Bacterial characterization of the snow cover at Spitzberg, Svalbard

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Abstract

A sampling campaign was organized during spring 2004 in Spitzberg, Svalbard, in the area around the scientific base of Ny-Ålesund, to characterize the snow pack bacterial population. Total bacteria counts were established by 4′,6-diamino-2-phenylindole (DAPI) in the seasonal snow pack bordering the sea. On the sea shore, bacterial concentration was about 6×10^4 cells mL^{-1}, without any significant variation according to depth. In the accumulation snow layer of the glacier, concentrations were about 2×10^4 cells mL^{-1}, except in the 2003 summer layer, where it reached 2×10^5 cells mL^{-1}, as the result of cell multiplication allowed by higher temperature and snow melting. Strains isolated from the seasonal snow pack were identified from their 16S rRNA gene sequences, and lodged in GenBank. They belong to the *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria*. They are closely related to cold environment bacteria, as revealed by phylogenetic tree constructions, and two appear to be of unknown affiliation. Using ^1H nuclear magnetic resonance, it was shown that these isolates have the capacity to degrade organic compounds found in Arctic snow (propionate, acetate and formate), and this can allow them to develop when snow melts, and thus to be actively involved in snow chemistry.

Introduction

Cold environments represent a large part of the Earth's biosphere, and their microbiota are of increasing interest. These can provide habitats for cells (Psenner & Sattler, 1998; Sattler *et al.*, 2001) that are already strongly suspected to play chemical roles in glaciers (Skidmore *et al.*, 2005), atmospheric clouds (Amato *et al.*, 2005) and more largely in water environments of these cold regions (Margesin & Schinner, 2001; Price & Sowers, 2004). It has been shown that bacterial activity can occur at subzero temperatures (Carpenter *et al.*, 2000; Junge *et al.*, 2004) and several physiological characteristics can allow such species to be active, for example spore formation, pigmentation (Fong *et al.*, 2001; Mueller *et al.*, 2005), increase in membrane fluidity (Seshu Kumar *et al.*, 2002) and production of enzymes active at low temperature (Groudieva *et al.*, 2004). The bacterial genera most commonly found in these cold environments belong to, in descending order of abundance, the *Proteobacteria* (especially *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*), *Cytophaga–Flavobacterium–Bacteroides* group, and low- and high-G+C Gram-positive genera (references above, and Christner *et al.*, 2001; Zhang *et al.*, 2002; Miteva & Brenchley, 2005).

Over the past few years, many studies have enabled the microbial content of various polar or mountain cold environments to be characterized, and the highest bacterial abundance values observed were reported from Arctic sea ice, where concentrations can reach 1×10^5 bacteria mL^{-1} of melted ice (Brinkmeyer *et al.*, 2003; Junge *et al.*, 2004). Fewer than 3×10^3 bacteria mL^{-1} were detected in the deep accretion ice of the subglacial Antarctic lake Vostok (*c.* 3500 m below the surface) (Karl *et al.*, 1999; Priscu *et al.*, 1999; Abyzov *et al.*, 2001). Intermediate bacterial concentration values were obtained for snow from glaciers (Skidmore *et al.*, 2000; Zhang *et al.*, 2002; Foght *et al.*, 2004), polar and alpine lakes (Alfreider *et al.*, 1996; Pearce *et al.*, 2003), and clouds (Bauer *et al.*, 2002; Amato *et al.*, 2005). For mid-latitude environments, mountain snow bacterial concentrations have been reported to range from 3×10^3 cells mL^{-1} (Bauer *et al.*, 2002) to *c.* 4×10^5 cells mL^{-1} (Alfreider *et al.*, 1996; Sattler *et al.*, 2001; Segawa *et al.*, 2005). However, the bacterial content of surface snow in polar regions has not thus far been investigated. Here we present the

microbiological characteristics of a seasonal snow pack from Ny-Ålesund (Spitzberg) with (1) its bacterial load, (2) the identification of cultivable bacteria and (3) their ability to degrade organic substances present in Arctic snow.

Materials and methods

Sampling sites

Samples were collected from two sites: the snow cover in the vicinity of the scientific base of Ny-Ålesund (78°56′N, 11°52′E, 10 m a.s.l., Spitzberg, see Fig. 1), and in the accumulation area of the Kongsvegen glacier (78°45′N, 13°19′E, 670 m a.s.l.), about 40 km east of Ny-Ålesund. The Ny-Ålesund site is located by the sea, along the south coast of the Kongsford, which is orientated east-west and open to the sea on its west side. The glacier is of north-west/south-east orientation, in the continuity of the Konsfjord.

Sampling method

All samples were taken with great care in order to avoid contamination, by wearing sterile gloves and a mask and using sterile material wrapped in two successive bags that had been sterilized by autoclaving. To collect snow, the surface in contact with the air was first systematically scratched using a sterile spoon and discarded. A sterile tube (50 mL) was then embedded into the snow to fill it without any need for extra manipulation, which could provide a source of contamination. Samples from different depths were taken at the two sites to establish total cell profiles, from pits dug into the Ny-Ålesund seasonal snow cover and in the annual layer of the Kongsvegen glacier (i.e. 170 cm deep). At each sampled depth, at least three samples were taken and two pits were investigated at each of the two sites. In addition, samples for cultures were collected on the day prior to our departure from the Ny-Ålesund site, at *c.* 3–15 cm snow depth, using the same protocol.

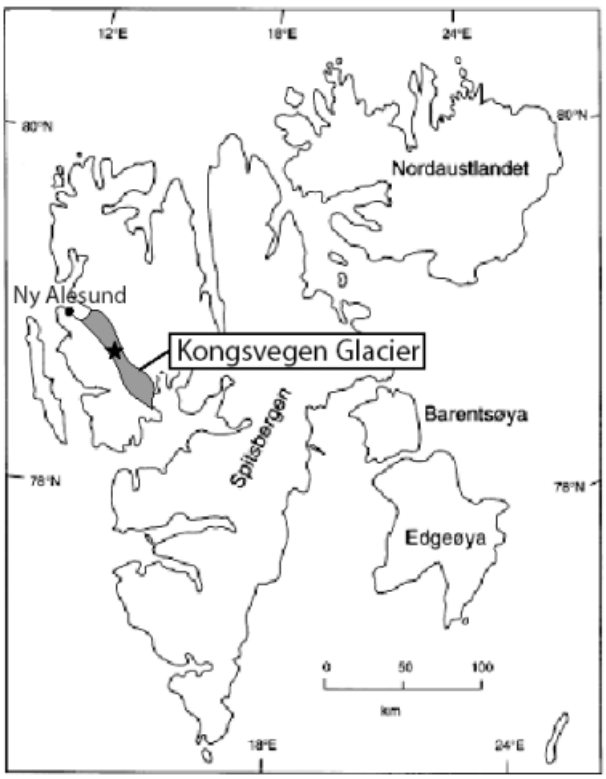

Fig. 1. Map of Spitzberg showing the locations of Ny-Ålesund and the Kongsvegen glacier.

Radionuclide measurements

To characterize the annual accumulation layer of the Kongsvegen glacier, radio nuclides were measured in snow samples collected for that purpose, just after those taken for microbial investigations.

Using a method developed by Delmas & Pourchet (1977), snow samples were melted (~500 mL, every 10 cm of snow equivalent), weighed, acidified and filtered on ion exchange paper, where all radionuclides were trapped. After drying, the filters were directly analysed by γ-spectrometry using a low-background germanium detector (germanium diode N type) (Pinglot & Pourchet, 1994). Because of low activity readings, some samples were combined for radioactivity analysis. For high-resolution gamma spectrometry, the analyser was protected against all interfering ambient radioactivity, in particular using an anti-Compton device. This system provides a lower detection threshold, especially for the isotopes of interest, such as ^{210}Pb (22.3-year half-life) and ^{7}Be (53 days) (Pinglot & Pourchet, 1994; Pourchet *et al.*, 2003). Standard ^{137}Cs, ^{210}Pb and ^{241}Am liquids from the CEA or Amersham laboratories (2% uncertainty at 95% confidence level) were used to calibrate the detector. The analytical procedures were the same as those used for the snow samples. The ^{210}Pb and ^{7}Be measurements were carried out with a time resolution between 24 and 72 h. Quantitative analysis software (GENIE 2000 v1.4; Canberra) was used to compute the activity of existing radionuclides and the associated accuracies. The total uncertainties due to both sampling procedures and counting statistics are of the order of 20% for ^{210}Pb and 50% for ^{7}Be. The ^{7}Be-specific activities were corrected for decay to the deposition time and counting. Blank and background values were checked regularly.

Total bacteria counts

Samples for total cell counts were treated immediately following collection. They were slowly melted at ambient temperature (about 15 °C) and fixed with an equal volume of prefiltered (0.22 μm; Millipore) 4–5% formaldehyde, and

stored at 4 °C before analysis. Each of the 30-mL fixed samples was incubated for 20 min in the dark with 2.5 μg mL^{-1} (final concentration) of 4′,6-diamino-2-phenylindole (DAPI) to stain cells (Porter & Feig, 1980), filtered (dark GTPB filters of 0.22 μm pore size) and rinsed with prefiltered (0.22 μm) distilled water. Filters were then mounted on slides, observed under epifluorescence microscopy (Olympus BH-2), × 1000 magnification, and cell numbers present on random fields were counted (at least 30 fields). Standard errors were calculated from at least three samples, from each of the two pits. Blanks, comprising filtered and distilled water only, were regularly checked.

Cultures and isolations of bacteria

Each sample to be used for cultivation was stored and transported frozen from Ny-Ålesund to the laboratory in France for analysis. It was then slowly and completely melted at ambient temperature, and divided to be incubated under different conditions. First, triplicates of 0.1 mL were directly plated onto solid R2A (Reasoner & Geldreich, 1983; Difco), and trypcase soy (TS) (bioMérieux) and Sabouraud (Difco) media supplemented with agar (20 g L^{-1}), to be incubated at 4, 15 and 27 °C. By contrast, liquid cultures were performed by enrichment of the melted snow with TS (1% and 50% v/v final concentration of the nutritive medium initially prepared at 30 g L^{-1}) and R2 (1% and 50% v/v final concentration of the nutritive medium initially prepared at 3.2 g L^{-1}) media. Liquid R2 was prepared according to the recipe of the commercial medium R2A, but free of agar. Triplicates of 100 mL of all these four media were made in 500-mL flasks incubated at 17 °C, under agitation (200 r.p.m.). When growth was visually detected, 0.1 mL was plated onto the corresponding solid medium for isolation of colonies. Each colony was differentiated from others on the basis of morphological criteria, and isolations were ensured by successive transfers onto the same medium if necessary. For all the experiments, prechilled filter tips and media were used to preserve cells from any heat shock.

Phylogenetic analysis and tree construction

Cell pellets obtained after centrifugation of liquid pure cultures of the isolated strains were resuspended into phosphate-buffered saline (PBS) solution and their total genomic DNA was extracted using the Easy DNA Kit (Invitrogen). Extracts were verified by gel electrophoresis, and 16S rRNA genes were amplified by PCR. This step was carried out using universal primers for *Eubacteria*: F8-Eub (5′-AGA GTTTGATCMTGGCTC-3′) and 1492r-Univ (5′-GNTACCTTGTTACGACTT-3′) (Humayoun *et al.*, 2003), in which M corresponds to A or C, and N to any one of the four nucleotides. About 100 ng of genomic DNA and 1.5 U of *Taq* polymerase (QBiogene) were employed. PCR was performed as follows: 25 cycles of 30 s at 94 °C for DNA, 30 s at 55 °C and 90 s at 72 °C, preceded by 5 min at 94 °C and ending with 7 min at 72 °C. PCR products were checked by gel electrophoresis, and purified using a Strataprep purification kit (Stratagene). They were finally freeze dried and sequenced by capillary electrophoresis (MWG-Biotech), using the previously described F8-Eub and 1492r-Univ primers, and a central primer, F500 (5′-CTAACT ACGTGC-CAGCAGC-3′), in order to obtain overlapping sequences. Comparisons of almost complete reconstructed 16S rRNA gene sequences with those included in GenBank were performed using the BLASTN interface, available at http://www.ncbi.nlm.nih.gov/BLAST/.

Multiple alignments of the 16S rRNA gene sequences obtained with some selected from among the closest related neighbours and others selected in the literature were achieved using CLUSTALW (http://www.ebi.ac.uk/clustalw/) and visually checked. Lengths of sequences were adjusted according to these multiple alignments and final lengths of 1367 and 1386 bp were thus compared for Gram-negative and Gram-positive isolates, respectively. One hundred trees generated by bootstrap were analysed by the neighbour-joining method of the PHYLIP 3.63 package (Felsenstein, 2004), after application of the Kimura two-parameter correction on calculated distances (correction of differences between rates of tranversion and transition) (Kimura, 1980). Two consensus trees were finally constructed to place both our Gram-negative and Gram-positive isolates.

Metabolic activity and ^{1}H NMR analyses

For each isolated strain tested, liquid precultures were first made at 17 °C in R2 broth for 48 h. Then, cultures were made under the same conditions, by transferring 3% v/v of preculture into fresh R2 broths. After 24–48 h growth, cells were harvested by centrifugation (4000 ***g***, 15 min, 4 °C), rinsed twice with NaCl 0.8%, and resuspended in test medium in order to obtain a final $OD_{580\,nm}$ of 10. The test medium was composed as follows: 25 mL of 0.1 M phosphate buffer adjusted to pH 7.0, containing 20 mM of propionate (C_3), acetate (C_2) or formate (C_1). Blanks, constructed by incubation of cells without added substance, were used to check that no compound was obtained from endogenous cell metabolism taking place in the incubation media.

For analysis, supernatants were separated from cells by centrifugation (12 000 ***g***, 3 min), and analysed by ^{1}H nuclear magnetic resonance (NMR) to evaluate biodegradation abilities of each strain for each of the three compounds (Grivet *et al.*, 2003). NMR samples were prepared as follows. Supernatants (450 μL) from biodegradation tests were supplemented with 50 μL of a 20 mM solution of $TSPd_4$ (sodium tetra deuteriated trimethylsilyl propionate;

Eurisotop) in D_2O (Eurisotop). D_2O was used for locking and shimming while $TSPd_4$ constituted a reference for chemical shifts (0 p.p.m.) and quantification. ^1H NMR spectra were recorded at 400.13 MHz on a Bruker Avance 400 spectrometer (Bruker) at 21 °C with 5-mm-diameter tubes containing 500 μL of sample. Thirty-two scans were collected (90°pulse, 4789.27 Hz SW, 655 36 data points, 6.84 min total acquisition time for one spectrum). Water signal was eliminated by presaturation. No filter was applied before Fourier transformation but a baseline correction was performed on spectra before integration with Bruker software. Under these conditions, the limit of quantification was in the range 0.05 mM. The concentration of metabolites was calculated as follows: $[m] = (9 \times A_0 \times [TSPd_4])/(b \times A_{ref})$, where $[m]$ is the concentration of metabolite, A_0 is the area of metabolite m resonance, A_{ref} is the area of reference resonance in the ^1H NMR spectrum, b is the number of protons of metabolite m in the signal integrated, and 9 is the number of protons resonating at 0 p.p.m.

For each of the three substrates, percentages of biodegradation over a period of 24 h were calculated from concentrations measured in the incubation media (supernatants) at $t_{0\,h}$ and $t_{24\,h}$ (respectively, $Ct_{0\,h}$ and $Ct_{24\,h}$), as follows: $[(Ct_{0\,h} - Ct_{24\,h})/Ct_{0\,h}] \times 100$.

Results and discussion

Bacterial concentration profiles and seasonal snow characteristics

Bacterial counts made all along the snow cover are shown in Fig. 2(a). The total snow depth at Ny-Ålesund was about 70 cm, and bacterial concentration was homogeneous at about 6×10^4 cells mL^{-1} over this depth. This concentration is about one order of magnitude lower than those found in sea ice (Brinkmeyer *et al.*, 2003), but slightly higher than the concentration measured in the snow cover of Alpine sites (1.1×10^4 cells mL^{-1} in Sattler *et al.*, 2001; 2×10^4 cells mL^{-1} in Bauer *et al.*, 2002).

The snow pack studied here corresponded to snow deposited from October/November 2003 to early spring 2004. The determination of bacterial abundance in a 200-cm profile (Fig. 2b) on the Kongsvegen glacier allowed us to determine the variability of bacterial snow content for 1 year's snow precipitation, given that 170 cm represented the snow deposits accumulated since the end of summer 2003 (i.e. the 2003–2004 period). Specific activity of ^7Be clearly showed the presence of two major types of snow [noted as snow (a) and snow (b)] along this annual profile. In the upper layer [e.g. snow (a)], from the surface to a depth of 80 cm, mean ^7Be activity ranged from around 242 to 920 mBq kg^{-1}. Deeper, from 80 to 165 cm, the low activity, reaching no more than 120 mBq kg^{-1}, supports a clear

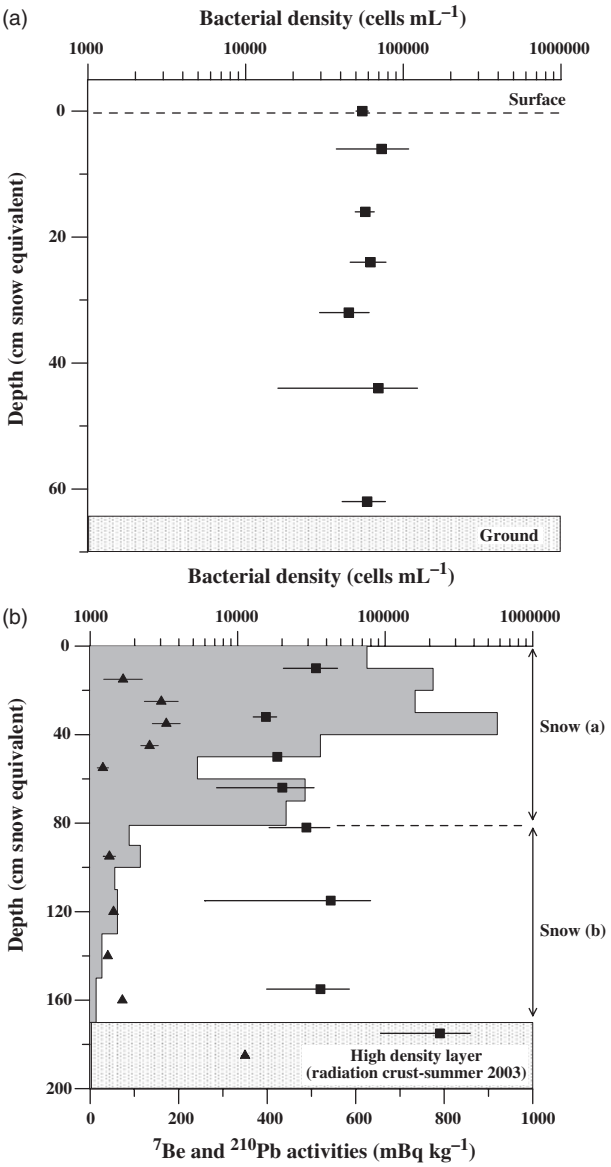

Fig. 2. Bacterial concentration (■) expressed as the number of cells mL^{-1} of melted sample, in the seasonal snow cover of the Ny-Ålesund site (a) and along the Kongsvegen glacier (b). Radionuclide-specific activities of ^7Be (shaded area) and ^{210}Pb (▲) were also measured along the annual accumulation snow layer of the glacier: snow (a) corresponds to fresh snow, deposited in winter (< 3 months), and snow (b) to that deposited between summer and winter; the deeper high-density layer (radiation crust) was the surface of the glacier during summer 2003.

difference of age between the two layers. Owing to radioactive decay properties and taking into account the variability of ^7Be atmospheric concentrations in this area, snow (a) was expected to be < 3 months old, and snow (b) corresponded to the period evaluated between the end of summer 2003 and January/February 2004. Below snow layer

Table 1. Isolated strains: conditions of isolation and description

Strain	Identification (sequence similarity)	GenBank accession number	Cell morphology	Colony pigmentation and morphology	Isolation temperature (°C)	Isolation media
A	*Chelatococcus* sp. (98.6%)	DQ497243	Short rods	Yellow, mucoid	27	Direct isolation, R2A
C	*Brevundimonas* sp. (99.5%)	DQ497234	Long, small thin rods	Orange, mucoid	17	Isolation after enrichment, 1% TS; 50% R2
D	*Bacillus* sp. (100%)	DQ497235	Thick rods	Translucent white, mucoid	17	Isolation after enrichment, 50% R2
E	*Micromonospora* sp. (99.7%)	DQ497236	Mycelium	Orange-red, raised, folded	17	Isolation after enrichment, 1% R2
H	*Hydrogenophaga* sp. (99.6%)	DQ497237	Small rods	Cream, mucoid	17	Isolation after enrichment, 50% TS; 1% TS
I	*Moraxella* sp. (99.7%)	DQ497238	Small rods	Translucent white, mucoid	17	Isolation after enrichment, 1% TS
L	Undetermined *Sphingomonadaceae* (95.2% with *Sandarakinorhabdus limnophila* AY902680)	DQ497240	Small rods	Orange, mucoid	17	Isolation after enrichment, 50% R2; 1% R2
N	Undetermined *Sphingomonadaceae* (94.5% with *Sandarakinorhabdus limnophila* AY902680)	DQ497241	Small rods	Orange-yellow, mucoid	17	Isolation after enrichment, 50% R2
P	*Paenibacillus* sp. (100%)	DQ497239	Very thick rods	White, matt	17	Isolation after enrichment, 1% R2
R	*Agromyces* sp. (100%)	DQ497242	Rods and mycelium	Pale yellow, mucoid	17	Direct isolation, TSA

Accession numbers of the 16S rRNA gene sequences submitted to GenBank are given. Strains are designated by nonconsecutive letters owing to redundancies of isolations in several cases.

(b), we found snow deposits from the autumn 2002 to summer 2003 period. This assumption was attested by:

(1) The nearly complete depletion of ^{7}Be activity below a depth of 170 cm. Indeed, we know that over 5–6 half-lives, more than 98% of the initial concentration of a radioactive element is depleted. In our case, because of the half-life of ^{7}Be fallout (53.4 days), 5–6 half-lives correspond to 250–300 days, i.e. about '1 year's' accumulation as previously defined. So, the nondetection of ^{7}Be activity below 170 cm indicated than '1 year' had been exceeded.

(2) The increase in snow density (not shown in Fig. 2) and of ^{210}Pb activity in the snow profile below 170 cm depth. These factors could result from metamorphism (i.e. the melting process during summer 2003) of 2002–2003 snow deposits, as attested to by variations in snow grain sizes and structure in this snow layer, defined as the radiation crust. As a consequence of this metamorphism, chemical species, such as ^{210}Pb, were concentrated in the resulting denser layer, as observed in Fig. 2(b).

The bacterial concentration varied from 1×10^4 to 4×10^4 cells mL^{-1} in the upper snow layers (Fig. 2b). These values are in the same range as those measured by Bauer *et al.* (2002) and by Segawa *et al.* (2005) in high mountain snow covers at midlatitude. This recorded concentration is consistent with the bacterial concentration found in the seasonal snow pack (Ny-Ålesund site), and it can thus be estimated that snow deposition in this region leads to a constant bacterial abundance in snow. The count performed in the radiation crust, corresponding to the surface of the glacier during summer, reached a significantly higher value of 2×10^5 cells mL^{-1} (Student's t-test, $P < 0.01$). It represents the previously explained melting/percolation processes, and could also result from cell multiplication occurring on the surface of the glacier during summer.

Isolated strains

Ten distinct bacterial strains were recovered by cultivation and successfully identified. Table 1 details the culture media in which they were detected, and the macroscopic characteristics of the colonies (pigmentation and morphology) once transferred onto solid media.

After about 1 month of cultivation, direct plating of melted snow on agar media only led to the recovery of two strains A and R, growing sparsely at 27 °C on R2A and at 17 °C on TS agar, respectively. At 4 °C and after 2 months of cultivation, only a single fungal colony was observed on R2A medium (not shown here) but no bacterial growth in any media was detected. As a consequence of the very low recovery of cells by direct plating, counts of cultivable cells were not performed. By contrast, enrichments of melted snow with nutrients (TS or R2A compounds) incubated at 17 °C provided conditions for growth for many more bacterial strains. Indeed, eight strains were recovered by this

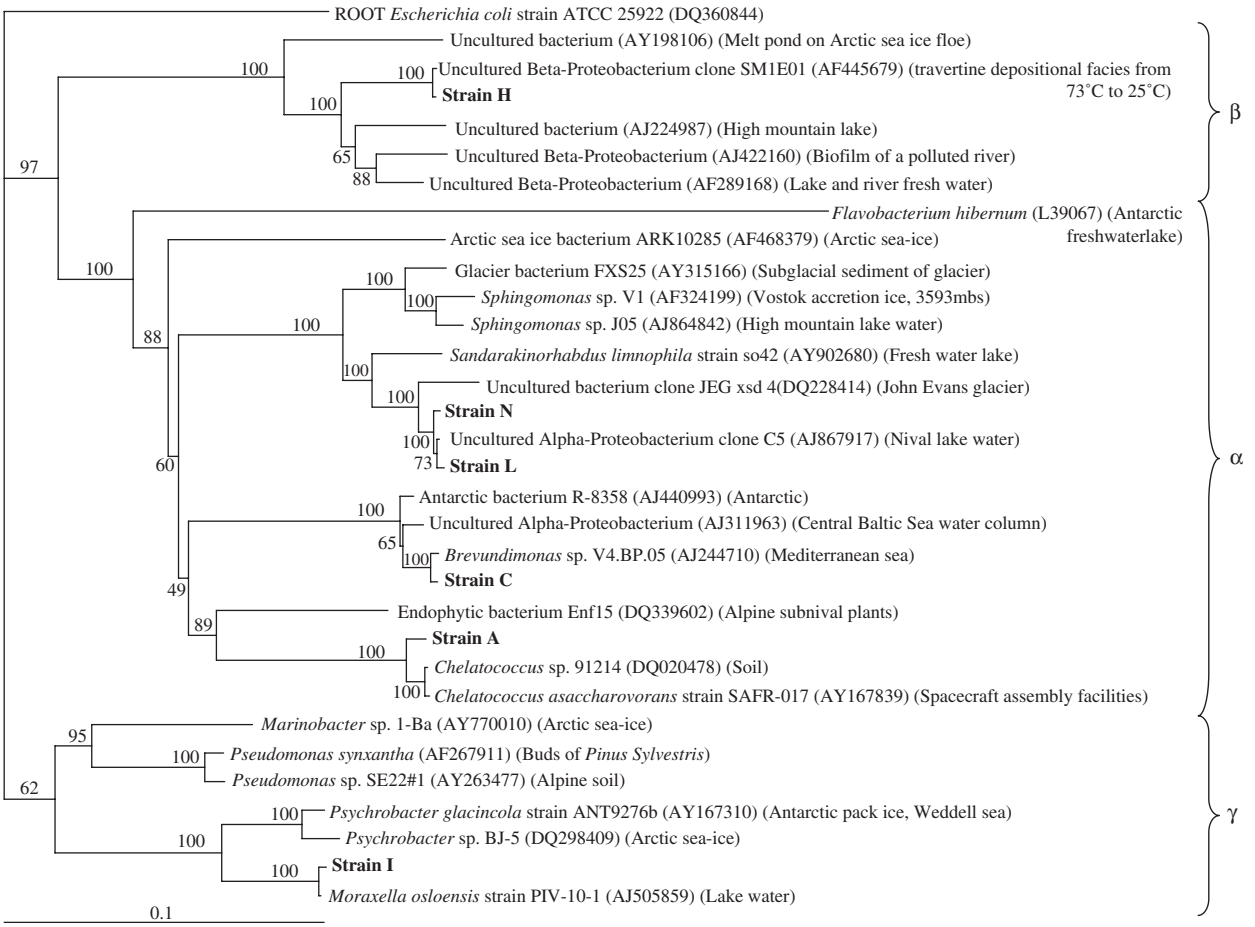

Fig. 3. Phylogenetic analysis of the 16S rRNA genes for the Gram-negative isolates and selected strains. The tree was generated by the neighbour-joining method after alignment of 1367 bp, and rooted with *Escherichia coli* ATCC 25922. Bootstrap values (100 replications) are specified for each node, and GenBank accession numbers and isolation sources are provided in parentheses. Scale bar indicates 0.1 substitutions per nucleotide.

method: one from flasks containing snow enriched with 50% of TS (TS 50%) (H), three from TS 1% (C, H and I), four from R2 50% (C, D, L and N) and three from R2 1% (E, L and P). In our case, these results suggest that the latter method is more appropriate to investigate the cultivable fraction that can be found in polar snow. This was directly inspired from works of Skidmore *et al.* (2000) and Bussmann *et al.* (2001). These authors have already successfully used such methods of amendment. Our incubation conditions allowed the isolation of strains able to develop under low nutrient conditions and at relatively low temperature, meaning they were oligotrophic and at least psychrotolerant microorganisms.

Cells of the strains were all rod shaped, except E, which was filamentous. More than the half of the isolated strains formed pigmented colonies. Four of them presented an orange pigmentation and two were yellow; others were paler, from cream to translucent white. Pigmented strains are frequently found at high proportions in such cold environments (Foght *et al.*, 2004; Mueller *et al.*, 2005). The presence of pigments has been suggested to play a role in maintaining membrane fluidity as an adaptation to life at low temperature (Fong *et al.*, 2001).

With regard to the taxonomy of the isolates, genera found were well distributed among the two Gram groups, with six strains belonging to the Gram-negative branch and four belonging to the Gram-positive branch. The phylogenetic trees constructed to determine their affiliations are shown in Figs 3 and 4. All the Gram-negative isolates belong to the *Proteobacteria*, of which the largest proportion (four out of six) belong to the *Alphaproteobacteria*. These isolates include the unambiguously ($>$ 99% homology) identified genus *Brevundimonas* (strain C), and uncertain genera in the cases of strains N and L. Their closest neighbours obtained by BLAST were found in a nival lake and in the John Evans glacier, attesting to their adaptation to cold environmental conditions. However, they show only poor homology (about 95%), with their closest recognized neighbour,

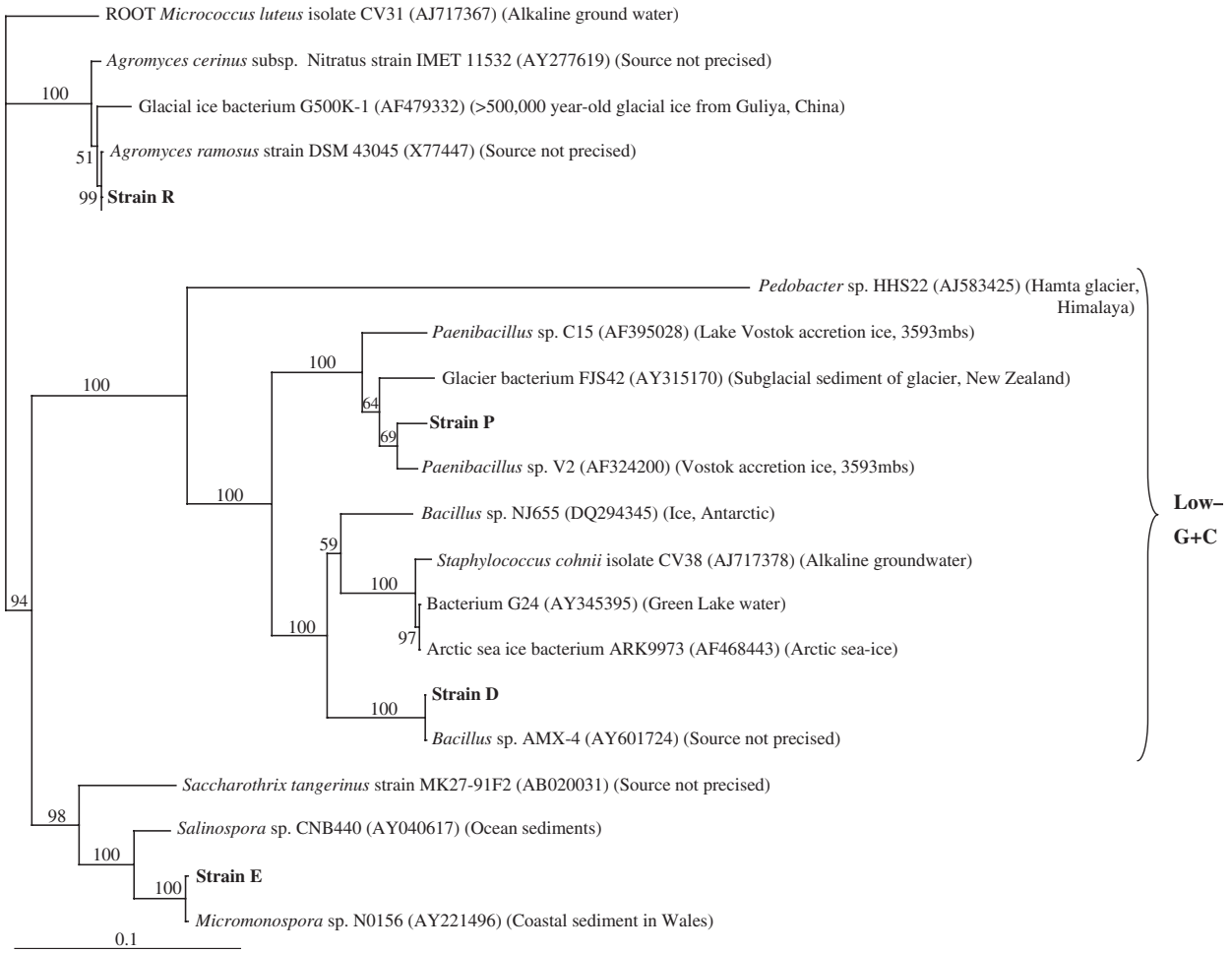

Fig. 4. Phylogenetic analysis of the 16S rRNA genes of the Gram-positive isolates and selected strains. The tree was generated by the neighbour-joining method after alignment of 1386 bp, and rooted with *Micrococcus luteus* CV31 from groundwater. Bootstrap values (100 replications) are specified for each node, and GenBank accession numbers and isolation sources are provided in parentheses. Scale bar indicates 0.1 substitutions per nucleotide.

Sandarakinorhabdus (Gich *et al*., 2005). They also show not more than 94% sequence homology with members of the genus *Sphingomonas*. As a consequence, these two strains are likely to be of as yet unknown affiliation. Strain C is more probably related to the sea, since it matches closely with isolates obtained from the Mediterranean and central Baltic seas. The 16S rRNA gene sequence of strain A shows $< 98.6\%$ homology with its nearest neighbours, *Chelatococcus* sp., found in soil and in the atmosphere (spacecraft). The *Betaproteobacteria* is also represented (strain H) by what appears to be a rare cultivated specimen of this affiliation, as shown by the phylogenetic tree: only uncultured bacteria are placed around strain H. This strain was identified as probably representing a species of the genus *Hydrogenophaga*. A member of the *Gammaproteobacteria* was cultivated (strain I), and identified as a species of the genus *Moraxella*, closely related to a strain isolated from lake water. Among the Gram-positive bacterial strains were two *Firmicutes* (low-G+C bacteria) and two *Actinobacteria* (high-G+C bacteria). The first are members of the genera *Bacillus* (strain D) and *Paenibacillus* (strain P), and the *Actinobacteria* are members of the genera *Micromonospora* (strain E) and *Agromyces* (strain R). Strains P and R appear to be related to bacteria found in deep ice cores, indicating their resistance to very cold conditions, while E is related to sea sediments, where the temperature generally encountered is around 4 °C.

All the genera we determined are well known to be widely spread in cold environments. *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* are abundantly found in pack ice at both poles, whether identified by cultivation or by molecular methods (Brinkmeyer *et al*., 2003; Groudieva *et al*., 2004). They are also among the dominant groups detected in the bacterial flora of the winter cover (snow and ice) and pelagic zone of a midlatitude high mountain lake (Alfreider *et al*., 1996). The *Betaproteobacteria*, in

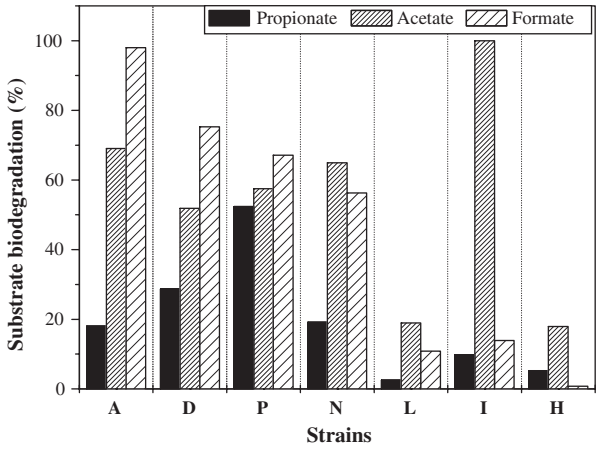

Fig. 5. Biodegradation abilities of the isolated strains on organic compounds at 17 °C. Concentrations measured by ^{1}H NMR were used to calculate the proportions of substrate biodegraded. Values are expressed as percentages of degraded compound after 24 h of incubation compared with the concentration measured at $t_{0\,h}$. Each strain was incubated with a single substrate at a time, in phosphate buffer at pH 7.0.

particular, was found to be highly dominant beneath several glaciers (Skidmore *et al.*, 2005). On the whole, Gram-negative bacteria are more often reported than Gram-positive bacteria from poles and glacier samples. Thus, Miteva & Brenchley (2005) isolated low- and high-G+C Gram-positive bacteria in an ice core, including members of the genus *Paenibacillus*, which was also detected in Lake Vostok accretion ice (Christner *et al.*, 2001). Finally, it can be noted that genera such as *Moraxella*, *Peanibacillus*, *Bacillus* and *Agromyces* were also found in cloud water samples at midlatitude during winter (P. Amato *et al.*, unpublished data).

Most of the studies performed on cold environments also report the presence of *Cytophaga–Flavobacterium* strains, but no cultivable specimen of this affiliation was detected in our snow sample.

The presence here in snow of strains closely related to others commonly found in cold environments testifies to their resistance to cold conditions, whether by their spore-forming ability (in the case of some Gram-positive bacteria) or by pigments related to membrane fluidity. Furthermore, their ability to develop under oligotrophic conditions and at moderate temperature suggest their activity when the snow melts, and so also their involvement in the chemical composition of runoff water.

Metabolic activity of the isolated strains

To characterize the isolated strains further, we investigated their metabolic potential with respect to organic compounds. Formate (C_1), acetate (C_2), and propionate (C_3) were chosen as they were found in Arctic snowfall (Toom-Sauntry & Barrie, 2002). Biodegradation of these compounds by seven of the isolated strains was monitored by ^{1}H NMR spectroscopy. Percentages of degraded substrates after 24 h of incubation at relatively moderate temperature (17 °C) are shown in Fig. 5. The results show that globally all the strains have good potential to metabolize the compounds tested.

Propionate is highly transformed (more than 20% of the total concentration) by the two low-G+C strains (D and P). For four strains (A, D, N, P), more than 50% of the acetate was metabolized in 24 h, and strain I appeared to be very efficient in degrading this compound, with no acetate remaining in its incubation medium. In the case of formate, strains A, D, P and N can be regarded as highly active, with between 55% (N) and 99% (A) of the initial amount being metabolized during the incubation period. More precisely, three types of responses were encountered among the tested strains under these laboratory experiments. Strains D, P and A had an increasing biodegradation capacity with decreasing carbonaceous chain length (propionate $<$ acetate $<$ formate). Others showed a preference for acetate, with relative variations concerning the degradation of formate. For strains N and L, formate was almost as well accepted as acetate, whereas in the case of strains I and H, almost exclusively acetate was transformed. Strain I seemed to be very specialized for the latter compound and strains L and H showed low activity ($<$ 20%) whatever the substrate considered.

On the whole, these results show that strains isolated from Arctic snow samples have the capacity to degrade organic compounds found there at moderate temperature. This suggests that bacteria present in snow are able to metabolize such substrates to develop and to sustain growth in this environment during melting periods. This is consistent with the cell multiplication suspected to occur on the summer layer of the glacier (as discussed previously). As a consequence, it demonstrates their capacity to be actively involved in the chemistry of the snow cover.

Conclusion

These investigations on the microbial content of the snow cover of an Arctic site, Spitzberg, performed in April 2004 show that, for a seasonal snow pack, the concentration of bacteria was constant with depth, at around 6×10^4 cells mL^{-1} of melted snow. A bacterial concentration of about 2×10^4 bacteria mL^{-1} was recorded along the annual accumulation layer of the Kongsvegen glacier. The concentration observed in the radiation crust, which constituted the glacier surface during the previous summer, was one order of magnitude higher. This certainly resulted from

percolation and bacterial growth on the snow surface during summer.

Direct plating and enrichment of snow samples with low levels of nutrients led to the isolation of ten strains, for which 16S rRNA gene sequences were lodged in GenBank. They were identified as belonging to the *Alphaproteobacteria* (one *Brevundimonas* sp., one *Chelatococcus* sp., and two *Sphingomonadaceaes* of unknown generic affiliation), *Betaproteobacteria* (one *Hydrogenophaga* sp.), *Gammaproteobacteria* (one *Moraxella* sp.), *Firmicutes* (one *Paenibacillus* sp. and one *Bacillus* sp.) and *Actinobacteria* (one *Agromyces* sp. and one *Micromonospora* sp.). Their physiological properties, such as spore-forming ability, pigmentation, and capacity to grow in low-nutrient media and at moderate temperature, would make them able to sustain growth and activity when snow melts in spring. This idea is also supported by their ability to metabolize organic compounds such as propionate, acetate or formate, which are found in Arctic snow, providing them with a substrate for growth. Such growth of microorganisms when snow is melting and during the following summer is consistent with cell counts made along the annual accumulation layer of the glacier.

Finally, the bacterial characterization of the snow cover in Svalbard reported here opens new perspectives concerning the potential role of microorganisms in the chemical reactions taking place in Arctic snow. First of all, they could be involved in carbon balance, and also participate in other geochemical cycles. For instance, it would be of interest to study the interaction of the strains isolated with divalent mercury, a toxic metal, so as to understand better the role of microbiology in snow towards this metal and thus understand whether bacteria can play a significant role in its cycle.

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