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Bacterial diversity of autotrophic enriched cultures from remote, glacial Antarctic, Alpine and Andean aerosol, snow and soil samples

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Abstract. Four different communities and one culture of autotrophic microbial assemblages were obtained by incubation of samples collected from high elevation snow in the Alps (Mt. Blanc area) and the Andes (Nevado Illimani summit, Bolivia), from Antarctic aerosol (French station Dumont d'Urville) and a maritime Antarctic soil (King George Island, South Shetlands, Uruguay Station Artigas), in a minimal mineral (oligotrophic) media. Molecular analysis of more than 200 16S rRNA gene sequences showed that all cultured cells belong to the *Bacteria* domain. Phylogenetic comparison with the currently available rDNA database allowed sequences belonging to *Proteobacteria* (*Alpha-*, *Beta-and Gamma-proteobacteria*) , *Actinobacteria* and *Bacteroidetes* phyla to be identified. The Andes snow culture was the richest in bacterial diversity (eight microorganisms identified) and the marine Antarctic soil the poorest (only one). Snow samples from Col du Midi (Alps) and the Andes shared the highest number of identified microorganisms (*Agrobacterium*, *Limnobacter*, *Aquiflexus* and two uncultured *Alphaproteobacteria* clones). These two sampling sites also shared four sequences with the Antarctic aerosol sample (*Limnobacter*, *Pseudonocardia* and an uncultured *Alphaproteobacteria* clone). The only microorganism identified in the Antarctica soil (*Brevundimonas* sp.) was also detected in the Antarctic aerosol. Most of the identified microorganisms had been detected previously in cold environments, marine sediments soils and rocks. Air current dispersal is the best model to explain the presence of very specific microorganisms, like those identified in this work, in environments very distant and very different from each other.

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1 Introduction

Long distance dispersal of biological particles produced by atmospheric circulation, ocean currents, birds, fish, mammals and human vectors, has been known since the mid 20th century (Gislén, 1948; Gregory, 1967; Schnell and Vali, 1972; Marshall, 1996a, b; Vincent, 2000). The small size of microorganisms makes them easily transportable by air masses. Eventually, these windborn particles are deposited on the ground and/or snow/ice, on very high mountains and in Polar Regions, respectively, in either dry or wet form (Chalmers et al., 1996). It has been proposed that microorganisms living in hot and/or cold terrestrial deserts are especially susceptible to dispersion due to their special adaptation to extreme and variable conditions (temperature, radiation, spectral quality, desiccation, etc.) (Flechtner, 1999; Van Thielen and Garbary, 1999; Garty, 1999; Elster and Benson, 2004).

Living microorganisms have been collected in the stratosphere (Imshenetsky et al., 1978), and there are several reports supporting the idea that microorganisms can live and reproduce on airborne particles (Dimmick et al., 1979). It has also been shown that microorganisms can actively grow and reproduce at temperatures near or below 0°C in cloud droplets collected at high altitudes (Sattler et al., 2001).

Both, viable and dead cells, or their remains, stored in the ice of continental icecaps and mountain glaciers are potential historical records of recent evolution of microbial life, as well as a record of the Earth's changing climate. In the wake of the recent discovery of the sub-glacial Antarctic Lake Vostok (Priscu et al., 1999; Siegert et al., 2001), and the possibility of recovering water samples containing fossil living microorganisms, there is a growing interest in investigating

the transport of living organisms via air over large distances, e.g. cold glacial regions.

Recently, the composition of micro-autotrophs (cyanobacteria and algae), micro-fungi (hyphae and spores), bacteria (rod, cocci and pigmented bacteria), yeast and plant pollen in remote aerosol, deposited snow, and ice have been evaluated (Elster et al., 2007). As a product of this study cultivable autotrophic pigmented microorganisms were obtained from alpine snow (Alps and Andes) and aerosol (Antarctic) samples.

It has been suggested (Imshsnetsky et al., 1978, Christner et al., 2000) that the presence of highly pigmented bacterial colonies in the mesosphere and in glacial ice was associated with the need to protect cells from harmful UV-radiation during atmospheric transport and exposure on the surface of the glaciers. Morphologically similar bacterial specimens have also been observed in soils in the Arctic Svalbard (unpublished data, Øehaková et al., 2009). These observations support the idea that this type of microorganisms commonly develop in cold desert ecosystems and are easily transported via aerosol to both, short and long distances.

In this work we report the identification, using molecular ecology methodologies, of autotrophic microorganisms able to grow oligotrophically in enrichment cultures of samples obtained from alpine snow (Alps and Andes) and aerosol (Antarctic). For comparison the culture of pigmented bacteria isolated from marine Antarctic soil (King George Island, South Shetland) was also analysed.

2 Material and methods

2.1 Site description and sample collection

The aerosol samples originated from a set of filters collected between 1994 and 2000 at the coastal Antarctic Station Dumont d'Urville (66°40′ S, 140°01′ E) for long-term atmospheric chemistry studies. This station is situated on a small island, a few hundred meters offshore from the mainland. Local meteorological conditions were described by Périard & Pettré (1992) and König-Langlo et al. (1998). The main features of the chemical composition of the aerosol can be found in Wagenbach et al. (1998), Minikin et al. (1998), and Legrand et al. (1998). All together, 13 Antarctic aerosol samples and controls were collected on Gelman Zefluor® filters (47 mm diameter, 0.5 μm pore size) by drawing in air at a flow rate of 1.5 $m^3\,h^{-1}$. The sampling interval was 20 h in summer (from November to February) and 40 h the rest of the year, which corresponds to 30 and 60 m^3 of air, respectively. All devices used were washed three times with 18.2 MΩ cm MilliQ® water, except for the filters. Collected filters were kept under cool, dark and dry conditions (−25°C). In 38% of analysed aerosol samples the bacteria that produced pigmented colonies were recorded (Elster et al., 2007). Samples with high content of bacteria producing pigmented colonies were chosen for this study.

In the year 2000, two snow pits were dug at very high elevation sites in the Mt. Blanc area of the Alps: one (0.6 m deep) at Col du Midi (elev. 3532 m a.s.l., 17 May) and the other (2 m deep) at Col du Dome (elev. 4250 m a.s.l., 31 August) for European sampling. Snow collected from recent deposits contained several visible dust layers. This dust is known to be transported airborne to the Alps from the Sahara desert (Oeschger et al., 1977; Wagenbach, 1989; Angelis and Gaudichet, 1991). The dust layers correspond to short events (outbursts) that occur under meteorological conditions such as a low descend over the western North Africa (Morocco) producing a southern wind over Italy and southern France. The time corresponds to a few hours up to some days. At Col du Dome the accumulation rate is variable, but from a pit, the age of the layer may be a few months up to a year.

In the Andes, surface snow blocks were collected in 2000 at the summit of Nevado Illimani, Bolivia (16°37′ S, 67°46′ W, Cordillera Real, elevation 6350 m). The Bolivian Andes are surrounded by the Altiplano, a high altitude desert (mean elevation: 3700 m a.s.l., Clapperton, 1993). This region, in particular, contains large salt flats called "salares" (Risacher, 1992), which are an important source of dust for the regional atmosphere during the dry season. From size and depth of snow-dust layer it has been estimated that the age of the collected sample from this site was the same or similar of the Mt. Blanc area.

Samples from the Alps (Col du Midi 6 samples+blank, Col du Dome, 9 samples + blank) and the Andes (2 samples) were analysed (Elster et al., 2007). Each snow sample contained about 0.5 to 1 kg of snow. The surface layer (about 3–5 cm) from the snow samples was cut out and only the central part of the snow blocks were taken for analysis (for more details on sample preparation see Elster et al., 2007). In this set 88% of analysed samples contained bacteria producing pigmented colonies (Elster et al., 2007). From these sets, the samples with the highest content of pigmented bacteria were concentrated by lyophilisation (two Alps and one Andes samples) for further analysis.

In the austral summer season of 2005, soil samples were collected from the vicinity of the Uruguay Antarctic Station Artigas, King George Island, South Shetlands. All samples were collected using pre-cleaned glass vials (aerosol) and sealed plastic bags (snow and soil), respectively, transported frozen to the laboratory in Grenoble and/or in Tebo and stored frozen until further analysis (for more details see Elster et al., 2007).

2.2 Sample preparation and cultivation

To minimize possible contaminations, all post-sampling manipulations were done in a UV-sterilized laminar flow hood using sterile glass vials. To retrieve samples from the snow pits an upper layer of about 2 cm was sliced with a sharp

knife. The samples were dried off at a temperature of −40°C in a special sterile lyophilization device (Lyovac GT2, Leybold-Heraeus, Germany). Glass vessels containing the solid deposits were rinsed with 10–15 ml of re-distilled water.

Aerosol filters were cut into quarters and one quarter was used for enrichment cultures. Each snow and aerosol sample was cultured in a sterile glass bottle (25 ml in volume) in BG-11 culture media (as described in Bischoff and Bold, 1963). Each glass bottle was filled with about 10 ml of sterile medium (for more details see Elster et al., 2007). Suspensions of 10 g soil+90 ml sterile water were homogenized using ultrasonication for 4 min for soil samples analysis. For enrichment cultures 1 ml of lyophilized snow or solid suspensions or a quarter of a filter were used. For colony isolation 0.1 ml of enrichment cultures were spread on Petri dishes containing 1.5% BG11 agar (Elster et al., 1999). Four replicates were performed for each agar culture. Glass bottles and Petri dishes were cultivated in an illuminated ($\sim$100 W/cm^2) refrigerator (temperature 5–8°C) with a light regime of 18 h of light, 2 h of UV-B radiation (germicide lamp) and 4 h of darkness. The germicide lamp was used to sterilize the culture growth area (UV-B light did not penetrate through the glass bottles). Experimental bottles were shaken every 2–3 days. After 1 and 2 months of cultivation, the contents of bottles and Petri dishes were analyzed under the light microscope (Olympus BX 60). Aliquots of samples were analyzed by fluorescence after staining with the DAPI fluorochrome (EFM Olympus BX 60) (Zachleder and Cepák, 1987) and transmission electron microscopy (Jeol equipment JEM 1010).

Five oligotrophic enrichment cultures, from each sampling station (Antartic Station Dumont dÚrville; Col du Midi, Col du Dome, Nevado Illimani and Uruguay Antarctic Station Artigas) were selected for phylogenetic analysis.

2.3 DNA extraction

One ml of each enrichment culture was used for DNA extraction. Fast DNA Spin kit for soil (Q-Bio Gene Inc., CA, USA) was used according to the manufacturer's instructions. To disrupt the cells, the mixture of ceramic and silica beads provided in the kit and three pulses of 40 s at speed 5.5 of the FastPrep bead-beating instrument (Bio 101) were applied. After the extraction, DNA was purified by passage through a GeneClean Turbo column (Q-Bio Gene Inc., CA, USA) and quantified by ethidium bromide-UV detection on an agarose gel (González-Toril et al., 2006).

2.4 16S ribosomal RNA clone library construction

PCR amplification of 16S rRNA gene fragments between *E. coli* positions 8 and 1507 for *Bacteria* domain (Lane, 1991), between *E. coli* position 25 and 1492 for *Archaea* domain (Achenbach and Woese, 1995), and between *E. coli* position 359 and 805 for *Cyanobacteria* phylum (Nübel et al., 1997) were performed. These genes were amplified by PCR in mixtures containing 20–30 ng of DNA per 50 μl reaction volume, 1×PCR buffer (Promega Biotech Iberica, Spain), 2.5 μM of each of the deoxynucleotides (Amersham Biosciences, UK), 2.5 mM $MgCl_2$, 1 mg mL^{-1} bovine serum albumin (BSA), 500 mM of each forward and reverse primers and 0.025 U/μl of Taq DNA polymerase (Promega Biotech Iberica, Spain) (Table 1). PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 1 min for the *Bacteria* domain, 56°C for the *Archaea* domain, and 60°C for the *Cyanobacteria* phylum. Positive and negative controls were always used in every PCR. Large 16S rRNA gene fragments (>1400 bp) were purified by GeneClean Turbo Column (Q-Bio Gene Inc., CA, USA) and cloned using the Topo Ta Cloning Kit (Invitrogen, CA, USA). Cloned inserts were amplified using PCR conditions described above and were directly sequenced with a Big-Dye sequencing kit (Applied Biosystem) following the manufacturer's instructions (González-Toril et al., 2006).

2.5 Clone library analysis

Sequences were analyzed using BLAST at the NCBI database (http://ncbi.nlm.nih.gov/BLAST) and added to the most important BLAST hits, to reach an alignment of 50 000 homologous bacterial 16S rRNA primary structures by using the ARB software package aligning tool (http://www.arb-home.de) (Ludwig et al., 2004). The rRNA alignments were corrected manually and alignment uncertainties were omitted in the phylogenetic analysis. Phylogenetic trees were generated using parsimony, neighbour-joining, and maximum-likelihood analyses with a subset of 200 nearly full-length sequences (>1400 bp). Filters, which excluded highly variable positions, were used. In all cases, general tree topology and clusters were stable and a consensus tree was generated (González-Toril et al., 2006). Sequences obtained in this study have been deposited in the EMBL sequence database under accession numbers from EU429484 to EU429508.

3 Results and discussion

Following the protocols for enrichment cultures of photoautotrophic microorganisms present in snow samples from the Alps and the Andes and in an aerosol sample from the Antarctica, pigmented non-photosynthetic prokaryotes grew successfully in the extremely poor nutritional conditions of the selected media (Fig. 1) (Elster et al., 2007). Similar pigmented bacteria have also been observed in soils in King George Island, South Shetland Island group as well in the Arctic Svalbard Islands (øehaková et al., 2008). It appears that this type of bacteria commonly develop in cold desert

Table 1. Primers used for PCR amplification

Primer [a]	Target site [b]	Sequence (5' to 3')	Specificity	Reference
8F	8–23	AGA GTT TGA TCM TGG C	*Bacteria* Domain	Lane, 1991
25F	9–25	CYG GTT GAT CCT GCC RG	*Archaea* Domain	Achenbach and Woese, 1995
1492R	1492–1513	TAC GGY TAC CTT GTT ACG ACT T	Universal	Achenbach and Woese, 1995
Cya359F	359–378	GGG GAA TYT TCC GCA ATG GG	*Cyanobacteria* and clhoroplasts	Nübel et al., 1997
Cya106F	106–127	CGG ACG GGT GAG TAA CGC GTG A	*Cyanobacteria* and clhoroplasts	Nübel et al., 1997
Cya781R(a)[c]	781–805	GAC TAC TGG GGT ATC TAA TCC CAT T	*Cyanobacteria* and clhoroplasts	Nübel et al., 1997
Cya781R(b)[c]	781–805	GAC TAC AGG GGT ATC TAA TCC CTT T	*Cyanobacteria* and clhoroplasts	Nübel et al., 1997

[a] F (foward) and R (reverse) indicate the orientations of the primers in relation to the rRNA. [b] Positions are given according to the *E. coli* numbering of Brosius et al., 1981. [c] Reverse primer CYA781R is an equimolar mixture of CYA781R(a) and CYA781R(b).

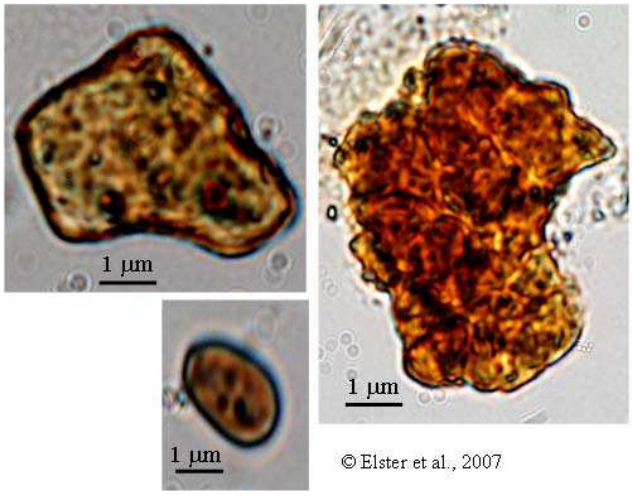

Fig. 1. Display of pigmented microorganisms enriched from snow and aerosol samples.

ecosystems and is easily transported with aerosols at both, short and long distances. In 1978, Imshsnetsky et al. had already studied pigmentation of viable bacteria recovered from the mesosphere. These authors suggested that the pigments produced by these bacteria are associated with the need to absorb harmful UV-radiation. Recently Du et al. (2006) and Mayilraj et al. (2006) have described the occurrence of pigmented bacteria from various marine and soil ecosystems, respectively.

Due to the characteristics of the cultures and the origin of the samples it was considered of interest to identify the microorganisms present in the different cultures, to evaluate the diversity corresponding to this very specific type of microorganisms and eventually to compare the results obtained in geographically dispersed sampling sites. A commercial soil DNA extraction kit was used to extract DNA from the enrichment cultures from particulate matter present in high elevation snow in the Alps and the Andes, from an Antarctic aerosol and a maritime Antarctic soil. No oxygenic phototrophic *Bacteria* were amplified using specific primers in any of the DNA extracted from the five enriched cultures. These results agree with the microscopy analysis of the cultures (Elster et al., 2007). Also, no *Archaea* were detected by amplification. PCR amplification of 16S rRNA genes using universal bacterial primers, followed by cloning and sequencing produced around 200 sequences, which were used to identify the different bacteria present in the enrichment cultures by comparison with the NCBI database. The retrieved sequences were added to a database of over 50 000 prokaryotic 16S rRNA gene sequences using the aligning tool of the ARB software package (http://www.arb-home.de) (Ludwig et al, 2004). Every sample was processed in the same way: positive PCR products were cloned and 50 positive colonies were chosen and sequenced. Around 50 sequences were obtained for every enrichment culture. All of them were aligned using the ARB software. With the sequences aligned we built a distances matrix that allowed different OTUs (operational taxonomic units) to be identified: 3 for Col du Dome, 7 for Col du Midi, 8 for Illimani, 1 for Artigas and 6 for aerosol (Antarctis). One representative sequence from every OTU was selected for phylogenetic analysis. With these selected sequences phylogenetic trees were generated using parsimony, neighbour joining, and maximum-likelihood analyses with a subset of 200 nearly full-length sequences (>1.400 bp). Filters excluding highly variable positions were used. In all cases general tree topology and clusters were stable. Thus, consensus trees were generated (Figs. 2–6).

After the analysis of the generated sequences, representatives of three bacterial phyla: *Proteobacteria* (*Alpha-*, *Beta-* and *Gamma-proteobacteria*), *Actinobacteria* and *Bacteroidetes*, were identified (Fig. 2). Seven close relative microorganisms were identified for the snow sample from Col du Midi (Alps). Three sequences corresponded to the *Alphaproteobacteria*: one as a possible member of the genus *Agrobacterium* (closest relative: clone IrT-J614-14, retrieved from an environmental sample of a uranium mine (Selenska-Pobell, 2002) (Fig. 3), the other two; close to clones B3NR69D12 and AP-12, detected in an underground cave (Northup et al., 2003) and in a marine estuary

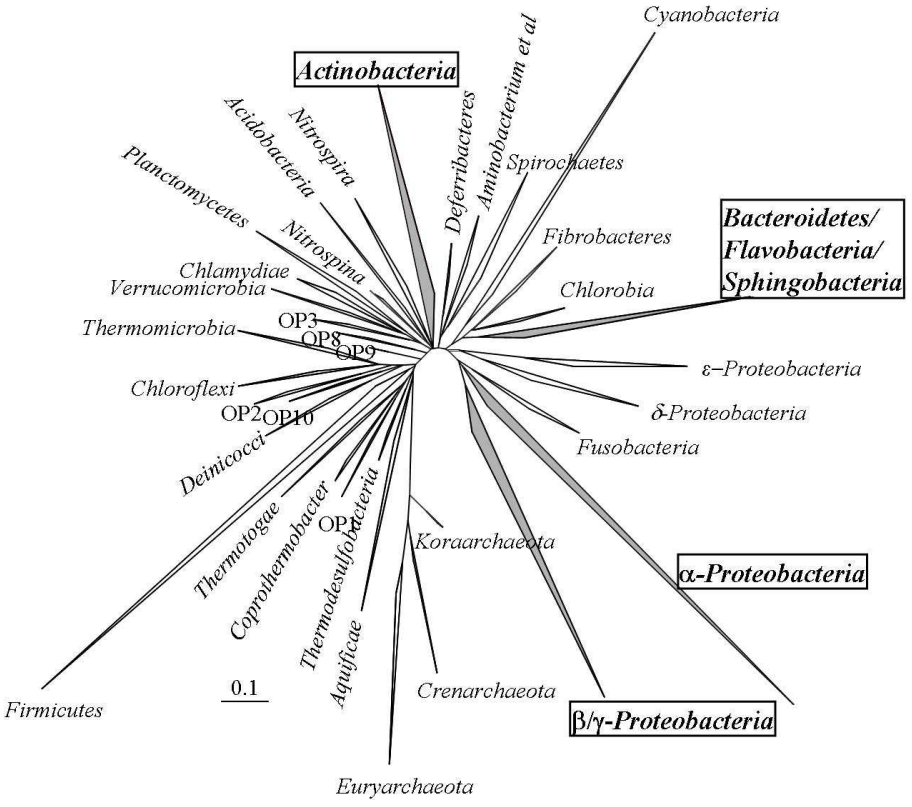

Fig. 2. Prokaryotic phylogenetic tree showing the phyla in which enriched microorganisms from snow, aerosol and soil have been identified.

(NCBI accession number AY145551, unpublished) respectively. Two sequences corresponded to the *Betaproteobacteria*: one a possible member of the species *Hydrogenophaga palleronii* (Fig. 4), identified by FISH in lake snow aggregates (Schweitzer et al., 2001), and the other close to a clone of the genus *Limnobacter*, D-15, isolated from underground mineral water (Loy et al., 2005). One sequence corresponding to the *Gammaproteobacteria* exhibited a high homology with *Pseudomonas pseudoalkaligenes*, which was isolated from a marine sediment (NCBI accession number AF286035, unpublished). One sequence corresponded to the phylum *Bacteroidetes*, with high homology with *Aquiflexus balticus* (Fig. 5), also retrieved from a marine sediment (Bretar et al., 2004).

The other sample from the Alpes (Col du Dome) exhibited much lower diversity than the one obtained from Col du Midi. In the Col du Dome sample only three representative sequences were retrieved. Two corresponded to uncultivated *Alphaproteobacteria* clones, AP-12 and O15B-H01, the first detected in a marine estuary (NCBI accession number AY145551, unpublished) and the other from a uranium mine ground water (NCBI accession number AY662032, unpublished). The other sequence corresponded to the class *Actinobacteria*, with a high level of homology with the species *Dietzia kujamensis*, which was isolated from the Himalaya (Mayilraj et al., 2006).

Eight closely related microorganisms were identified from the Andean snow sample (Nevado Illimani). Three sequences corresponded to the *Alphaproteobacteria* class: two related to the *Agrobacterium* (clone IrT-J614-14) (Fig. 3) and the clone B3NR69D12, both identified in Col du Midi; and one related to the uncultured EV818CFSSAHH29 clone, which was identified from subsurface water in the Kalahari Shield, South Africa (NCBI accession number DQ336984, unpublished). Two sequences corresponded to the *Betaproteobacteria* class: *Hydrogenophaga palleronii* (Fig. 4) and *Limnobacter* clone D-15, both also identified in Col du Midi. One sequence exhibiting high homology with *Aquiflexus balticus* (Fig. 5) of the phylum *Bacteroidetes*, previously described in Col du Midi, was retrieved from the Andes enrichment culture. The last two sequences corresponded to the *Actinobacteria* class: one with high homology to *Microbacterium thalassium* (Richert et al., 2007) and the other with *Pseudonocardia* sp. (Fig. 6) which was isolated from marine sediments (NCBI accession number AY974793, unpublished).

Seven sequences retrieved from the aerosol sample from Antarctica were identified by phylogenetic analysis. Three sequences corresponded to the *Alphaproteobacteria* class: one related with clone B3NR69D12, which has been also identified in the Andes and the Alps (Col du Midi); another related with clone AP-12 which has been also identified in

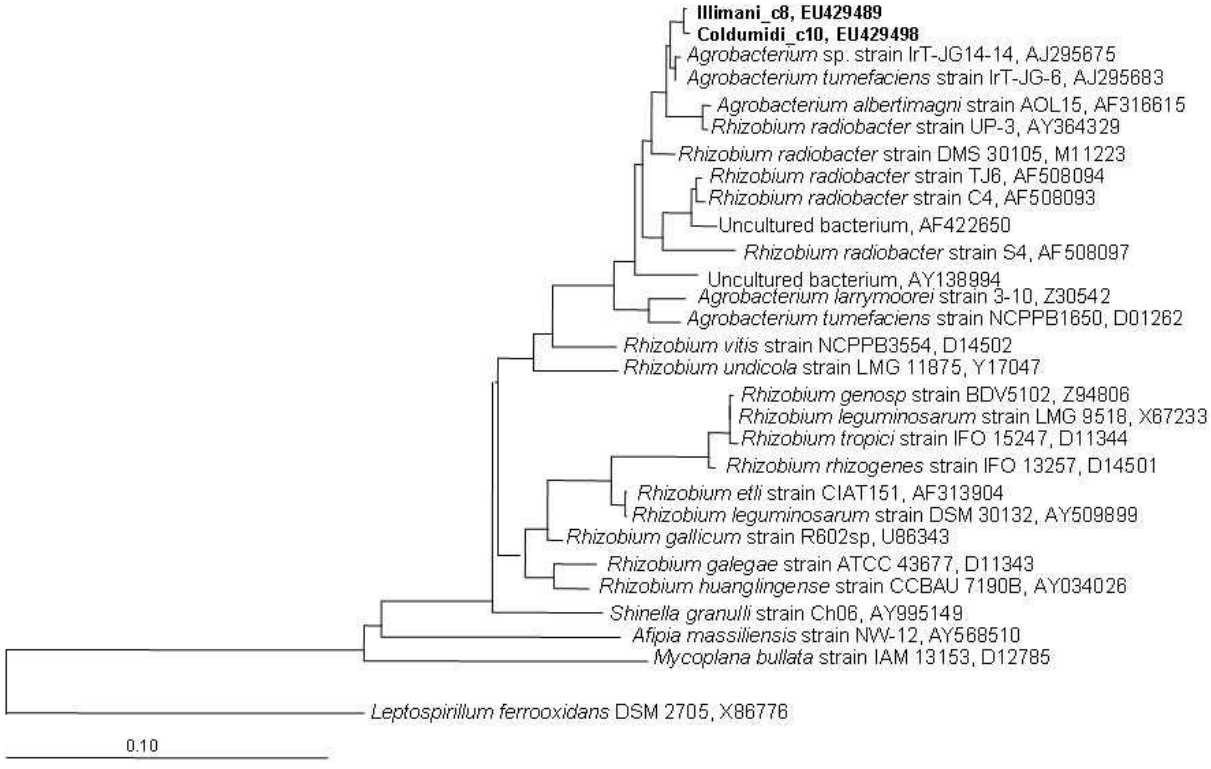

Fig. 3. Phylogenetic tree of *Agrobacterium* and related bacteria. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from Col du Midi (Alps) are designated by "Coldumidi" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence.

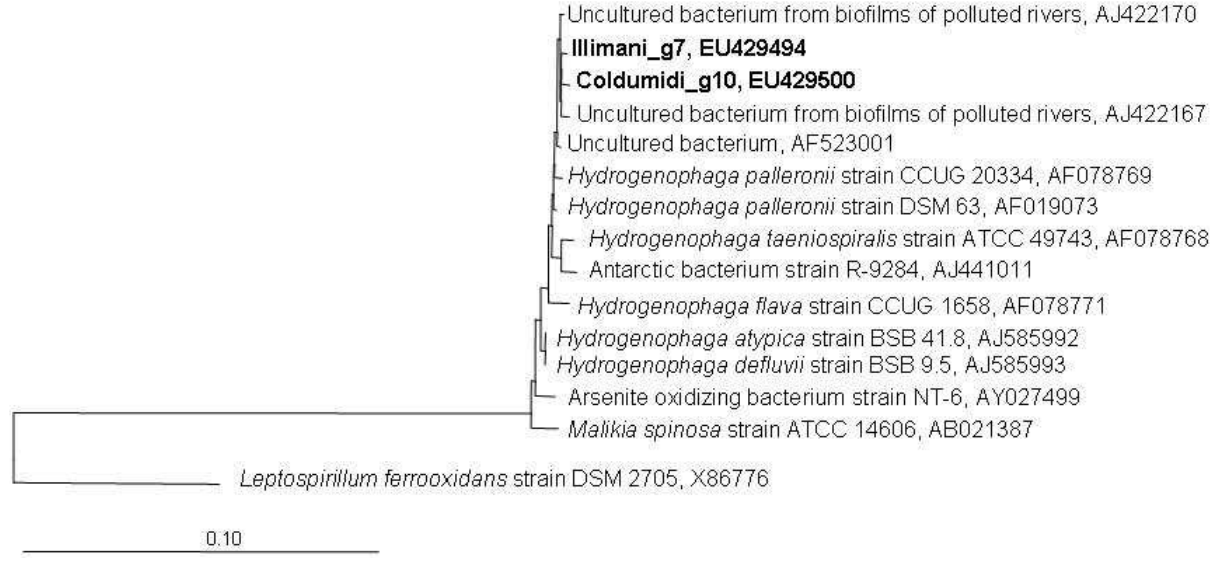

Fig. 4. Phylogenetic tree of *Hydrogenophaga* and related bacteria. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from Col du Midi (Alps) are designated by "Coldumidi" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence.

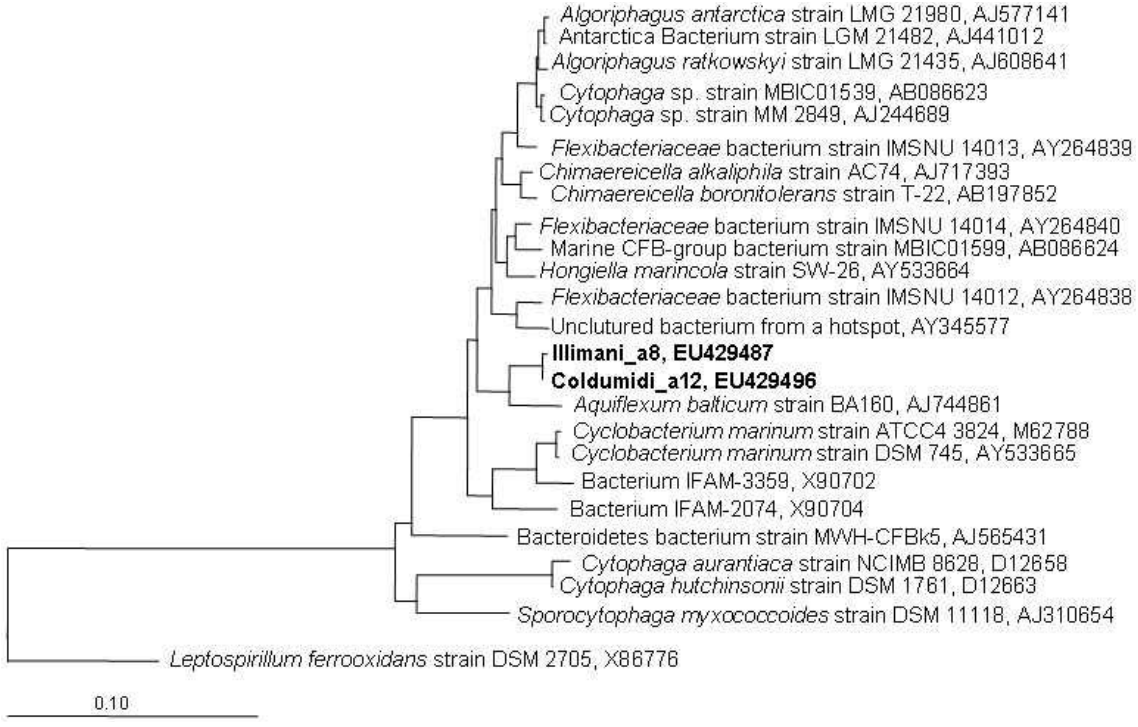

Fig. 5. Phylogenetic tree of *Aquiflexus* and related bacteria. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from Col du Midi (Alps) are designated by "Coldumidi" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence.

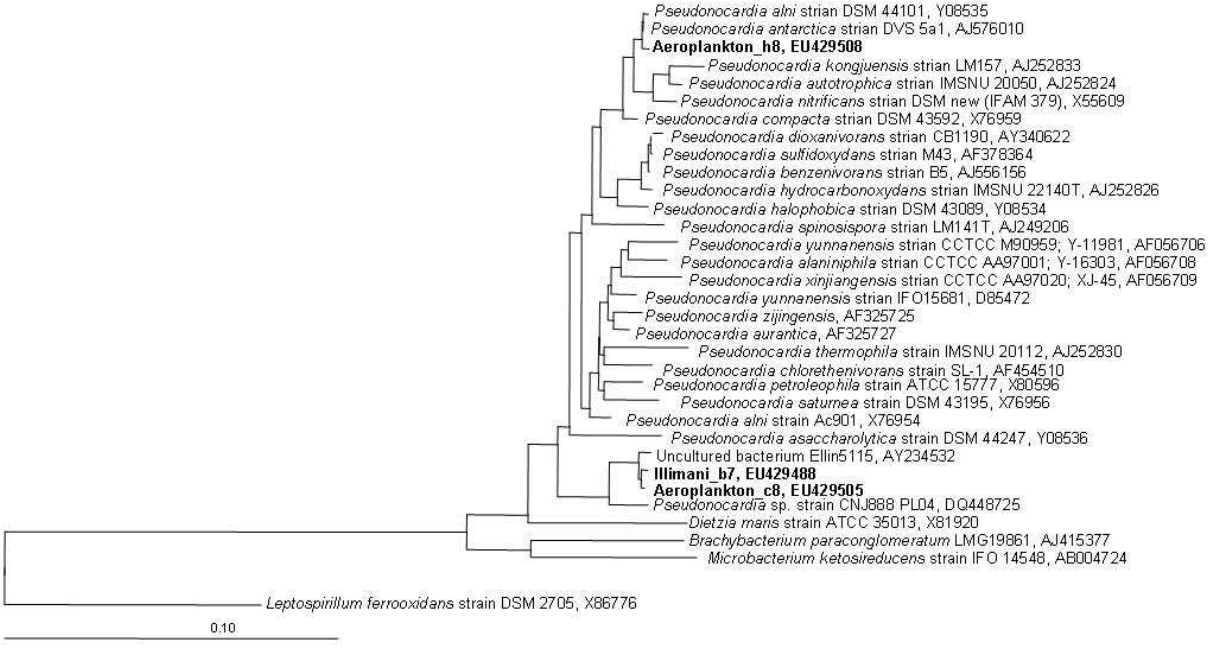

Fig. 6. Phylogenetic tree of *Pseudonocardia* and related bacteria. Cloned 16S rRNA sequences from enrichment cultures are indicated in bold. Sequences retrieved from Nevado Illimani (Andes) are designated by the word "Illimani" followed by the number of the sequence. Sequences from aerosol sample collected in Antarctica are designated by "Aeroplankton" followed by the number of the sequence. Bar represents 10% estimated phylogenetic divergence.

Table 2. Analysis of OTUs. Microorganisms and sequences closely related with clones obtained in this study and some of their characteristics.

Col du Dome (snow – the Alps)	Col du Midi (snow – the Alps)	Illimani (snow – the Andes)	Uruguay Antarctic Station Artigas (soil – King George Island)	French Antarctic Station Dumont d'Urville (aerosol)	Interesting characteristics	NCBI[a]
Clone AP-12 Near *Bradyrhizobium* *Alphaproteobacteria*	Clone AP-12 Near *Bradyrhizobium* *Alphaproteobacteria*			Clone AP-12 Near *Bradyrhizobium* *Alphaproteobacteria*	Uncultured bacterium from Weser Estuary	AY145551 (98%)
	Clone B3NR69D12 Near *Afipia massiliensis* *Alphaproteobacteria*	Clone B3NR69D12 Near *Afipia massiliensis* *Alphaproteobacteria*			Un cultured bacterium from an inhabiting ferromanganese deposits in Lechuguilla and Spider Caves	AY186080 (99%)
Clone O15B-H01 Near *Sinella granuli* *Alphaproteobacteria*					Uncultured bacterium from a groundwater contaminated with high levels of nitric acid-bearing uranium water	AY662032 (100%)
	Clone IrT-J614-14 Near *Agrobacterium* *Alphaproteobacteria*	Clone IrT-J614-14 Near *Agrobacterium* *Alphaproteobacteria*			Uncultured bacterium from a uranium mining waste piles	AJ295675 (99%)
		Clone EV818CFSSHH29 Near *Brevundimonas* *Alphaproteobacteria*	*Brevundimonas* sp. *Alphaproteobacteria*	*Brevundimonas vesicularis* *Alphaproteobacteria*	– Uncultured bacterium from subsurface water of Kalahari Shield, South Africa – Bacteria isolated from permafrost. – Anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core - Bacteria detected in lake snow aggregates by FISH	DQ336984 (97%) DQ177489 (99%) AY169433 (99%)
	Clone D-15 Near *Limnobacter* *Betaproteobacteria*	Clone D-15 Near *Limnobacter* *Betaproteobacteria*		Clone D-15 Near *Limnobacter* *Betaproteobacteria*	Uncultured bacterium from natural mineral water	AF522999 (99%)
	Hydrogenophaga palleronii *Betaproteobacteria*	*Hydrogenophaga palleronii* *Betaproteobacteria*			Bacteria detected in lake snow aggregates by FISH	AF078769 (98%)

[a] Accession Number in NCBI of the closest phylogenetic relatives and percentage of similarity.

Col du Midi; and a third one corresponding to the *Brevundimonas vesicularis*, which was isolated from a Greenland ice core (Sheridan et al., 2003). One sequence corresponded to *Betaproteobacteria* and had a high level of homology with the uncultured clone of *Limnobacter* D-15, already identified in the enrichment cultures of snow samples from the Andes and the Alps (Col du Midi). Three sequences corresponded to the *Actinobacteria* class: one related with *Pseudonocardia antarctica* (Fig. 3), a psychrophilic microorganisms isolated from McMurdo Valley in the Antarctica (Prabahar et al., 2004); a second one corresponding to a member of the *Pseudonocardia* genus (Fig. 6) isolated from marine sediments and also identified in the Andes; and a third one similar to *Brachybacterium conglomeratum*, which was isolated from a spacecraft assembly facility (NCBI accession number AY145551, unpublished).

The high mountain snows from the Alps and Andes were richer in bacterial diversity than the Antarctic aerosol (Elster et al., 2007). This observation agrees with the relatively large amounts of dust transported in the free troposphere of these regions. Saharan dust layers are very common in high altitude alpine snow (De Angelis AND Gaudichet, 1991). The blocks of Alps snow used in this study even recorded a Sahara dust event. Dust concentration was also relatively high in the snow deposited on the summit of Illimani (Clapperton, 1993), despite the absence of bare rocks near the sampling site. Similar results of microbial abundances in snow were also demonstrated from various Antarctic localities (Wynn-Williams, 1991).

The sample from marine Antarctica soil was a pure culture because only one sequence was retrieved with a high level of 16S rRNA gene sequence homology with a member of the *Brevundimonas* sp. identified previously in permafrost samples and close to the *Alphaproteobacteria* species of *Brevundimonas* identified in the Antarctic aerosol.

All the identified microorganisms in the enrichment cultures belong to the bacterial domain. Snow samples from the Alps (Col du Midi) and the Andes (Illimani), as well as the Antarctic aerosol sample exhibited a similar level of bacterial diversity in the corresponding enrichment cultures

Table 2. Continued.

Col du Dome (snow – the Alps)	Col du Midi (snow – the Alps)	Illimani (snow – the Andes)	Uruguay Antarctic Station Artigas (soil – King George Island)	French Antarctic Station Dumont d'Urville (aerosol)	Interesting characteristics	NCBI[a]
	Clone LCP-79 *Pseudomonas Gammaproteobacteria*				Uncultured bacterium from marine sediments	AF286035 (98%)
Dietzia spp. *Actinobacteria*					Bacteria isolated in cold environments (Himalaya and artic Ocean)	DQ060378 (99%)
		Pseudonocardia sp. *Actinobacteria*		*Pseudonocardia antarctica Actinobacteria and Pseudonocardia* sp. *Actinobacteria*	– Bacteria isolated from marine sediments (depths of 500 m). – Bacteria isolated from McMurdo Dry Valleys, Antarctica. Filamentous and produce brown colour substrate mycelia and aerial mycelia Vich form a white conglomerate.	AY234532 (99%) AJ576010 (99%)
		Microbacterium thalassium Actinobacteria			In young cultures, cells are small irregular rods. In old cultures, rods become shorter or spherical elements. Colonies are yellowish while, yellow or orange.	AM181507 (98%)
				Brachybacterium sp. *Actinobacteria*	Bacteria isolated from spacecraft assembly facilities	AY167842 (99%)
	Aquiflexum balticum Bacteroidetes	*Aquiflexum balticum Bacteroidetes*			Bacterium isolated from surface water of the Central Baltic Sea (depth of 5 m). Red and transparent colonies when young, but turn opaque with ongoing incubation. Cells contain carotenoids.	AJ744861 (94%)

(Table 2). The marine Antarctic soil (King George Island, Uruguay Antarctic Station Artigas) enrichment culture contained only one sequence (Table 2). This is probably due to the strict oligotrophic protocol used for its enrichment. Only one type of bacteria was able to grow on mineral agar plate. On the contrary, in the case of snow and aerosol samples, growth in liquid mineral media was always observed. In the supplementary information http://www.biogeosciences.net/6/33/2009/bg-6-33-2009-supplement.pdf figures of more phylogenetic tree are provided.

The distribution of detected bacteria was related to cold environments (21.4%), marine environments (35.7%) and soils/subsurface (35.7%) (Table 2). No single cosmopolitan bacterium was found in all the analyzed sites, although three geographically distant sampling sites (Alps, Illimani and Antarctic aerosol) shared three microorganisms (*Limnobacter* clone D-15, and two uncultured *Alphaproteobacteria*, clones B3NR69D12 and AP-12). Different bacteria were found in two locations: *Agrobacterium* sp. (Col du Midi and Illimani), *Brevundimonas* sp. (Antarctic aerosol and Antarctic maritime soil), *Hydrogenophaga palleroni* (Col du Midi and Illimani), *Aquiflexus balticus* (Col du Midi and Illimani) and *Pseudonocardia* sp. (Illimani and Antarctic aerosol) (Table 2). Six out of fourteen identified genera or species are found only in a single location (Table 2). Interestingly enough, much more diversity was found in very close locations in the Alps (Col du Dome and Col du Midi, only one common sequence out of ten) than in locations in different hemispheres, altitudes or matrix (snow versus aerosol) (Table 2). Concerning the pigments that color to some of the colonies, several bacteria such as *Dietzia*, *Aquiflexum*, *Microbacterium* and *Pseudonocardia*, related with those present in the enrichment cultures, have been described as having this peculiar phenotypic property. In addition, members of the *Actinobacteria* class are well known for their ability to synthesize pigments for radiation protection purposes.

It is evident that the experimental procedure used in this study is not contamination-free (see Elster et al., 2007). Careful analysis of contamination was done through the

use of blank samples (e.g. vials without sample but merely opened in the field for the same period of time, distilled or re-distilled water used throughout the project, filters for aerosol collection, negative controls for DNA extraction and PCR amplification, etc.). No growth could be detected in any of the controls performed. In addition, most of the identified bacteria fit quite well with bacteria isolated or identified from similar habitats around the world (e.g. Castro et al., 2004; Shivaji et al., 2004; Mayilraj et al., 2006; Després et al., 2007; Georgakopoulos et al., 2008). These bacterial communities represent individuals frequently occurring in remote terrestrial cold or hot deserts/semi-deserts, and/or marginal soil-snow-ice ecosystems.

Concerning the different models for long distance microbial dissemination (atmospheric circulation, ocean currents, birds, fish, mammals and human vectors), if we consider the habitats in which related microorganisms have been detected, we can rule out ocean currents and animal vectors, leaving atmospheric circulation as the most plausible means of dissemination, especially given the microbial diversity detected in the Antarctic aerosol and shared with other distant locations (71.4%), which underlines the importance of microbes attached to dust particles for their disseminations.

Of course, our data can not rule out the possible model of "everything is everywhere", although considering the experimental constraints introduced by the use of extremely oligotrophic selective media, we think that atmospheric dispersion can explain adequately the observed results. One of the problems related with this type of experiments is to rule out the possibility of contamination. Considering the location of the selected sampling sites and the obtained results, we believe we demonstrated by the protocols used that this possibility is improbable. Although the differences observed among the samples obtained during the same campaign in two close sites in the Alps is not obvious, we think that it is a very useful internal control for the lack of contamination in the sampling manipulation because they were collected by the same team and analyzed together in the same conditions. We strongly believe that the common microbial patterns of the particular microorganisms observed at distant locations reflects true airborne microbial dissemination, and therefore we would like to propose the protocol of enrichment in oligotrophic media for further testing of airborne microbial dispersion to reduce the amount of contaminants that could interfere with the analysis of the results.

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