pufLM* sequences grouping among the reference data of closely related taxa with a known photoheterotrophic phenotype. Nevertheless, the newly obtained *pufLM* sequences were not highly similar to the sequences of their clos-

est neighbors (Fig. 1). Perhaps more closely related neighbors have not yet been cultured or, since many anoxygenic phototrophs can grow heterotrophically, their phototrophic potential may not yet have been recorded [58]. Interestingly, the *pufLM* sequence of the *Hymenobacter* isolate (R-68361) grouped in a separate branch among the reference data from *Sphingomonas* spp., with a high bootstrap value supporting the branch (Fig. 1).

BchL/bchX could be amplified from 41 of the 330 representative isolates, 17 of which also tested positive for *pufLM* (Table S2). Known anoxygenic phototrophs contain both *bchL* and *bchX* [41], which co-amplified with the primers used here [4]. Therefore, these PCR products could not be sequenced directly. Since the IGK3 and DVV primers used for amplification of *bchL* and *bchX* also amplify *nifH* [4], an additional PCR was carried out with broad range *nifH* primers (F2 and R6) in order to verify that no *nifH* were amplified (Table 2). This primer set, designed by Marusina et al. [66] was reported to amplify strictly *nifH* and resulted in no positive amplifications [42].

In previous research, the diversity of key bacterial protein encoding genes in the Calvin-Benson-Bassham cycle (RuBisCO) was investigated using clone libraries and Illumina MiSeq sequencing [101,Tahon et al. Submitted for publication]. The results revealed a large diversity of RuBisCO types IA, IB and IC (*cbbL* gene) grouping with *Proteobacteria* and *Actinobacteria*. The type II RuBisCO (*cbbM* gene) could not be amplified from the samples. Therefore, the 330 representative isolates were additionally checked for the presence of *cbbL* and *cbbM* genes (Table 1) but this resulted in no positive amplifications.

Discussion

Bacteria in Antarctic soils are subjected to a range of extreme environmental conditions, including sub-zero temperatures with repeated cycles of freezing and thawing, low transient precipitation [114], very low availability of organic matter [107] and strong solar radiation at exposed sites. In these environmental conditions, some bacteria may have adopted a phototrophic lifestyle, converting sunlight into chemical energy. The analysis in the present study is the first to report on the diversity of culturable aerobic anoxygenic phototrophic bacteria relying on a type 2 photochemical reaction center in soils from the SRM (East Antarctica). Previous studies of environmental DNA have revealed that a broad diversity of (aerobic) anoxygenic phototrophs, particularly those using the aforementioned reaction center, was present in the exposed soils in the proximity of the Princess Elisabeth Station, whereas the relative abundance of oxygenic photosynthetic microorganisms was found to be low in many samples [101,102,107]. Therefore, the main objective was to isolate and characterize AAP with a type 2 reaction center, which is a group that has, to date, almost exclusively been studied in aquatic environments [58].

AAP relying on a type 2 photochemical reaction center have predominantly been found in the *Alpha-* and *Gammaproteobacteria*, and to a lesser extent in the *Betaproteobacteria*. A single representative is known in the *Gemmatimonadetes* and in the *Firmicutes* [58,79,121]. The isolation strategy using oligotrophic media and a light regime simulating the increasing day length during transition from Antarctic winter to summer gave access to a range of bacteria known to be common inhabitants of soils, including those from Antarctica [2,77,80,105,115]. Following MALDI-TOF MS and 16S rRNA gene analysis, ~52% of the 892 isolates identified grouped among known alphaproteobacterial AAP taxa, particularly with *Sphingomonas* (~45%). The majority of these alphaproteobacterial AAP were retrieved from liquid enrichments incubated at 4 °C (Table S3). A total of 20 isolates, many obtained from PA medium incubated at 4 °C, grouped with *Betaproteobacteria*, whereas only a single *Firmicutes* isolate was picked up. However, none of these isolates grouped among known AAP taxa and no *Gammaproteobacteria* or *Gemmatimonadetes* were isolated. This observation was in accordance with previous observations made on these samples with culture-independent approaches. Clone libraries and Illumina MiSeq sequencing of the *puf(L)M* and *bchL/bchX* genes revealed a dominance of alphaproteobacterial AAP, including many of the groups isolated here [101,102]. Only 0.65% of the ~680,000 *pufM* Illumina reads grouped with beta- and gammaproteobacterial *pufM*, while 16S rRNA gene sequencing also revealed a high relative abundance of *Alphaproteobacteria* and, in particular, AAP taxa. On the other hand, *Beta-* and *Gammaproteobacteria*, *Gemmatimonadetes* and *Firmicutes* were (almost) absent in these samples and the SRM in general [101,Tahon et al. Submitted for publication]. Although similar patterns could be observed in both approaches (i.e. dominance of alphaproteobacterial AAP taxa), the comparison of culture-dependent and culture-independent 16S rRNA and *puf(L)M* gene datasets only revealed a limited overlap, indicating that both approaches provided complementary information concerning a community's diversity that might be missed when using a single approach. However, the low overlap may be explained by the limitations of these methods. Amplicon sequencing is dependent on DNA extraction and the use of primers, and these steps do not retrieve all diversity. Indeed, recent metagenome analysis has led to the discovery of a novel bacterial phylum that had always remained hidden because of mismatches in the most commonly used primers for 16S rRNA gene sequencing [34]. While cultivation might overcome this problem, many isolates are still resistant to the commonly used cultivation techniques. The low number of types shared will have also been biased by the cultivation setup. Firstly, although specific cultivation conditions for the targeted growth of aerobic phototrophic bacteria were used, in addition to oxygenic and anoxygenic phototrophic bacteria, a high number of non-phototrophs lacking *pufLM* and *bchL/bchX* were also isolated. A portion of these isolates may have phototrophic capacities that depend on rhodopsins or BchL; however, these features may have been missed as a result of primer mismatch, or the presence of a different rhodopsin type or a type 1 photochemical reaction center. For example, very little rhodopsin data has been retrieved from currently available terrestrial Antarctic metagenomes with MG-RAST [113] and IMG [65]. Most rhodopsin data, however, originates from aquatic environments [15]. Since it is well known that this protein family contains an enormous diversity [8,35], currently available primers may be unsuitable for capturing terrestrial Antarctic rhodopsin variants, whereas annotation pipelines may be unable to detect these variants in terrestrial metagenomes. Nevertheless, future advances in physiological characterization and genome analyses may resolve this question. Secondly, the unexpected and abundant growth of *Cyanobacteria* in the liquid enrichments may have restricted the growth of other phototrophic bacteria in these setups. Thirdly, when applying a culture-independent approach, the presence of DNA from dead cells, which is capable of persisting in cold soil for long periods of time, may inflate the bacterial diversity observed, hence reducing the overlap observed with a culture-dependent approach [21]. Finally, the high number of colonies, their very slow growth and miniscule colony size, in combination with manual picking, introduced an additional bias, since it was not possible to isolate every single colony. Additionally, the limited biomass production impeded many of the analyses and called for a modification of the standard operating procedures used for fast growing organisms producing high biomass. Development of new innovative and high throughput strategies will be necessary to cultivate and characterize a larger proportion of the Antarctic biodiversity.

Of the 75 isolates showing phototrophy potential (i.e. positive PCR for a phototrophy gene), 67 grouped with alphaproteobacterial

AAP taxa (Table S2). Although the abundance of alphaproteobacterial AAP is well recognized in various aquatic environments all over the planet [16,29,58,84], to date, little is known about their diversity, distribution and role in terrestrial ecosystems. The majority of potential phototrophs, and ~45% of the isolates in general, grouped with *Sphingomonas* (Table 2, Figs. 1 and S5). The presence of *Sphingomonas* in Antarctica is not unusual, since several members of this group have previously been isolated from a range of cold ecosystems, including Antarctica [18,46,77], and they are well known for their phototrophic capacities [48,88]. In recent years, analyses have also revealed the metabolic diversity of several *Sphingomonas* strains, as well as their capacity to adapt to extreme cold, high UV-B radiation and arid conditions, indicating that they are ideal candidates for survival in extreme oligotrophic Antarctic systems [9,46,51,52]. The second most recovered group of alphaproteobacterial phototrophs was highly related to *Methylobacterium* (Fig. 1, Table 2). Some *Methylobacterium* strains are well known for their tolerance to high UV radiation and dehydration, which are common environmental conditions in Antarctic soils [75]. Indeed, Romanovskaya et al. [85] reported this genus, and in particular *Methylobacterium extorquens*, a bacterium known to possess a type 2 photochemical reaction center, in terrestrial biotopes on several Antarctic islands [85,109], whereas PufM, BchL and BchX sequences from members of this taxon have previously been reported from terrestrial locations in the Arctic [36] and Antarctic [102]. Interestingly, no *pufLM* genes could be amplified from strain R-67878 (*Rhodopseudomonas* sp.). Given that all known *Rhodopseudomonas* species are phototrophic [100], the negative result may have been caused by primer mismatch. Indeed, we have previously performed an *in silico* comparison of multiple primers targeting different regions of the *pufM* gene [102], and the results in fact showed that none of the primers investigated targeted all known diversity.

Based on 16S rRNA sequence data combined with the MALDI-TOF MS dendrogram, 87 of the isolates grouped with the *Bacteroidetes*, and especially with *Hymenobacter* (Table S3, Fig. S4). The type species of this genus, *Hymenobacter roseosalivarius*, was originally isolated from exposed areas in the McMurdo Dry Valleys [50], and has been commonly reported from several terrestrial and aquatic Antarctic locations [2,57,77]. To our knowledge, no anoxygenic phototrophic members have ever been reported in the phylum *Bacteroidetes* [53]. Remarkably, the *pufLM* genes encoding the conserved proteins of the type 2 phototrophic reaction center could be amplified from *Hymenobacter* isolate R-68361 (Fig. 1). The 16S rRNA gene sequence of strain R-68361 was found to be 93.96% identical to that of *Hymenobacter terrae* DG7A, a recently described strain isolated from soil samples in Seoul (South Korea) [98]. The *pufLM* sequence of R-68361 was identical to cloned sequences previously obtained from the same sample [101]. Phylogenetic analysis of this sequence grouped it among reference data for *Sphingomonas* spp. with a known photoheterotrophic phenotype (Fig. 1). This grouping may be the result of a horizontal gene transfer event, since similar observations have previously been made for phototrophic *Gemmatimonadetes* [121]. In addition, a positive result for *bchL*/*bchX* was obtained from one of the *Spirosoma* isolates (R-67957) (Table S2). It should also be noted that both rhodopsin-dependent and Bchl-dependent phototrophy require the transcription of unique multiple genes [17]. Thus, to assess whether these isolates are the first phototrophic representatives of the *Bacteroidetes*, genome and transcriptome analysis will be needed in order to verify the presence and expression of all the required phototrophic genes.

In addition to taxa known to contain AAP, ~31% of the 892 isolates were identified as *Actinobacteria*, and they were predominantly isolated from solid media (Table S3). Six of these isolates tested positive in the PCR for *bchL*/*bchX*, although these products were co-amplified and therefore were not sequenced. None of the *Actinobacteria* tested positive for rhodopsins or *pufLM* (Table 2). This might be explained by primer mismatch, since currently available primers may be unable to target all the rhodopsin and photoreaction center diversity. However, future physiological characterization and genome analyses may resolve this question but, until then, the phototrophic potential of these *Actinobacteria* remains unknown. All actinobacterial isolates grouped with the *Actinobacteridae*, which is similar to observations made in other Antarctic soils [1,2,77]. Most of the *Actinobacteridae* isolates were identified as *Arthrobacter*, *Nocardioides*, *Modestobacter* and *Rhodococcus*. Members of these genera have previously been isolated from several cold regions, such as Greenland, the Arctic and Antarctica [7,68,78,90,95]. The presence of *Modestobacter*, some of which are known to be cold tolerant and radiation resistant, may be linked to its possible involvement in the weathering of rocks [45,74]. On the other hand, *Arthrobacter* is a genus generally associated with the soil compartment and is recognized for its physiological versatility (e.g. altering of its cell wall fatty acid composition in response to lowered growth temperatures) and its ability to use a wide range of substrates [2,111].

Conclusions

This study provided the first data on the culturable aerobic anoxygenic phototrophic bacterial diversity in the Sør Rondane Mountains, East Antarctica. In general, the conditions used resulted in slow growing isolates producing extremely small colonies and minimal amounts of biomass. The isolation strategy resulted in approximately 52% of isolates belonging to known alphaproteobacterial AAP taxa, while other isolates were distributed over six phyla, including a candidate phylum. In addition, enrichment cultures revealed the presence of *Cyanobacteria* and even green algae. It was also demonstrated for the first time that a single *Bacteroidetes* isolate may have phototrophic potential. Overall, the results suggested that the ability to adopt a photoheterotrophic lifestyle may provide an advantage in the oligotrophic Antarctic soils surrounding the Princess Elisabeth Station.

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Author contributions

Conceived and designed the experiments: GT, AW. Performed the experiments: GT. Analyzed the data: GT. Wrote the paper: GT, AW. Both authors approved the final manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2017.05.007.

References

[1] Aislabie, J.M., Chhour, K.-L., Saul, D.J., Miyauchi, S., Ayton, J., Paetzold, R.F., Balks, M.R. (2006) Dominant bacteria in soils of Marble Point

and Wright Valley, Victoria Land, Antarctica. Soil Biol. Biochem. 38, 3041–3056.
[2] Aislabie, J.M., Lau, A., Dsouza, M., Shepherd, C., Rhodes, P., Turner, S.J. (2013) Bacterial composition of soils of the Lake Wellman area, Darwin Mountains, Antarctica. Extremophiles 17, 775–786.
[3] Allgaier, M., Uphoff, H., Felske, A., Wagner-Döbler, I. (2003) Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. Appl. Environ. Microbiol. 69, 5051–5059.
[4] Ando, S., Goto, M., Meunchang, S., Thongra-ar, P., Fujiwara, T., Hayashi, H., Yoneyama, T. (2005) Detection of *nifH* sequences in sugarcane (*Saccharum officinarum* L.) and pineapple (*Ananas comosus* [L.] Merr.). Soil Sci. Plant Nutr. 51, 303–308.
[5] Atamna-Ismaeel, N., Finkel, O.M., Glaser, F., Sharon, I., Schneider, R., Post, A.F., Spudich, J.L., von Mering, C., Vorholt, J.A., Iluz, D., Béjà, O., Belkin, S. (2012) Microbial rhodopsins on leaf surfaces of terrestrial plants. Environ. Microbiol. 14, 140–146.
[6] Atamna-Ismaeel, N., Sabehi, G., Sharon, I., Witzel, K.-P., Labrenz, M., Jurgens, K., Barkay, T., Stomp, M., Huisman, J., Béjà, O. (2008) Widespread distribution of proteorhodopsins in freshwater and brackish ecosystems. ISME J. 2, 656–662.
[7] Babalola, O.O., Kirby, B.M., Le Roes-Hill, M., Cook, A.E., Cary, S.C., Burton, S.G., Cowan, D.A. (2009) Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. Environ. Microbiol. 11, 566–576.
[8] Bamann, C., Bamberg, E., Wachtveitl, J., Glaubitz, C. (2014) Proteorhodopsin. Biochim. Biophys. Acta: Bioenerg. 1837, 614–625.
[9] Baraniecki, C.A., Aislabie, J., Foght, J.M. (2002) Characterization of *Sphingomonas* sp. Ant 17, an aromatic hydrocarbon-degrading bacterium isolated from Antarctic soil. Microb. Ecol. 43, 44–54.
[10] Béjà, O., Lanyi, J.K. (2014) Nature's toolkit for microbial rhodopsin ion pumps. Proc. Natl. Acad. Sci. U. S. A. 111, 6538–6539.
[11] Béjà, O., Suzuki, M.T., Heidelberg, J.F., Nelson, W.C., Preston, C.M., Hamada, T., Eisen, J.A., Fraser, C.M., DeLong, E.F. (2002) Unsuspected diversity among marine aerobic anoxygenic phototrophs. Nature 415, 630–633.
[12] Bellenger, J.P., Wichard, T., Kraepiel, A.M.L. (2008) Vanadium requirements and uptake kinetics in the dinitrogen-fixing bacterium *Azotobacter vinelandii*. Appl. Environ. Microbiol. 74, 1478–1484.
[13] Benemann, J.R., McKenna, C.E., Lie, R.F., Traylor, T.G., Kamen, M.D. (1972) The vanadium effect in nitrogen fixation by azotobacter. Biochim. Biophys. Acta: Gen. Subj. 264, 25–38.
[14] Bockheim, J.G. (2015) Soil-forming factors in Antarctica. In: Bockheim, G.J. (Ed.), The Soils of Antarctica, Springer International Publishing, Cham, pp. 5–20.
[15] Boeuf, D., Audic, S., Brillet-Guéguen, L., Caron, C., Jeanthon, C. (2015) MicRhoDE: a curated database for the analysis of microbial rhodopsin diversity and evolution. Database 2015, bav080.
[16] Boeuf, D., Cottrell, M.T., Kirchman, D.L., Lebaron, P., Jeanthon, C. (2013) Summer community structure of aerobic anoxygenic phototrophic bacteria in the western Arctic Ocean. FEMS Microbiol. Ecol. 85, 417–432.
[17] Bryant, D.A., Frigaard, N.-U. (2006) Prokaryotic photosynthesis and phototrophy illuminated. Trends Microbiol. 14, 488–496.
[18] Busse, H.-J., Denner, E.B.M., Buczolits, S., Salkinoja-Salonen, M., Bennasar, A., Kämpfer, P. (2003) *Sphingomonas aurantiaca* sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas*. Int. J. Syst. Evol. Microbiol. 53, 1253–1260.
[19] Cameron, R.E. (1969) Abundance of microflora in soils of desert regions. NASA Tech. Rep. 32, 1–16.
[20] Campbell, B.J., Waidner, L.A., Cottrell, M.T., Kirchman, D.L. (2008) Abundant proteorhodopsin genes in the North Atlantic Ocean. Environ. Microbiol. 10, 99–109.
[21] Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N. (2016) Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat. Microbiol. 2, 16242.
[22] Chew, A.G.M., Bryant, D.A. (2007) Chlorophyll biosynthesis in bacteria: the origins of structural and functional diversity. Ann. Rev. Microbiol. 61, 113–129.
[23] Cho, J.-C., Giovannoni, S.J. (2004) Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. Appl. Environ. Microbiol. 70, 432–440.
[24] Chong, C.-W., Pearce, D.A., Convey, P. (2015) Emerging spatial patterns in Antarctic prokaryotes. Front. Microbiol. 6, 1058.
[25] Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B.K., Lim, Y.-W. (2007) EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int. J. Syst. Evol. Microbiol. 57, 2259–2261.
[26] Cleenwerck, I., Camu, N., Engelbeen, K., De Winter, T., Vandemeulebroecke, K., De Vos, P., De Vuyst, L. (2007) *Acetobacter ghanensis* sp. nov., a novel acetic acid bacterium isolated from traditional heap fermentations of Ghanaian cocoa beans. Int. J. Syst. Evol. Microbiol. 57, 1647–1652.
[27] Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J.R.W., Kersters, K., Vandamme, P. (1999) Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. Int. J. Syst. Bacteriol. 49, 405–413.
[28] Convey, P., Gibson, J.A.E., Hillenbrand, C.-D., Hodgson, D.A., Pugh, P.J.A., Smellie, J.L., Stevens, M.I. (2008) Antarctic terrestrial life – challenging the history of the frozen continent? Biol. Rev. 83, 103–117.
[29] Cottrell, M.T., Mannino, A., Kirchman, D.L. (2006) Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre. Appl. Environ. Microbiol. 72, 557–564.
[30] Daubin, V., Szollosi, G.J. (2016) Horizontal gene transfer and the history of life. Cold Spring Harb. Perspect. Biol. 8.
[31] Delwiche, C.F., Palmer, J.D. (1996) Rampant horizontal transfer and duplication of rubisco genes in eubacteria and plastids. Mol. Biol. Evol. 13, 873–882.
[32] DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072.
[33] Edwards, U., Rogall, T., Blöcker, H., Emde, M., Böttger, E.C. (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. 17, 7843–7853.
[34] Eloe-Fadrosh, E.A., Paez-Espino, D., Jarett, J., Dunfield, P.F., Hedlund, B.P., Dekas, A.E., Grasby, S.E., Brady, A.L., Dong, H.L., Briggs, B.R., Li, W.J., Goudeau, D., Malmstrom, R., Pati, A., Pett-Ridge, J., Rubin, E.M., Woyke, T., Kyrpides, N.C., Ivanova, N.N. (2016) Global metagenomic survey reveals a new bacterial candidate phylum in geothermal springs. Nat. Commun. 7.
[35] Ernst, O.P., Lodowski, D.T., Elstner, M., Hegemann, P., Brown, L.S., Kandori, H. (2014) Microbial and animal rhodopsins: structures, functions, and molecular mechanisms. Chem. Rev. 114, 126–163.
[36] Feng, Y., Grogan, P., Caporaso, J.G., Zhang, H., Lin, X., Knight, R., Chu, H. (2014) pH is a good predictor of the distribution of anoxygenic purple phototrophic bacteria in Arctic soils. Soil Biol. Biochem. 74, 193–200.
[37] Feng, Y., Lin, X., Mao, T., Zhu, J. (2011) Diversity of aerobic anoxygenic phototrophic bacteria in paddy soil and their response to elevated atmospheric CO_2. Microb. Biotechnol. 4, 74–81.
[38] Ferrer, M., Golyshina, O., Beloqui, A., Golyshin, P.N. (2007) Mining enzymes from extreme environments. Curr. Opin. Microbiol. 10, 207–214.
[39] Fritsen, C.H., Grue, A.M., Priscu, J.C. (2000) Distribution of organic carbon and nitrogen in surface soils in the McMurdo Dry Valleys, Antarctica. Polar Biol. 23, 121–128.
[40] Fu, L., Niu, B., Zhu, Z., Wu, S., Li, W. (2012) CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28, 3150–3152.
[41] Fujita, Y., Bauer, C.E. (2003) The light-independent protochlorophyllide reductase: a nitrogenase-like enzyme catalyzing a key reaction for greening in the dark. In: Guilard, R., Kadish, K., Smith, K.M. (Eds.), The Porphyrin Handbook, Academic Press, Amsterdam, pp. 109–156.
[42] Gaby, J.C., Buckley, D.H. (2012) A comprehensive evaluation of PCR primers to amplify the *nifH* gene of nitrogenase. PLoS One 7, e42149.
[43] Gorodetskaya, I.V., Van Lipzig, N.P.M., Van den Broeke, M.R., Mangold, A., Boot, W., Reijmer, C.H. (2013) Meteorological regimes and accumulation patterns at Utsteinen, Dronning Maud Land, East Antarctica: analysis of two contrasting years. J. Geophys. Res. Atmos. 118, 1700–1715.
[44] Goujon, M., McWilliam, H., Li, W., Valentin, F., Squizzato, S., Paern, J., Lopez, R. (2010) A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic Acids Res. 38, W695–W699.
[45] Gtari, M., Essoussi, I., Maaoui, R., Sghaier, H., Boujmil, R., Gury, J., Pujic, P., Brusetti, L., Chouaia, B., Crotti, E., Daffonchio, D., Boudabous, A., Normand, P. (2012) Contrasted resistance of stone-dwelling Geodermatophilaceae species to stresses known to give rise to reactive oxygen species. FEMS Microbiol. Ecol. 80, 566–577.
[46] Gunnigle, E., Ramond, J.-B., Guerrero, L.D., Makhalanyane, T.P., Cowan, D.A. (2015) Draft genomic DNA sequence of the multi-resistant *Sphingomonas* sp. strain AntH11 isolated from an Antarctic hypolith. FEMS Microbiol. Lett. 362.
[47] Gupta, R.S. (2012) Origin and spread of photosynthesis based upon conserved sequence features in key bacteriochlorophyll biosynthesis proteins. Mol. Biol. Evol. 29, 3397–3412.
[48] Hiraishi, A., Kuraishi, H., Kawahara, K. (2000) Emendation of the description of *Blastomonas natatoria* (Sly 1985) Sly and Cahill 1997 as an aerobic photosynthetic bacterium and reclassification of *Erythromonas ursincola* Yurkov et al. 1997 as *Blastomonas ursincola* comb. nov. Int. J. Syst. Evol. Microbiol. 50, 1113–1118.
[49] Hirose, S., Nagashima, K.V.P., Matsuura, K., Haruta, S. (2012) Diversity of purple phototrophic bacteria, inferred from *pufM* gene, within epilithic biofilm in Tama River, Japan. Microbes Environ. 27, 327–329.
[50] Hirsch, P., Ludwig, W., Hethke, C., Sittig, M., Hoffmann, B., Gallikowski, C.A. (1998) *Hymenobacter roseosalivarius* gen. nov., sp. nov. from continental Antarctic soils and sandstone: bacteria of the Cytophaga/Flavobacterium/Bacteroides line of phylogenetic descent. Syst. Appl. Microbiol. 21, 374–383.
[51] Hortnagl, P., Perez, M.T., Sommaruga, R. (2010) Living at the border: a community and single-cell assessment of lake bacterioneuston activity. Limnol. Oceanogr. 55, 1134–1144.
[52] Huang, J., Huang, Z., Zhang, Z.-D., He, L.-Y., Sheng, X.-F. (2014) *Sphingomonas yantingensis* sp. nov., a mineral-weathering bacterium isolated from purplish paddy soil. Int. J. Syst. Evol. Microbiol. 64, 1030–1034.
[53] Huang, Y., Zeng, Y., Lu, H., Feng, H., Zeng, Y., Koblížek, M. (2016) Novel acsF gene primers revealed a diverse phototrophic bacterial population including *Gemmatimonadetes* in the Lake Taihu. Appl. Environ. Microbiol. 82, 5587–5594.
[54] Jeanthon, C., Boeuf, D., Dahan, O., Le Gall, F., Garczarek, L., Bendif, E.M., Lehours, A.-C. (2011) Diversity of cultivated and metabolically active aerobic anoxygenic phototrophic bacteria along an oligotrophic gradient in the Mediterranean Sea. Biogeosciences 8, 1955–1970.

[55] Jensen, H.L. (1942) Nitrogen fixation in leguminous plants. I. General characters of root-nodule bacteria isolated from species of *Medicago* and *Trifolium* in Australia. Proc. Linn. Soc. N. S. W. 67, 98–108.
[56] Kato, S., Nakawake, M., Ohkuma, M., Yamagishi, A. (2012) Distribution and phylogenetic diversity of *cbbM* genes encoding RubisCO form II in a deep-sea hydrothermal field revealed by newly designed PCR primers. Extremophiles 16, 277–283.
[57] Klassen, J.L., Foght, J.M. (2011) Characterization of *Hymenobacter* isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. Extremophiles 15, 45–57.
[58] Koblížek, M. (2015) Ecology of aerobic anoxygenic phototrophs in aquatic environments. FEMS Microbiol. Rev. 39, 854–870.
[59] Koh, E.Y., Atamna-Ismaeel, N., Martin, A., Cowie, R.O.M., Béjà, O., Davy, S.K., Maas, E.W., Ryan, K.G. (2010) Proteorhodopsin-bearing bacteria in Antarctic sea ice. Appl. Environ. Microbiol. 76, 5918–5925.
[60] Koh, E.Y., Phua, W., Ryan, K.G. (2011) Aerobic anoxygenic phototrophic bacteria in Antarctic sea ice and seawater. Environ. Microbiol. Rep. 3, 710–716.
[61] Lee, K.C.Y., Herbold, C.W., Dunfield, P.F., Morgan, X.C., McDonald, I.R., Stott, M.B. (2013) Phylogenetic delineation of the novel phylum *Armatimonadetes* (former candidate division OP10) and definition of two novel candidate divisions. Appl. Environ. Microbiol. 79, 2484–2487.
[62] Letunic, I., Bork, P. (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23, 127–128.
[63] Letunic, I., Bork, P. (2011) Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. Nucleic Acids Res. 39, W475–W478.
[64] Li, W., Godzik, A. (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22, 1658–1659.
[65] Markowitz, V.M., Chen, I.-M.A., Chu, K., Szeto, E., Palaniappan, K., Pillay, M., Ratner, A., Huang, J., Pagani, I., Tringe, S., Huntemann, M., Billis, K., Varghese, N., Tennessen, K., Mavromatis, K., Pati, A., Ivanova, N.N., Kyrpides, N.C. (2014) IMG/M 4 version of the integrated metagenome comparative analysis system. Nucleic Acids Res. 42, D568–D573.
[66] Marusina, A.I., Boulygina, E.S., Kuznetsov, B.B., Tourova, T.P., Kravchenko, I.K., Gal'chenko, V.F. (2001) A system of oligonucleotide primers for the amplification of *nifH* genes of different taxonomic groups of prokaryotes. Microbiology 70, 73–78.
[67] McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P. (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6, 610–618.
[68] Mevs, U., Stackebrandt, E., Schumann, P., Gallikowski, C.A., Hirsch, P. (2000) *Modestobacter multiseptatus* gen nov., sp nov., a budding actinomycete from soils of the Asgard Range (Transantarctic Mountains). Int. J. Syst. Evol. Microbiol. 50, 337–346.
[69] Mizrahi-Man, O., Davenport, E.R., Gilad, Y. (2013) Taxonomic classification of bacterial 16S rRNA genes using short sequencing reads: evaluation of effective study designs. PLoS One 8, e53608.
[70] Nagashima, K., Hiraishi, A., Shimada, K., Matsuura, K. (1997) Horizontal transfer of genes coding for the photosynthetic reaction centers of purple bacteria. J. Mol. Evol. 45, 131–136.
[71] Nanba, K., King, G.M., Dunfield, K. (2004) Analysis of facultative lithotroph distribution and diversity on volcanic deposits by use of the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase. Appl. Environ. Microbiol. 70, 2245–2253.
[72] Nichols, D. (2007) Cultivation gives context to the microbial ecologist. FEMS Microbiol. Ecol. 60, 351–357.
[73] Niemann, S., Puhler, A., Tichy, H.V., Simon, R., Selbitschka, W. (1997) Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population. J. Appl. Microbiol. 82, 477–484.
[74] Normand, P., Gury, J., Pujic, P., Chouaia, B., Crotti, E., Brusetti, L., Daffonchio, D., Vacherie, B., Barbe, V., Medigue, C., Calteau, A., Ghodhbane-Gtari, F., Essoussi, I., Nouioui, I., Abbassi-Ghozzi, I., Gtari, M. (2012) Genome sequence of radiation-resistant *Modestobacter marinus* strain BC501, a representative actinobacterium that thrives on calcareous stone surfaces. J. Bacteriol. 194, 4773–4774.
[75] Onofri, S., Selbmann, L., Zucconi, L., Pagano, S. (2004) Antarctic microfungi as models for exobiology. Planet. Space Sci. 52, 229–237.
[76] Osanai, Y., Nogi, Y., Baba, S., Nakano, N., Adachi, T., Hokada, T., Toyoshima, T., Owada, M., Satish-Kumar, M., Kamei, A., Kitano, I. (2013) Geologic evolution of the Sør Rondane Mountains, East Antarctica: collision tectonics proposed based on metamorphic processes and magnetic anomalies. Precambrian Res. 234, 8–29.
[77] Peeters, K., Ertz, D., Willems, A. (2011) Culturable bacterial diversity at the Princess Elisabeth Station (Utsteinen, Sør Rondane Mountains, East Antarctica) harbours many new taxa. Syst. Appl. Microbiol. 34, 360–367.
[78] Peeters, K., Verleyen, E., Hodgson, D., Convey, P., Ertz, D., Vyverman, W., Willems, A. (2012) Heterotrophic bacterial diversity in aquatic microbial mat communities from Antarctica. Polar Biol. 35, 543–554.
[79] Perreault, N.N., Greer, C.W., Andersen, D.T., Tille, S., Lacrampe-Couloume, G., Lollar, B.S., Whyte, L.G. (2008) Heterotrophic and autotrophic microbial populations in cold perennial springs of the high Arctic. Appl. Environ. Microbiol. 74, 6898–6907.
[80] Pointing, S.B., Chan, Y., Lacap, D.C., Lau, M.C.Y., Jurgens, J.A., Farrell, R.L. (2009) Highly specialized microbial diversity in hyper-arid polar desert. Proc. Natl. Acad. Sci. 106, 19964–19969.
[81] Price, P.S. (2010) FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS One 5, e9490.
[82] Prosser, J.I. (2015) Dispersing misconceptions and identifying opportunities for the use of 'omics' in soil microbial ecology. Nat. Rev. Microbiol. 13, 439–446.
[83] Ranganayaki, S., Mohan, C. (1981) Effect of sodium molybdate on microbial fixation of nitrogen. Z. Allg. Mikrobiol. 21, 607–610.
[84] Ritchie, A.E., Johnson, Z.I. (2012) Abundance and genetic diversity of aerobic anoxygenic phototrophic bacteria of coastal regions of the Pacific Ocean. Appl. Environ. Microbiol. 78, 2858–2866.
[85] Romanovskaya, V., Tashyrev, O., Rokitko, P., Shilin, S., Chernaya, N., Tashyreva, A. (2009) Microbial diversity in terrestrial Antarctic biotopes. Ukr. Antarct. J. 8, 364–369.
[86] Sabehi, G., Béjà, O., Suzuki, M.T., Preston, C.M., DeLong, E.F. (2004) Different SAR86 subgroups harbour divergent proteorhodopsins. Environ. Microbiol. 6, 903–910.
[87] Sabehi, G., Massana, R., Bielawski, J.P., Rosenberg, M., Delong, E.F., Béjà, O. (2003) Novel proteorhodopsin variants from the Mediterranean and Red seas. Environ. Microbiol. 5, 842–849.
[88] Salka, I., Srivastava, A., Allgaier, M., Grossart, H.-P. (2014) The draft genome sequence of *Sphingomonas* sp. strain FukuSWIS1, obtained from acidic Lake Grosse Fuchskuhle, indicates photoheterotrophy and a potential for humic matter degradation. Genome Announc. 2, e01183–01114.
[89] Salka, I., Wurzbacher, C., Garcia, S.L., Labrenz, M., Juergens, K., Grossart, H.-P. (2014) Distribution of acl-Actinorhodopsin genes in Baltic Sea salinity gradients indicates adaptation of facultative freshwater photoheterotrophs to brackish waters. Environ. Microbiol. 16, 586–597.
[90] Saul, D.J., Aislabie, J.M., Brown, C.E., Harris, L., Foght, J.M. (2005) Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. FEMS Microbiol. Ecol. 53, 141–155.
[91] Schloss, P.D. (2010) The effects of alignment quality, distance calculation method, sequence filtering, and region on the analysis of 16S rRNA gene-based studies. PLoS Comput. Biol. 6, 1–16.
[92] Schloss, P.D., Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl. Environ. Microbiol. 71, 1501–1506.
[93] Sharma, A.K., Sommerfeld, K., Bullerjahn, G.S., Matteson, A.R., Wilhelm, S.W., Jezbera, J., Brandt, U., Doolittle, W.F., Hahn, M.W. (2009) Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater *Actinobacteria*. ISME J. 3, 726–737.
[94] Sharma, A.K., Zhaxybayeva, O., Papke, R.T., Doolittle, W.F. (2008) Actinorhodopsins: proteorhodopsin-like gene sequences found predominantly in non-marine environments. Environ. Microbiol. 10, 1039–1056.
[95] Shivaji, S., Begum, Z., Shiva Nageswara Rao, S.S., Vishnu Vardhan Reddy, P.V., Manasa, P., Sailaja, B., Manasa, M.S., Prathiba, M.S., Thamban, M., Krishnan, K.P., Singh, S.M., Srinivas, T.N.R. (2013) Antarctic ice core samples: culturable bacterial diversity. Res. Microbiol. 164, 70–82.
[96] Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soeding, J., Thompson, J.D., Higgins, D.G. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539.
[97] Spiridonova, E.M., Berg, I.A., Kolganova, T.V., Ivanovsky, R.N., Kuznetsov, B.B., Tourova, T.P. (2004) An oligonucleotide primer system for amplification of the ribulose-1,5-bisphosphate carboxylase/oxygenase genes of bacteria of various taxonomic groups. Microbiology 73, 316–325.
[98] Srinivasan, S., Lee, J.-J., Park, K.R., Park, S.-H., Jung, H.-Y., Kim, M.K. (2015) *Hymenobacter terrae* sp. nov., a bacterium isolated from soil. Curr. Microbiol. 70, 643–650.
[99] Storelli, N., Peduzzi, S., Saad, M.M., Frigaard, N.U., Perret, X., Tonolla, M. (2013) CO_2 assimilation in the chemocline of Lake Cadagno is dominated by a few types of phototrophic purple sulfur bacteria. FEMS Microbiol. Ecol. 84, 421–432.
[100] Swingley, W.D. (2009) Evolutionary relationships among purple photosynthetic bacteria and the origin of proteobacterial photosynthetic systems. In: Hunter, C.N., Daldal, F., Thurnauer, M.C., Beatty, J.T. (Eds.), The Purple Phototrophic Bacteria, Springer Netherlands, Netherlands.
[101] Tahon, G., Tytgat, B., Stragier, P., Willems, A. (2016) Analysis of *cbbL*, *nifH*, and *pufLM* in soils from the Sør Rondane Mountains, Antarctica, reveals a large diversity of autotrophic and phototrophic bacteria. Microb. Ecol. 71, 131–149.
[102] Tahon, G., Tytgat, B., Willems, A. (2016) Diversity of phototrophic genes suggests multiple bacteria may be able to exploit sunlight in exposed soils from the Sør Rondane Mountains, East Antarctica. Front. Microbiol. 7, 2026.
[103] Tamames, J., Abellán, J.J., Pignatelli, M., Camacho, A., Moya, A. (2010) Environmental distribution of prokaryotic taxa. BMC Microbiol. 10, 85.
[104] Tayeh, M.A., Madigan, M.T. (1987) Malate-dehydrogenase in phototrophic purple bacteria: purification, molecular weight, and quaternary structure. J. Bacteriol. 169, 4196–4202.
[105] Teixeira, L.C.R.S., Peixoto, R.S., Cury, J.C., Sul, W.J., Pellizari, V.H., Tiedje, J., Rosado, A.S. (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. ISME J. 4, 989–1001.
[106] Tsukamoto, T., Demura, M., Sudo, Y. (2014) Irreversible trimer to monomer transition of thermophilic rhodopsin upon thermal stimulation. J. Phys. Chem. B 118, 12383–12394.
[107] Tytgat, B., Verleyen, E., Sweetlove, M., D'hondt, S., Clercx, P., Van Ranst, E., Peeters, K., Roberts, S., Namsaraev, Z., Wilmotte, A., Vyverman, W., Willems, A. (2016) Bacterial community composition in relation to bedrock type and

macrobiota in soils from the Sør Rondane Mountains, East Antarctica. FEMS Microbiol. Ecol. 92, fiw126.
[108] Vasileva-Tonkova, E., Romanovskaya, V., Gladka, G., Gouliamova, D., Tomova, I., Stoilova-Disheva, M., Tashyrev, O. (2014) Ecophysiological properties of cultivable heterotrophic bacteria and yeasts dominating in phytocenoses of Galindez Island, maritime Antarctica. World J. Microbiol. Biotechnol. 30, 1387–1398.
[109] Vuilleumier, S., Chistoserdova, L., Lee, M.-C., Bringel, F., Lajus, A., Zhou, Y., Gourion, B., Barbe, V., Chang, J., Cruveiller, S., Dossat, C., Gillett, W., Gruffaz, C., Haugen, E., Hourcade, E., Levy, R., Mangenot, S., Muller, E., Nadalig, T., Pagni, M., Penny, C., Peyraud, R., Robinson, D.G., Roche, D., Rouy, Z., Saenampechek, C., Salvignol, G., Vallenet, D., Wu, Z., Marx, C.J., Vorholt, J.A., Olson, M.V., Kaul, R., Weissenbach, J., Médigue, C., Lidstrom, M.E. (2009) *Methylobacterium* genome sequences: a reference blueprint to investigate microbial metabolism of C1 compounds from natural and industrial sources. PLoS One 4, e5584.
[110] Wahlund, T.M., Woese, C.R., Castenholz, R.W., Madigan, M.T. (1991) A thermophilic green sulfur bacterium from new-zealand hot-springs, *Chlorobium tepidum* sp. nov. Arch. Microbiol. 156, 81–90.
[111] White, P.L., Wynn-Williams, D.D., Russell, N.J. (2000) Diversity of thermal responses of lipid composition in the membranes of the dominant culturable members of an Antarctic fellfield soil bacterial community. Antarct. Sci. 12, 386–393.
[112] Wieme, F., Spitaels, M., De Bruyne, K., Van Landschoot, A., Vandamme, P. (2014) Effects of growth medium on matrix-assisted laser desorption–ionization time of flight mass spectra: a case study of acetic acid bacteria. Appl. Environ. Microbiol. 80, 1528–1538.
[113] Wilke, A., Bischof, J., Gerlach, W., Glass, E., Harrison, T., Keegan, K.P., Paczian, T., Trimble, W.L., Bagchi, S., Grama, A., Chaterji, S., Meyer, F. (2016) The MG-RAST metagenomics database and portal in 2015. Nucleic Acids Res. 44, D590–D594.
[114] Wynn-Williams, D.D. (1990) Microbial colonization processes in Antarctic fellfield soils: an experimental overview (Eleventh Symposium on Polar Biology). Proc. NIPR Symp. Polar Biol. 3, 164–178.
[115] Yergeau, E., Newsham, K.K., Pearce, D.A., Kowalchuk, G.A. (2007) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. Environ. Microbiol. 9, 2670–2682.
[116] Yuan, H., Ge, T., Chen, C., O'Donnell, A.G., Wu, J. (2012) Significant role for microbial autotrophy in the sequestration of soil carbon. Appl. Environ. Microbiol. 78, 2328–2336.
[117] Yurkov, V., Csotonyi, J.T. (2009) New light on aerobic anoxygenic phototrophs. In: Hunter, C.N., Daldal, F., Thurnauer, M.C., Beatty, J.T. (Eds.), The Purple Phototrophic Bacteria, Springer Netherlands, Dordrecht, pp. 31–55.
[118] Yurkov, V.V., Beatty, J.T. (1998) Aerobic anoxygenic phototrophic bacteria. Microbiol. Mol. Biol. Rev. 62, 695–724.
[119] Yutin, N., Suzuki, M.T., Béjà, O. (2005) Novel primers reveal wider diversity among marine Aerobic anoxygenic phototrophs. Appl. Environ. Microbiol. 71, 8958–8962.
[120] Zazovskaya, E., Fedorov-Davydov, D., Alekseeva, T., Dergacheva, M. (2015) Soils of Queen Maud Land. In: Bockheim, J.G. (Ed.), The Soils of Antarctica, Springer International Publishing, Basel, pp. 21–44.
[121] Zeng, Y., Feng, F., Medová, H., Dean, J., Koblížek, M. (2014) Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum *Gemmatimonadetes*. Proc. Natl. Acad. Sci. 111, 7795–7800.

Supplementary Material

Isolation and characterization of aerobic anoxygenic phototrophs from exposed soils from the Sør Rondane Mountains, East Antarctica

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1. Supplementary Figures and Tables

1.1. Supplementary Figures

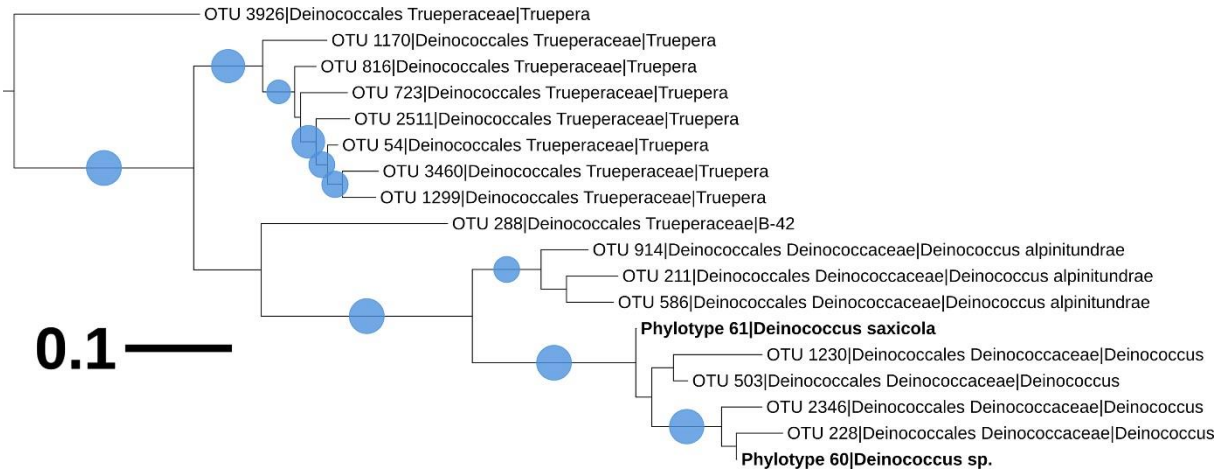


Figure S1. Detailed view of the Deinococcus-Thermus cluster of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

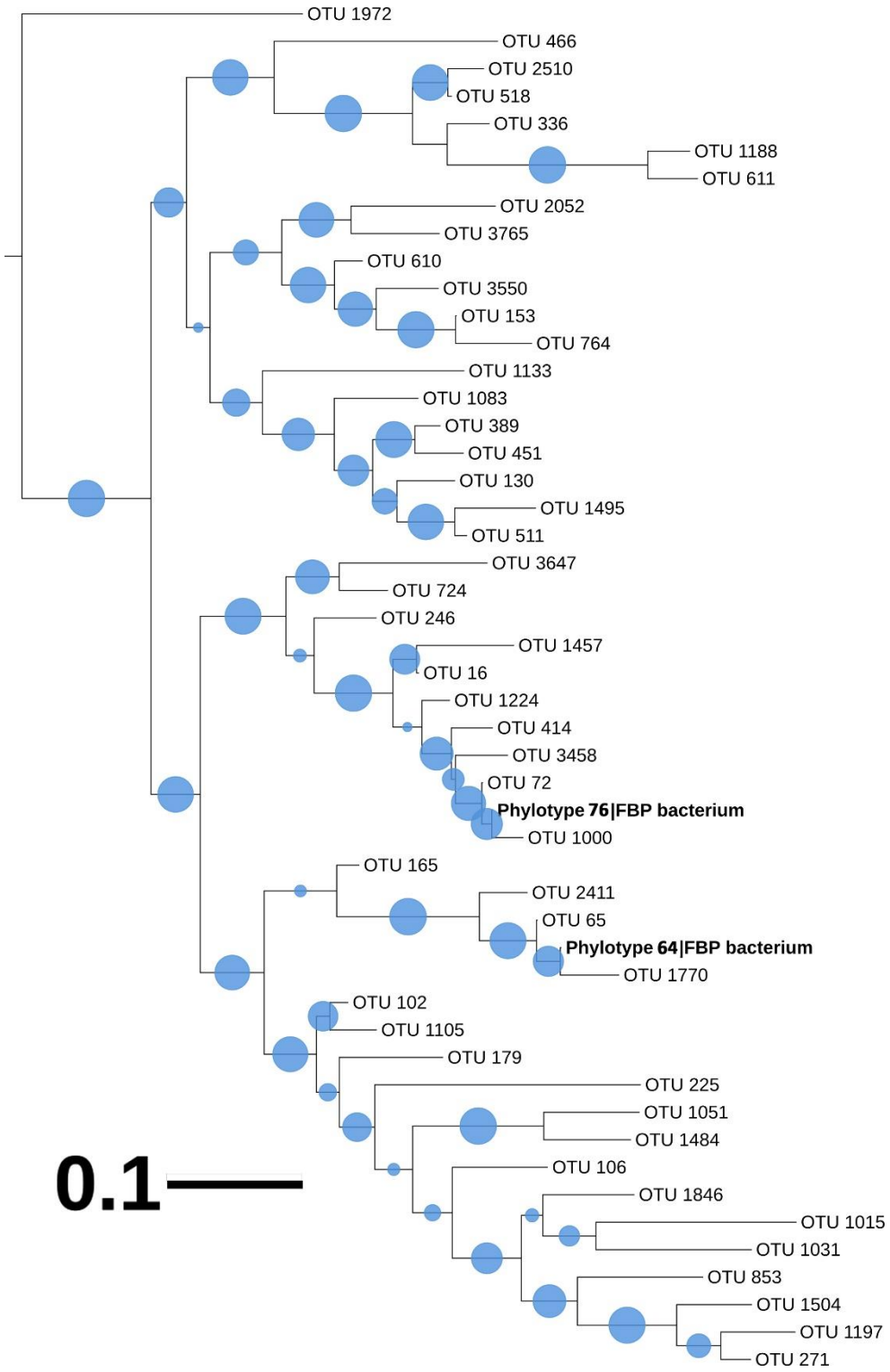


Figure S2. Detailed view of the FBP cluster of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

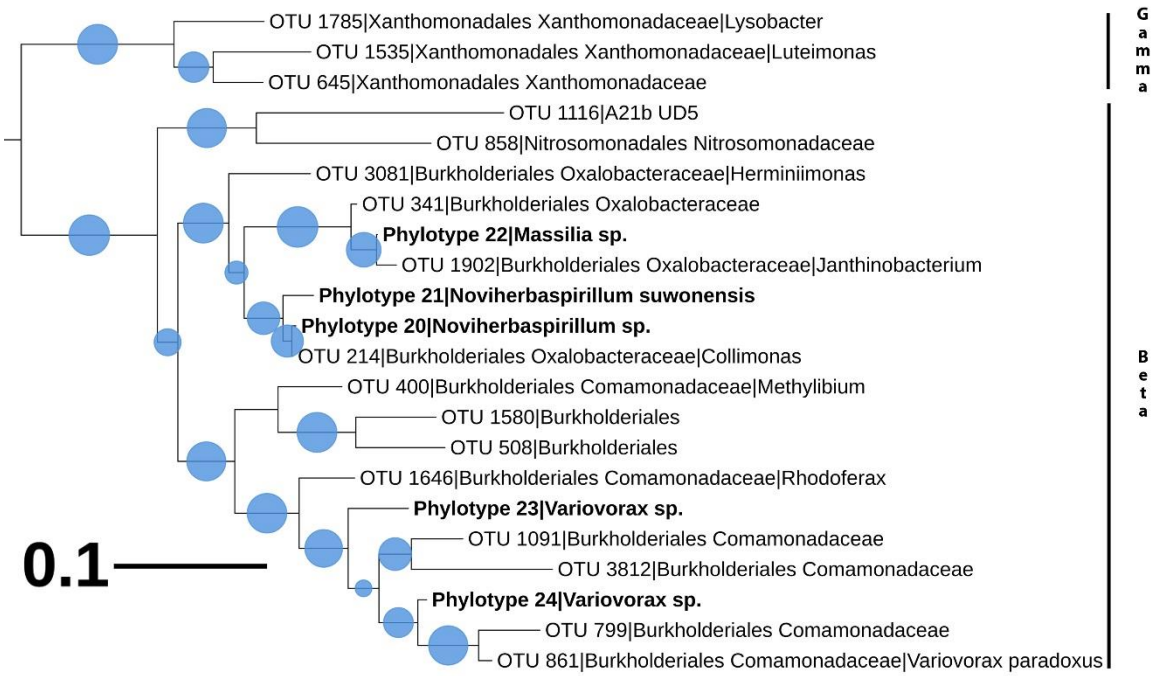


Figure S3. Detailed view of the Beta- and Gammaproteobacteria cluster of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

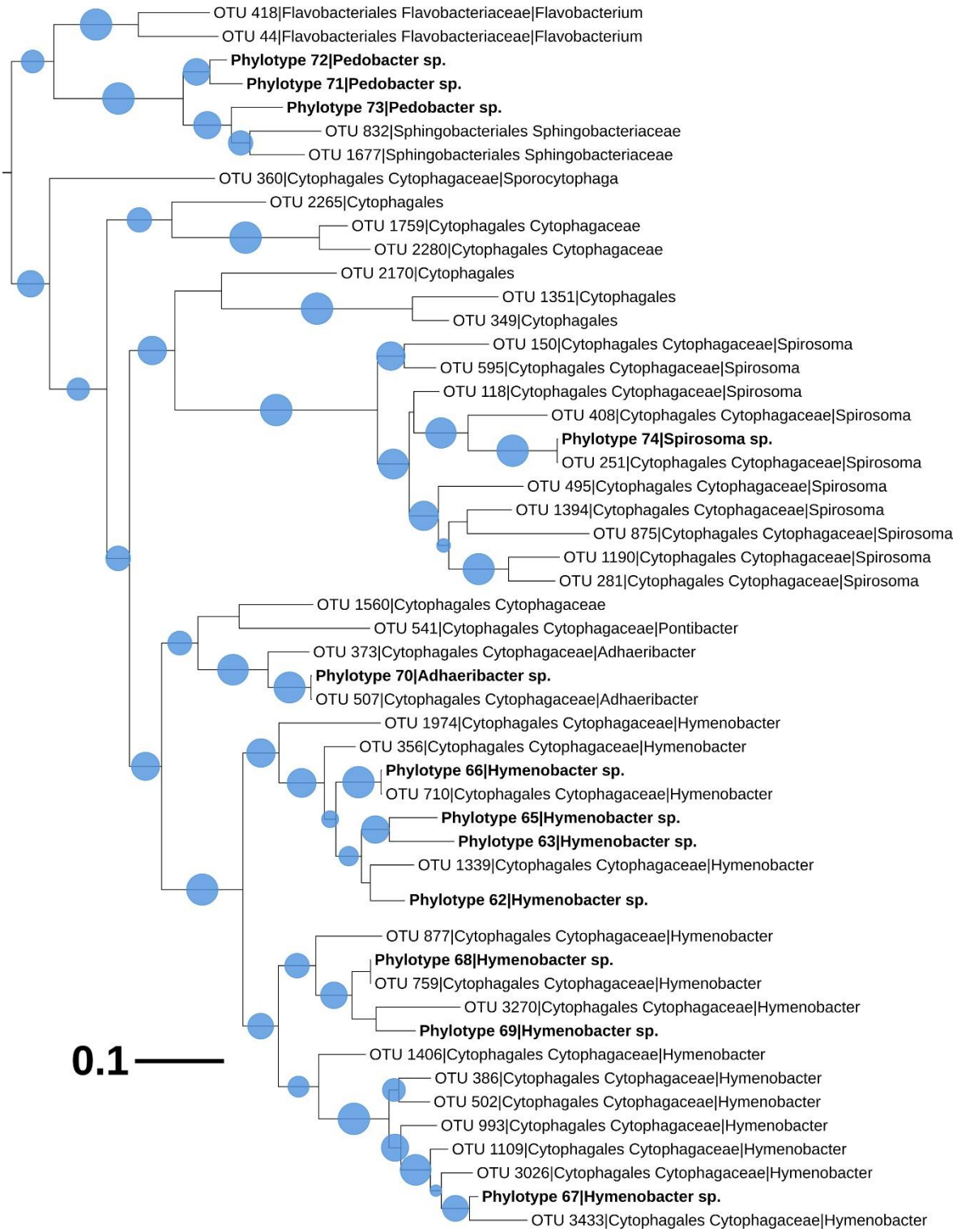


Figure S4. Detailed view of the Bacteroidetes cluster of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Illumina sequences grouping with the orders Saprospirales and Rhodothermales are not included in the view. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

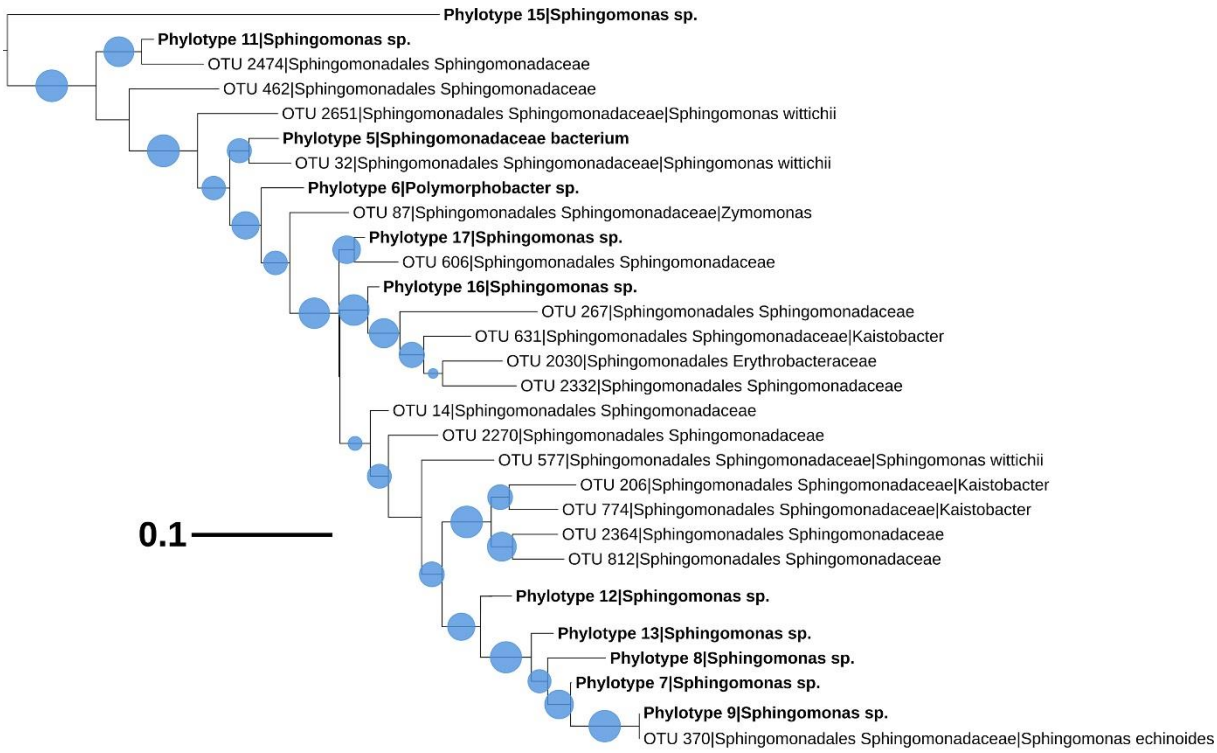


Figure S5. Detailed view of the Sphingomonadales cluster (Alphaproteobacteria) of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

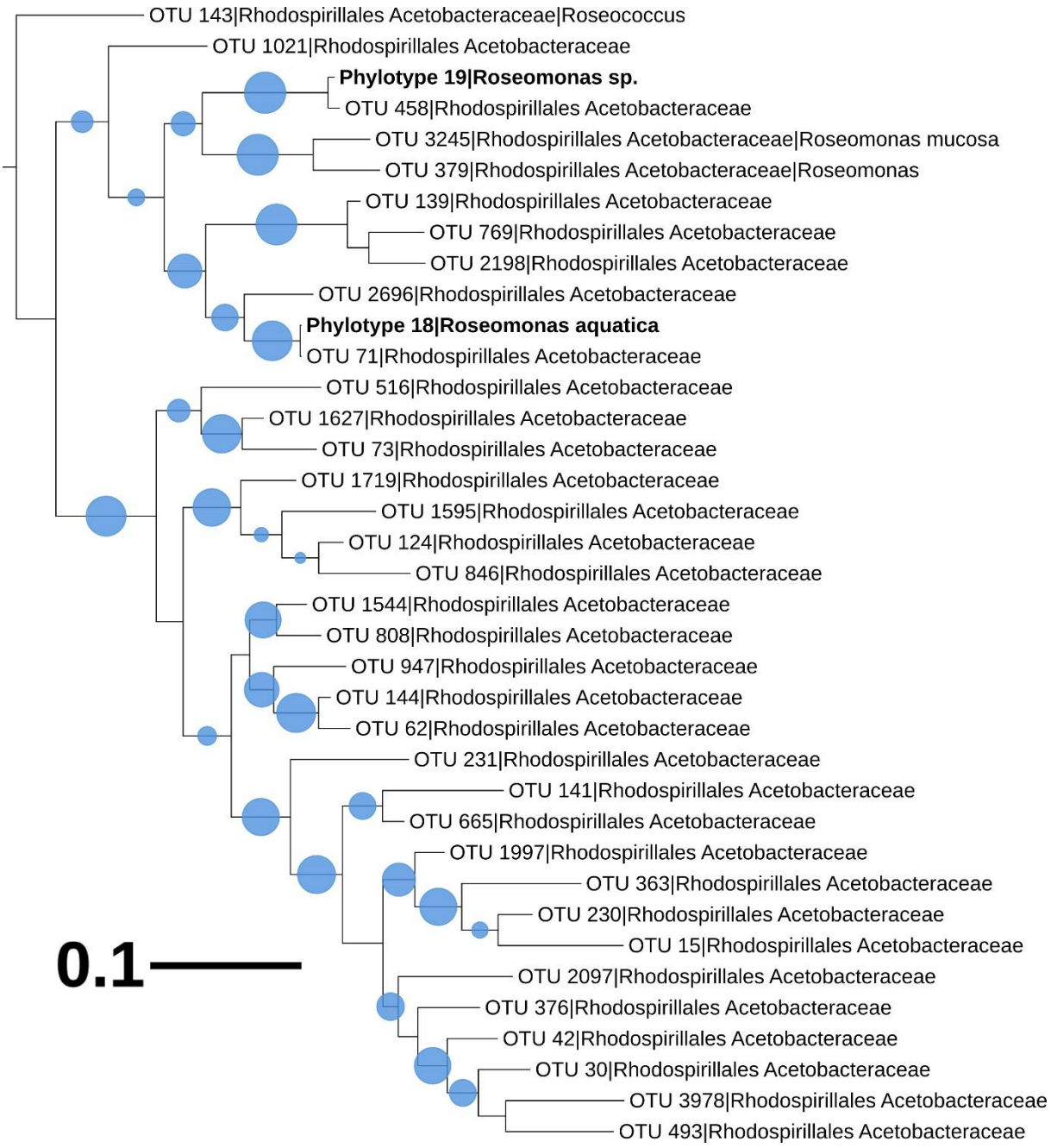


Figure S6. Detailed view of the Rhodospirillales cluster (Alphaproteobacteria) of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

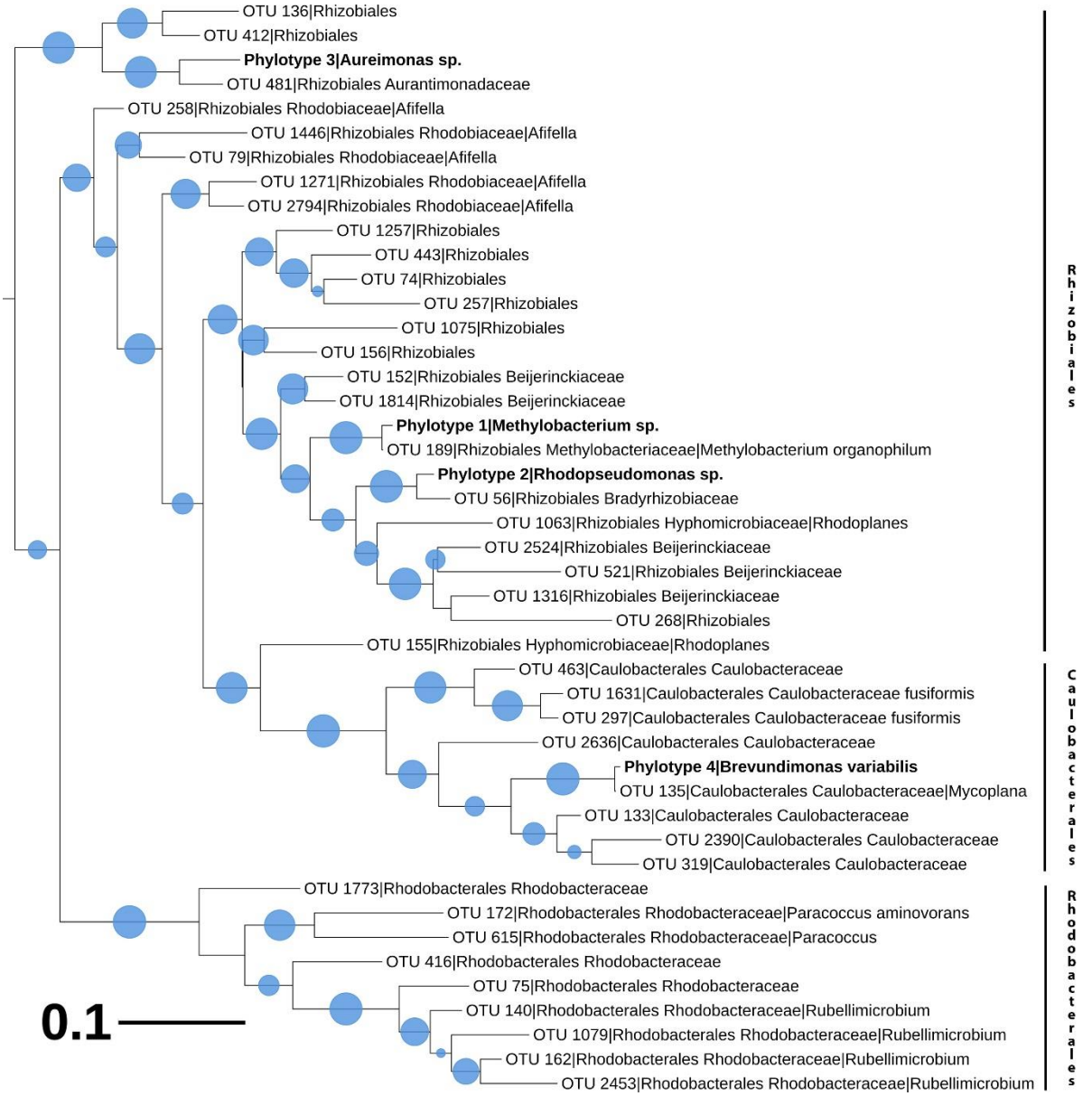


Figure S7. Detailed view of the Caulobacterales, Rhizobiales and Rhodobacterales clusters (Alphaproteobacteria) of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

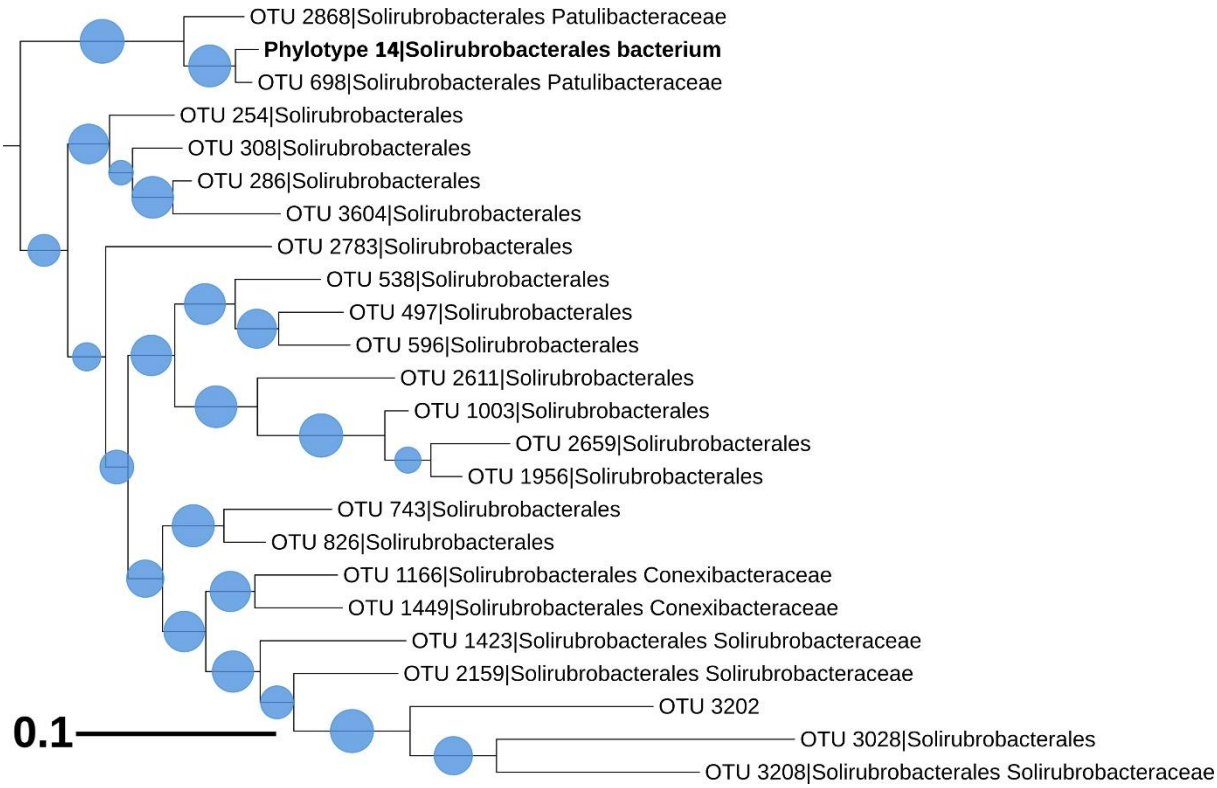
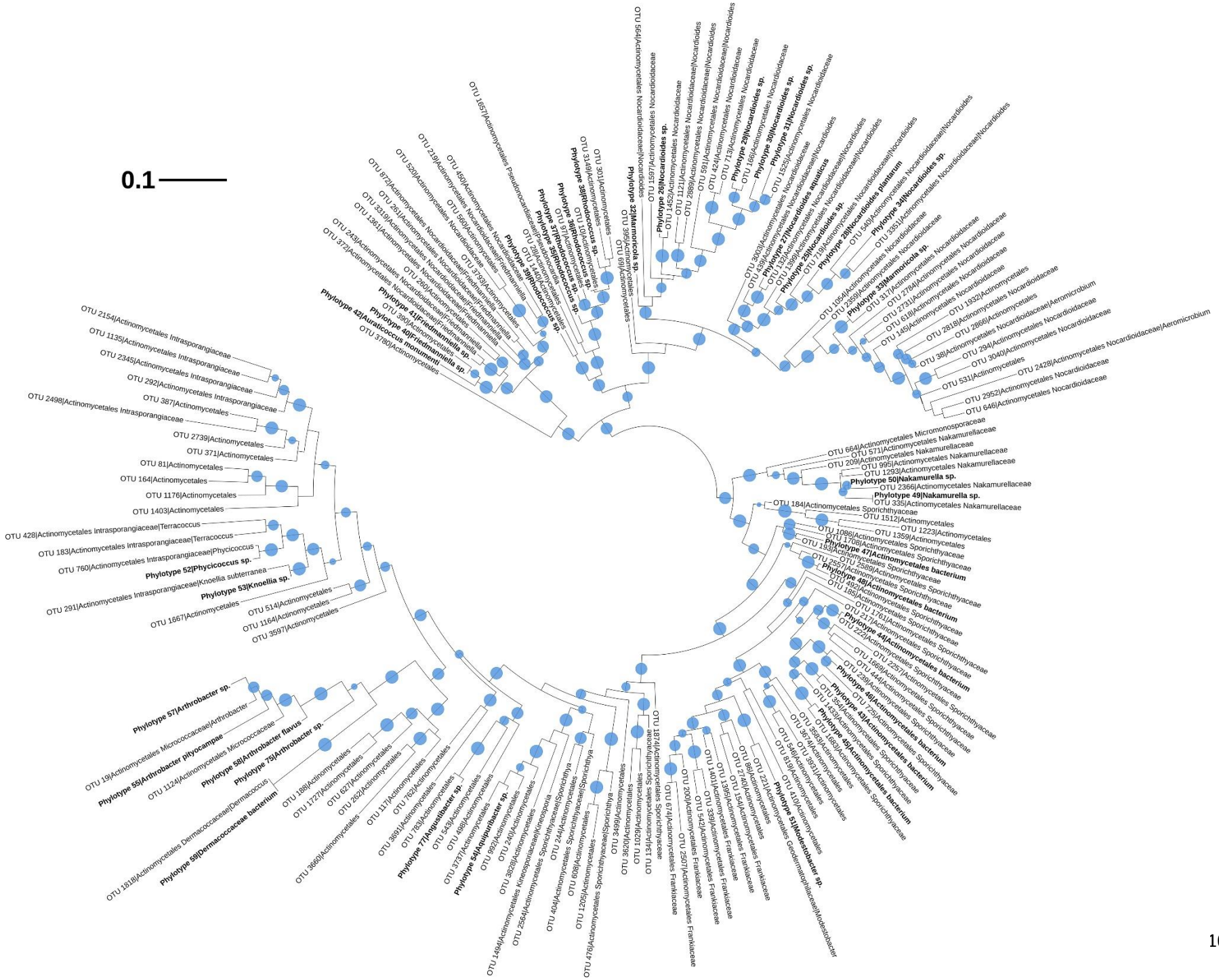


Figure S8. Detailed view of the Solirubrobacterales cluster (Actinobacteria) of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.


0.1
OTU 1654|Actinomycetales Nocardioidaceae|Nocardioides
OTU 1597|Actinomycetales Nocardioidaceae
Phylotype 26|Nocardioides sp.
OTU 1452|Actinomycetales Nocardioidaceae
OTU 1121|Actinomycetales Nocardioidaceae|Nocardioides
OTU 2869|Actinomycetales Nocardioidaceae|Nocardioides
OTU 591|Actinomycetales Nocardioidaceae|Nocardioides
OTU 424|Actinomycetales Nocardioidaceae|Nocardioides
OTU 713|Actinomycetales Nocardioidaceae
Phylotype 29|Nocardioides sp.
OTU 166|Actinomycetales Nocardioidaceae
Phylotype 30|Nocardioides sp.
OTU 1525|Actinomycetales Nocardioidaceae
Phylotype 31|Nocardioides sp.
OTU 1399|Actinomycetales Nocardioidaceae
Phylotype 27|Nocardioides aquaticus
OTU 1324|Actinomycetales Nocardioidaceae|Nocardioides
Phylotype 25|Nocardioides sp.
OTU 719|Actinomycetales Nocardioidaceae
Phylotype 28|Nocardioides plantarum
OTU 540|Actinomycetales Nocardioidaceae|Nocardioides
OTU 3093|Actinomycetales Nocardioidaceae|Nocardioides
OTU 459|Actinomycetales Nocardioidaceae|Nocardioides
Phylotype 34|Nocardioides sp.
OTU 3351|Actinomycetales Nocardioidaceae
OTU 1054|Actinomycetales Nocardioidaceae
OTU 2359|Actinomycetales Nocardioidaceae
Phylotype 35|Marmoricola sp.
OTU 3171|Actinomycetales Nocardioidaceae
OTU 2784|Actinomycetales Nocardioidaceae
OTU 2731|Actinomycetales Nocardioidaceae
OTU 618|Actinomycetales Nocardioidaceae
OTU 1445|Actinomycetales Nocardioidaceae
OTU 1932|Actinomycetales
OTU 2818|Actinomycetales
OTU 2866|Actinomycetales
OTU 38|Actinomycetales Nocardioidaceae|Aeromicrobium
OTU 294|Actinomycetales Nocardioidaceae
OTU 3040|Actinomycetales Nocardioidaceae
OTU 531|Actinomycetales
OTU 2428|Actinomycetales Nocardioidaceae|Aeromicrobium
OTU 2952|Actinomycetales Nocardioidaceae
OTU 646|Actinomycetales Nocardioidaceae
OTU 1857|Actinomycetales Pseudonocardiaceae|Pseudonocardia
OTU 459|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 219|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 520|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 872|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 261|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 3219|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 1361|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 243|Actinomycetales Nocardioidaceae|Friedmanniella
OTU 372|Actinomycetales Nocardioidaceae|Friedmanniella
Phylotype 42|Auraticoccus monumenti
Phylotype 41|Friedmanniella sp.
OTU 380|Actinomycetales
Phylotype 40|Friedmanniella sp.
OTU 3780|Actinomycetales
Phylotype 39|Rhodococcus sp.
Phylotype 37|Rhodococcus sp.
Phylotype 36|Rhodococcus sp.
Phylotype 38|Rhodococcus sp.
OTU 3149|Actinomycetales
OTU 3011|Actinomycetales
OTU 395|Actinomycetales
OTU 69|Actinomycetales
OTU 2154|Actinomycetales Intrasporangiaceae
OTU 1135|Actinomycetales Intrasporangiaceae
OTU 2345|Actinomycetales Intrasporangiaceae
OTU 292|Actinomycetales Intrasporangiaceae
OTU 2498|Actinomycetales Intrasporangiaceae
OTU 387|Actinomycetales
OTU 2739|Actinomycetales
OTU 371|Actinomycetales
OTU 81|Actinomycetales
OTU 164|Actinomycetales
OTU 1176|Actinomycetales
OTU 1403|Actinomycetales
OTU 428|Actinomycetales Intrasporangiaceae|Terracoccus
OTU 183|Actinomycetales Intrasporangiaceae|Terracoccus
OTU 760|Actinomycetales Intrasporangiaceae|Phycicoccus
Phylotype 52|Phycicoccus sp.
OTU 291|Actinomycetales Intrasporangiaceae|Knoellia subterranea
Phylotype 53|Knoellia sp.
OTU 1667|Actinomycetales
OTU 514|Actinomycetales
OTU 1164|Actinomycetales
OTU 3597|Actinomycetales
OTU 664|Actinomycetales Micromonosporaceae
OTU 571|Actinomycetales Nakamurellaceae
OTU 209|Actinomycetales Nakamurellaceae
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OTU 1293|Actinomycetales Nakamurellaceae
Phylotype 50|Nakamurella sp.
OTU 2366|Actinomycetales Nakamurellaceae
Phylotype 49|Nakamurella sp.
OTU 335|Actinomycetales Nakamurellaceae
OTU 184|Actinomycetales Sporichthyaceae
OTU 1512|Actinomycetales
OTU 1223|Actinomycetales
OTU 1359|Actinomycetales
OTU 1086|Actinomycetales
OTU 1708|Actinomycetales Sporichthyaceae
Phylotype 47|Actinomycetales bacterium
OTU 1853|Actinomycetales Sporichthyaceae
OTU 2589|Actinomycetales Sporichthyaceae
OTU 2557|Actinomycetales Sporichthyaceae
Phylotype 48|Actinomycetales bacterium
OTU 1495|Actinomycetales Sporichthyaceae
OTU 1861|Actinomycetales Sporichthyaceae
OTU 2171|Actinomycetales Sporichthyaceae
OTU 1761|Actinomycetales Sporichthyaceae
Phylotype 44|Actinomycetales bacterium
OTU 2225|Actinomycetales Sporichthyaceae
OTU 2257|Actinomycetales Sporichthyaceae
OTU 1669|Actinomycetales Sporichthyaceae
OTU 1444|Actinomycetales Sporichthyaceae
Phylotype 46|Actinomycetales bacterium
OTU 725|Actinomycetales Sporichthyaceae
OTU 354|Actinomycetales Sporichthyaceae
OTU 1453|Actinomycetales Sporichthyaceae
Phylotype 45|Actinomycetales bacterium
OTU 1683|Actinomycetales Sporichthyaceae
OTU 3883|Actinomycetales
OTU 3674|Actinomycetales
OTU 399|Actinomycetales
OTU 5469|Actinomycetales
OTU 419|Actinomycetales
OTU 410|Actinomycetales
Phylotype 51|Modestobacter sp.
OTU 1395|Actinomycetales Geodermatophilaceae|Modestobacter
OTU 2740|Actinomycetales Frankiaceae
OTU 154|Actinomycetales Frankiaceae
OTU 98|Actinomycetales Frankiaceae
OTU 221|Actinomycetales
OTU 1401|Actinomycetales Frankiaceae
OTU 339|Actinomycetales Frankiaceae
OTU 542|Actinomycetales Frankiaceae
OTU 200|Actinomycetales Frankiaceae
OTU 2507|Actinomycetales Frankiaceae
OTU 674|Actinomycetales Frankiaceae
OTU 1874|Actinomycetales Sporichthyaceae
OTU 1134|Actinomycetales Sporichthyaceae
OTU 1029|Actinomycetales
OTU 3620|Actinomycetales
OTU 3499|Actinomycetales
Phylotype 24|Sporichthya
OTU 476|Actinomycetales Sporichthyaceae|Sporichthya
OTU 1205|Actinomycetales
OTU 608|Actinomycetales
OTU 244|Actinomycetales Sporichthyaceae|Sporichthya
OTU 404|Actinomycetales
OTU 2564|Actinomycetales Sporichthyaceae|Kineosporia
OTU 1494|Actinomycetales Kineosporiaceae|Kineosporia
OTU 3823|Actinomycetales
OTU 240|Actinomycetales
OTU 992|Actinomycetales
Phylotype 54|Aquipuribacter sp.
OTU 543|Actinomycetales
OTU 496|Actinomycetales
OTU 3737|Actinomycetales
Phylotype 77|Angustibacter sp.
OTU 783|Actinomycetales
OTU 3691|Actinomycetales
OTU 762|Actinomycetales
OTU 117|Actinomycetales
OTU 3660|Actinomycetales
OTU 262|Actinomycetales
OTU 627|Actinomycetales
OTU 1727|Actinomycetales
OTU 188|Actinomycetales
OTU 1818|Actinomycetales Dermacoccaceae|Dermacoccus
Phylotype 59|Dermacoccaceae bacterium
Phylotype 75|Arthrobacter sp.
Phylotype 58|Arthrobacter flavus
OTU 1124|Actinomycetales Micrococcaceae
Phylotype 55|Arthrobacter pityocampae
OTU 19|Actinomycetales Micrococcaceae|Arthrobacter
Phylotype 57|Arthrobacter sp.

Figure S9. Detailed view of the Actinomycetales cluster (Actinobacteria) of the maximum likelihood phylogenetic tree (1000 bootstraps) combining partial 16S rRNA gene sequences obtained using a culture-independent and –dependent approach. Representatives of the isolate phylotypes are labelled in bold. For Illumina OTUs [Tahon et al. Submitted for publication], identifications at the most detailed taxonomic level are given. Scale bar indicates 0.1 substitutions per position. Bootstrap values are displayed as circles with a diameter reflecting the height of the bootstrap value. Smallest circles represent the lower cutoff of 50%.

1.2. Supplementary Tables

Table S1. Number of isolates per condition.

	4 °C			15 °C		
PH medium	Solid	Liquid shaken	Liquid not-shaken	Solid	Liquid shaken	Liquid not-shaken
KP2	45	30	30	45	20	30
KP15	30	30	30	45	20	20
KP43	45	32	30	45	30	30
KP53	30	32	30	45	30	25
	4 °C			15 °C		
PA medium	Solid	Liquid shaken	Liquid not-shaken	Solid	Liquid shaken	Liquid not-shaken
KP2	35	38	38	45	20	20
KP15	41	44	34	45	20	30
KP43	45	30	30	45	20	20
KP53	30	30	28	45	20	20

Table S2. Detailed overview of all 330 reference isolates enclosed in the 77 phylotypes listed in Table 2. For each representative strain, strain number, 16S rRNA phylotype, classification, results of protein encoding gene PCRs (+: positive, -: negative) and information on the nearest phylogenetic neighbor are given. For the representative strain of each phylotype, the percentage of 16S rRNA gene sequence similarity with the closest phylogenetic neighbor is given as well as the accession number of the 16S rRNA gene of the neighbor.

Table S2 is provided in a separate file (Table S2.xlsx).

Table S3. Recovery of isolates from the different isolation conditions. Identifications based on the MALDI-TOF MS dendrogram combined with 16S rRNA gene sequencing. PH: medium with carbon sources (glucose, sucrose, sodium succinate, sodium pyruvate, sodium acetate and malate, 0.5 mM each). PA: medium without carbon sources. A: agar, S: shaken liquid setup, NS: non-shaken liquid setup.

Identification	**Together**												
	4 °C						**15 °C**						
	PH			**PA**			**PH**			**PA**			
	A	**S**	**NS**	**A**	**S**	**NS**	**A**	**S**	**NS**	**A**	**S**	**NS**	**Total no. of isolates**
Proteobacteria													
Alphaproteobacteria													
Aureimonas sp.	0	2	2	0	0	1	0	0	0	0	0	0	5
Brevundimonas variabilis.	0	1	3	0	3	5	0	0	14	1	0	0	27
Methylobacterium sp.	0	0	5	0	14	6	1	0	0	0	0	0	26
Polymorphobacter sp.	0	0	0	0	0	0	0	0	0	0	0	1	1
Rhodopseudomonas sp.	0	0	0	0	0	0	0	0	0	0	0	1	1
Roseomonas sp.	0	0	0	0	0	1	1	0	0	0	0	0	2
Sphingomonadaceae bacterium	0	0	0	0	0	0	0	0	0	0	0	4	4
Sphingomonas sp.	35	62	51	25	34	30	26	40	73	5	7	9	397
Betaproteobacteria													
Noviherbaspirillum sp.	0	0	0	1	2	1	0	0	0	0	0	0	4
Massilia sp.	0	0	0	3	0	0	0	0	0	0	0	0	3
Variovorax sp.	0	2	0	1	1	2	0	0	0	0	4	3	13
Actinobacteria													
Actinomycetales bacterium	0	0	0	0	0	2	13	0	0	6	0	3	24
Angustibacter sp.	0	0	0	0	0	0	1	0	0	0	0	0	1
Aquipuribacter sp.	0	0	0	0	0	0	1	0	0	6	0	0	7
Arthrobacter sp.	12	1	4	19	5	6	17	0	0	19	0	0	83
Auraticoccus monumenti	0	0	0	0	0	0	2	0	0	0	0	0	2
Dermacoccaceae bacterium	0	0	0	0	0	0	4	0	0	0	0	0	4
Friedmanniella sp.	0	0	2	0	1	0	2	0	0	5	0	0	10
Geodermatophilus sp.	1	0	0	0	0	0	0	0	0	0	0	0	1
Knoellia sp.	3	0	0	0	0	0	0	0	0	0	0	0	3
Marmoricola sp.	0	0	0	0	0	1	2	0	0	8	0	0	11
Modestobacter sp.	1	3	0	0	3	0	3	0	0	6	0	0	16
Nakamurella sp.	0	0	0	0	0	0	1	0	0	8	0	0	9
Nocardioides sp.	11	5	1	5	9	11	11	3	5	20	0	2	83
Phycicoccus sp.	3	3	0	7	0	0	1	4	0	0	5	0	23
Rhodococcus sp.	0	0	0	9	1	5	3	5	0	1	0	2	26
Solirubrobacterales bacterium	0	0	0	0	0	0	1	0	0	0	0	0	1

Identification	Together												
	4 °C						15 °C						
	PH			PA			PH			PA			
	A	S	NS	A	S	NS	A	S	NS	A	S	NS	Total no. of isolates
Deinococcus-Thermus													
Deinococcus sp.	0	0	0	0	9	4	2	0	0	0	0	0	15
Firmicutes													
Paenibacillus sp.	0	0	0	0	0	0	1	0	0	0	0	0	1
FBP													
FBP bacterium	0	0	0	0	0	0	2	0	0	0	0	0	2
Bacteroidetes													
Adhaeribacter sp.	0	0	0	0	0	0	1	0	0	0	0	0	1
Hymenobacter sp.	4	5	3	3	9	11	3	0	0	1	1	0	40
Pedobacter sp.	1	0	0	3	0	0	4	1	0	0	1	0	10
Spirosoma sp.	2	2	4	5	3	3	0	0	0	1	10	6	36

			Nearest phylogenetic neighbor					Taxonomy			
Phylotype	Strain	Accession no.	ID	Sequence similarity (%)	Accession number	*nifH /bchL /bchX*	*pufLM*	Phylum	Class	Order	Family
1	R-68457	KY386309	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	KP15.18.4.PA.S	KY386323	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	R-68525	KY386327	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	KP15.21.4.PA.S	KY386331	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	R-68173	KY386380	Methylobacterium iners 5317S-33	98,52	EF174497	+	-	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	R-68388	KY386396	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	R-68391	KY386399	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	R-68554	KY386402	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	R-68669	KY386414	Methylobacterium iners 5317S-33			+	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
1	KP2.9b.4.PA.S	KY386471	Methylobacterium iners 5317S-33			-	+	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteraceae
2	R-67878	KY386506	Rhodopseudomonas pseudopalustris DSM123	98,67	AB498818	-	-	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae
3	R-68373	KY386318	Aureimonas ferruginae CC-CFT023	96,64	JQ864240	+	-	Proteobacteria	Alphaproteobacteria	Rhizobiales	Aurantimonadaceae
4	R-68476	KY386398	Brevundimonas variabilis ATCC15255			-	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-68394	KY386404	Brevundimonas variabilis ATCC15255			-	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	KP2.19.15.PH.NS	KY386407	Brevundimonas variabilis ATCC15255			-	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-68473	KY386409	Brevundimonas variabilis ATCC15255			+	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-68297	KY386412	Brevundimonas variabilis ATCC15255			-	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-68298	KY386416	Brevundimonas variabilis ATCC15255			+	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-68299	KY386422	Brevundimonas variabilis ATCC15255			+	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-68483	KY386436	Brevundimonas variabilis ATCC15255			+	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-67742	KY386439	Brevundimonas variabilis ATCC15255			+	-	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
4	R-68295	KY386469	Brevundimonas variabilis ATCC15255	99,79	AJ227783	+	+	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae
5	R-67883	KY386496	Polymorphobacter multimanifer 262-7	94,94	AB649056	+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
5	KP53.16.15.PA.NS	KY386580	Polymorphobacter multimanifer 262-7			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
6	R-68699	KY386562	Polymorphobacter multimanifer 262-7	95,99	AB649056	-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68084	KY386306	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	KP15.16.4.PA.V	KY386315	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-67991	KY386320	Sphingomonas faeni MA-olki			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68089	KY386319	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68414	KY386322	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68418	KY386339	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68527	KY386348	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68539	KY386359	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-67789	KY386360	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68448	KY386370	Sphingomonas faeni MA-olki			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68170	KY386374	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-67846	KY386377	Sphingomonas faeni MA-olki			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-67984	KY386379	Sphingomonas faeni MA-olki	99,62	AJ429239	+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	KP2.34b.15.PH.V	KY386451	Sphingomonas faeni MA-olki			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-67744	KY386468	Sphingomonas faeni MA-olki			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	KP43.10b.4.PH.V	KY386477	Sphingomonas faeni MA-olki			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68208	KY386485	Sphingomonas faeni MA-olki			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
7	R-68340	KY386497	Sphingomonas faeni MA-olki			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
8	R-68700	KY386453	Sphingomonas oligophenolica JCM 12082	98,24	AB018439	-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68087	KY386314	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68461	KY386351	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	KP2.23.15.PH.NS	KY386419	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68261	KY386466	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68499	KY386473	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68344	KY386472	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68493	KY386479	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68425	KY386482	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68427	KY386487	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68341	KY386490	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68103	KY386491	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68279	KY386493	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae

9	R-68104	KY386499	Sphingomonas aerolata NW12			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68435	KY386501	Sphingomonas aerolata NW12			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68105	KY386502	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68335	KY386511	Sphingomonas aerolata NW12			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68336	KY386515	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	KP43.28.4.PH.NS	KY386528	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68566	KY386531	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68274	KY386532	Sphingomonas aerolata NW12	99,09	AJ429240	-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68308	KY386534	Sphingomonas aerolata NW12			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68023	KY386540	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68025	KY386544	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68275	KY386549	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68232	KY386553	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	KP43.5.15.PH.S	KY386557	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68305	KY386561	Sphingomonas aerolata NW12			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68495	KY386563	Sphingomonas aerolata NW12			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68343	KY386567	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68309	KY386568	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-67894	KY386575	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	KP53.14.4.PH.S	KY386576	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68345	KY386579	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68383	KY386582	Sphingomonas aerolata NW12			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68291	KY386584	Sphingomonas aerolata NW12			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68245	KY386586	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68129	KY386587	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68246	KY386589	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68445	KY386594	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68042	KY386595	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68381	KY386598	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68380	KY386601	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68046	KY386603	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68578	KY386606	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68351	KY386607	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68574	KY386613	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68036	KY386622	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68349	KY386621	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68512	KY386624	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68037	KY386625	Sphingomonas aerolata NW12			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	KP53.9.15.PH.S	KY386626	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	R-68348	KY386628	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
9	KP53.9a.4.PH.NS	KY386629	Sphingomonas aerolata NW12			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
11	R-68222	KY386535	Sphingomonas yunnanensis YIM 003	97,52	AY894691	-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68269	KY386302	Sphingomonas cynarae SPC-1			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68271	KY386310	Sphingomonas cynarae SPC-1			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68375	KY386329	Sphingomonas cynarae SPC-1			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68304	KY386332	Sphingomonas cynarae SPC-1	98,24	HQ439186	-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-67996	KY386342	Sphingomonas cynarae SPC-1			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68199	KY386373	Sphingomonas cynarae SPC-1			+	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68171	KY386378	Sphingomonas cynarae SPC-1			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68355	KY386381	Sphingomonas cynarae SPC-1			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68392	KY386400	Sphingomonas cynarae SPC-1			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	R-68317	KY386417	Sphingomonas cynarae SPC-1			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	KP2.25b.4.PA.NS	KY386428	Sphingomonas cynarae SPC-1			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
12	KP2.32b.4.PA.NS	KY386447	Sphingomonas cynarae SPC-1			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
13	R-68091	KY386335	Sphingomonas mucosissima CP173-2			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
13	R-67999	KY386352	Sphingomonas mucosissima CP173-2			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
13	R-68524	KY386357	Sphingomonas mucosissima CP173-2	98,27	AM229669	-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
15	R-67917	KY386305	Sphingomonas yantingensis 1007			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae

15	R-68270	KY386304	Sphingomonas yantingensis 1007	96,79	JX566547	+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
15	KP15.19.15.PH.S	KY386324	Sphingomonas yantingensis 1007			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
15	KP15.20.15.PH.V	KY386328	Sphingomonas yantingensis 1007			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
15	R-67859	KY386337	Sphingomonas yantingensis 1007			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
15	R-68007	KY386366	Sphingomonas yantingensis 1007			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
15	R-68267	KY386368	Sphingomonas yantingensis 1007			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68405	KY386312	Sphingomonas hunanensis JSM 083058			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-67790	KY386362	Sphingomonas hunanensis JSM 083058			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	KP15.4.15.PH.NS	KY386367	Sphingomonas hunanensis JSM 083058			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68410	KY386375	Sphingomonas hunanensis JSM 083058			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68292	KY386383	Sphingomonas hunanensis JSM 083058			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68553	KY386406	Sphingomonas hunanensis JSM 083058			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68552	KY386415	Sphingomonas hunanensis JSM 083058			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68400	KY386424	Sphingomonas hunanensis JSM 083058			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68370	KY386440	Sphingomonas hunanensis JSM 083058			-	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68161	KY386455	Sphingomonas hunanensis JSM 083058			+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68260	KY386460	Sphingomonas hunanensis JSM 083058	98,52	FJ527417	+	+	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
16	R-68242	KY386578	Sphingomonas hunanensis JSM 083058			-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
17	R-67954	KY386585	Sphingomonas pituitosa EDIV	95,58	AJ243751	-	-	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae
18	R-68475	KY386403	Roseomonas aquatica TR53	99,93	AM231587	-	+	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae
19	R-68165	KY386462	Roseomonas tokyonensis K-20	98,6	AB297501	+	-	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae
20	R-67997	KY386345	Noviherbaspirillum psychrotolerans PB1	98,62	JN390675	-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
20	R-68490	KY386488	Noviherbaspirillum psychrotolerans PB1			-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
21	R-68579	KY386599	Noviherbaspirillum suwonense 54105-65	98,95	JX275858	-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
22	R-67978	KY386448	Massilia eurypsychrophila B528-3	97,7	KJ361504	-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
22	R-67982	KY386454	Massilia eurypsychrophila B528-3			-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
23	R-67959	KY386465	Variovorax boronicumulans BAM-48			-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
23	R-67932	KY386486	Variovorax boronicumulans BAM-48	97,55	AB300597	-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
23	KP43.23.4.PA.S	KY386522	Variovorax boronicumulans BAM-48			-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
23	R-68338	KY386543	Variovorax boronicumulans BAM-48			-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
23	R-67927	KY386558	Variovorax boronicumulans BAM-48			-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
24	R-67871	KY386393	Variovorax ginsengisoli Gsoil 3165	99,26	AB245358	-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
24	R-67891	KY386627	Variovorax ginsengisoli Gsoil 3165			-	-	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
25	KP15.17.15.PA.V	KY386317	Nocardioides ginsengagri BX5-10	97,38	GQ339904	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
25	R-68562	KY386518	Nocardioides ginsengagri BX5-10			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
26	R-68482	KY386444	Nocardioides salarius CL-Z59	97	DQ401092	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
27	R-68162	KY386458	Nocardioides aquaticus EL-17K	99,38	X94145	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
28	KP43.13.15.PH.NS	KY386484	Nocardioides plantarum NCIMB 12834	100	AF005008	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
28	R-68307	KY386526	Nocardioides plantarum NCIMB 12834			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
28	KP43.9.15.PH.NS	KY386564	Nocardioides plantarum NCIMB 12834			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	KP2.10.4.PA.S	KY386388	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68053	KY386389	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-67906	KY386390	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	KP2.14.4.PH.V	KY386397	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68364	KY386401	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68474	KY386405	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68550	KY386421	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68154	KY386423	Nocardioides terrigena DS-17	97,79	EF363712	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-67971	KY386425	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68065	KY386430	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-67865	KY386438	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-67756	KY386443	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68140	KY386461	Nocardioides terrigena DS-17			+	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
29	R-68014	KY386492	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
30	R-67827	KY386592	Nocardioides terrigena DS-17	96,06	EF363712	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
31	R-68372	KY386385	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
31	R-68145	KY386386	Nocardioides terrigena DS-17	97,52	EF363712	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
31	R-67747	KY386391	Nocardioides terrigena DS-17			+	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae

31	KP2.23.15.PH.V	KY386420	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
31	R-68158	KY386446	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
31	R-68160	KY386452	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
31	R-68167	KY386463	Nocardioides terrigena DS-17			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
32	R-67776	KY386307	Marmoricola korecus Sco-A36			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
32	R-67781	KY386333	Marmoricola korecus Sco-A36	98,67	FN386723	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
33	R-68467	KY386387	Marmoricola aquaticus CBMAI 1089			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
33	R-67804	KY386524	Marmoricola aquaticus CBMAI 1089	97,75	JN615437	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-67800	KY386498	Nocardioides antarcticus M-SA3-94	97,76	KM347967	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68564	KY386504	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68503	KY386512	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68432	KY386521	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68485	KY386533	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68233	KY386554	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68486	KY386559	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68497	KY386560	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
34	R-68352	KY386605	Nocardioides antarcticus M-SA3-94			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocerdioidaceae
35	R-68019	KY386523	Rhodococcus aerolatus PAMC 27367	96,34	KM044053	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
35	R-68235	KY386556	Rhodococcus aerolatus PAMC 27367			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
36	R-68187	KY386338	Rhodococcus aerolatus PAMC 27367	95,64	KM044053	+	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
37	R-68509	KY386574	Rhodococcus aerolatus PAMC 27367	95,92	KM044053	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
38	R-67872	KY386395	Rhodococcus aerolatus PAMC 27367	95,85	KM044053	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
38	R-68258	KY386617	Rhodococcus aerolatus PAMC 27367			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
39	R-68460	KY386303	Rhodococcus fascians LMG 3623			+	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
39	R-68273	KY386321	Rhodococcus fascians LMG 3623	98,77	JMEN01000010	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
39	R-68530	KY386341	Rhodococcus fascians LMG 3623			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
39	R-68463	KY386344	Rhodococcus fascians LMG 3623			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
39	KP15.30.4.PA.NS	KY386358	Rhodococcus fascians LMG 3623			+	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
39	R-68196	KY386364	Rhodococcus fascians LMG 3623			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
39	R-68515	KY386604	Rhodococcus fascians LMG 3623			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae
40	R-68537	KY386361	Friedmanniella sagamiharensis FB2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae
40	R-67749	KY386408	Friedmanniella sagamiharensis FB2	96,09	AB445456	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae
40	R-67754	KY386434	Friedmanniella sagamiharensis FB2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae
40	R-67745	KY386470	Friedmanniella sagamiharensis FB2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae
40	R-68220	KY386525	Friedmanniella sagamiharensis FB2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae
41	R-68221	KY386527	Friedmanniella luteola FA1	98,22	AB445453	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae
42	R-68201	KY386505	Auraticoccus monumenti MON 2.2	99,58	FN552748	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae
43	R-67786	KY386350	Jatrophihabitans endophyticus S9650	93,6	JQ346802	-	-	Actinobacteria	Actinobacteria	Actinomycetales	
43	R-68511	KY386570	Jatrophihabitans endophyticus S9650			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
44	R-68701	KY386494	Jatrophihabitans soli KIS75-12	94,37	KP017569	-	-	Actinobacteria	Actinobacteria	Actinomycetales	
45	R-68182	KY386325	Frankia alni ACN14A			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
45	R-68183	KY386330	Frankia alni ACN14A	94,11	CT573213	-	-	Actinobacteria	Actinobacteria	Actinomycetales	
45	R-67752	KY386426	Frankia alni ACN14A			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
45	R-67882	KY386481	Frankia alni ACN14A			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
45	R-68215	KY386514	Frankia alni ACN14A			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
45	R-67881	KY386566	Frankia alni ACN14A			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
45	R-67888	KY386611	Frankia alni ACN14A			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
46	R-67810	KY386542	Cryptosporangium minutisporangium IFO15962	94,41	AB037007	-	-	Actinobacteria	Actinobacteria	Actinomycetales	
47	R-68148	KY386394	Modestobacter versicolor CP153-2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
47	R-67836	KY386609	Modestobacter versicolor CP153-2	94,28	AJ871304	-	-	Actinobacteria	Actinobacteria	Actinomycetales	
47	R-68237	KY386619	Modestobacter versicolor CP153-2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
48	R-68223	KY386537	Sporichthya brevicatena IFO 16195	93,78	AB006164	-	-	Actinobacteria	Actinobacteria	Actinomycetales	
48	R-68238	KY386569	Sporichthya brevicatena IFO 16195			-	-	Actinobacteria	Actinobacteria	Actinomycetales	
49	KP15.26.15.PA.V	KY386343	Nakamurella lactae DLS-10			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae
49	R-68216	KY386516	Nakamurella lactae DLS-10	98,07	AM778124	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae
50	R-67826	KY386590	Nakamurella lactae DLS-10			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae
50	R-67832	KY386596	Nakamurella lactae DLS-10			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae
50	R-67838	KY386610	Nakamurella lactae DLS-10	98,57	AM778124	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae

51	R-68190	KY386347	Modestobacter lapidis MON 3.1			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
51	R-68535	KY386365	Modestobacter lapidis MON 3.1			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
51	R-68354	KY386382	Modestobacter lapidis MON 3.1			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
51	R-67763	KY386459	Modestobacter lapidis MON 3.1			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
51	R-68492	KY386483	Modestobacter lapidis MON 3.1			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
51	R-68563	KY386513	Modestobacter lapidis MON 3.1			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
51	R-68230	KY386550	Modestobacter lapidis MON 3.1	97,28	LN810544	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
52	R-68264	KY386392	Phycicoccus ochangensis L1b-b9	98,09	GQ344405	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	R-68327	KY386427	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	R-68064	KY386429	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	R-67977	KY386445	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	KP2.38.4.PH.V	KY386456	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	R-67902	KY386464	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	R-68010	KY386474	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	R-68028	KY386548	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
52	KP43.40.4.PA.V	KY386551	Phycicoccus ochangensis L1b-b9			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
53	R-68061	KY386418	Knoellia sinensis DSM12331	98,7	AVPJ01000034	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
54	R-67798	KY386475	Aquipuribacter hungaricus IV-75			+	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
54	R-67807	KY386530	Aquipuribacter hungaricus IV-75			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
54	KP43.37.15.PA.V	KY386545	Aquipuribacter hungaricus IV-75	97,88	FM179321	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
54	R-68204	KY386565	Aquipuribacter hungaricus IV-75			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae
55	R-68518	KY386301	Arthrobacter pityocampae Tp2	99,34	EU885749	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
55	R-68186	KY386336	Arthrobacter pityocampae Tp2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
55	R-67785	KY386346	Arthrobacter pityocampae Tp2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
55	R-67998	KY386349	Arthrobacter pityocampae Tp2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
55	R-68224	KY386538	Arthrobacter pityocampae Tp2			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68156	KY386431	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68157	KY386442	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68477	KY386457	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68144	KY386467	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68102	KY386476	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	KP43.11.4.PH.V	KY386478	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-67801	KY386510	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68017	KY386519	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-67803	KY386520	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-67808	KY386536	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68027	KY386546	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68229	KY386547	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68231	KY386552	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68508	KY386577	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68247	KY386593	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68043	KY386597	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68045	KY386600	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	KP53.28.4.PA.V	KY386602	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-67839	KY386614	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-68257	KY386616	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-67817	KY386620	Arthrobacter agilis DSM20550			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
57	R-67818	KY386623	Arthrobacter agilis DSM20550	99,46	X80748	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
58	R-67793	KY386372	Arthrobacter flavus TB 23	99,25	ALPM01000083	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
58	R-68397	KY386413	Arthrobacter flavus TB 23			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
59	R-68209	KY386489	Calidifontibacter indicus PC IW02			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Dermacoccaceae
59	R-68253	KY386608	Calidifontibacter indicus PC IW02	94,71	EF187228	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Dermacoccaceae
59	KP53.41.15.PH.V	KY386615	Calidifontibacter indicus PC IW02			-	-	Actinobacteria	Actinobacteria	Actinomycetales	Dermacoccaceae
60	R-68180	KY386316	Deinococcus marmoris DSM12784			-	-	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae
60	R-68561	KY386437	Deinococcus marmoris DSM12784	100	JNIV01000230	-	-	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae
60	R-68498	KY386507	Deinococcus marmoris DSM12784			-	-	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae
61	R-68571	KY386571	Deinococcus saxicola AA-1444	99,93	AJ585984	-	-	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae
61	KP53.17.15.PH.V	KY386581	Deinococcus saxicola AA-1444			-	-	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae

61	R-68514	KY386612	Deinococcus saxicola AA-1444			-	-	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae
62	R-68702	KY386340	Hymenobacter roseosalivarius AA718			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
62	R-68471	KY386441	Hymenobacter roseosalivarius AA718	98,01	Y18833	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
62	R-68240	KY386573	Hymenobacter roseosalivarius AA718			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
62	R-68582	KY386591	Hymenobacter roseosalivarius AA718			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
63	R-68402	KY386432	Hymenobacter roseosalivarius AA718	97,94	Y18833	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
65	R-68403	KY386435	Hymenobacter roseosalivarius AA718	98,89	Y18833	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
66	R-68243	KY386583	Hymenobacter roseosalivarius AA718	94,75	Y18833	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
66	R-68122	KY386588	Hymenobacter roseosalivarius AA718			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
67	R-67758	KY386449	Hymenobacter arcticus R2-4	98,05	KC213491	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	R-68178	KY386311	Hymenobacter terrae DG7A	93,96	KF862488	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	R-68353	KY386313	Hymenobacter terrae DG7A			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	R-68361	KY386326	Hymenobacter terrae DG7A			-	+	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	KP15.29.4.PA.NS	KY386354	Hymenobacter terrae DG7A			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	KP15.29b.4.PA.V	KY386355	Hymenobacter terrae DG7A			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	R-68447	KY386356	Hymenobacter terrae DG7A			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	R-68536	KY386363	Hymenobacter terrae DG7A			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
68	R-68359	KY386369	Hymenobacter terrae DG7A			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
69	R-68030	KY386555	Hymenobacter terrae DG7A	91,29	KF862488	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
70	R-68225	KY386541	Adhaeribacter aquaticus DSM16391	96,37	AXBK01000007	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
71	R-68113	KY386539	Pedobacter panaciterrae Gsoil 042			-	-	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
71	R-68289	KY386572	Pedobacter panaciterrae Gsoil 042	97,59	AB245368	-	-	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
72	R-67967	KY386410	Pedobacter duraquae WB2.1-25	98,36	AM491368	-	-	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
73	R-68191	KY386353	Pedobacter ruber W1	95,43	HQ882803	-	-	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
73	R-68207	KY386480	Pedobacter ruber W1			-	-	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae
74	R-68376	KY386334	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-68523	KY386371	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-68079	KY386376	Spirosoma rigui WPCB118	97,94	EF507900	-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-67957	KY386411	Spirosoma rigui WPCB118			+	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-68424	KY386495	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-68502	KY386503	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-67885	KY386508	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-67936	KY386509	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-68108	KY386517	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
74	R-68505	KY386529	Spirosoma rigui WPCB118			-	-	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
75	R-68384	KY386384	Arthrobacter oxydans DSM 20119	99,25	X83408	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
77	R-68259	KY386618	Angustibacter aerolatus 7402J-48	96,64	JQ639056	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiaceae
10	R-68085	KY386308	Geodermatophilus terrae PB261	99,02	JN033773	-	-	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae
14	R-68159	KY386450	Conexibacter arvalis KV-962	94,02	AB597950	-	-	Actinobacteria	Thermoleophilia	Solirubrobacterales	
56	R-68670	KY386433	Paenibacillus frigoriresistens YIM 016	97,66	JQ314346	-	-	Firmicutes	Bacilli	Bacillales	Paenibacillaceae
64	R-68168	KY386300	Nocardioides echinoideorum CC-CZW004	78,7	KM085325	-	-	FBP			
76	R-68213	KY386500	Hippea Maritima DSM 10411	79,07	CP002606	-	-	FBP			