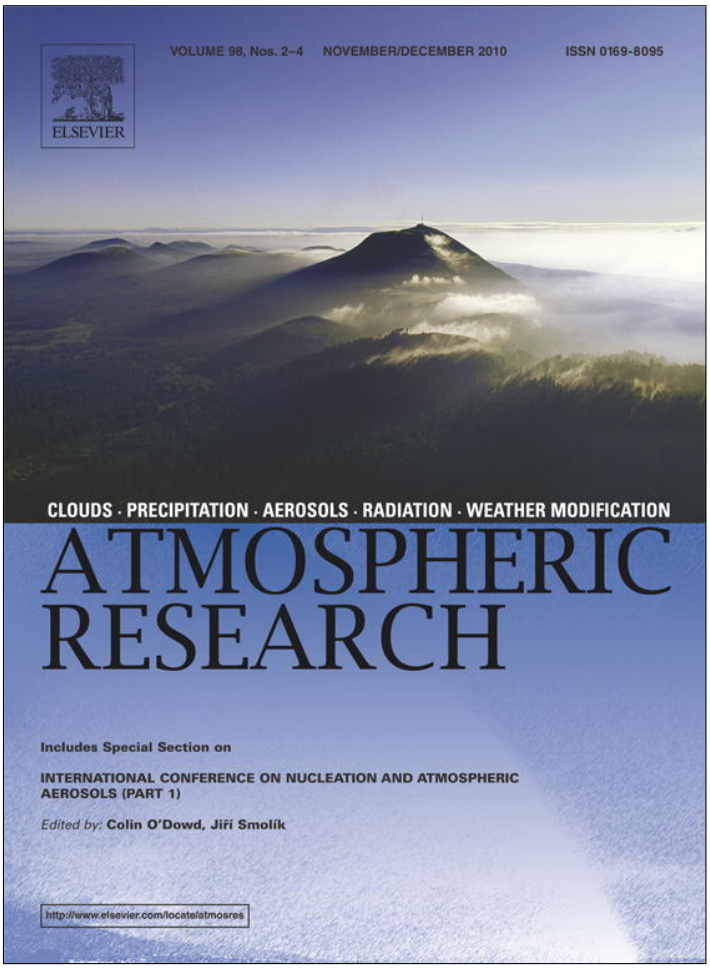

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A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes

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ABSTRACT

Recent studies showed that living microorganisms, including bacteria, fungi and yeasts, are present in the atmospheric water phase (fog and clouds) and their role in chemical processes may have been underestimated. At the interface between atmospheric science and microbiology, information about this field of science suffers from the fact that not all recent findings are efficiently conveyed to both scientific communities. The purpose of this paper is therefore to provide a short overview of recent work linked to living organisms in the atmospheric water phase, from their activation to cloud droplets and ice crystal, to their potential impact on atmospheric chemical processes. This paper is focused on the microorganisms present in clouds and on the role they could play in atmospheric chemistry and nucleation processes. First, the life cycle of microorganisms *via* the atmosphere is examined, including their aerosolization from sources, their integration into clouds and their wet deposition on the ground. Second, special attention is paid to the possible impacts of microorganisms on liquid and ice nucleation processes. Third, a short description of the microorganisms that have been found in clouds and their variability in numbers and diversity is presented, emphasizing some specific characteristics that could favour their occurrence in cloud droplets. In the last section, the potential role of microbial activity as an alternative route to photochemical reaction pathways in cloud chemistry is discussed.

1. Introduction

Biological material is ubiquitous in the atmospheric environment. Until recently, most aerobiology studies have focused on pollens and vegetal or animal debris, in particular in relation to public health issues. Biological particles also contain microorganisms (virus, bacteria and fungi), a fraction of which is able to survive and develop under conditions such as those encountered in the atmosphere. The presence of living organisms has been documented in almost all atmospheric compartments, from indoor air to the stratosphere. While the role of living organisms in biogeochemical cycles is clearly established in soils and waters, much less information is available about their behaviour in the atmosphere, in particular in the atmospheric waters (clouds, rain).

Microorganisms are present in the atmospheric water phase including fogs and clouds (Alfreider et al., 1996; Fuzzi et al., 1997; Sattler et al., 2001; Bauer et al., 2002; Segawa et al., 2005; Amato et al., 2005, 2007b; Ahern et al., 2007; Bowers et al., 2009). It should be stressed that the majority of

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the papers focusing on the microbiological content of the atmosphere has provided a description of the microbial population found in the air (Di Giorgio et al., 1996; Lighthart, 1997; Jones and Harrison, 2004; Fierer et al., 2008, Maron et al., 2005, Brodie et al., 2007; Després et al., 2007, Fröhlich-Nowoisky et al., 2009), while only a few of them discussed about their global distributions and their importance in atmospheric physico-chemical processes. The objective of this paper is to present a short review of our knowledge about the microorganisms present in clouds and their potential role in atmospheric chemistry and nucleation processes.

2. Cycling of microorganisms *via* the atmosphere

Microorganisms are ubiquitous on Earth including water, soil and vegetation, and they can experience a life cycle *via* the atmosphere as illustrated in Fig. 1. The first step consists of the aerosolization of microorganisms into the air. During their emission into the atmosphere, microorganisms are subject to a wide range of conditions: from climatic factors (temperature, relative humidity, wind, solar radiation, etc.) to atmospheric composition (trace gases, pH, presence of chemicals, etc.). Their release to the atmosphere depends on the type of surface cover (rural, urban, forest, ocean, etc.) and varies temporally with seasonal and daily periodicities (Lighthart, 1997). The mechanisms involved in these processes remain still poorly known; in the case of water, especially oceans, microorganisms are thought to be emitted by bubble bursting. In an interesting review, Blanchard (1989) reported that bubbles are produced at the surface of the sea by whitecaps and breaking waves and rainfalls. It was shown under laboratory conditions that the bubbles existing in water volume collect bacteria; when the bubble bursts, the cells are ejected upward with jet drops. Depending on several factors like the size of the drop, the length of the bubble path though the liquid, the hydrophobicity of the cell surface and the chemical composition of the liquid, the concentration of the bacteria within jet drops can be increased by several orders of magnitude compared with their concentration in the bulk liquid. Wind speed has been identified as the major factor responsible for the aerosolization of microorganisms from solid surfaces (soil, vegetation); bacteria are present on soil particles and are lifted by the wind, eventually along with dust (Griffin, 2007). Spores of some fungal conidia can be aerosolized by wind bursts depending on meteorological factors including wind speed, temperature, humidity, etc (Jones and Harrison, 2004). The microorganisms present in the phyllosphere (up to 10^7 bacteria per cm^2 of the leaf surface) are though to be ejected in the air by the action of wind, under the influence on factors such as humidity and temperature which allow leaves to dry and be fragmented into fine particles (Hirano and Upper, 2000; Morris et al., 2004). Aerosols can also result from the fragmentation of dried biofilms at the surface of leaves on which bacteria are established. Also, rain drops impacting the vegetation are probably responsible for the aerosolization of epiphytic bacteria. Biological emission of virus, fungi and bacteria organisms is very difficult to assess and was considered much lower than other primary aerosol sources. Jaenicke (2005) proposed that the source intensity of the biogenic aerosol in general must be corrected and should be estimated in the same order of magnitude than other major aerosol sources.

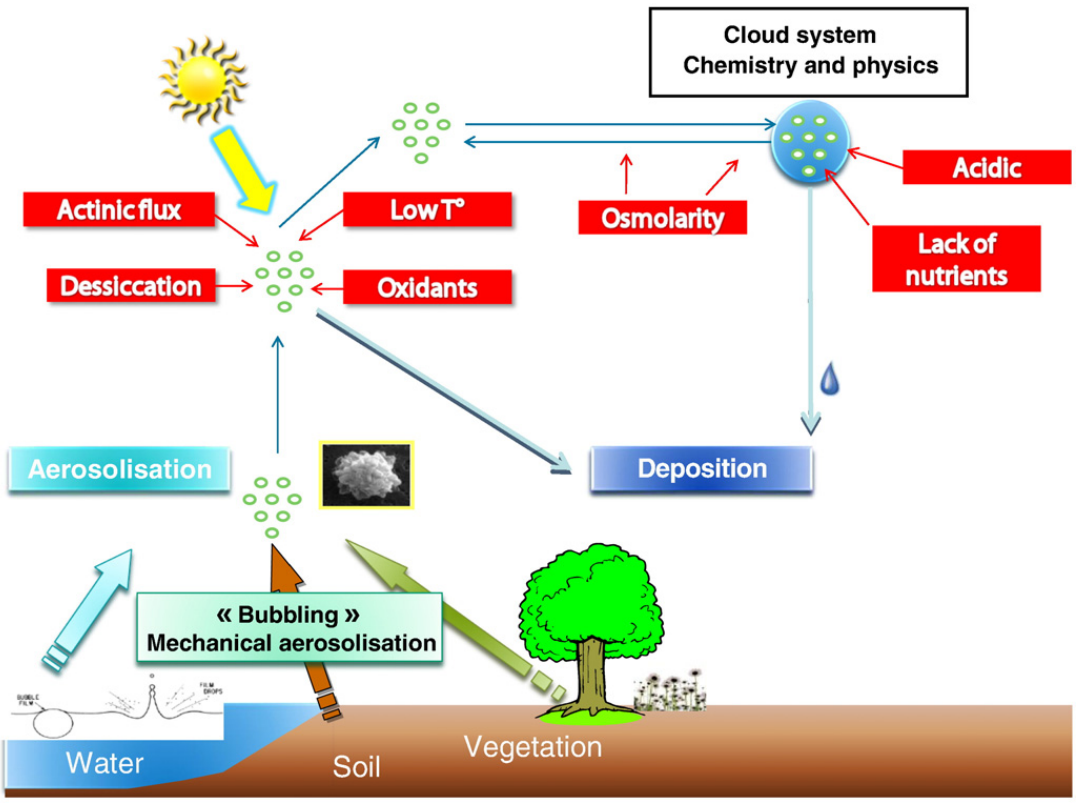

Fig. 1. Schematic representation of the life cycle of microorganisms in the atmosphere, summarizing the factors that likely limit their survival and activity. Adapted from Amato, 2006.

Recently, mechanisms of emission to the atmosphere and estimates of bacteria concentrations for some ecosystems (natural and human-influenced) have been evaluated by Burrows et al. (2009a).

The source origins of bacteria have been discussed by Amato et al. (2007d) for specific conditions observed in cloud at the puy de Dôme. This study suggested that the ocean is an important source of microorganisms and that the microorganisms emitted from seawater could be preferentially included into cloud droplets. This is to be connected to the study of Burrows et al. (2009b) in which the transport of microorganisms in the atmosphere has been simulated with investigations on the factors influencing their residence time including the type of surface of emission, and the CCN (Cloud Condensation Nuclei) and IN (Ice Nuclei) activities.

The variation of microbial abundance in clouds at the puy de Dôme with seasons was also investigated by Amato et al., (2007b,d). A seasonal dependence was observed, with a general increase of the concentrations of cultivable microorganisms and of the total fungal cells during summer and autumn. Additional sources (such as development of vegetation in spring/summer) could also explain the higher concentration of the representatives of epiphytic genera such as *Pseudomonas*. Another hypothesis is linked to the changing atmospheric conditions impacting on the nature of living microorganisms in the atmosphere.

Once aloft, living cells are in fact exposed to hostile conditions including solar radiation (especially UV), desiccation, low temperatures, interactions with oxidants (H_2O_2, O_3, $HO^{\bullet}$, etc.), low nutrient availability, acidity and rapid variations of salinity. So, specific physiological characteristics seem necessary for surviving in the atmosphere. In fact, seasonal dependency of physiologic properties was also found by Amato et al. (2007b) with psychrotolerant microorganisms more numerous during winter than summer at the puy de Dôme station. During summer instead, the proportion of Gram-positive cultivable bacteria (42.1% of the isolates) was lower than that of Gram-negative (57.9% of the isolates). From summer to winter, the relative proportion of each group (Gram-positive and Gram-negative) changed, and the Gram-negative group became dominant. A more detailed analysis of the bacterial composition for the two periods (Fig. 2) showed that the observed difference between winter and summer is in great part the result of a decrease in the proportion of Firmicutes from 21% to 5%, combined with an increase in the proportion of Gamma-Proteobacteria from 8% to 37%. Such a fact can be supported by the higher resistance of Gamma-Proteobacteria to solar radiation.

3. Role of microorganisms in cloud droplet and ice crystal activation

Microorganisms can be incorporated into cloud droplets by nucleation processes: by offering a surface condensation to water vapour and acting as CCN (Cloud Condensation Nuclei) or by inducing ice formation and act as IN (Ice Nuclei). First, microorganisms can be considered as CCN, as a particular case of aerosol particles presenting some specific physico-chemical properties due to their biological nature (Ariya et al., 2009). Second, the properties of microorganisms able to initiate the formation of ice (Ice Nucleation Active) concern

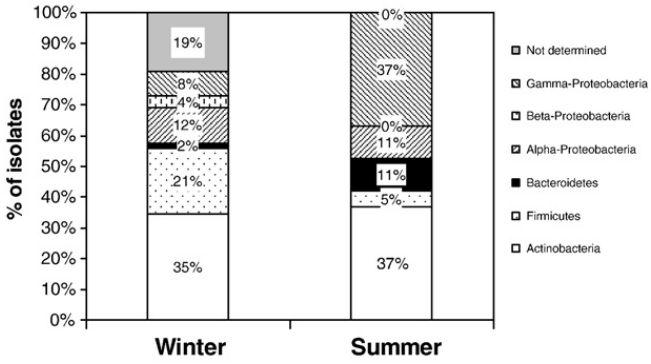

Fig. 2. Typical relative composition of the cultivable bacterial community during winter and summer in cloud water collected at the puy de Dôme (France). Figure from Amato et al. (2007b).

notably bacteria belonging to the genus *Pseudomonas*. The precise mechanisms involved are still under controversy in the scientific community (Möhler et al., 2007; Sun and Ariya 2006).

3.1. Microorganisms as Cloud Condensation Nuclei (CCN)

Bacteria are spherical or rod-shaped particles of 1 μm and as so they can participate in the formation of clouds by offering a surface for the condensation of water vapour when the relative humidity exceeds saturation with respect to the pertinent phase over the particle surface. This kinetic process is controlled by the size, composition and surface properties of the particle (Köhler's theory — Köhler, 1936). Considering bacteria, their surface are characterized by a specific negative charge and hydrophilicity/hydrophobicity balance which depends on the strain. Even the small presence of a hygroscopically active material onto the particle surface can substantially increase its ability to activate to cloud droplets. For instance, the presence of lipopolysaccharides or the production of EPS (ExoPolymeric Substances) composed of polysaccharidic and proteic structures can enhance the hydrophilicity of the cell surface and thus its "wettability" and hygroscopy. These bacterial surface properties are important considering the processes of CCN activation as it was shown that the critical supersaturation (RH-100%), the hygroscopicity and the dry diameter of the particles are correlated (Möhler et al., 2007; Petters and Kreidenweis, 2007). Only a few papers reported experimental data using bacterial cells. A fairly large fraction of bacterial strains of the phyllosphere belonging to the *Erwinia carotovora* species was shown to be activated at 1% supersaturation (Franc and DeMott, 1998). Bauer et al. (2003) found that *Micrococcus agilis*, *Mycoplana bullata* or *Brevundimonas diminuta* were activated at <0.1% supersaturation.

These experiments showed that the results obtained for bacteria are highly variable and depend on the surface properties of these organisms but they are consistent with values expected for the aerosol particle of the same size (around 1 μm) (Möhler et al., 2007). However, extrapolating such data to natural conditions remains a challenge.

In addition to the direct role that microorganisms could play as bioaerosols, they could also contribute or modify the formation of CCN by producing organic compounds that will

enhance the formation of cloud droplets. This is the case of biosurfactants, produced by many microorganisms which can modify very efficiently surface tension due to their amphiphilic nature. Organic surfactants present in droplet solutions can lower surface tension and lead to a significant decrease of critical supersaturations when present in sufficient quantities (Prisle et al., 2009). Examples of microbial biosurfactant structures are presented in Fig. 3. Very recently, Ekström et al. (2009) suggested the presence of bioaerosols in the atmosphere sampled at different locations. These biosurfactants could be emitted by microorganisms present at the Earth's surface or directly in cloud water. Ahern et al. (2007) showed that 70 fluorescent *Pseudomonas* strains isolated from Hebridean cloud and rain samples were biosurfactant producers. Various strains of *Pseudomonas*, *Rhodococcus* and *Bacillus* producing biosurfactants, were also found in cloud water sampled at the puy de Dôme station (unpublished results). Other compounds could also play a role in the CCN formation such as organic acids, sugars, and polysaccharides (Ariya et al., 2009).

3.2. Microorganisms as Ice Nuclei (IN)

The history of the ice nucleation activity of bacteria started with the pioneering work of Soulage in 1957 who found bacteria cells in ice crystals and that of Vali et al. (1976) who made a link between ice nucleation processes and epiphytic bacteria. It was understood later that the bacteria living on the

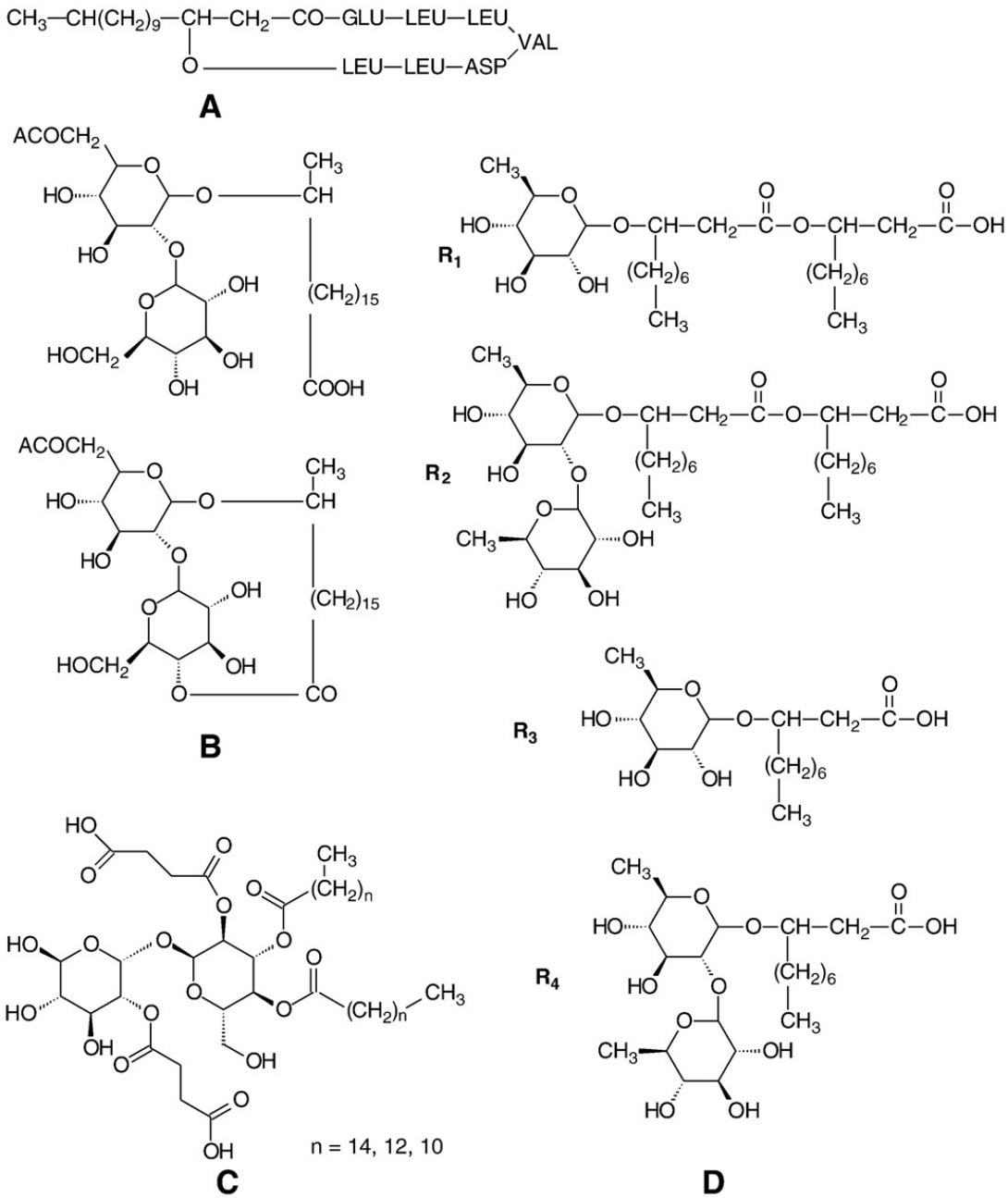

Fig. 3. Molecular structure of some biosurfactants (A) surfactin produced by *Bacillus subtilis*, (B) sophorolipids produced by *Candida bombicola*, (C) succinoyltrehalose lipid produced by *Rhodococcus erythropolis*, (D) rhamnolipids produced by *Pseudomonas aeruginosa*.

surface of leaves (mainly *Pseudomonas*, and more particularly *Pseudomonas syringae),* were responsible for inducing ice nucleation at a temperature warmer than the usual (see for review Morris et al., 2004; Hirano and Upper, 2000; Möhler et al., 2007). Indeed, laboratory experiments showed, using cultured bacteria that ice formation in pure water could occur at T≥−10 °C instead of −39 °C for homogeneous ice nucleation processes. In parallel, biologists showed that the same type of bacteria is damaging plants by freezing plant leaves. In some cases, ice nucleation occurred at −2 °C which was a several degrees warmer than for other ice nuclei such as silver iodide (−5 °C) under the same conditions. In addition to *P. syringae*, some other bacteria from the phyllosphere were shown to be INA (Ice Nucleation Active) bacteria, including *P. fluorescens*, *P. viriflava*, *Pantoea agglomerans* (former called *Erwinia herbicola*) and *Xanthomonas campestis*. Also the phytopathogen fungi of the genus *Fusarium* can exhibit IN properties. The development of molecular biology and biochemistry tools allowed understanding the molecular basis of the ice nucleation activity in bacteria: IN is due to the expression of a protein of 150 to 180 kD anchored on the outer part of the cell membrane (and thus exposed to the extracellular medium). This protein (INP) acts as a template for ice formation by orienting water molecules at the surface of the cell. The amount and type of anchor of the INA proteins forming aggregates in the membrane can modulate the freezing efficiency: Bacterial ice nucleation efficiency has been classified into three groups according to the temperature of freezing: Type I (−5 °C≥T°≤−2 °C), Type II (−7 °C≥T°≤−5 °C) and Type III (T°≤−7 °C) (Morris et al., 2004). It was also demonstrated that the purified protein can initiate ice nucleation but in a less efficient way that embedded in the membrane of bacteria.

Although the molecular basis of ice nucleation activity is now established, the main question remains: do these bacteria really play a role in atmospheric microphysics, i.e. in cloud and precipitation formation? To answer this question, scientists have tested different strategies.

The first strategy is based in creating "artificial clouds" using bacterial isolate sprays and observing the formation of ice crystals. Some experiments were conducted with the commercial product Snomax™ derived from *P. syringae*, largely used in ski resorts to make artificial snow. Simulations of cloud forming conditions were carried out in a cloud chamber at Colorado State University (Ward and DeMott, 1989) as well as in the AIDA (Aerosol Interaction and Dynamics in the Atmosphere) chamber in Karslruhe (Möhler et al., 2008). A droplet fall tower experiment was also conducted at the University of Washington (Wood et al., 2002). Results obtained with Snomax™ in these three installations were in good agreement: strong ice nucleation efficiency was found between −7 and −9 °C, a few % of the cells were active around −5 °C. However, experiments performed with five different *P. syringae*, *P. virififlava* and *E. herbicola* bacterial species in the AIDA facility (Möhler et al., 2008) showed only ice nucleation activity at temperatures between −7 and −11 °C with an ice nucleation active fraction of about one nucleus for every 10^4 cells. It can be noticed in these experiments that bacterial cells first acted as CCN, then induced freezing. However we should not conclude that bacterial cells are not candidates for IN processes. In particular, it was shown that INA protein expression is very dependant on bacterial metabolism, culture medium, growth phase, bacterial genome, bacterial origin, etc. (Morris et al., 2004); therefore many parameters must be tested before final conclusions.

The second strategy is not based on laboratory experiments but on the direct study of cloud and precipitation samples. The idea is to look at the presence of INA bacteria in these natural environments, especially in remote places far from agricultural contexts; and thus to track dissemination of bacteria *via* the water cycle. Ahern et al. (2007) did not find any IN gene or activity in any of the numerous isolates of *Pseudomonas* using both PCR and DSC (Differential Scanning Calorimetry) in Hebridean cloud and rainwater. Also Junge and Swanson (2008) did not detect any INA in several representative Arctic and Antarctic sea-ice bacterial isolates using a free-fall freezing tube technique; this study was carried out to support the role that marine psychro-active bacteria could play in the formation of sea-ice and ice in polar clouds. However, positive results were obtained with cloud water isolates sampled from the puy de Dôme summit. Indeed our group found two cultivable microbial strains of *Fusarium avenaceum* and *Pseudomonas pv atropurpurea* with ice nucleation activity at −5 °C and −6 °C respectively using immersion freezing testing (unpublished data). Bowers et al. (2009) compared total microbial and IN counts in clear air, cloudy air and snow samples collected in Colorado. They showed that for similar abundances within these samples, the number of IN was increasing with relative humidity. Also the microbial communities were quite different in the air compared to snow. Surprisingly the abundance of *Pseudomonads* (known for its INA properties) in all samples was very low. However, the genus *Psychrobacter* was one of the dominant groups and it was shown that some isolates of this genus could be INA bacteria (Ponder et al., 2005). Even though these results were found using molecular tools and that no test on cultivated bacteria could be made, these results demonstrate the presence of INA bacteria in the atmosphere. Very interesting results were obtained by Christner et al. (2008a,b) who examined the concentration and nature of IN over a large number of rain and snow samples collected in various locations: Montana and Louisiana (USA), the Alps and Pyrenees (France), Ross Island (Antarctica) and Yukon (Canada). They found that half of the samples were ice nucleation active at temperatures − 5 °C based on an immersion freezing test. In addition, they showed the influence of geographic, seasonal and precipitation chemistry on IN abundance and activity. To prove the biological, and more precisely the bacterial and/or proteinaceous, nature of these ice nuclei, samples were treated with lysozyme (enzyme that hydrolyses the bacterial cell wall) or by heat (denaturation of all proteins). These treatments greatly reduced the amount of INA in all samples demonstrating their biological and bacterial nature.

Pratt et al. (2009) detected *in situ* biological particles in ice crystals in clouds sampled by aircraft at a high altitude in Wyoming (7 to 8 km; T° −31° to −34 °C). The chemical composition of individual CVI (Counterflow Virtual Impactor) ice residual particles was measured by A-ATOFMS (Aircraft Aerosol Time of Flight Mass Spectrometry). This technique allowed to record the representative mass spectra of biological (Fig. 4A) and dust particles (Fig. 4B) which can be differentiated by the presence of some specific ions:

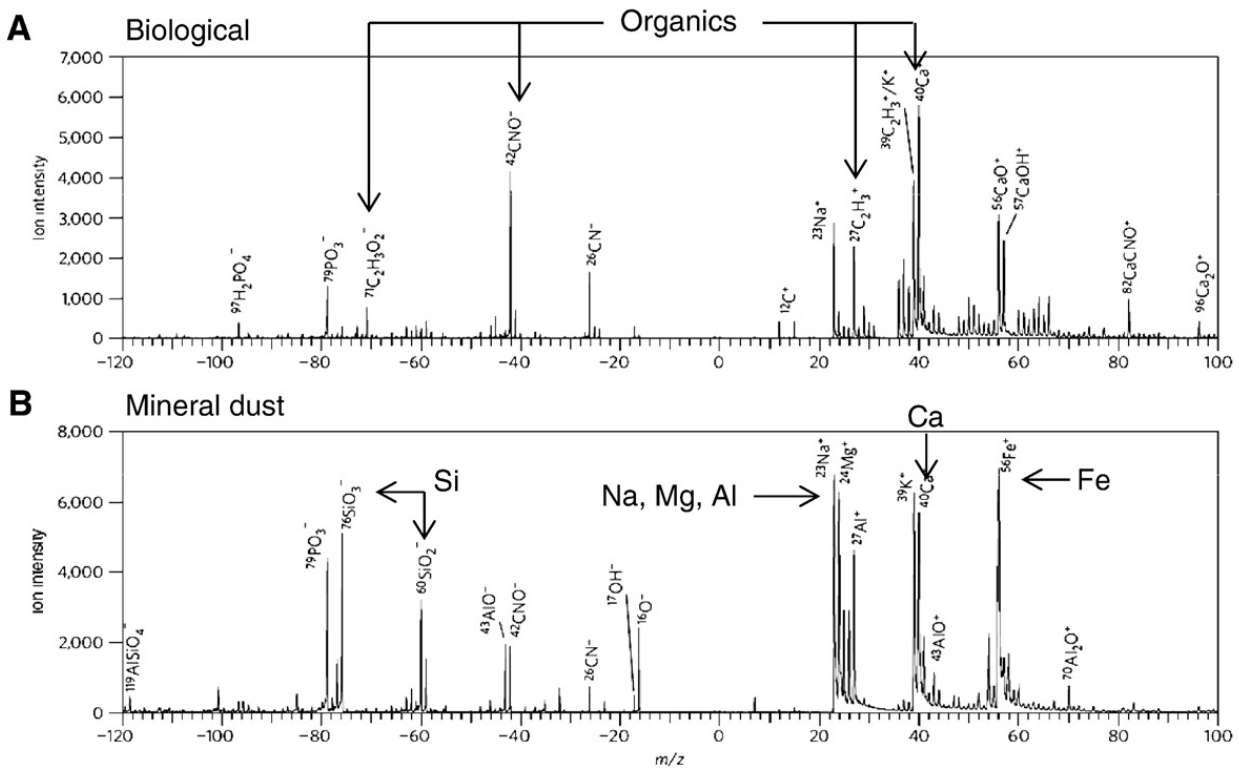

Fig. 4. Mass spectra of individual CVI (Counterflow Virtual Impactor) residual particles measured by A-ATOFMS (Aircraft Aerosol Time of Flight Mass Spectrometry). A) Biological particles. B) Mineral dust particles. Figure adapted from Pratt et al. (2009).

phosphorus and organics ions can be considered as a biological signature while silicon, sodium, calcium, magnesium and iron ions are of mineral origin. Biological particles accounted for 33% of the residues and mineral dust particles for 55%. In addition, the spectra of the biological aerosols were rather close to those measured on *P. syringae*. This is consistent with the presence of bacterial cells in cloud ice crystals present at a high altitude.

Finally, in order to quantify the actual role of microorganisms as IN, the experimental data obtained with INA bacteria need to be considered in cloud process models that simulate mixed-phase clouds (Leroy et al., 2006; Phillips et al., 2007). The objective is to consider in numerical models the activation of microorganisms into ice crystals through parameterizations that are developed based on laboratory investigations. The study carried out by Levin et al. (1987) was the first to evaluate biological particles as effective immersion IN that can freeze larger droplets. They used a time dependent one and a half dimensional model that treats cloud microphysics in detail, but assumes a constant cloud radius and allows entrainment. When they considered the data of biogenic ice nucleation from their laboratory study, their simulations resulted in even larger amounts of rain on the ground. Larger scale models that couple emissions, transports and microphysical processes of microorganisms should be developed on the basis of *in situ* measurements (quantifications and characterization of sources), of laboratory investigations (which microorganisms are efficient IN?) and results obtained with the cloud process model (development of parameterizations for IN activity of microorganisms). Recent simulations from Phillips et al. (2009) were performed with an aerosol-cloud model that doubles moment bulk microphysics for a month-long period of summertime convective activity over Oklahoma. This study is the first attempt at evaluating the impacts from biological aerosols on mesoscale cloud and demonstrated that INA bacteria modify the extent and properties of clouds. Very recently, Hoose et al. (2010) reported that the role of bacterial IN in inducing precipitation is likely negligible on the global scale, but may impact the behaviour of clouds on the local scale.

4. Microorganisms in clouds

4.1. Number, activity and type of microorganisms

Table 1 summarizes some of the major sampling sites where the numbers of microorganisms were determined for cloud water and rainwater. This is very heterogenic and strongly depends on a wide range of factors listed in the introduction. Total bacterial concentration in atmospheric waters varies from 10^3 to 10^5 cell mL^{-1}; this is basically decreasing with increasing altitude. In cloud water, the concentration of fungi and yeasts is one order of magnitude lower than that of bacteria (10^2 to 10^4 cell mL^{-1}); however, the difference in size between eukaryotic cells and bacteria (the former being in general about 10 times larger) results in equivalent biomasses.

Less than 1% of the bacteria and less than 50% of the fungi and yeasts collected from clouds sampled at the puy de Dôme station could be cultivated in non selective media. However, it was shown by the measures of the concentration in adenosine triphosphate (ATP) in cloud samples that a large majority of the cells counted by microscopy were likely alive (Amato et al., 2007d).

Table 1
Concentrations of microorganisms in atmospheric water samples.

Localization	Type of atmospheric water sample	Concentration of bacteria	Concentration of fungi and yeasts	References
puy de Dôme mountain — France 1464 m.a.s.l.	Cloud	1.7×10^{4}–2.4×10^{5} mL^{-1} §	8.9×10^{2}–2.5×10^{4} mL^{-1} §	Amato et al., 2007d
Rax mountain — Austria 1644 m.a.s.l.	Cloud	2×10^{4} mL^{-1} +	5.9×10^{3} mL^{-1} + /	Bauer et al., 2002, 2003
Sonnblick mountain — Austria, 3106 m.a.s.l	Cloud	1.5×10^{3} mL^{-1} &	/ /	Sattler et al., 2001
Kleiner Feldberg mountain — Germany, 857 m.a.s.l	Cloud Rain	1.05×10^{8} mL^{-1} a 3.57×10^{3} mL^{-1} a		Matthias-Maser et al., 2000
Po valley — Italy	Fog	30–150 mL^{-1} b	30–60 mL^{-1} b /	Fuzzi et al., 1997
Shizuoka and Tsukuba — Japan	Rain	1.6×10^{4}–2.4×10^{4} mL^{-1} a		Casareto et al., 1996
Charlottesville — Virginia, USA	Rain	2×10^{5}–6×10^{5} mL^{-1} a		Herlily et al., 1987

[a] Total number of Primary Biological Aerosol Particle (PBAP).
[b] CFU (colony forming units), i.e. cultivable microorganisms.
[+] Average from 3 cloud events.
[&] Average from 12 cloud events.
[§] Average from 14 cloud events.

So far, very few data have provided a detailed description of the microbial species found in water droplets. Three studies reported the identification of cultivable microorganisms: Fuzzi et al. (1997) reported the presence of three bacterial genera including *Pseudomonas*, *Bacillus* and *Acinetobacter* in fog droplets sampled in the Pô Valley, an area highly polluted by industrial activities. Amato et al. (2005, 2007b) observed a more diversified population distributed among various phyla or sub-phyla (*Alpha*-, *Beta*- and *Gamma*-*Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes*), and belonging mainly to the genera *Pseudomonas*, *Sphingomonas*, *Staphylococcus*, *Streptomyces* and *Arthrobacter*. They also found an important diversity among various fungal phyla (*Ascomycota, Basidiomycota, Deuteromycota*). Only one paper reports the description of a bacterial population present in clouds collected in Scotland using direct analysis of DNA by PCR (polymerase chain reaction) amplification and cloning the 16 S rDNA genes. They found many strains belonging to the *Pseudomonas* and *Acinetobacter* genera (Ahern et al., 2007).

4.2. Some specificities for living in clouds

Concerning cloud water, Amato et al. (2007b) observed that about half of the isolated microorganisms (20 over 40 tested isolates) was able to develop at a low temperature (5 °C). It included Gram-negative bacteria, among which some of them were psychrophiles (faster growth at 5 °C than at 17 °C or 27 °C). Sattler et al. (2001) showed that microorganisms could develop in cloud water at 0 °C. The presence of microorganisms active at low temperatures encountered in clouds is not surprising since psychrophilic microbial strains have been recovered and demonstrated to be active in many cold environments like glacier, polar snow and ice, permafrost (Carpenter et al., 2000; Christner et al., 2001, 2003; Junge et al., 2004; Foght et al., 2004; Groudieva et al., 2004; Amato et al., 2007a; Steven et al., 2006).

In these studies, many pigmented bacteria (about 60% of the cultivated fraction) were observed in cloud water (Amato et al., 2005; 2007b). Red, orange and yellow pigments are probably carotenoïds and could protect organisms in the atmosphere: first, it was shown that pigmented bacteria were more hydrophobic and better aerosolized than non pigmented bacteria (Blanchard, 1989). Second, the fluidity of the membranes was increased by the presence of such pigments and this balanced the low temperature effects; pigmented microorganisms have notably been isolated from polar areas (Fong et al., 2001; Mueller et al., 2005). They are also known to protect cells against free radicals (Gourmelon et al., 1994) and low osmotic pressures (Fong et al., 2001). Interestingly, these pigments are also present in bacteria exposed to UV light on the plant leaf surface and they are "prepared" to cloud conditions.

Finally, spore forming microorganisms have been found in a large number in the atmosphere: Elbert et al. (2007) and Heald and Spracklen (2009) estimate the spore concentrations from 10^{4} to 10^{6} m^{-3} with a huge spatial and temporal variability. Elbert et al. (2007), based on measurements in the Amazon region, attributed an average of 35% of the total aerosol mass to be fungal spores. At a continental background site in the Austrian Alps, Bauer et al. (2002) quantified biological components in atmospheric samples and estimated that bacteria contributed to 0.03% and fungal spores 0.9% of the total OC (Organic Carbon) mass. However, at a suburban site, Bauer et al. (2008) showed that the contribution of fungal spores to OC was 6% and 14% of the OC mass concentration observed in spring and summer, respectively. More recently, Huffman et al. (2010) used an ultraviolet aerodynamic particle sizer (UV-APS) to measure the concentrations of biological particle and size distribution in the air in Central Europe. They found a peak of abundance at around 3 μm corresponding to fungal spores or agglomerated bacteria; this peak exhibited a low absolute concentration ($<2.10^{-3}$ cm^{-1}). In addition, there was a pronounced diel cycle (24-h) in the abundance of bioaerosols in the air, with a maximum intensity during early/mid morning. In cloud and fog water, yeast and fungi as well as spore forming bacteria such as *Bacillus* strains have been found in a relatively higher concentration when the water pH was lower (Fuzzi et al., 1997; Amato et al., 2005). One of the reasons could be a

selection of the resistant forms, i.e. the spores, by the hostile conditions encountered in the atmosphere under an anthropogenic influence.

5. Role of microorganisms in atmospheric chemistry

The detection of microorganisms in cloud water raised a fundamental and innovative scientific question brought to attention very recently by Ariya et al. (Ariya et al., 2002; Ariya and Amyot, 2004; Côté et al., 2008), Amato et al. (Amato et al., 2005, 2007c; Vaïtilingom et al., 2010) and Deguillaume et al., 2008: do microorganisms play a role in atmospheric chemistry? Since cloud droplets are a liquid solution which contains carbon and nitrogen sources, it could be thus a privileged medium for microbial activity in the atmosphere.

5.1. Biotransformation of organic compounds by microorganisms isolated from the atmosphere

A few research groups have investigated the enzymatic potential of microorganisms isolated from the atmosphere to biotransform organic compounds of the atmosphere water phase. In cloud water, the more remarkable point is the high concentration of carboxylic acids and aldehydes with a short carbonaceous chain of which concentrations can reach more than 1 mg L^{-1} (Marinoni et al., 2004). Carboxylic acids are produced by the oxidation of the organic matter in clouds (Herrmann et al., 2005).They contribute to the acidification of the aqueous phase and this in cloud organic mass production by oxidation processes represents a new route for Secondary Organic Aerosol (SOA) formation (Blando and Turpin, 2000). Therefore, these latter compounds have been chosen for biodegradation studies.

The first study showing a possible impact of microorganisms on atmospheric multiphase chemistry was the degradation of formic and acetic acids by bacteria from rainwater (Herlihy et al., 1987). Ariya and collaborators isolated fungal strains of *Geotrichum, Penicillium, Eupenicillium and Thysanophora* from air samples and showed that four of these strains rapidly degraded some of the major dicarboxylic acids of the atmospheric aqueous phase. This included malonic, succinic, glutaric, adipic, pimelic, oxalic and pinic acids (Ariya et al., 2002; Côté et al., 2008). Amato et al. (2005, 2007c) showed that most of the bacterial strains isolated from cloud water sampled at the puy de Dôme Mountain had the enzymatic equipment necessary for degrading monoacid and diacid compounds (acetic, lactic, formic and succinic acids) as well as formaldehyde and methanol. Those compounds are present in relatively large concentrations in cloud water (Marinoni et al., 2004) and play a major role in atmospheric chemistry (Ervens et al., 2005).

5.2. Comparison between biological pathways and photochemical reaction pathways

Solar light is considered as the predominant catalyser for chemical reactions occurring in the atmosphere, so called photochemistry. It induces the formation of free radicals, like $HO^{\bullet}$ and $HO_2^{\bullet}$, which mainly result from the presence of hydrogen peroxide H_2O_2. They at their turn oxidize organic compounds, notably those emitted by human activities. Hydrocarbons are oxidized into alcohols, aldehydes, carboxylic acids and, as a final step, into CO_2. Oxygen, metals (especially iron) and ions like nitrate and chloride also contribute to the oxidation of organic compounds in the atmosphere.

Most of the intermediate photoproducts are very similar to metabolites produced by microorganisms. This is depicted in Fig. 5 with the degradation of lactic acid by photo- and biodegradation (Amato et al., 2007d; Parazols, 2007). In the two processes, acetic and pyruvic acids are formed *via* the degradation of lactic acid. Methylglyoxal can only result from biological degradation of lactic acid, but not from its photochemical transformation, which leads instead to the formation of pyruvic acid. The main differences observed between photo and biodegradation are i) the reversibility of some biologically driven reactions and ii) the stoechiometry of the by-products: in biodegradation processes the main intermediate is pyruvic acid, while photochemical processes mainly involve acetic acid as intermediate species, iii) contrary to photochemical reactions, enzymatic reactions depend on the chirality of the substrates, for instance different enzymes are involved in the transformation of L and D lactate. The similarity of by-products is not only observed with the degradation of lactic acid but can be generalised to other carboxylic acids found in the cloud aqueous phase of the atmosphere. Hence, this raises the question: does microbial degradation of organic compounds compete with radical chemistry (photochemistry) in cloud water? To answer this problem, two different approaches are currently in progress.

The first consists of comparing experimentally the chemical and biological kinetic constants measured under bulk conditions and estimating the corresponding lifetimes of the studied compounds (Table 2). Ariya et al. (2002), while studying the degradation of dicarboxylic acids by *Geotrichum* sp. isolated from air samples, showed that most of the lifetimes related to biodegradation were comparable to the lifetimes related to the presence of $HO^{\bullet}$; these were much shorter than other major atmospheric oxidants such as O_3 and $HO_2^{\bullet}$ (Table 2). Vaïtilingom et al. (2010) measured the biodegradation rates at 5 °C of acetic, formic, and succinic acids by *Pseudomonas graminis* isolated from cloud water. Biodegradation related lifetimes were compared with the reactions taking place in clouds during daytime and night, involving the presence of $^{\bullet}OH + NO_3^{\bullet}$ and of $NO_3^{\bullet}$, respectively. This showed that although photochemistry is the most efficient process during daytime, microorganisms could be driving the reactivity of cloud water at night (Table 2). For instance *P. graminis* degraded formic acid about ten times faster than $NO_3^{\bullet}$ and fifty times slower than $HO^{\bullet}$. The results obtained for *Geotricum* sp and *P. graminis* were consistent with each other, with estimated lifetimes in the range of a few days.

The second approach is part of a long-term strategy, but seems actually to be the method that will allow evaluating the impact of the microbiological process in cloud chemistry. The idea is to introduce both biological and radical reactions into numerical models and perform sensitivity tests on different parameters such as metabolic kinetic constants, number of cells, temperature, pH, light flux, etc. Cloud chemistry models take into account the exchanges existing between the interstitial phases (gases and particles) and cloud droplets and crystals (Leriche et al., 2007; Long et al., 2010). Numerous simulations

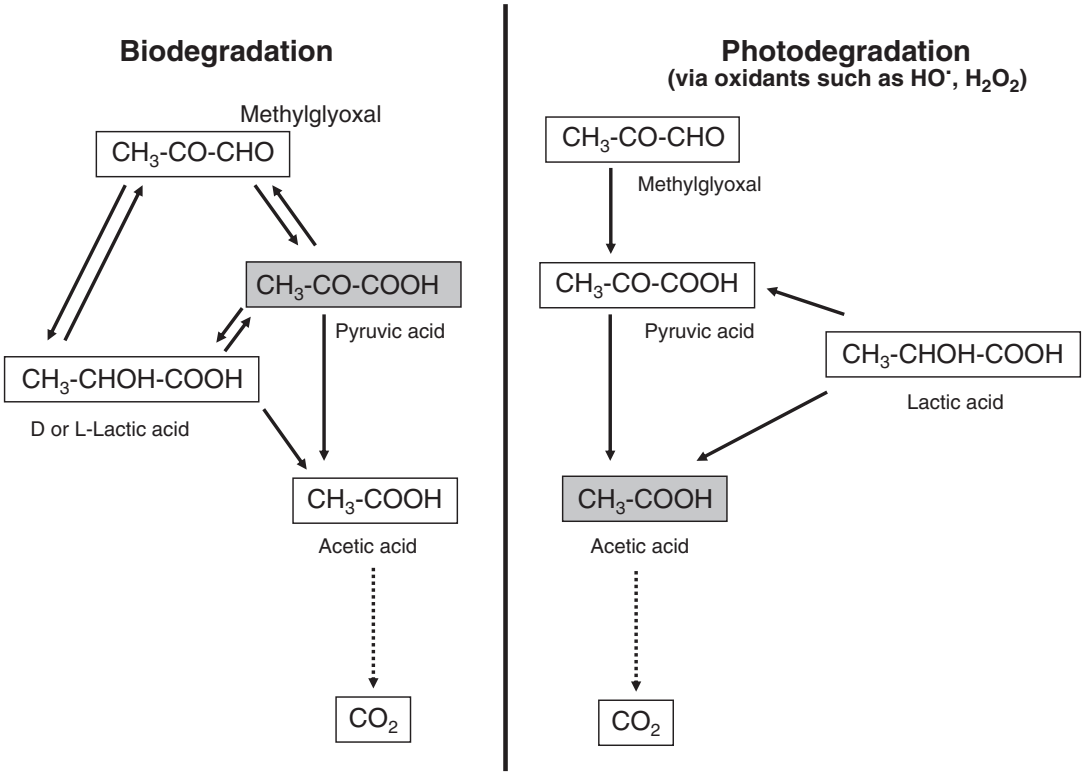

Fig. 5. Comparison of the photochemical and biochemical degradation pathways of lactic acid. Grey squares indicate the main intermediates observed under laboratory conditions (Data from Amato et al., 2007c; Parazols 2007).

under various environmental conditions (different cloud types, air-masses, metrological conditions, seasons, etc.) will be required in order to evaluate the relative contribution of microbial activity and photochemical pathways.

6. Perspectives

The atmospheric life cycle of microorganisms has been under the scope of numerous recent studies. It appears that the characterization of the sources and strengths of airborne microorganisms would be useful notably for the numeric investigations of their dissemination. A major limitation in proposing reliable fluxes is linked to limited understanding of emission mechanisms and the effect of meteorological variables (Jones and Harrison, 2004; Burrows et al., 2009a). These should be studied in more detail in order to build inventories of the emission of airborne microorganisms.

Because clouds offer very specific living conditions in the atmosphere, a number of recent studies have investigated the behaviour of microorganisms in cloud water. Again, many questions remain unanswered and would be investigated in the future. Additional information is needed to better characterize the microbial population in clouds and precipitations. Up to now only a few samples have been analyzed, and the choice of the methods used can be discussed: i) On the one hand, molecular tools avoid culture problems and are thought to provide a better view of the actual biodiversity existing in a sample. A great number of techniques are now available (DNA/RNA isolation, PCR, ARDRA, T-RFLP, RISA, qPCR, etc.) (for review see Georgakopoulos et al., 2009). Nevertheless, even molecular technologies cannot describe all the species: because of the very low concentration of microorganisms in atmospheric waters, a large number of PCR cycles are needed to amplify DNA and false positive can occur or minor groups

Table 2
Estimated lifetimes of some organic acids in cloud water, considering rates of degradation related to the presence of either $NO^{\bullet}_3$, $HO^{\bullet}$, $HO^{\bullet}_2$, O_3 or of microorganisms. Data are adapted from Ariya et al., 2002[(a)] or Vaïtilingom et al., 2010[(b)].

Estimated lifetime (days)						
Organic acid	$NO^{\bullet}_3$	$HO^{\bullet}$	$HO^{\bullet}_2$	O_3	*Geotricum* sp	*Pseudomonas graminis*
• Malonic acid	–	[a]6–24	[a]>643	[a]2.1×10^3	[a]1.5	–
• Glutaric acid	–	[a]1.3–5.2	–	[a]8.9×10^6	[a]5.0	–
• Adipic acid	–	[a]0.1–0.4	–	[a]6.8×10^6	[a]2.0	–
• Pimelic acid	–	[a]0.03–0.12	–	[a]2.6×10^6	[a]3.5.0	–
• Pinic acid	–	–	–	[a]4.6×10^5	[a]4.0	–
• Succinic acid	[b]14.0	[a]3–12/[b] 0.3	[a]>771	[a]17.3×10^6	[a]10.0	[b]1.5
• Formic acid	[b]18.7	[b]0.04	–	–	–	[b]2.0
• Acetic acid	[b]665.2	[b]2.1	–	–	–	[b]69.1

cannot be detected. ii) On the other hand, cultivable bacteria represent less than 1% of the total population but the viability of the isolates in the atmospheric environment is indubitable. In addition, isolated strains can be used for laboratory investigations. In conclusion, both approaches (culture and molecular) are complementary and should be used in parallel. These combined approaches notably allowed the detection of two separate clusters of *Pseudomonads* in cloud water samples (Georgakopoulos et al., 2009).

More experiments should be performed in laboratories with a great variety of microbial isolates from clouds to get qualitative and quantitative data. Concerning atmospheric chemistry, biodegradation (and photodegradation) pathways and kinetic constants must be investigated under conditions closer to cloud conditions, for instance using real cloud water. For nucleation processes, basic knowledge is needed to understand the relationships between: i) the bacterial surface hygroscopy and CCN properties, ii) the conditions of bacterial IN protein expression and nucleation properties, and iii) the production of bacterial metabolites (biosurfactants, saccharides, and alcohols) and aerosol properties. More generally, there is a current need for characterizing bioaerosol particles using various methods; these have been reviewed by Georgakopoulos et al. (2009) and Ariya et al. (2009).

The main issue for evaluating the influence of microorganisms in atmospheric physico-chemical processes is actually the practical impossibility to describe the exact physiology and metabolism of microorganisms *in situ*, i.e. while they are in clouds. There is also an extreme difficulty to reproduce a cloud environment under laboratory conditions, so most of the work that has been done so far is based on bulk conditions, which are very far from being realistic because: i) multiphase systems (solid/liquid/gas) are in constant exchange and ii) microbial population and physical and chemical conditions are different from one cloud event to another. Numeric models developed to study physical and chemical processes within clouds need to be improved to consider biological processes in the complex multiphase cloud system. Clearly, this will require improved integration of expertise from different fields of science that have historically been working independently.

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