Community Variability of Bacteria in Alpine Snow (Mont Blanc) Containing Saharan Dust Deposition and Their Snow Colonisation Potential

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Microorganisms uplifted during dust storms survive long-range transport in the atmosphere and could colonize high-altitude snow. Bacterial communities in alpine snow on a Mont Blanc glacier, associated with four depositions of Saharan dust during the period 2006–2009, were studied using 16S rRNA gene sequencing and flow cytometry. Also, sand from the Tunisian Sahara, Saharan dust collected in Grenoble and Mont Blanc snow containing no Saharan dust (one sample of each) were analyzed. The bacterial community composition varied significantly in snow containing four dust depositions over a 3-year period. Out of 61 phylotypes recovered from dusty snow, only three phylotypes were detected in more than one sample. Overall, 15 phylotypes were recognized as potential snow colonizers. For snow samples, these phylotypes belonged to Actinobacteria, Proteobacteria and Cyanobacteria, while for Saharan sand/dust samples they belonged to Actinobacteria, Bacteroidetes, Deinococcus-Thermus and Proteobacteria. Thus, regardless of the time-scale, Saharan dust events can bring different microbiota with no common species set to alpine glaciers. This seems to be defined more by event peculiarities and aeolian transport conditions than by the bacterial load from the original dust source.

Key words: snow, bacterial community composition, Mont Blanc glacier, Saharan dust, 16S rRNA genes

In recent years, high mountain and polar glacier snow fields have been recognized as highly diverse microbial community habitats (45). Microbial processes, such as heterotrophy, photosynthesis, and nutrient cycling have been shown to occur in melting snow (23). Several studies suggest that biological activity related with algal-bacterial associations growing on snow and ice, substantially lowers the albedo of the glacier surface (51). Snow algae are primary producers that can sustain bacterial heterotrophic communities in this ecosystem (50). Nevertheless, studies of bacterial diversity in mountains as well as polar glacial snowpacks are rather limited.

Mountain snow bacterial loads have been reported to range from \(3 \times 10^4\) cells mL\(^{-1}\) (2, 30) to \(4 \times 10^5\) cells mL\(^{-1}\) (1, 47), the higher value being usually associated with wind-born mineral particles (30, 47). A study of the diversity of culturable microbial assemblages from glacier snow in the Alps (Mt. Blanc area) and the Andes (Nevado Illimani summit) revealed only a few bacterial species, some having pigmentation which could help protect them from harsh UV radiation (15). Evidence of bacterial growth in surface snow during the melting season has been shown for the psychrophilic bacterium Cryobacterium psychrophilum, and for two psychrotrophic bacteria, Variovorax paradoxus and Janthinobacterium lividum, by Segawa et al. (47). Cell concentrations were shown to positively correlate with the density of mineral particles in snow (47). Liu et al. (30) found that bacterial diversity in the snow of the Tibetan Plateau is related to the surrounding environment. In the case of the Guoqu Glacier, it was shown that bacterial abundance in snow was strongly affected by dust input from arid and mid-arid regions (30).

There is a strong link between aeolian dust deposition registering seasonal atmospheric fluctuations and microbial community structures in mountain glacier snow or ice (30, 50). Layered distribution of bacterial isolates within ice cores was thought to originate from diverse bacterial sources which could have been different in the past (55, 57). It has been shown that dust concentration is more important in determining bacterial diversity than bacterial abundance in the Himalayan glaciers (57), a fact which could highlight the importance of aeolian transport conditions; therefore, the study of microorganisms trapped in ice might be useful for the reconstruction of past climatic and environmental changes (57).

Dust of Saharan origin seasonally affects high-elevation Alpine sites by disturbing the chemical composition of snow deposits (37). De Angelis and Guidichet (10) who studied dust depositions on Mont Blanc for a period of 30 years, showed that Saharan dust deposition dominates other dust sources. Studies performed at high-alpine stations (9, 49)
considered the main source of Saharan dust to be North Africa, namely, Algeria, Libya, Morocco and Tunisia.

Sporadic inputs of Saharan dust from late spring to early summer constitute an important source of base ions and nutrients (predominantly iron, phosphorus [P], nitrogen and sulfate) for terrestrial and aquatic ecosystems, and this can improve biological productivity (29, 38, 44). The addition of P and DOC (dissolved organic carbon) from Saharan dust to sea water was shown to increase the abundance and activity of heterotrophic bacteria (20, 39). Despite physical stresses associated with atmospheric transport (UV radiation, desiccation, low temperature, etc.), many species of bacteria and fungi associated with Saharan dust events are capable of surviving (19, 21). To date, a wide range of dust-borne microorganisms has been identified, including pathogenic microorganisms, that can cover great distances through the atmosphere (19). The bacterial community structure was shown to depend on the particle size of Saharan dust (36). Perfumo and Marchant (35) have argued that African dust storms are probably the source of thermophilic geobacilli found in cool soils in Northern Ireland. Moreover, a high similarity in microbial community structure has been observed between the alpine lake bacterioneuston and the airborne bacteria associated with Saharan dust deposited on the snow cover of alpine lakes (21). Nevertheless, only a few studies have been conducted on the effect of dust-borne bacteria inputs on the autochthonous community structure and their ecological consequences (21, 29, 35, 43).

The objective of this study was to assess uncultured bacterial abundance and diversity in the snowpack of the Mont Blanc (MtBl) glacier containing Saharan dust deposited during four dust events during 2006–2009. The final goal was to identify the bacteria that could be involved in the establishment of snow microbiota. The hypothesis put forward was that all microbial communities derived from snow containing Saharan dust, excepting those originating from the MtBl “clean” snowpack (containing no Saharan dust) which served as a control, should contain a core set of phylotypes involved in snow colonization. To decipher the microbial cell/dust grain relationship, we analyzed the Saharan dust depositions from the same May 2008 dust event collected at low (~200 m a.s.l., Grenoble, France) and high (4,250 m a.s.l., MtBl glacier) altitudes. In trying to trace the microbial load source, our study included Saharan sand collected in Tunisia (one of the probable regions of dust uplift) as well as the MtBl “clean” snowpack (with no sign of Saharan dust). In addition, we made an attempt to culture radiation-resistant species from the MtBl snow contaminated with Saharan dust since these organisms have been detected previously in desert soils (41, 56).

Materials and Methods

Sample collection

Four snow samples with Saharan dust layers and one sample of “clean” snow (hereafter designated as snow containing no Saharan dust) were collected at Col du Dôme (4,250 m a.s.l.), located close to the MtBl summit (MtBl Massif, Alps, France), during several field trips (Fig. 1, Table 1). The selected site is characterized by snow accumulation ranging from 3.8 to 6 m snow yr⁻¹ with an average annual temperature of −11°C (54). Due to the expected low biomass in snow samples, especially that with no Saharan dust (6), all precautions were taken to exclude possible contamination. The surface snow (~2–5 cm) in contact with air was always removed using a clean shovel. Samples were collected using sterile or decontaminated plastic containers by experimenters wearing single-use Tyvek coveralls and vinyl gloves. Reddish-brown dust layers were identified in the snow upon digging four snow pits of a depth ranging from 4–140 cm (Table S1). Dust layers corresponding to the June 2006, May 2008 and June 2008 events were obtained using decontaminated containers embedded into the pit walls at randomly selected points (10–15 cm intervals) whereas a snow sample with the dust layer corresponding to the May 2009 event, as well as “clean” snow, was collected into a sterile crate using a strictly decontaminated plastic (polyethylene) shovel for appropriating snow layers (5 to 15 cm and 2 to 10 cm deep, correspondingly) at the sampling sites (50 by 100 cm). The length of the Saharan dust preservation period in snow varied for different dust events from approximately one week to 3.5 months (Table 1). While the MtBl site receives dust and air-borne particles continuously along with snow precipitation, it is possible to distinguish the background concentration (a mix of dust from various sources, local, regional or more distant, with a concentration level of 0.1–1 ppm) from the Saharan dust event, whose concentration value was higher by three orders of magnitude. The most salient indices are given by the reddish-brown colour of the snow layer, which was not observed at the time of the “clean” snow sampling. Furthermore, the “clean” snow was fresh, as a result of some very recent precipitation events during the three days before sampling. The containers and crates with collected samples were kept frozen in metallic insulated boxes and transported rapidly to the laboratory (LGGE). Before subsequent treatment, the samples were stored in a freezer room at −15°C.

Fig. 1. Map of the Col du Dôme area, the Mont Blanc Massif with positions of sample points listed in Table 1. Glaciers are represented with elevation lines by Vincent et al. (54).
Table 1. Surface snow with Saharan dust deposition samples under study and 16S rRNA gene clone libraries (V3-V5 region)

<table>
<thead>
<tr>
<th>Dust event (date)</th>
<th>Sample</th>
<th>Collection place/altitude (m a.s.l.)</th>
<th>Dust preservation period in snow (month)</th>
<th>Clone library</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral type— Illite</td>
<td>May 2008 SDm05/2008</td>
<td>MtBI P2/4,250</td>
<td>1.0</td>
<td>MB-SD</td>
</tr>
<tr>
<td>May 2008</td>
<td>SD05/2008</td>
<td>Groenlen/200</td>
<td>NA</td>
<td>SD</td>
</tr>
<tr>
<td>June 2008</td>
<td>Sdm06/2008</td>
<td>MtBI P3/4,250</td>
<td>0.25</td>
<td>MB-SD2</td>
</tr>
<tr>
<td>Mineral type— Smectite</td>
<td>June 2006</td>
<td>SDm06/2006</td>
<td>MtBI P1/4,250</td>
<td>3.5</td>
</tr>
<tr>
<td>May 2009</td>
<td>SDm05/2009</td>
<td>MtBI P4/4,250</td>
<td>0.25</td>
<td>MS5</td>
</tr>
<tr>
<td>NA</td>
<td>Dm08/2009</td>
<td>MtBI P5/4,250</td>
<td>NA</td>
<td>MS7</td>
</tr>
<tr>
<td>Mineral type— ND</td>
<td>NA</td>
<td>SS03/2008</td>
<td>Ksar Ghilane, Sahara, Tunisia</td>
<td>NA</td>
</tr>
</tbody>
</table>

The dust was collected as rainy deposit following the May 2008 event and the relevant dust layer was collected one month later on MtBI (SDm05/2008).

The sample was used for a culturing approach.

The sample was collected as “clean” snow but in fact proved to be containing a blackish fine dust, probably, of local source.

ND: no data.

2008 in the Saharan desert in the vicinity of the Ksar Ghilane oasis, Tunisia, 2,000 km away from MtBl. Overall, 0.1 g of sand was used for analysis.

Sample treatment

The snow samples were melted at room temperature within class 10,000 (ISO 7) dust-free room facilities inside a class 100 (ISO 5) laminar flow hood (LGGE, Grenoble, France). The obtained meltwater was concentrated several thousand times using Centriprep YM3 or Centricon Plus-70 columns (Millipore, Bedford, MA, USA) featuring 3,000 and 5,000 Da pore membranes, respectively (Table S1). Concentrated water subsamples were used for cell enumeration and DNA extraction as well as archiving. The laboratory personnel always used special “clean-room” certified sterile single-use supplies (sterile cloths, gloves, face masks, etc.). Surfaces and tools were treated with a decontamination solution (e.g., Proline Biocontrol, Biohit, Helsinki, Finland).

Bacterial cell enumeration

Bacterial cell concentrations were determined by flow cytometry using water phase subsamples. Samples were fixed with 0.2% glutaraldehyde at a final concentration of 0.5%, incubated for 2 h before being frozen in liquid nitrogen and then stored at ~80°C until analysis. Samples were thawed at room temperature and incubated for 15 min in the presence of SYBR-Green I (Invitrogen, Carlsbad, CA, USA) in a class 10,000 (ISO 7) clean room (LGGE). The V3-V5 variable region of bacterial 16S rRNA genes was amplified with bacterial primers 338Fb and 518Rb (Invitrogen). The presence and size of inserts were assessed by PCR using a vector M13 sequence-based primers flanking insert. The restriction fragments were separated by electrophoresis with a Coolidge tube (Imax =125 mA, Vmax=60 kV) with a Rh anode. Analysis was performed according to the protocol described in Rajot et al. (42). Samples were prepared for X-ray analysis by suspending a known volume of each snow or rain sample in osmosis water. Suspensions were filtered on 47 mm polycarbonate membranes (Nuclepore AOX, 0.4 μm pore size, Whatman, Maidstone, UK) that were then rapidly analyzed. The Ksar Ghilane sand was first crushed in an agate mortar and a known weighted sample was suspended in osmosis water. As the other samples, the suspensions were filtered on 47 mm polycarbonate membranes (Nuclepore AOX, 0.4 μm pore size, Whatman).

The concentration and volume-size distribution of the insoluble dust from snow and blood rain samples were analyzed using a particle counter (Coulter Counter Multisizer Ilen, 256 channels, Beckman Coulter, Brea, CA, USA) set up in a class 10,000 (ISO 7) clean room (LGGE). The procedures for sample preparation were described earlier (11).

DNA extraction and amplification

Genomic DNA (gDNA) was extracted using a PowerSoil DNA Isolation Kit according to the manufacturer’s instructions (MoBio Laboratories, Carlsbad, CA, USA) in a class 10,000 (ISO 7) dust-free room facility (LGGE). The V3-V5 variable region of bacterial 16S rRNA genes was amplified with bacterial primers 338Fb and 518Rb (a bit modified version of primer com2) as described previously (5, 28). The PCR protocol included 2.0 units of the FastStart DNA polymerase (Roche, Paris, France) in 20 μL and was run for 43 cycles at 53°C primer annealing temperature using a TProfessional thermocycler (Biometra, Goettingen, Germany). Sham DNA extraction and DNA-free PCR were always run in parallel as contamination controls.

Clone libraries, sequence and phylogenetic analyses, and statistics

The V3-V5 amplicons (about 590 bp for E. coli) were cloned into the TOPO TA cloning vector (TOPO TA Cloning Kits for Sequencing; Invitrogen) using chemically competent TOP10 cells (Invitrogen). The presence and size of inserts were assessed by PCR using a vector M13 sequence-based primers flanking insert. The samples and relevant clone libraries are listed in Table 1.

For each constructed clone library, 20 or more randomly selected clones were lysed and analyzed by amplified rDNA restriction analysis (ARDRA). Aliquots (2.5 μL) of amplicon were digested for 2 h at 37°C with Alul, HpaII and HaeIII (NEB, Ipswich, MA, USA). The restriction fragments were separated by electrophoresis in 4.5% agarose gel (a mixture of Pronadisa Agarose D1 [Conda, Madrid, Spain] and MetaPhor agarose [Lonza, Rockland, ME, USA] along with Synergel [Diversified Biotech, Boston, MA, USA] as agarose clarifier additive) in a 0.5×Tris-borate-EDTA buffer and...
visualized after ethidium bromide staining. Similar restriction band profiles were combined into ribogroups or ribotypes.

As a preliminary estimate of library diversity, Good’s coverage index (16) was computed, and additional clones were analyzed, if needed, in order to improve library coverage and to reach the ≥50% threshold. One to three clones representing a ribogroup were amplified by PCR with M13-based primers and amplicons generated were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced by LGK Genomics GmbH (Berlin, Germany). The sequences were grouped into phylotypes (OTUs) using a threshold of ≥98% sequence similarity. The same value was used for phylotype identification amongst the BLAST-found closest relatives in GenBank. Since only representative clones of unique ARDRA ribotype were sequenced, all the clones within the group were assigned to the same phylotype.

The findings from control reactions were used to update our contaminant library (hereafter designated as CL) established previously (5). The phylotypes discovered in each clone library were initially tested against CL entries and only those not present in the CL (so called “true” phylotypes) were further analyzed using the GenBank database with BLAST software in binary mode: closest environmental clone and closest known taxon.

Those phylotypes showing less than 90% sequence similarity with known taxa were assigned to UNIDENTIFIED entries. If such phylotypes proved to be impossible to assign to a known phylum by phylogenetic reconstruction, they were finally defined as UNCLASSIFIED. Those phylotypes showing con-specificity (≥98% similarity) with normal human microbiome findings (e.g., skin, intestine etc.) (18, 53) were designated human-associated phylotypes (hereafter HA-phylotypes). Furthermore, phylotypes which demonstrated con-specificity with Genbank clones/isolates recovered from various cold environments were assigned as cold-loving icy phylotypes.

Multiple sequence alignment was generated using CLUSTALW (52). For phylogenetic tree inference, the maximum parsimony algorithm as implemented in the MEGA4 software (27) was used. The branch support was calculated for 500 bootstrap replicates of the data.

Clone library coverage was determined using Good’s coverage (16). A Shannon–Weaver index was generated through the DOTUR 1.53 program (46), using the formula $H = -\sum p_i \ln p_i$, where $p_i$ is the proportion of the $i$th phylotypes, calculated from $n/N$ (where $n$ is the number of sequences in each phylotype and $N$ is the total number of sequences in the data set). Chaol richness estimates were calculated also in DOTUR 1.53 (46) using the formula $\text{Chaol} = \sum n_i(1-n_i)/\sum n_i$, where $n_i$ is the number of phylotypes observed, $n_i$ is the number of singletons (phylotypes represented by a single clone), and $n_i$ is the number of doubletons (phylotypes represented by two clones). Rarefaction curves were computed based on the analytical approximation algorithm of Hurlbert (24) using the rarefactWin (version 1.3, http://www.uga.edu/~strata/software/anRarefactWin.html). The 16S rRNA clone libraries were compared using the LIBSHUFF option in Mothur (version 1.5.0, http://www.mothur.org/wiki/Main_Page) in order to estimate whether microbial communities from different samples were overlapping or non-overlapping.

Culturing radiation-resistant bacteria

A culturing approach was implemented in an attempt to recover radiation-resistant species (e.g., Deinococcus sp. and Geodermatophilus sp.) in a snowpack on MtBI contaminated with Saharan dust. Briefly, culturing followed standard procedures using serial tenfold aqueous dilutions on standard methods agar (SMA; Difco-BD, Franklin Lakes, NJ, USA) and tryptcase soy agar (TSA; Difco-BD) plates incubated at 28°C for 4 days with and without a UV irradiation step (1 min) at 254 nm in the laminar flow hood. The UV lamp was a standard Sylvania mercury vapor 30 W fluorescent tube delivering 254 nm centered radiation. The tube was at a distance of 10 cm, which would yield a theoretical output of 13 kJ m⁻² after 60 s of exposure. DNA isolation was as described before (22), and bacterial 16S rRNA genes were amplified by PCR with pA and pH' primers (4) and cloned as described above prior to sequence determination and analysis.

Nucleotide sequence accession numbers

The nucleotide sequence data determined in this study were deposited in the GenBank/EMBL/DDBJ databases under accession numbers HQ396620–HQ396624 for isolates and HM104591–HM104622, HQ396557–HQ396558, HQ396567–HQ396619 and HQ400256–HQ400266 for clones.

Results

Identification of dust source

Back-trajectories of all studied dust events indicated basically the same source area located in North Africa (Fig. S1).

Analysis of the elemental composition measured by X-ray fluorescence did not reveal any major differences in composition among various samples. The only discriminating factor was the representation of the $K_2O/(SiO_2 + Al_2O_3)$ ratio vs. $SiO_2/Al_2O_3$ ratio. These ratios, calculated from elemental concentrations of Al, Si and K converted into oxides (by weight), are used to define the Al and Si content in aluminosilicates, mainly clays and feldspsars. The higher than 2 $SiO_2/Al_2O_3$ ratio suggests clays either in the form of illite or smectite, the former being characterized by a higher $K_2O/(SiO_2+Al_2O_3)$ ratio. Thus, regarding the clay mineral composition of the dust, our samples could be categorized as either illite-type or smectite-type samples (Table 1). The mineral composition of the sand sample was dominated by quartz (as inferred from the very high Si/Al ratio), so its elemental composition did not assist in identifying the remaining minerals.

Dust concentrations and volume size distributions

While the total dust concentration within snow and blood rain samples varied by more than 3 orders of magnitude (0.5 ppm to 15 ppm for MtBI snow, up to >350 ppm for the blood rain sample), they shared a common pattern of size distribution with relatively minor variations with respect to their wide spectrum (between 0.8 μm to 25 μm). The mean modal size of the dust carried to MtBI was about 5.5 μm (Fig. 2), the dust particles deposited on MtBI in June 2006 and June 2008 showed a larger mode around 6–6.5 μm, and the May 2009 event a smaller mode ~4 μm (Fig. 2). The smallest mode of ~3 μm was observed for the insoluble particles of non-Saharan (local, regional, etc.) origin (Dom08/2009, Fig. 2) as it came from a snow sample collected at altitude, just after heavy snow episode during the summertime when intense atmospheric convection uplifts the air and anthropogenic pollution from the valley. In the case of the May 2008 Grenoble blood rain event, the size distribution mode was about 8–9 μm.

Bacterial cell enumeration

Cell concentration could be assessed only for 4 out of 5 MtBI snow samples: 110 and 214 cells mL⁻¹ for samples SDm06/2008 and SDm05/2008, respectively, and 0 and 2 cells mL⁻¹ for samples SDm05/2009 and Dom08/2009, respectively. The first two samples were collected in small volumes, while the latter two were collected in much larger
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volumes. Sample SDm06/2006 as well as two other Saharan dust and sand samples showed high background fluorescence due to abundant mineral particles, making cells hard to discern.

Clone library features and statistics

A total of 295 clones from 7 clone libraries were grouped into ribotypes by AluI, HaeIII and HpaII digestion of the PCR-amplified V3-V5 region of bacterial 16S rRNA genes. Representative clones of each ribotype were sequenced and sequences were grouped into 129 phylotypes (Table 2). The “true” phylotypes amounted to 121 entries after removing those that were found to have counterparts in our CL (Table 2). Although the majority of phylotypes showed ≤95% similarity with sequence entries deposited in GenBank, 16 of them were initially unidentified, thus representing novel taxa (Table S2). Seven out of the 16 phylotypes have since been assigned as Bacteroidetes, Alphaproteobacteria, Actinobacteria and chloroplast DNA based on phylogenetic reconstructions, while 9 others have remained unidentified and unclassified.

Moreover, 13 phylotypes have proved to be con-specific to clones or taxa of normal human microflora, thus representing HA-phylotypes. These were all recovered from samples of Saharan dust transported a great distance and were not found in the desert sand or in the “clean” snow containing no Saharan dust.

On the whole, the analyzed clone libraries were quite well covered with a Good’s coverage index average of about 72% (Table 2). The rarefaction curves clearly approached the plateau only in the case of the MB5 snow-derived clone library (Fig. 3). The SD and SS clone libraries (Saharan dust collected in Grenoble and Saharan sand, respectively) were the most diverse and also the most under-described libraries, as measured by indices and supported by rarefaction analysis (Table 2).

The LIBSHUFF analyses revealed significant differences between all snow-derived clone libraries (P<0.0001 for almost all pairwise comparisons, Table S4). The Saharan dusty snow libraries were also significantly different from the Saharan sand library, as well as the Saharan dust library corresponding to May 2008 even at lower altitude (all P values were <0.0001, Table S4).

Table 2. 16S rRNA clone libraries and their characteristics

| Clone library | Clones/ phylotypes | CL foreclosure/ phylotypes | “True” clones/ phylotypes | Shannon-Weaver indexb (95% CI) | Chao 1 (95% CI) | Good’s coverage (%) | HAc phylotypes | Icyd phylotypes |
|---------------|--------------------|--------------------------|---------------------------|-------------------------------|----------------|-------------------|----------------|----------------|---|
| Mineral type—illite | MB-SD 38/19 0 38/19 2.67 (2.40–2.93) 24 (19.33–45.1) 74 3 3 | | | | | | | |
| | SD 39/24 0 39/24 2.88 (2.59–3.17) 68.33 (36.08–180.03) 54 1 2 | | | | | | | |
| | MB-SD 38/15 2 29/19 2.18 (1.81–2.55) 20 (14.45–46.75) 72 2 1 | | | | | | | |
| Mineral type—smectite | MB5 40/12 1 39/11 2.04 (1.75–2.34) 12 (11.09–21.68) 92 2 1 | | | | | | | |
| | MS5 60/20 2 57/18 1.67 (1.23–2.02) 22.2 (16.6–47.37) 74 5 3 | | | | | | | |
| | MS7 40/17 3 36/14 1.92 (1.5–2.35) 31 (16.99–94.31) 75 0 0 | | | | | | | |
| Mineral type—ND | SS 40/22 0 40/22 2.8 (2.52–3.08) 30.43 (23.48–56.91) 65 0 5 | | | | | | | |
| Total | 295/129 8 278/121 NA NA NA | | | |

a CL: contaminant phylotypes from our contaminant library.

b Diversity indices as well as library coverage were calculated using only “true” phylotypes.

c HA: human-associated phylotypes.

d Icy phylotypes mean phylotypes closely related to cold-loving bacteria.
Taxonomic assignment of 61 phylotypes discovered in the four Saharan dusty snow samples showed differences in the species affiliation and phylum distribution pattern among all four samples (Fig. 4, Table S2). The smectite-type clay SDm06/2006 sample was dominated by *Deinococcus-Thermus*, *Bacteroidetes* and *Alphaproteobacteria*, unlike the other smectite-type clay sample (SDm05/2009) that had an abundance of *Actinobacteria* and chloroplast DNA. Interestingly, all abundant *Deinococcus-Thermus* sequences observed in SDm06/2006 sample were affiliated only with the two *Deinococcus*-like species (Table S2). The illite-type clay samples collected in 2008 within a 1-month time period (SDm06/2008 and SDm06/2008) demonstrated completely different species profiles and phylum distribution patterns from those observed in the smectite-type. Nevertheless, both SDm05/2008 and SDm06/2008 samples displayed a predominance of *Actinobacteria* and *Alphaproteobacteria* (Fig. 4). Also, samples of smectite-type clay collected in 2009 over a 3-month time period (SDm05/2009 and Dm08/2009) but differing in dust source (Saharan vs. non-Saharan), showed some similarities at the phylum level. Both samples were dominated by *Actinobacteria* and chloroplast DNA (Fig. 4).

Differences in the taxonomic composition of microbial assemblages became more evident after comparing the above samples with the MtBl snow sample containing no Saharan dust as well as samples of Saharan sand and Saharan dust collected in Grenoble (Fig. 4). The Saharan dust samples after the same May 2008 event (SDm05/2008 and Dm05/2008) collected at low altitude (as dust Grenoble) and high altitude (as snowpack with dust on MtBl) showed different species composition, dominated by *Cyanobacteria*, *Alphaproteobacteria* and chloroplast DNA in the first case and by *Actinobacteria* and *Alphaproteobacteria* in the second (Fig. 4). It is worth noting that the Saharan sand sample was dominated by *Bacteroidetes* (Fig. 4). Finally,
representatives of the family **Geodermatophilaceae** (genera **Geodermatophilus**, **Blastococcus** and **Modestobacter**) were found in all samples with the exception of the snow sample containing Saharan dust from the June 2006 event and the Saharan dust sample collected in Grenoble.

Although no common species set was observed among studied samples, at least three phylotypes were detected in more than one sample (Fig. 4, Table S2). For example, the phylotype related to **Sphingomonas kaiensis** was found in snow samples that received Saharan dust from the May and June 2008 events, respectively (SDm05/2008 and SDm06/2008). Snow samples containing Saharan dust from the May 2008 and May 2009 events (SDm05/2008 and SDm05/2009) had one shared phylotype related to **Blastococcus saxobsidens**. Phylotype related to **Rubellimicrobium mesophilum** was detected in two dusty snow samples (SDm06/2008 and SDm05/2009) and the Saharan sand sample.

**Snow-colonizing bacterial phylotypes**

All 121 obtained phylotypes except for HA-phylotypes were evaluated *in silico* for their ability to colonize MtBl snow below zero degrees temperature, through comprehensive consideration of their closest relatives in GenBank. As a result, a total of 15 phylotypes (hereafter called icy phylotypes) were recognized in all samples studied except for the "clean" snow sample (Fig. 5, Table S3). The majority of these icy phylotypes were grouped with **Actinobacteria** (31.3%) and **Alphaproteobacteria** (26.7%) while the remaining phylotypes were assigned to **Cyanobacteria**, **Bacteroidetes**, **Deinococcus-Thermus**, **Betaproteobacteria** with one phylotype remaining unclassified. It is noteworthy that 8 icy phylotypes recognized in the dusty snow samples were related to chemoorganotrophic (87.5%) and photolithoautotrophic (12.5%) bacteria while 6 icy phylotypes (excluding unclassified phylotype) from the Saharan dust collected in Grenoble and Saharan sand samples were related only to the chemoorganotrophic species (Table S2).

With respect to the growth temperature, only one icy phylotype derived from the Saharan dusty snow sample (SDm05/2009) could be considered psychrotolerant as indicated by its closest strain characterization within the species range. At least 7 other icy phylotypes were con-specific with mesophilic organisms, while the remaining 7 phylotypes showed no particular temperature preference (Table S2). The closest relatives of icy phylotypes recognized in the dusty snow samples were found in alpine/polar snow, cold soil and sediments, alpine lakes and even clouds. In comparison, the majority of the icy phylotypes derived from the Saharan dust collected in Grenoble and Saharan sand samples were closely related to bacteria found in cold soil (Fig. 5, Table S3).

**Culturing snow melt**

An attempt to culture snow-melt microbes was made, specifically targeting radiation-resistant **Deinococci**, but with no success (Table 3). Two colony-forming clones were recovered from the non-irradiated assay, and these were found to belong to **Actinobacteria** and to **Firmicutes**. A similar result was obtained from the UV-irradiated assay,
with 3 colony-forming clones recovered, and all belonged to Actinobacteria and to Firmicutes. The latter two were represented by bacterial species that possess UV-resistant features such as spore formation and pigmentation.

**Discussion**

The desert constitutes a major source of dust that can be uplifted by strong winds and carried for thousands of kilometers, thus fertilizing faraway downwind regions with nutrients and bringing microorganisms. The Sahara is the most important natural source of dust that can be transported across the Mediterranean towards the Alpine region several times a year (17, 49); however, mobilization areas of dust in the Sahara, transport pathways and meteorological conditions can vary and thus affect the associated bacterial load. The clay mineralogy of Saharan dust is known to be characteristic of different source regions (17). In this study, the dust elemental composition indicates the presence of two mineral types in our samples, namely illite and smectite. Both detected minerals are rather common in the soils of North and West Sahara, especially in the Tunisian and Libyan deserts (40).

It has been suggested that variations in the dust particles size distribution may be related to changes in the source and/or transport pathways (25). Variability in size distribution and in the concentration of aeolian dust, which is deposited over the Grenoble region and the southeastern part of France, is highly dependent on the peculiar atmospheric circulation and synoptic conditions driving the northward advection of North African dust plumes. The size distribution of particles reaching low and high altitudes is different, and larger particles have a lower chance of being observed on a glacier. Namely, this result was observed with Saharan dust that had been deposited in Grenoble and on MtBl during the May 2008 dust event. Furthermore, the results from this study demonstrated variations in the size distribution of the dust particles associated with the Saharan dust depositions of the same clay type on MtBl. We suggest that this may be an indication of differences in the atmospheric transport pathway and/or dust travelling time.

The bacterial cell concentrations in mountain snow can reach up to $10^9$ cells mL$^{-1}$ when associated with dust particles (30, 47) and the lowest values ever reported ($6.8 \times 10^5$ cells mL$^{-1}$) were shown for the Everest snow (30). Cell concentrations observed in our dusty snow samples appear to be the lowest known for mountain snowpack. This result can be explained by the treated volume of the snow sample and by the high level of precautions against contamination during snow sample collection. Although some bacteria could be attached to the dust particles (8), it has been shown that mineral particles with attached bacteria made up only 10% of the total mineral particles (31). Hence, it appears that the obtained cell counts were not largely underestimated.

Based on rarefaction analysis and coverage estimators, the number of screened clones was sufficient to reveal the entire diversity only in the dusty snow sample from the June 2006 dust event. The coverage of other dusty snow clone libraries (>70%) also provided a good inventory of the actual diversity in these samples. The highest potential for discovering major new groups by analyzing the additional clones was detected for Saharan dust from Grenoble and Saharan sand libraries. Liu et al. (30) found that the bacterial diversity was much higher in the case of glaciers having high Ca$^{2+}$ concentrations, which is an indication of dust deposition. Our data also demonstrate higher diversity indices for microbial communities derived from the snow samples containing Saharan dust, as compared to those detected in the complementary “clean” snow sample.

LIBSHUFF analysis and comparison of phytype content/distribution amongst gene libraries showed that microbial communities in alpine snow that received four Saharan dust depositions over the period 2006–2009 are distinct in their composition. This indicates that dust events even of the same clay type and during month-long time periods can bring completely novel species to the Alpine glaciers. The May 2008 dust event recorded at different altitudes also demonstrated that depending on the dust grain distribution and local aeolian conditions for dust transport, the microbial taxa occurrence and distributions can be different. We hypothesize that the variability in bacterial community composition in alpine snow associated with Saharan dust results probably more from the conditions of dust mobilization in a source area, its transport and the preservation period in snow, than from dust sources. As a complementary finding, we suggest that the same factors may partly explain the differences in bacterial fingerprints observed between the Saharan dust and Saharan sand containing samples. Given that some microorganisms are capable of further (albeit slow) development in snow, the length of the Saharan dust preservation period (seasonally adjusted) in snow becomes an important time

<table>
<thead>
<tr>
<th>Isolate (Acc.Number)</th>
<th>Closest cultured match in Genbank (Acc.Number)</th>
<th>similarity (%)</th>
<th>Comments (information from Genbank submission data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN21am (HQ396622)</td>
<td><em>Arthrobacter oxydans</em> 1663 (EU086792)</td>
<td>99</td>
<td>Isolated from human clinical specimens.</td>
</tr>
<tr>
<td>PN13a (HQ396621)</td>
<td><em>Terrabacillus saccharophilus</em> RB589 (AB243847)</td>
<td>98</td>
<td>Spore-forming bacterium isolated from field soil in Japan.</td>
</tr>
<tr>
<td>UV+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN24UVa (HQ396620)</td>
<td><em>Curtobacterium flaccumfaciens pv. flaccumfaciens</em> LMG 3645 (NR_025467)</td>
<td>99</td>
<td>Isolated from the phyllosphere and litter layer after mulching the sward.</td>
</tr>
<tr>
<td>PN25UVam (HQ396623)</td>
<td><em>Bacillus simplex</em> WN570 (DQ275175)</td>
<td>99</td>
<td>Spore-forming bacterium. Isolated from granite.</td>
</tr>
<tr>
<td>PN26UVa (HQ396624)</td>
<td><em>Bacillus pumilus</em> SAFR-032 (CP000813)</td>
<td>100</td>
<td>Spore-forming bacterium. Exhibit elevated resistance to UV radiation and H$_2$O$_2$ compared to other Bacillus species.</td>
</tr>
</tbody>
</table>
factor for microbial community composition, selecting for more advanced and adapted species. For example, the abundance of Deinococcus-like phylotypes observed in the sample containing Saharan dust deposited during 3.5 months in the snowpack (June, July and August), could be related to their development in snow. Also, the fact that out of 11 detected phylotypes in this sample, 6 phylotypes accounted for 82% in a library, suggests that this might be the consequence of their propagation or survival in snow.

In the present study, all the dominant phylotype divisions detected in different snow samples containing Saharan dust are known to be abundant in arid regions. For example, Actinobacteria and Proteobacteria are frequently represented in soils, and the former may constitute up to 50% of the total bacterial populations in desert soils (3, 19). Molecular microbial diversity in Tatsahouine sand was dominated by Proteobacteria, Actinobacteria and Acidobacteria with Bacteroidetes easily cultured (7). In addition, the Actinobacteria, namely those belonging to family Geodermatophilaceae, were found in almost all of our samples. They are predominantly recovered from extreme environments such as the Antarctic or hot desert soils (33), rocks and monument surfaces (12, 13, 34). Furthermore, some of the genera/phyla that have been identified in our dusty snow samples were also found previously in high mountain snow. For example, the study of mountain snow with Saharan dust deposition in the Central Pyrenees (2,240 m a.s.l.) showed the predominance of Betaproteobacteria and Actinobacteria (21). A study of bacterial abundance and biomass in mountain snow from the Tateyama Mountains (2,450–2,700 m a.s.l.) in Japan revealed that Betaproteobacteria dominated, while Actinobacteria, Firmicutes and Bacteroidetes formed the subdominant taxa (47). Here we detected only three minor Betaproteobacteria phylotypes. Interestingly, one of those phylotypes was closely related to Massilia spp. detected in “red snow” in an Antarctic oasis (14). Liu et al. (30) found that the Hymenobacter (Cytophagaceae) is rather abundant in the mountain snow from the Guoqu and Zadang Glacier, where the dust impact from China deserts occurred seasonally. In our dusty snow sample, only two minor Hymenobacter-like phylotypes were detected. In addition, the Guoqu and Zadang Glacier snow communities also showed a high abundance of Proteobacteria (Alpha-, Beta- and Gamma-) and Actinobacteria, paralleling our own findings. Three of our minor phylotypes were found to be similar to those reported by Liu et al. (30) for the above-mentioned glaciers. Interestingly, two belonged to the widespread chemoorganotrophic Sphingomonas genus, which was rather common in four Everest snow samples (30).

With respect to survival abilities, the genus Geodermatophilus along with Deinococcus, Hymenobacter and Methylobacterium detected in the present study, are known to be resistant to high doses of ionizing radiation (41). Radiation-resistant bacteria originating from the desert soil could have a certain advantage to survive during their aeolian transport with dust and subsequent deposition on high mountain snow and being distessed by harsh UV-radiation. Taking this into account, attempts were made to culture radiation-resistant bacteria, notably Deinococcus; however, if the radiation level was sufficient to produce a high number of radiation-resistant lineages, the DNA was in all likelihood too damaged or culture conditions were not sufficient to permit growth of Deinococcus-like bacteria recovered using a molecular approach. Yet, all three clones recovered from the UV-treated sample were characterized by radiation resistance. Two belonged to Bacillus (B. pumilus and B. simplex), a genus that features spore formation. Such spores are more resistant to UV radiation than vegetative cells because desiccation, the presence of small acid-soluble spore proteins and the spore core’s large pool of dipicolinic acid do mitigate DNA damage (48). The third clone, Curtobacterium flaccumfaciens, is a phytopathogenic actinobacterium that has been shown to colonize the phyllosphere, thus a UV-exposed biotope. Jacobs and Sundin (26) described it as UV-resistant, the feature which is most probably related to the ability of this bacterium to synthesize pigments as do many other actinobacteria.

Dust-borne bacteria originating from hot or cold terrestrial deserts can be viewed as possible snow colonists because they are able to withstand increased radiation and desiccation (15), and tolerate low temperatures. Fifteen phylotypes recognized in the present study presumably possess survival/colonization abilities in a snowpack, as inferred from their closest strain physiology or closest relative sources (different cold habitats). Based on the first criterion, only one phylotype proved to be psychrotolerant. Still, we suggest that these seven icky phylotypes related to mesophilic species may survive/grow in snow during the summertime on the MtBl glacier. Seven remaining phylotypes which showed lower similarity in a sequence with known taxa (<98%) were included in the list of possible snow colonizers mostly based on their closest relative sources. At least four icky phylotypes identified in dusty snow samples were closely related to those reported from mountain and polar snow (14, 30). This observation supports their cosmopolitan distribution in different snow eoniches.

In the present study, 13 phylotypes were categorized as HA-phylotypes based on their con-specificity (≥98% similarity) with normal (non-pathogenic) human microbiome representatives. These phylotypes could represent contaminants resulting from labware while they were absent from our contaminant library.

From our study, it appears that the seasonally abundant deposition of Saharan dust on the snow surfaces of Alpine glaciers is not only a source of nutrients but also a vehicle for diverse bacteria, which could thrive on or within snow. The difference in microbial community composition among four Saharan dust depositions in alpine snow over the period 2006–2009 indicates that dust events of the same clay types and in rather short time periods, can bring completely different taxa to Alpine glaciers. Such a difference can be caused by the conditions of aeolian dust transport. To prove this assumption, it will be necessary to investigate a much larger number of snow samples containing Saharan dust from known sources, soil samples representing these sources and snow samples containing no Saharan dust. Meteorological conditions experienced during dust transport, as well as the description of pathways will also be required for each of the events. The data on microbial diversity in mountain snow are still limited as well as data on the potential
for bacteria associated with Saharan dust to colonize icy environments. Hence, further studies using high-throughput sequencing and RNA-based approach should help us gain more insight into the temporal and spatial scales over which bacterial communities change and become adapted to grow in mountain snow.

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References


