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Microbial population in cloud water at the Puy de Dôme: Implications for the chemistry of clouds

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Abstract

Airborne micro-organisms are ubiquitous in the atmosphere where they can remain alive and be transported over long distances, thus colonizing new environments. Despite their great importance in relation to ecological and socio-economical issues (bio-terrorism, health, etc.) very few studies have been carried out in this field.

In this study, the structure of the microbial community present in atmospheric water samples from clouds at the Puy de Dôme (alt 1465 m, Massif Central, France) is described and the metabolic potential of some bacteria is investigated. The total microflora has been quantified by epifluorescence microscopy, while the cultivable aerobic micro-organisms were isolated. Bacteria were identified by 16S DNA sequencing and fungi by morphological criteria. The total bacterial count reached about 3×10^4 cells m^{-3} of cloud volume (1×10^5 cells mL^{-1} of cloud water), of which less than 1% are cultivable. Most of the isolated micro-organisms, including 12 fungal and 17 bacterial strains, are described here for the first time in atmospheric water. Many bacterial strains seem to be adapted to the extreme conditions found in cloud water (pH, $T°$, UV radiations, etc.). Comparison of the two samples (March 2003) shows that pH can be a major factor controlling the structure of this community: an acidic pH (Sample 1: pH = 4, 9) favours the presence of fungi and spore-forming bacteria, while a more neutral pH (Sample 2: pH = 5, 8) favours greater biodiversity. We have also shown, using in situ ^{1}H NMR, that most of the isolated bacteria are able to degrade various organic substrates such as formate, acetate, lactate, methanol and formaldehyde which represent the major organic compounds present in cloud water. In addition, the detection of intermediates indicated preferential metabolic routes for some of the strains.
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Keywords: Cloud water; Micro-organisms; NMR; Organic acids; Formaldehyde; Methanol; Biodegradation

1. Introduction

Airborne micro-organisms have long been considered as inert particles transported by the atmospheric circulation that can colonize new environments. In this context, their importance is mostly related to ecological

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and socio-economical issues (bio-terrorism, health, etc.). Recently, the potential role of micro-organisms in controlling the physical and chemical properties of atmospheric waters was investigated (Ariya and Amyot, 2004). However, the structure and function of microbial communities in cloud and precipitation remain largely unknown.

Atmospheric water represents, in some respects, an extreme environment characterized by low temperatures, relatively low pH and complex mixtures of organic and inorganic compounds. However, bacteria, fungi, yeast and protozoa not only can survive in such media (Matthias-Maser and Jaenicke, 1995; Matthias-Maser et al., 1995, 2000; Fuzzi et al., 1997; Sattler et al., 2001; Bauer et al., 2002) but some of them can also develop and potentially modify the physical and chemical properties of cloud and rain. This is firstly due to their hygroscopic and ice-forming nuclei properties that can affect the initial process of droplets and crystal formation (Szyrmer and Zawadzki, 1997; Cochet and Widehem, 2000). Secondly, micro-organisms can be considered as biocatalysts able to transform organic and inorganic compounds in cloud water. Recent studies indicate that airborne bacteria and fungi can transform dicarboxylic acids (Ariya et al., 2002). In addition Sattler et al. (2001) showed that micro-organisms collected from supercooled cloud droplets are able to survive and grow at low temperatures ($\leqslant 0\,^{\circ}C$), having thus an active metabolism under conditions close to those of clouds.

Chemical reactions in clouds significantly influence the transformation and the removal of particulate and gaseous compounds in the atmosphere and hence lead to a degree of control over the atmospheric oxidizing capacity (Lelieveld and Crutzen, 1991). This is because many of the reactive species can dissolve and react in the liquid phase where reaction kinetics are often faster than corresponding reactions in the gas phase. Up to now only mechanisms involving chemical or photochemical reactions have been taken into account to describe these processes.

The first objective of this work was to present sampling and analytical methodologies used to describe the structure of microbial communities in two cloud water samples sampled at the Puy de Dôme station (alt. 1465 m, 48°N; 2°E, Massif Central, France). Preliminary results are presented in relation to air mass characteristics and cloud chemical composition. The second objective was to investigate the metabolic potential of certain bacteria towards organic substrates such as formate, acetate, lactate, methanol and formaldehyde, which are the major organic compounds present in cloud water at the Puy de Dôme (Marinoni et al., 2004). This was achieved by quantifying the mass fraction of each substrate consumed by individual strains and identifying metabolic intermediates by NMR spectroscopy (Delort and Combourieu, 2000; Grivet et al., 2003).

2. Materials and methods

2.1. Physicochemical measurements

Cloud samples were taken at the Puy de Dôme summit during two sessions in March 2003. Experimental studies were carried out at the Puy de Dôme (PDD) station (48°N, 2°E; 1465 m a.s.l.), in the Massif Central Region (France). It is a strategic point to observe warm and mixed clouds that are present 50% of the time between November and March. Clouds are frequently formed at the top of the site, either during advection of frontal systems or by the orographic rising of moist air. During the winter/spring months, the station lies in the free troposphere and air masses are usually free from the influence of local pollution. Road access to the site was restricted to authorized personnel during all sampling periods; cars were stopped 5 km before the summit at 850 m a.s.l. to prevent local contamination. A small military base is located to the north of the station, but fuel combustibles are limited exclusively to winter storm events. The most frequent air mass pathways were investigated on the basis of back-trajectory analyses and air mass properties by Sellegri et al. (2003) and Marinoni et al. (2004), showing predominantly three types of air masses labelled Maritime (Westerly flow), Polluted (Northerly Flow) and Continental (variable).

Meteorological parameters are monitored at the station (wind speed and direction, temperature, pressure, and relative humidity (RH)). In addition, monitoring of a variety of atmospheric gas concentrations (O_3, NOx, SO_2), black carbon (Magee Aethalometer AE 42), and total particle number concentrations (CN), measured in the range from 10 nm to 3 μm (condensation particle counter model 3010, TSI) takes place throughout the year. The station is equipped for cloud microphysical measurements LWC (cloud liquid water content) and effective radius (R_e) of droplets as obtained with a Gerber PVM-100 probe. An overall description of measurements performed at the station can be found at http://wwwobs.univ-bpclermont.fr/observ/chimie/pdd_listedata.html

2.2. Cloud water sample collection

Cloud droplets were collected with single-stage cloud collectors with a cut-off diameter of 7 μm (Kruisz et al., 1993; Marinoni et al., 2004). One collector was used for the detection of micro-organisms while the second was used for chemical analyses. All the material needed for sampling was previously sterilized by autoclaving.

Sterile mask and gloves were worn during sample collection to avoid any contamination. The sterility of the apparatus was tested by pouring autoclaved deionized water through the apparatus and treating it as a cloud water sample. Samples were kept cold (4 °C) for a few hours until laboratory experiments. Cleaning procedures for chemical analyses were similar to those described by Marinoni et al. (2004).

2.3. Total identified micro-organism count

Cloud water samples were fixed by formaldehyde (2% vol/vol final concentration). Ten milliliters of these solutions were stained by 4′,6-diamino-2-phenylindole (DAPI) (2.5 μg ml^{-1} final concentration, 20 min incubation in the dark), filtered on black GTBP 0.22 μm filters (Millipore). Filters were mounted on slides and observed under epifluorescence microscopy (Olympus BH-2), with ×100 objective lens and immersion oil. Total cell numbers were estimated by counts of at least 1000 cells, i.e. observation of 30 fields, on random microscopic fields.

The concentration of micro-organisms in cloud volume was calculated by converting the liquid phase concentration into an atmospheric concentration (cloud water load (CWL)). This was simply computed using the average concentration of cloud water (LWC) as measured by the PVM probe:

$$N_{\text{microorganism in cloud volume}} = \text{LWC}_{\text{g m}^{-3}} * N_{\text{microorganism in cloud water volume}}.$$

This expression of cell numbers allows data from one sample to another to be compared, avoiding any dilution effect.

2.4. Count, isolation and identification of aerobic cultivable micro-organisms

Micro-organism cultures were made on solid non-selective media for bacteria (Trypcase-Soy, bioMérieux) and fungi (Sabouraud, Difco) by applications of 0.1 mL of 1–10^{-3} dilutions of cloud water. Colony forming units (CFUs) were then counted after at least one week at 28 °C under aerobic conditions, to estimate the cultivable ratio of micro-organisms in the sample. From these mixed cultures, pure strains were isolated from individual colonies on Petri dishes. Their genomic DNA was extracted by 5 ml filtration on GTTP 0.2 μm filters (Millipore) of thick re-suspended CFUs onto NaCl 0.8%. Cell lysis was carried out by addition of Tris-EDTA buffer (560 μL) and lysozyme (7 μL, final concentration 250 μg mL^{-1}) and by 30 min incubation at 37 °C. Then sodium dodecyl sulphate 10% solution (SDS, 30 μL) and proteinase K (3 μL, final concentration 100 μg mL^{-1}) were added. After another 37 °C incubation for at least 60 min, 5 M sodium chloride (100 μL) and cethyltrimethyl ammonium bromide/sodium chloride (CTAB/NaCl, 1% wt./vol., 80 μL) were added, and the solution was incubated again for 10 min at 65 °C. DNA was purified by subsequent phenol–chloroform treatment and alcohol precipitations (Sambrook et al., 1989). 16S rRNA gene amplification was carried out by PCR, using 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) primers, 50–100 ng of the total DNA extract of each strain, and 1.5 unit of Taq DNA polymerase. Each PCR product was purified by gel electrophoresis using QIAquick Gel Purification kits (Qiagen). 16S rRNA gene sequence was obtained by capillary electrophoresis (MWG-Biotech, Courtaboeuf, France) using the 27f DNA primer described above. Gene sequence similarities with known sequences from genomic banks were estimated using the BLASTN application (http://www.ncbi.nlm.nih.gov/BLAST/).

Identification of fungi strains was made on the basis of morphological criteria by CBS (Centraalbureau voor Schimmelcultures—Fungal Biodiversity Centre, The Netherlands).

2.5. Biodegradation tests

Pure bacterial strains isolated from cloud water were grown in 100 mL of Trypcase soy broth in 500 mL Erlenmeyer flasks incubated at 28 °C with agitation at 200 rpm. After 24 or 48 h growth, cells were harvested from 25 mL of culture by centrifugation at 9000*g* for 15 min at 5 °C. The supernatant was discarded and the pellet was washed twice with NaCl 0.8%. Finally, cells were resuspended in 0.1 M pH 7.0 phosphate buffer for acetate, lactate and formate, or pH 8.0 for the mixture formaldehyde + methanol (Kato et al., 1983). Resting cells were incubated in 25 mL of this buffer with 20 mM formate, acetate or lactate, or with 2 mM formaldehyde + 0.8 mM methanol, in 100 mL Erlenmeyer flask at 28 °C with agitation (200 rpm). Incubation of cells under the same conditions in the absence of the substrate constituted a reference, as did incubation of the substrate in the buffer without cells. Samples (1 mL) were taken regularly and were centrifuged at 12,000*g* for 5 min. Supernatants were immediately frozen until NMR analysis.

2.6. ^1H NMR spectroscopy analyses

NMR samples were prepared as follows: Supernatants (450 μL) issued from biodegradation tests were supplemented with 50 μL of a 20 mM solution of sodium tetra deuteriated trimethylsilyl propionate ($TSPd_4$, Eurisotop) in D_2O (Eurisotop). D_2O was used for locking and shimming while $TSPd_4$ constituted a reference for chemical shifts (0 ppm) and quantification. ^1H NMR spectra were recorded at 400.13 MHz on a Bruker

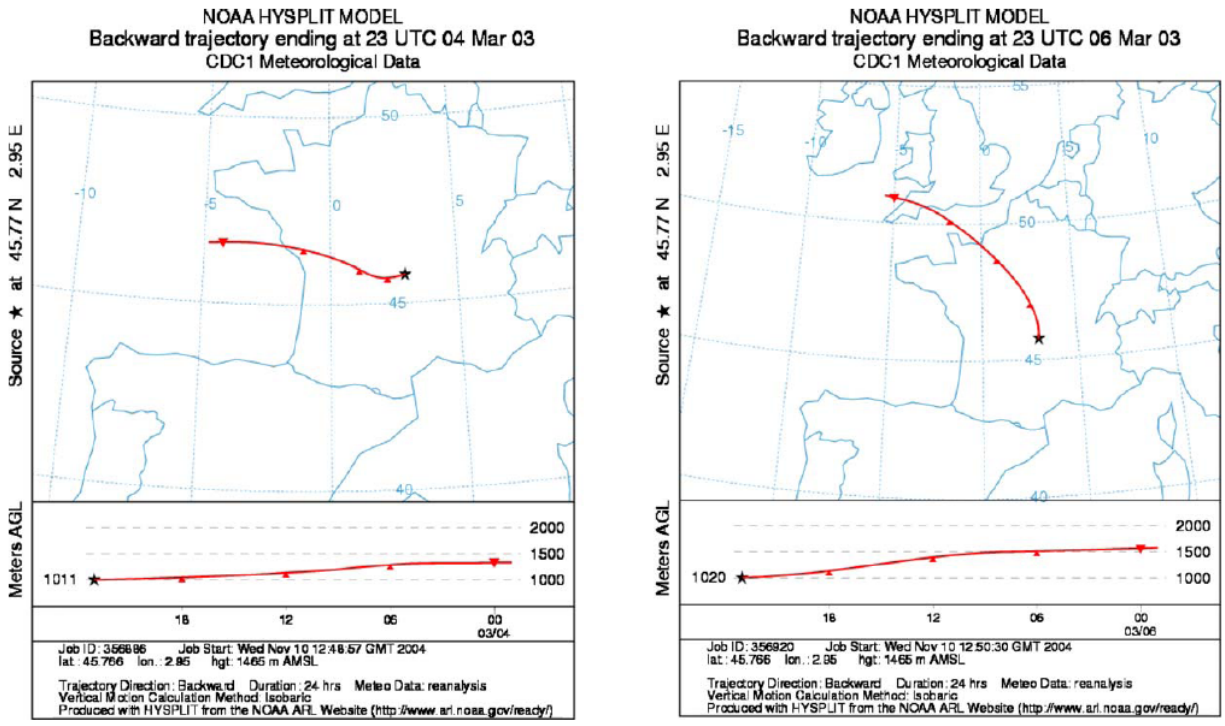
Avance 400 spectrometer at 21 °C with 5 mm-diameter tubes containing 500 µL of sample. Thirty two scans were collected (90° pulse, 4789.27 Hz SW, 65,536 data points, 6.84 min total acquisition time for one spectrum). Water signal was eliminated by presaturation. No filter was applied before Fourier transformation but a baseline correction was performed on spectra before integration with Bruker software. Under these conditions, the limit of quantification is in the range of 0.05 mM. The concentration of metabolites was calculated as follows: $[m] = (9 \times A_0 \times [\mathrm{TSPd_4}])/(b \times A_{\mathrm{ref}})$, 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 ^{1}H 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 ppm.

3. Results and discussion

Two cloud water samples were collected at the Puy de Dôme: sample 1 (4 March 2003) and sample 2 (6 March 2003). Samples 1 and 2 were collected from air masses from west and northwest fluxes, respectively (Fig. 1) and can be considered as non-polluted (Marinoni et al., 2004), regarding low organic carbon level ($<1\,\mathrm{mg\,L^{-1}}$, not shown). The pH and the temperature of these two samples were different (see Table 1).

Table 1
Physico-chemical and biological characteristics of cloud samples

Sample	1	2
Date	4 March 2003	6 March 2003
LWC ($\mathrm{g\,m^{-3}}$)	0.3	0.2
Temperature (°C)	5	−2
pH	4.9	5.8
Cultivable bacteria (Number $\mathrm{m^{-3}}$)	10±2	395±64
Cultivable fungi (Number $\mathrm{m^{-3}}$)	390±45	53±9
Total bacteria (Number $\mathrm{m^{-3}}$)	$2.5\pm0.5\times10^4$	$7.1\pm1.4\times10^4$
Bacterial cultivability (%)	0.02–0.06 %	0.38–0.81 %
Number of spore-forming bacterial strains	2	3
Number of non-spore forming bacterial strains	0	12
Number of fungal strains	7	5



03/04/2004 03/06/2004

Fig. 1. Twenty-four hours backward trajectories for the two air masses sampled (HYSPLIT model).

Epifluorescence microscopy measurements using DAPI-stained bacteria showed the presence of 2.5×10^4 cells m^3 and 7.1×10^4 cells m^{-3} of cloud volume in samples 1 and 2, respectively (see Table 1). These numbers were in the same range as those reported in the literature (Fuzzi et al., 1997; Bauer et al., 2002) for atmospheric samples but much lower than usually measured in lakes or soils.

Counts of isolated colonies of aerobic cultivable micro-organisms showed that sample 1 contained mainly fungi (390 CFUs m^{-3}) and few bacteria (10 CFUs m^{-3}) while the reverse situation was found in sample 2 (fungi: 53 CFUs m^{-3}, bacteria: 395 CFUs m^{-3}). From these numbers it can also be deduced that only a small fraction of bacteria were cultivable (less than 1%; see Table 1). Such cultivability is usually reported in studies of microbial communities from natural environments (Amann et al., 1995; Lighthart, 1997; Bauer et al., 2002).

16S DNA of isolated bacterial colonies was sequenced and 16S rRNA gene sequence similarities allowed the nearest species to be identified and a genus proposed for most of the bacterial strains (see Table 2):

- Strains belonging to Actinobacteria phylum were the most abundant: the identified genera were *Streptomyces* (3), *Microbacterium* (2), *Micrococcus* (1), *Arthrobacter* (1), *Clavibacter* (1) and *Kocuria* (1). They are typical of the phyllosphere or soil biotope, where they play an important role in degrading organic matter.
- Two strains from the Bacteroidetes phylum (*Cytophaga–Flavobacterium* group) were also found. These bacteria are known to be psychrophile and form biofilms that confer resistance to extreme conditions. They are found on the surface of particulate material in Artic ice (Junge et al., 2002; Brinkmeyer et al., 2003).
- One strain of *Pseudomonas* (gamma-Proteobacteria) was found; it is well-known that ice-forming nuclei bacteria belong to this genus (Cochet and Widehem, 2000) and that *Pseudomonas* species are usually psychrotolerant. This genus is also found in high altitude glaciers (Christner et al., 2003; Zhang et al., 2002) and in fog droplets (Fuzzi et al., 1997). Interestingly two strains (2-18 and 2-20) from the Proteobacteria phylum (beta-Proteobacteria group) showed close homologies to the Antarctic bacterium R-7687 strain isolated from Antarctic ice.
- Two *Bacillus* strains (Firmicutes phylum) were present in cloud water at the Puy de Dôme. They form very resistant endospores and are also found in high altitude glaciers (Christner et al., 2003; Zhang

Another striking point is that most bacteria collected were pigmented, mainly orange or yellow (10 strains out of 17, see Table 2). A predominance of pigmented isolates has also been described in Artic ice (Fong et al., 2001). These pigments are likely to belong to the carotenoid family. Carotenoids have been described to protect bacteria from low temperatures, low salt concentration and UV exposure (Fong et al., 2001; Vincent et al., 2000).

Fungi genera were determined from physiological and morphological criteria; they are listed in Table 3. They belonged to Ascomycota (5) and Basidiomycota (6) phyla. These species are currently found in the natural environment, like soils or shafts. Some strains of Basidiomycota are difficult to identify when they are not in their natural surroundings. *Aspergillus* genus was also described in fog droplets (Fuzzi et al., 1997).

Although only two samples were collected and a small number of micro-organisms were isolated, three remarks can be made:

(i) species close to bacteria usually found in cold environment were present in sample 2 (bacterial strains no. 2-1, 2-4, 2-15, 2-18, 2-20) where the temperature was lower,
(ii) sample 1 contained only spore-forming species (including 2 bacterial and 7 fungal strains) while sample 2 was more diverse, containing 12 non-spore-forming bacteria species and 8 spore-forming bacterial or fungal strains. This could be due to the influence of pH; spore-forming organisms can resist more acidic conditions or generally extreme conditions. Bauer et al. (2002) showed the large contribution of bacterial and fungal spores to the organic carbon content of cloud water, precipitations and aerosols. Also Zhang et al. (2002) reported that spore-forming bacteria represented up to 40% of total bacteria in high altitude glacier ice.
(iii) the different origin of the air masses (west and northwest) could also contribute to the different biodiversity of the microbial population in the two samples.

3.2. Biodegradation of organic compounds

In order to investigate the role of bacteria in cloud chemistry, we tested the biodegradative potential of some cultivable aerobic bacteria isolated from sample 2 with respect to certain organic substances present in

Table 2
Bacterial strain identifications

Date	No. of strains	Sequenced 16sDNA fragment length	Sporing ability	CFU colour	Phylum	Genus	Nearest species	% of 16sDNA analog (pb)
03/04/2003	1-1	624pb	Yes	White-yellow	Actinobacteria	*Streptomyces*	*streptomyces somaliensis* strain DSM 40267	99% (622/624)
							S. albidoflavus strain DSM 46452	99% (622/624)
							S. canescens strain DSM 40001T	99% (622/624)
	1-2	Non-sequenced	Yes	White-yellow	Actinobacteria	*Streptomyces*	*Streptomyces* sp. (according to morphological characteristics)	—
								—
								—
03/06/2003	2-1	693pb	No	Orange	Bacteroidetes	*Cytophaga or Flavobacterium*	*Cytophaga* sp. P1	97% (658/675)
							Flavobacterium sp. EP300	98% (631/642)
							Flavobacterium sp. EP286	98% (631/642)
	2-2	567pb	No	Yellow	Actinobacteria	*Micrococcus*	*Micrococcus luteus*	99% (560/561)
							Micrococcus sp. 98TH11321	99% (560/561)
							Variovorax sp. Amico6	99% (560/561)
	2-3	415pb	No	Yellow	Actinobacteria	*Microbacterium*	*Microbacterium phyllosphaerae* strain DSM 13468	99% (401/403)
							Rape rhizosphere bacterium tsb034	99% (400/403)
							Microbacterium sp. BM-12_4	99% (400/403)
	2-4	735pb	No	Orange-red	Bacteroidetes	*Flavobacterium*	*Bacteroidetes* bacterium EC2	99% (710/717)
							Flavobacterium sp. MTN11	99% (710/717)
							Flavobacterium sp. 36	97% (681/699)
	2-5	1031pb	Yes	Cream	Firmicutes	*Bacillus*	Bacillus sp. 19489	99% (1020/1025)
							Bacillus sp. LMG 21002	99% (1020/1025)
							Bacillus sp. 19491	99% (1019/1025)
	2-9	630pb	Yes	White	Firmicutes	*Bacillus*	*Bacillus cereus* strain G8639	99% (625/626)
							Bacillus cereus strain AH527	99% (625/626)
							Bacillus cereus biovar toyoi	99% (625/626)
	2-11	449pb	Yes	Pink-grey	Actinobacteria	*Streptomyces*	*Streptomyces* sp. 428DO9	100% (385/385)
							Streptomyces sp. 428DO10	100% (385/385)
							Streptomyces sp. 428DO11	100% (385/385)
	2-12	403pb	No	Yellow	Actinobacteria	*Microbacterium*	*Microbacterium phyllosphaerae*	99% (401/403)
							Rape rhizosphere bacterium tsb034	99% (400/403)
							Microbacterium sp. GWS-BW-H75M	99% (400/403)
	2-13	503pb	No	Yellow	Actinobacteria	*Kocuria*	*Kocuria palustris*	100% (468/468)
							Gram positive bacterium Wuba45	100% (468/468)
							Kocuria sp. GIC66	100% (462/462)
	2-14	901pb	No	Yellow	Actinobacteria	*Clavibacter*	*Clavibacter michiganensis sepedonicus* LMG 2889	100% (800/800)
							Clavibacter michiganensis	99% (799/800)
							Clavibacter michiganensis insidiosum LMG 3663	99% (799/800)
	2-15	457pb	No	Cream	Gamma-proteobacteria	*Pseudomonas*	*Pseudomonas* sp. HF3/S21027	100% (441/441)
							Pseudomonas sp. isolate EK1	100% (441/441)
							Pseudomonas sp. PsH	100% (441/441)
	2-16	643pb	No	White	Actinobacteria	*Arthrobacter*	*Arthrobacter* sp. GWS-BW-H157	99% (623/625)
							Arthrobacter sp. A3Z-18	99% (621/625)
							Arthrobacter sp. A3Z-17	99% (621/625)
	2-18	554pb	No	Cream	Beta-Proteobacteria	Uncertain genera	*Janthinobacterium lividum*	99% (514/518)
							Antarctic bacterium R-7687	99% (514/518)
							Pseudomonas mephitica	99% (514/518)
	2-20	454pb	No	Cream	Beta-Proteobacteria	Uncertain genera	*Oxalobacteraceae* bacterium Tf 246	99% (450/451)
							uncultured eubacterium WD293	99% (449/451)
							Antarctic bacterium R-7687	99% (449/451)
	2-22	878pb	No	Cream	Actinobacteria	Uncertain genera	Bacterium CAGY3 strain CAGY-3	99% (856/857)
							Rape rhizosphere bacterium tsb086	99% (854/857)
							Curtobacterium sp. VKM Ac-2061	99% (852/857)

Table 3
Fungal strain identifications

Date of sampling	Strains	Phylum	Identification
03/04/2003	1-3	Ascomycota	*Eurotium amstelodami*
	1-4	Basidiomycota	*Stereum cf. rugosum*
	1-5	Basidiomycota	Unidentified
	1-6	Basidiomycota	*Stereum cf. sanguinolentum*
	1-7	Basidiomycota	Unidentified
	1-8	Ascomycota	*Aspergillus fumigatus*
	1-9	Basidiomycota	*Perenniporia* sp.
03/06/2003	2-6	Ascomycota	*Aspergillus fumigatus*
	2-7	Ascomycota	*Emericella nidulans*
	2-8	Basidiomycota	*Agricalis* sp.
	2-17	Ascomycota	*Beauveria bassiana*
	2-21	—	Unidentified

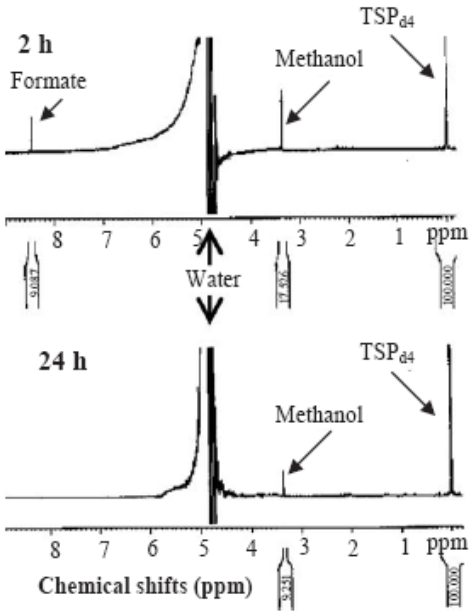


Fig. 2. ^{1}H in situ NMR spectra of incubation media of bacterial strain no. 2-15 with 2 mM formaldehyde and 0.8 mM methanol collected after 2 and 24 h of incubation.

cloud water at the Puy de Dôme (Marinoni et al., 2004): acetate, lactate, formate, methanol and formaldehyde. Two lactate enantiomers (D and L) were tested as the metabolic pathways involved are different.

Resting cells of each strain were incubated at 28 °C under aerobic conditions in a phosphate buffer containing one of the organic substrates as sole carbon source. Formaldehyde (2 mM) and methanol (0.8 mM) concentrations were lower than that of lactate, formate or acetate (20 mM) as these compounds are usually toxic for bacteria. Although cell and organic compound concentrations were high, the bacterial concentration/substrate concentration ratio was in the same range as that found in clouds. The kinetics of degradation was monitored by ^{1}H NMR spectroscopy. The spectra were directly collected on the incubation media after elimination of the bacterial pellet by centrifugation. This analysis is very rapid (less than 7 min). An example of ^{1}H NMR spectra collected after 2 and 24 h of incubation of the *Pseudomonas* strain no. 2-15 with 2 mM formaldehyde and 0.8 mM of methanol is presented in Fig. 2. The integral of methanol resonance (singlet at $\delta = 3.36$ ppm) decreased with time as a result of bacterial degradation (the signal of formaldehyde at δ 4.82 ppm is masked by the huge signal of water and cannot be quantified under these conditions). The concentration of methanol was measured from the ratio of its integral to that of the internal reference ($TSPd_4$) as described previously. The same procedure was used to quantify the degradation of acetate ($\delta = 1.92$ ppm, singlet), formate ($\delta = 8.46$ ppm, singlet) L-lactate ($\delta = 1.33$, doublet and $\delta = 4.24$, quadruplet) and D-lactate ($\delta = 1.45$, doublet and $\delta = 4.46$, quadruplet). Fig. 3 shows the percentage of degradation of each substrate by the different bacterial strains after 24 h of incubation.

The general finding is that most of the strains degraded the four substrates, but each strain presented a specific metabolic profile. As expected, acetate was easily degraded as it is a common intermediate in the Krebs cycle in aerobic bacteria (KEGG metabolic pathways). However, strain no. 2-18 only slightly degraded this compound. Formate was 100% degraded by bacterial strains no. 2-4, 2-5, 2-9, 2-15 and efficiently by most of the strains too, except by bacterial strains no. 2-18 and 2-22. Formate is an intermediate that can be involved in many metabolic routes, including the glyoxylate cycle, or can be transformed into CO_2 and H_2 via formate dehydrogenase activity (KEGG metabolic pathways). With respect to lactate, the two isomers were degraded differently according to the strains tested: bacterial strains no. 2-2, 2-4, 2-5, 2-15, 2-16 degraded L-lactate better than D-lactate. The reverse situation was observed for bacterial strain no. 2-9, while the degradation rate of the two isomers was similar for bacterial strains no. 2-3 and 2-14. Note that only L-lactate was metabolized by strains no. 2-12 and 2-18. Strain 2-13 was able to metabolize neither L-lactate nor D-lactate. These results show a difference in the activity of the various enzymes involved in the D- or L-lactate pathways, including pyruvate dehydrogenase, lactate racemase, D-lactate dehydrogenase (KEGG metabolic pathways).

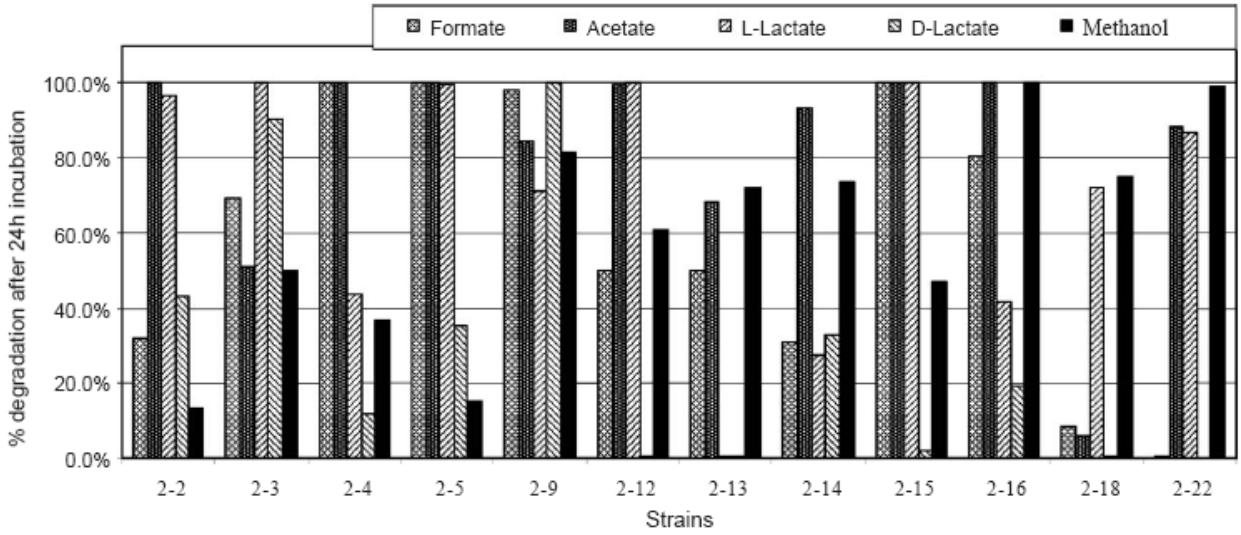


Fig. 3. Percentage of degradation of acetate, formate, L- and D-lactate and methanol by bacterial strains after 24 h of incubation. D-lactate was not tested with strain 2-22.

Methanol is, on the whole, degraded relatively less efficiently if we consider that its concentration is more than 20 times lower. However, it was degraded by all the bacterial strains tested. This consumption can be very efficient, as noted for strains no. 2-9, 2-12, 2-13, 2-14, 2-16, 2-18 and 2-22 (>60% degradation). Methanol degradation leads to the formation of formaldehyde (KEGG metabolic pathways) which can be assimilated or dissimilated by various routes.

Apart from quantitative data expressed as the % degradation of the various substrates, in situ ^{1}H NMR provides qualitative data about certain metabolic intermediates that can accumulate during the kinetics of degradation. For instance, formate ($\delta = 8.46$ ppm) as an intermediate of formaldehyde degradation was detected in the incubation media of the bacterial strain no. 2-15 after 2 h of incubation (see Fig. 2); it was no longer present after 24 h. So, although formaldehyde efficiency could not be quantified at this point, the presence of formate implies that formaldehyde is clearly degraded. Formate was also present at the end of incubation (24 h) of formaldehyde with bacterial strains no. 2-2, 2-3, 2-12, 2-13 and 2-18; this accumulation could be due to the relatively low degradability of formate by these strains (unlike bacterial strain no. 2-15 which is a very good degrader of formate). The detection of formate derived from formaldehyde is consistent with the presence of formaldehyde dehydrogenase and alcohol dehydrogenase (KEGG metabolic pathways). The degradation of formaldehyde has been shown previously in bacteria from other environments methylotrophic bacteria (Vorholt, 2002), *Pseudomonas putida*, *Staphylococcus aureus* (Mason and Sanders, 1988, Adroer et al., 1990) and *Escherichia coli* (Hunter et al., 1984). Acetate was also detected as a metabolic intermediate during the degradation of L-lactate by bacterial strain no. 2-18; it resulted from the successive activities of lactate aldolase, carboxylate reductase and aldehyde dehydrogenase (KEGG metabolic pathways). The accumulation of acetate is consistent with the very poor degradability of acetate by this strain.

4. Conclusions

To our knowledge, this work is the first report combining a detailed description of microbial communities in cloud water and the enzymatic activity of some of the identified strains towards acetate, L- and D-lactate, formate, methanol and formaldehyde. Although the total number of bacteria was in the same range of magnitude in the two samples (10^4–10^5/m^3), the biodiversity of aerobic cultivable micro-organisms was very different, especially the bacteria/fungi or spore-forming/non-spore-forming micro-organisms ratios. It is likely that pH plays an important role in regulating this biodiversity as previously shown in fog water (Fuzzi et al., 1997) although additional measurements are clearly needed to confirm these results.

Some of the identified strains belong to genera already described in fog droplets such as *Pseudomonas*, *Bacillus* and *Aspergillus* (Fuzzi et al., 1997). In addition, we found bacteria and fungi belonging to various kinds of phylum that had never been described in atmospheric waters. Generally, they were species from natural environments (vegetation and soil) as previously observed for bacterial aerosols (Lighthart, 1997), and their occurrence in clouds indicates that these micro-organisms are

re-suspended and transported in a similar way to terrigeneous primary particles.

The use of 16S DNA sequences allowed the strains isolated in this work to be compared with the nearest species in data banks. Interestingly we found bacteria closely related to a strain from the Antarctic. As already stated we found bacteria usually described in Polar sea ice and high altitude glaciers. We can suspect that these types of bacteria are well-adapted to cold and extreme conditions (Thomas and Dieckman, 2002; Staley and Gosink, 1999) and could thus survive and be active in clouds. This idea is reinforced by the fact that we found microorganisms able to form biofilms on particulates, to synthesize pigments or to form endospores; all these properties can help resistance to unfavourable conditions present in clouds (low temperature and salinity, UV exposure, low pH, etc.).

The potential role of bacteria in the chemistry of clouds was investigated as a first approach under laboratory conditions. We used pure cultures with only one carbon substrate and under rather warm temperatures. The objective was to establish a rapid and simple protocol to test the enzymatic equipment of each strain, or in other words to check the metabolic potential of these bacteria. For this purpose, we used in situ ^{1}H NMR which is a convenient method that is both qualitative and quantitative. We showed that most of the isolated strains that could be cultured, meaning those which are alive in clouds, are able to efficiently degrade various substrates. In addition, the detection of intermediates indicated preferential metabolic routes for some of the strains.

The next step will now be to work under conditions closer to those of cloud water including lower temperature, and mixture of organic and inorganic compounds. In situ ^{1}H NMR will be a useful tool under these conditions as a single spectrum will give information about mixtures of organic substrates and metabolites. The structure of microbial community will also be investigated using molecular techniques such as FISH or TRFLP, in order to gain a complementary insight in microbial biodiversity.

Our results together with those obtained by Ariya et al. (2002) show that investigation of the metabolism of organic compounds in the atmosphere is a real challenge and not a dream.

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