# Chapter 10
# Clouds: A Transient and Stressing Habitat for Microorganisms

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**Abstract** In this chapter, we synthesized the current knowledge about clouds as ecosystems which have been discovered very recently. First, we briefly described the cloud habitat. Cloud physics chemistry and microphysics are described, showing that this environment is extreme. Microorganisms are exposed to a dynamic medium changing extremely rapidly (evaporation/condensation of the cloud droplets, quick temperature and pressure changes, freeze/thaw cycle) and also to chemical stresses (strong oxidants, acidic pHs and toxics). Then the life cycle of microorganisms in the atmosphere is detailed showing that cloud is a transient habitat: microorganisms are aerosolized, transported in the air, integrated in cloud droplets and deposited back to the ground with precipitation. Finally the cloud microbiome is described; it appears that it remains largely unknown and based mainly on culture techniques. In the second part of the chapter, the abilities of these microorganisms to survive in this stressing environment are described in details. Microbes can adapt their metabolism as it was shown that the majority of the community is metabolically active and that they metabolize organic compounds in cloud water. They have also developed general strategies that help resisting to atmospheric constraints, such as the production of extracellular polymeric substances and pigments, or the formation of spores. Finally they can respond to specific stresses such as oxidative, osmotic and temperature

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stresses thanks to protecting metabolites such as osmo- and thermo-protectants, anti-oxidants or by using specific enzymes.

## 10.1 Introduction

Unlike most environments such as soil, freshwaters, and oceans, microbiology of the atmosphere has only recently been studied. Indeed, the presence of living cells in clouds opens new routes in understanding the biogeochemistry of this medium. This lack of knowledge is certainly related to the fact that this environment is not easy to study: clouds are difficult to sample, specific sites are needed to get in parallel all the meteorological and physico-chemicals parameters, the number of microbial cells is low making investigations particularly difficult. As cloud environment is very difficult to reproduce in lab conditions, microcosm experiments are not easy to design. Finally working on cloud microorganisms requires knowledge in atmospheric sciences and strong interactions with physicists in this field. However, for a long time, a gap existed between atmospheric sciences and biology, where physicists considered microorganisms as simple particles and studied them only for their microphysical properties. The fact that they can be alive and can actively impact the atmospheric processes was not yet considered.

The question remains to know whether the cloud microbiome is a real "ecosystem". Indeed its major specificity is the fact that it is transient and highly diluted. This means that microorganisms are dispersed in a few droplets for a rather short time, and are thus not very likely to communicate. However they can exchange metabolites thanks to evaporation-condensation mechanisms leading to concentration modifications and exchanges between droplets.

Cloud environment can be considered as "extreme" mainly because it is an unstable medium, where physico-chemical conditions changes are large and fast. In addition this medium is highly oxidative due to the presence of strong oxidants and UV light. To face these stresses, some microorganisms have developed specific features allowing them to survive during their journey in the atmosphere.

## 10.2 Clouds as a Transient and Stressing Habitat for Microorganisms

### *10.2.1 The Cloud Habitat*

#### 10.2.1.1 The Physics of Cloud

Cloud lifetime is controlled by the dynamics of the atmosphere at the synoptic scale and, in close interaction, by microphysical processes (i.e. nucleation of cloud

droplets, condensation and evaporation, collision/coalescence processes, sedimentation of hydrometeor etc.) at small scale.

Clouds are made of microscopic droplets of liquid water ("warm clouds"), crystals of ice ("cold clouds"), or both ("mixed phase clouds"). Cloud droplets are formed by the condensation of water vapor onto cloud condensation nuclei (CCN) when the supersaturation of air exceeds a critical value which is described by the "Köhler theory" (Köhler 1936). Cloud condensation nuclei are needed during the formation of cloud droplets because of the Kelvin effect, which describes the variation in saturation vapor pressure due to a curved surface. When the cloud droplet radius is small, the amount of supersaturation needed for condensation to occur is so important, that it is impossible to occur naturally in the atmosphere. The second important factor is the concentration of solute that is described by the Raoult's Law: at high solute concentrations, when the droplets are small, the supersaturation needed is smaller than without the presence of a nucleus. The cloud lifetime depends on the way the cloud is evolving in the atmosphere. A cloud droplet can evaporate or fall to the Earth as precipitation. In warm clouds, larger cloud droplets fall at a higher terminal velocity than the smaller ones; the large droplets then can collide with small ones and combine to form larger drops. When drops become large enough so that the acceleration due to gravity is much larger than the acceleration due to drag, the drops can fall down as precipitation. These processes are called "collision/coalescence". In mixed phase cloud, this effect is not as important because other complex mixed phase processes occurs and lead to precipitation formation. Mixed-phase clouds and cold clouds are composed of various iced hydrometeors (pristine, snow, graupel) with a large size range and with various complex shapes. Important processes that form precipitation in mixed-phase clouds are riming, when a supercooled liquid drop collides with a solid snowflake, and also aggregation, when two solid snowflakes collide and combine together.

At synoptic scale, the formation of clouds strongly depends on updrafts. Water droplets that group together are quickly pulled down to the ground by gravity, so that they would quickly dissipate and the cloud never forms. An updraft can form if warm air interacts with cold air which can be cause by topography. As the warm air rises in the atmosphere, the moisture in the updraft will condense into liquid form and add to the amount of water available for precipitation. Violent updrafts can reach speed up to 290 km/h. The droplets can also freeze during through one of these updrafts and can cycle through several updrafts before finally becoming so heavy that it falls to the ground.

In this context, clouds are harsh environments for microorganisms because they have to deal with rapid changes in osmotic pressure when the liquid water of the cloud varies and/or when evaporation of cloud droplets occurs. Pruppacher and Jaenicke (1995) estimated that atmospheric water condensates and evaporates in average 10 times before being removed by precipitation (Pruppacher and Jaenicke 1995). The solute concentration can vary by several orders of magnitude: assuming neither precipitation nor gasification of the solutes, a 20 μm cloud droplet that evaporates to a diameter of 2 μm would concentrate chemical species by a factor of $10^3$. For instance, ion species measured at the puy de Dôme station, the observed

concentrations of total ion content ranged from 1 to 1.9 mM (Deguillaume et al. 2014). Evaporation of cloud droplets would thus increase ion concentration to up to 1 M and create high osmotic shocks for the microorganisms.

Clouds also have to deal with strong pressure and temperature variation as well as supporting several freeze-thaw cycles, especially when strong updrafts are present. For example, in the troposphere, temperature generally decreases with increasing altitude, by 0.6 to 1 °C every 100 m. This, associated with vertical winds of 50 km $h^{-1}$ (14 m $s^{-1}$), potentially exposes airborne cells to thermal variations of up to approximately 1 °C every 7 s, and eventually also to freeze-thaw cycles.

#### 10.2.1.2 The Chemistry of Cloud

The cloud aqueous phase is a very complex mixture of inorganic and organic chemical compounds. These chemical compounds found in cloud droplets originate from various sources: from the soluble fraction of the aerosol particles which can also act as cloud condensation nuclei (CCN), from the dissolution of soluble trace gases as well as from scavenging processes. Cloud reactivity also forms new chemical compounds. Since cloud water contains strong acids, potentially toxic molecules (formaldehyde for example), and strong oxidants like hydrogen peroxide or radicals, it can represent a stressful medium for microorganisms.

The major inorganic ions found in cloud water are the sulfate ($SO_4^{2-}$), chloride ($Cl^-$) and nitrate ($NO_3^-$) anions and the alkali and alkaline Earth metals cations ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$), in addition to ammonium ($NH_4^+$). These chemical compounds results from various sources. For example, the major fraction of the potassium, magnesium and calcium ions come from the mineral part of aerosol particles originating from soil; nitrate and ammonium can enter into cloud water as constituents of condensation nuclei as well as by gas to liquid scavenging of gaseous $HNO_3$ and $NH_3$; the sulfate arises from the oxidation of gaseous precursors that are dissolved into cloud droplets such as $SO_2$. In polluted region, the oxidation of $SO_2$ and $NO_2$ is a major source of strong acids $H_2SO_4$ and $HNO_3$ that control cloud water acidity i.e. the pH. In coastal regions and over the ocean, sodium chloride constitutes the largest part of all ions. The acidity of the cloud water strongly depends on the air mass origin. The pH can vary between 2.2 and 7 (Aleksic et al. 2009; Collett Jr et al. 2002; Deguillaume et al. 2014; Hill et al. 2007). For example, for polluted clouds, the pH at the puy de Dôme station (France) is the most acidic due to the higher amount of nitrate and sulfate (pH around 4). pH values encountered in clouds are far from the known limits of growth of microorganisms that can develop at pH close to 0 for certain species (Schleper et al. 1995). However, beyond these limits, their survival strongly depends of their capacity to maintain their pH cytoplasmic at values compatible to their metabolism. Most of the microorganisms have optimal pH for their growth between 5 and 9 (Padan et al. 2005). During the cloud lifetime, the microorganisms have to deal with strong variations of the pH of the cloud droplets due to the condensation/evaporation processes.

The organic matter also represents a major fraction of the soluble matter in cloud droplets (Herckes et al. 2013). The total Dissolved Organic Carbon (DOC) is highly variable depending on the history of the air mass. For highly polluted clouds, DOC values can reach 200 mgC $L^{-1}$ as reported by Wang et al. (2011) at Mount Tai in China (Wang et al. 2011). Currently, DOC values are between 5 and 10 mgC $L^{-1}$ in average for continental clouds (Anastasio et al. 1994; Löflund et al. 2002; Ervens et al. 2013; Hutchings et al. 2009) and below 5 mgC $L^{-1}$ for clouds from marine origin (Marinoni et al. 2004; Deguillaume et al. 2014). Carboxylic acids represent around 10% of the dissolved organic carbon in the cloud droplets (Table 10.1). The carboxylic acids can be produced in the gaseous phase and dissolved in the aqueous phase (main source of acetic and formic acid); they can also result from the dissolution of soluble particles (main source of oxalic, succinic, malonic, and maleic acids); or produced via the aqueous phase reactivity (Herrmann et al. 2015). Due to the presence of free radicals such as the hydroxyl radical $^{\bullet}OH$ in the aqueous phase, the oxidation of organic matter is considered as an important source of carboxylic acids (Tilgner and Herrmann 2010); they also represent one of their main sinks. Carbonyl compounds are also present in cloud water and they essentially result from their dissolution from the gas phase into the aqueous phase depending on their Henry's law constants (Ervens et al. 2003). In the aqueous phase, the oxidation of aldehydes produces carboxylic acids but also lead potentially to the formation of oligomers (Ervens et al. 2003, 2015). Concentration levels of carbonyl compounds such as formaldehyde, acetaldehyde, glyoxal and methylglyoxal have been measured in cloud water (Houdier et al. 2011; van Pinxteren et al. 2005; Matsumoto et al. 2005). Formaldehyde in the gas phase is produced by biomass burning and fossil fuel combustion, and also by photochemical oxidation of methane and non-methane hydrocarbons. This compound is efficiently transferred into the aqueous phase due to its efficient Henry's law constant, explaining its higher concentration in cloud water (Table 10.1). In the case of foggy event occurring in polluted area, the aqueous formaldehyde concentration can reach up to 710 µM (Jacob et al. 1984). This kind of high concentration of formaldehyde can be partially toxic for the biological content of the cloud/fog droplets.

The proportion of undetermined organic matter in cloud water is still high and represents more than 90% of the total dissolved organic matter. Among this complex organic matter in cloud water, HUmic LIke Substances (HULIS) have been identified and correspond to large multifunctional compounds such as proteins, cellulose, dicarboxylic acids, polyols, amino acids, fatty acids, sugars, polysaccharides or aliphatic and aromatic hydrocarbons (LeClair et al. 2012; Ekström et al. 2010).

This description of cloud carbon content shows that this medium is rather poor, with low DOC concentrations (Table 10.1). In addition, methanol and formaldehyde (C1 compounds) could be toxic for many microbial species.

Cloud water is an oxidizing environment with a redox potential of up to more than 200 mV (Deguillaume et al. 2014), notably due to the presence of radicals ($^{\bullet}OH$ and $HO_2^{\bullet}/O_2^{\bullet-}$) and their precursors ($H_2O_2$, metals) (Table 10.1). $H_2O_2$ concentrations are ranging from 0 to 3.2 µM at Kleiner Feldberg [Germany; Sauer et al. (1996)], from 0.1 to 57.7 µM at the puy de Dôme, with an average of 7.8 µM

**Table 10.1** Observed concentration of carboxylic acids, formaldehyde, hydrogen peroxide, iron and dissolved organic carbon in cloud samples

| Chemical compounds | Concentrations (μM) | References |
|---|---|---|
| **Acetic acid** | 30.0–84.0 | Watanabe et al. (2001) |
| $CH_3CO(OH)$ | 4.0–37.8 | Löflund et al. (2002) |
| | 1.6–41.4 | van Pinxteren et al. (2005) |
| | 0.3–57.6 | Deguillaume et al. (2014) |
| **Formic acid** | 1.3–34.3 | Löflund et al. (2002) |
| CHO(OH) | 36.0–51.2 | Decesari et al. (2001) |
| | 4.9–39.1 | van Pinxteren et al. (2005) |
| | 0.2–52.8 | Deguillaume et al. (2014) |
| **Succinic acid** | 0.8–2.6 | Löflund et al. (2002) |
| $CO(OH)CH_2CH_2CO(OH)$ | 0.1–4.1 | Deguillaume et al. (2014) |
| Malonic acid | 0.7–2.9 | Löflund et al. (2002) |
| $CO(OH)CH_2CO(OH)$ | 0.4–1.8 | van Pinxteren et al. (2005) |
| | 0.3–7.0 | Deguillaume et al. (2014) |
| **Oxalic acid** | 0.7–12.6 | Löflund et al. (2002) |
| CO(OH)CO(OH) | 0.1–15.2 | Decesari et al. (2001) |
| | 2.4–11.6 | van Pinxteren et al. (2005) |
| | 0.2–19.4 | Deguillaume et al. (2014) |
| **Formaldehyde** | 8.0–14.0 | Collett et al. (1990) |
| $CH_2O$ | 13.6–61.5 | Igawa et al. (1989) |
| | 0.1–4.8 | van Pinxteren et al. (2005) |
| | 0.1–14.2 | Deguillaume et al. (2014) |
| **Hydrogen peroxide** | 0–247 | Olszyna et al. (1988) |
| $H_2O_2$ | 1–167 | Richards (1995) |
| | 0–14 | Valverde-Canossa et al. (2005) |
| | 0–19 | Marinoni et al. (2011) |
| **Iron** | 0.1–1.6 | Deutsch et al. (2001) |
| $Fe^{2+}$ and $Fe^{3+}$ | 0.6–6.3 | Pehkonen et al. (1992) |
| | 0.3–22.6 | Erel et al. (1993) |
| | 0.1–11.9 | Parazols et al. (2007) |
| **D**isolved **O**rganic **C**arbon (**DOC**) | **Concentration** (**mgC** $\mathbf{L^{-1}}$) | |
| | 3.0–18.0 | Anastasio et al. (1994) |
| | 1.8–8.1 | Ervens et al. (2013) |
| | 2.0–35.0 | Wang et al. (2013) |
| | 0.3–25.0 | Deguillaume et al. (2014) |

[France; Deguillaume et al. (2014)], and extremely high concentrations (up to 247 μM) were reported from Whitetop Mountain [U.S.A.; Olszyna et al. (1988)]. Their aqueous concentrations result from both various chemical interactions

(photolysis processes and chemical reactions) and from the phase transfer exchange between the gas and aqueous phase. $^{\bullet}OH$ radicals, can be either taken up from the gas phase or in situ produced in the aqueous phase.

One of the most relevant in situ sources of $^{\bullet}OH$ in the aqueous phase is the so-called "Fenton" reaction between $H_2O_2$ and iron(II) (Eq. 10.1). The produced iron(III)-hydroxy complexes are also photolyzed (mostly the $[Fe(OH)]^{2+}$ aqua-complexe that is dominant for atmospheric pH between 3 and 5) accelerating the formation of $^{\bullet}OH$ in the aqueous phase (Eq. 10.2).

$$Fe^{2+} + H_2O_2 \rightarrow [Fe(OH)]^{2+} + {}^{\bullet}OH \tag{10.1}$$

$$[Fe(OH)]^{2+} + h\nu \rightarrow Fe^{2+} + H^{+} + {}^{\bullet}OH \tag{10.2}$$

The photolysis of $H_2O_2$, $NO_3^-$ and iron(III)-hydroxy complexes are also effective sources of $^{\bullet}OH$ in the aqueous phase (Bianco et al. 2015) together with the phase transfer from the gas phase. Additionally, the reactions of oxidized TMIs (Transition Ion Metals) with $H_2O_2$ and the photolysis of metal-organic acid complexes such as iron(III)-oxalate complexes can act as source for $HO_2^{\bullet}/O_2^{\bullet-}$ in the aqueous phase (Weller et al. 2014). The presence of such an oxidizing environment represents a strong stress that microorganisms have to cope with.

### *10.2.2 The Cycle of Microorganisms via the Cloud Habitat*

The concept of "cloud microbiome" which was recently suggested is unique mainly due to the specific status of cloud microorganisms compared to the other stable environmental ecosystems (waters, soil, plants, etc.). Cloud is a transient habitat lasting from a few hours to a few days as it is part of the life cycle of the microorganisms in the atmosphere. Microorganisms are aerosolized, transported in the air and deposited further or integrated in clouds by nucleation and scavenging processes and can be back to the earth by wet deposition using precipitation as shuttles (Fig. 10.1). During their travel they are exposed to very strong stresses, especially in cloud itself, which likely alter viability (see Sect. 10.1).

The sources of microorganisms are very wide including water, soil and vegetation; Burrows et al. (2009a) have evaluated that, globally, $\sim 10^{24}$ bacterial cells are aerosolized from surface environments each year. Large spatial and temporal heterogeneities exist in the distribution of microorganisms in the air, notably in relation with the type of surface cover (rural, urban, forest, ocean, etc.) and it varies temporally with seasonal and daily periodicities (Burrows et al. 2009a; Lighthart 1997). The mechanisms of aerosolization are still not completely understood. In the case of oceans, microorganisms are emitted by bubble bursting. Bubbles are produced at the surface of the sea by whitecaps and breaking waves and rainfalls; since microorganisms are concentrated at the water-air interface, their concentration within jet drops can be increased by several orders of magnitude compared with

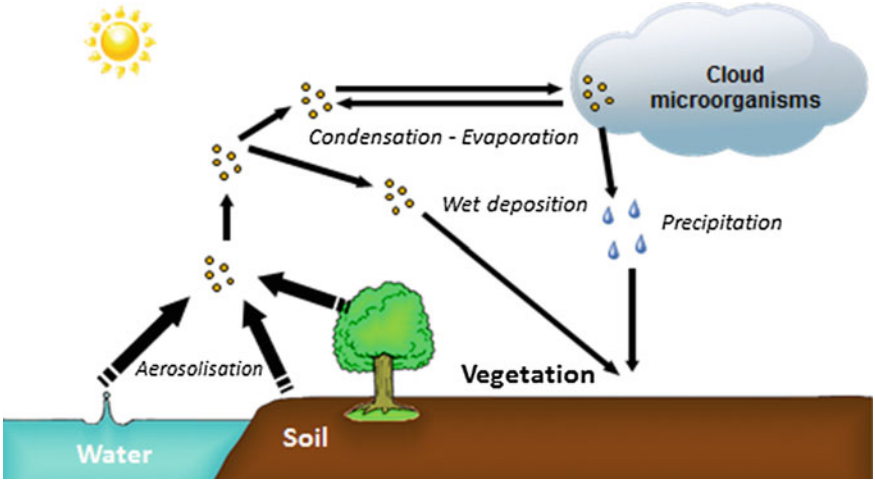


**Fig. 10.1** Clouds acts as a transient habitat during the life cycle of microorganisms. Microorganisms are aerosolized, transported *vertically* and *horizontally*, integrated in clouds by condensing water and shuttled down to the ground mainly by precipitation

their concentration in the bulk liquid (Blanchard 1989; Marks et al. 2001; Aller et al. 2005; Mayol et al. 2014). Microorganisms can be also lifted up in the air by the wind as part of solid aerosols, particularly on dust particles (Griffin 2007). Fungi can emit spores specially designed to be aerosolized by the wind depending on meteorological factors including wind speed, temperature, humidity, (Jones and Harrison 2004). Finally vegetation is a major source of bioaerosols; the microbial density on plant leaves is $\sim 10^3$–$10^8$ bacteria $g^{-1}$ (Lindow et al. 1978; Lindemann et al. 1982; Morris et al. 2008). In that case different mechanisms can be involved in aerosolization: microorganisms can be ejected by direct impact of rain drops on leaves or can be transported upward by turbulent flows (sensible heat flux); wind can lift dry leaves particles or dry biofilm fragments where bacteria are imbedded (Hirano and Upper 2000; Morris et al. 2004).

Measurement of emission fluxes is difficult and few data are available. It is supposed to vary largely with the type of sources: while Lindemann et al. (1982) have measured net upward fluxes in the range of 100–1000 CFU $m^{-2}$ $s^{-1}$ over agricultural areas, Burrows et al. (2009a, b) has evaluated emission rates as low as $\sim 1$ CFU $m^{-2}$ $s^{-1}$ from seas and glaciated ecosystems based on near-surface concentration measurements, however it should be noted that this evaluation is very rough and still questionable.

Once aloft, micron-sized particles like microorganisms are dispersed vertically and horizontally. They can go up in the atmosphere along the different layers (troposphere 0–12 km, stratosphere 12–50 km, and mesosphere 50–85 km). Although most studies have been performed in the troposphere, which is the major place where clouds form and exist; viable microorganisms have been collected up

to 77 km of altitude (Imshenetsky et al. 1978). Concerning horizontal dispersion microorganisms can be transported thousands of kilometers away from their emission source (Prospero et al. 2005; Kellogg and Griffin 2006; Smith et al. 2013), and eventually reach the most remote regions of the planet. For instance, Asian dust storms can take 7–9 days to cross the Pacific Ocean, while African dusts can reach the Caribbean and America within 3–5 days (Griffin 2007). The residence time of particles in the atmosphere depends on their size, large particles (>10 μm in diameter) can settle by dry deposition. For particles in the range of 0.1–10 μm the prominent process of deposition is wet: it requires the presence of condensed water. Finally small particles (from $10^{-4}$ to $10^{-1}$ μm) must aggregate within each other and reach a size large enough to be deposited (Renoux and Boulaud 1998). This means that the prominent manner to descend back to the surface for microorganisms, which are in the size range of 1 μm is wet deposition. The residence time of microorganisms in the atmosphere is thus largely dependent on meteorological conditions (dry vs. humid) and was modelled to be between 10 and 2 days, respectively (Burrows et al. 2009b). Very recently Amato et al. (2015) have measured the residence time of selected bacterial strains in a cloud simulated chamber (AIDA, Karlsruhe, Germany) which might improve the numerical models of bacterial dissemination in the future. The cultivability of airborne bacterial cells over time showed an exponentially decreasing function with a half-life time of about 3.5–4.5 h. In other words, considering the average residence time of 3.4 days of bacteria in the atmosphere estimated by models (Burrows et al. 2009b), our results indicate that the proportion of cells surviving aerial transport is only 1 cell out of $10^6$ cells aerosolized from the surface. The distance of transportation of bioaerosols depends on the wind speed and can be simulated to be between several hundreds to thousand kilometers.

As explained above, microorganisms can be deposited to the ground using precipitation as shuttles. However falling rain drops are largely inefficient in scavenging particles of this size, so microorganisms have to be first integrated in cloud droplets by nucleation or scavenging processes into clouds. First microorganisms themselves can act as CCN (Cloud Condensation Nuclei) offering a surface to condensation of water vapour; it is as a particular case of aerosol particles presenting some specific physico-chemical properties due to their biological nature (Sun and Ariya 2006). Furthermore, some specific microorganisms, notably some bacteria belonging to the genus *Pseudomonas*, can initiate the formation of ice at relatively warm temperatures (−2 to −12 °C) thanks to a surface protein (Möhler et al. 2007; Ariya et al. 2009; Hoose and Möhler 2012). Such biological Ice Nuclei Active (INA) bioaerosols are present in precipitation (Christner et al. 2008a, b; Stephanie and Waturangi 2011). More recently they have also been described in cloud waters (Joly et al. 2013, 2014). Although the role of microorganisms as CCN is general and admitted, simply due to their particle size, the quantitative implication of INA bacteria in forming clouds and precipitations is still controversial (DeMott and Prenni 2010; Hoose et al. 2010).

### 10.2.3 The Cloud Microbiome

Before being precipitated, cloud is thus a transient habitat where microorganisms can live and survive for a few hours to a few days. However, it remains a rather unexplored extreme environment.

First descriptions of microorganisms in cloud droplets refers to the works of Sattler et al. (2001) and Bauer et al. (2002), it was then largely completed by recent studies (Amato et al. 2005, 2007d; Vaïtilingom et al. 2012). The concentration of microbial cells in warm clouds in the free troposphere was evaluated by microscopy or flow cytometry, it is in the range of $10^2$–$10^5$ cells $mL^{-1}$ depending on the sampling site, see Table 10.2.

The biodiversity of the cloud microbiome is still rather unknown. This is because only a few studies have been made due to the difficulties in sampling clouds in sterile conditions suitable for microbial study. In addition, most of the studies were performed using culture methods which is known to cover between <1% (of the bacteria) to ~10% (of the fungi) of the total communities. Fuzzi et al. (1997) first reported the presence of cultivable strains of *Pseudomonas*, *Bacillus and Acinetobacter* in fog water sampled in the Pô valley; Ahern et al. (2007) isolated a number of *Pseudomonas* strains in Hebridean clouds. The largest description of cloud isolates has been done from cloud waters sampled at the puy de Dôme station (1465 m a.s.l.) (Vaïtilingom et al. 2012) a site internationally recognized for atmospheric research (Global Atmospheric Watch, GAW labelled). 185 heterotrophic bacteria and 150 yeasts were isolated from 32 cloud events and identified, including -Alpha, -Beta and Gamma-Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria, and Basidiomycetous and Ascomycetous yeasts. The results show that major genera dominate among cultivable cells (Fig. 10.2). Concerning bacteria, the most frequently encountered genera are *Pseudomonas* (γ-Proteobacteria), *Sphingomonas*

**Table 10.2** Microbial concentration in cloud waters

| Sites | Altitude (m.a.s.l.) | Number of sampled clouds | Bacterial concentration (cells $mL^{-1}$) | Fungal concentration (cells $mL^{-1}$) | This study |
|---|---|---|---|---|---|
| Puy de Dôme, France | 1465 | 34 | $3.3 \times 10^3$–$2.5 \times 10^5$ | $8.9 \times 10^2$–$3.2 \times 10^4$ | Amato et al. (2007c), Vaïtilingom et al. (2012) |
| Mont Rax, Austria | 1644 | 3 | $4.9 \times 10^4$–$8.1 \times 10^4$ | $5.9 \times 10^3$ | Bauer et al. (2002) |
| Mont Sonnblick, Austria | 3106 | 12 | $7.9 \times 10^2$–$2.5 \times 10^3$ | – | Sattler et al. (2001) |
| Aircraft sampling, Michigan, U. S.A | 2240–3320 | 5 | $9.2 \times 10^4$–$4.3 \times 10^5$ | – | Kourtev et al. (2011) |

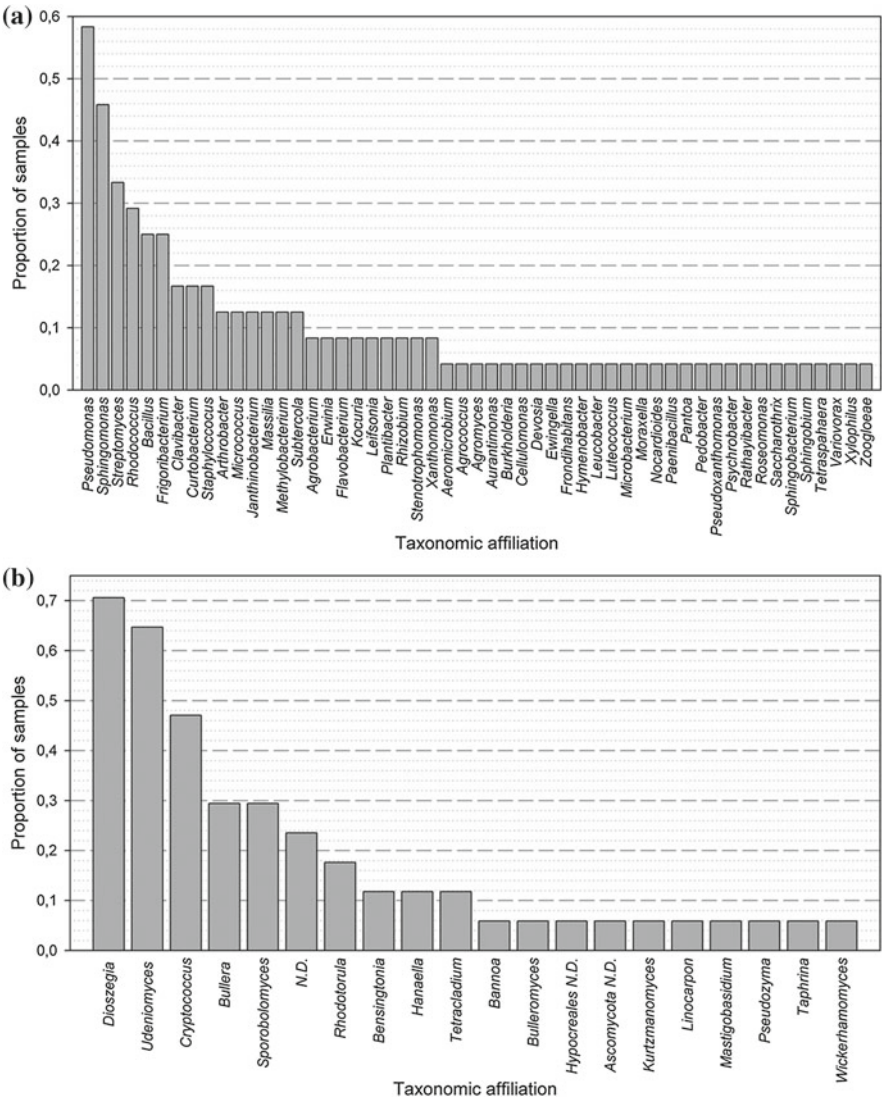


**Fig. 10.2** The frequency of cloud events collected at the puy de Dôme submit in which the different genera of bacteria (**a**) or yeasts (**b**) were detected viable by culture over the period 2003–2010 (adapted from Vaïtilingom et al. 2012)

(α-Proteobacteria), *Streptomyces* (Actinobacteria), *Rhodococcus* (Actinobacteria), and *Bacillus* (Firmicutes), while for yeasts *Dioszegia* (Basidimycota), *Udeniomyces* (Basidimycota) and *Crytococcus* (Basidimycota) are the most frequent ones.

Although DNA-based analyses of air samples have been recently reported (Bowers et al. 2009; Brodie et al. 2007; Fierer et al. 2008; Gandolfi et al. 2013;

Garcia et al. 2012; Smith et al. 2013; Zweifel et al. 2012; Maki et al. 2010, 2013; Fröhlich-Nowoisky et al. 2009; Bottos et al. 2014; Fahlgren et al. 2010; Jeon et al. 2011; Maron et al. 2005; Després et al. 2007), very few studies have focused on cloud communities. Up to very recently it was very difficult to apply DNA-based techniques to assess this biodiversity due to sample volume limitations and a very low microbial density; new molecular tools which are very sensitive should provide more information. Kourtev et al. (2011) showed a high genetic diversity in Lower peninsula of Michigan (USA) by Denaturing Gradient Gel Electrophoresis (DGGE); they found the same major bacterial phyla (Proteobacteria > Actinobacteria > Firmicutes) than in clouds collected at the puy de Dôme (Vaïtilingom et al. 2012), but the major difference was the prevalence of Cyanobacteria (around 50% of the sequences). Actually cyanobacteria were not identified using culture-based methods as there were not looked for. More recently Šantl-Temkiv et al. (2013a, b) described the microbial population in a storm cloud (hailstones); the major bacterial groups belonged to Actinobacteria, Bacteroidetes, gamma and alpha Proteobacteria phyla as described in warm clouds (Vaïtilingom et al. 2012), however the major genus of alpha Proteobacteria in hailstones was *Mycobacterium* instead of *Sphingomonas.* Using SSU rRNA gene sequence analysis of microorganisms living in cloud-free, cloudy and tropical storm environments of the upper troposphere (8–15 km altitude) also revealed the prevalence of Proteobacteria (alpha and beta Proteobacteria) (DeLeon-Rodriguez et al. 2013). Interestingly two of the core families of these different samples were the *Methylobacteriacae* and *Oxalobacteraceae*. The difficulty to compare the biodiversity described in all these studies lays in the fact that of course the techniques used are different (culture *versus* uncultured-based approaches) but also because the samplings were performed in different geographical sites or altitudes. The heterogeneity at low altitude can result directly from the sources influences as described by Burrows et al. (2009b). However when the whole atmosphere is considered, one could think microorganisms circulate freely around the earth, opposite to microorganisms present in other closed environment compartments such as soil, lakes and rivers. The question of the biogeography of the atmosphere is still a non-resolved question (Womack et al. 2010).

These studies show that although the cloud microbiome is diverse, some major groups are dominating; this could result of a selection of the type of microorganisms occurring during the aerosolization process or thanks to their survival abilities in clouds.

## 10.3 Microorganisms Are Surviving in Clouds

### *10.3.1 Microorganisms Are Metabolically Active in Clouds*

Physicists of the atmosphere had considered for a long time that microorganisms were just inert particles, however it has been discovered recently that at least a

fraction of the microorganisms are actually alive in clouds. Although a small fraction of cloud microorganisms can be cultivated, most of them were shown to be still viable. Sattler et al. (2001) measured the incorporation of [methyl-$^{3}$H]thymidine and [$^{14}$C]leucine demonstrating for the first time that bacteria could be maintained and grow in super-cooled cloud droplets. Amato et al. (2007a, b) showed the ability of microorganisms isolated from cloud to grow in cloud water incubated in laboratory. Looking at individual strains, their doubling times at 17 and 5 °C were in the range of 5–20 h depending on the strains. It is rather long compared to cloud life time, meaning that the multiplication of cells only happens during long cloud events, and probably not more than once in the cloud's lifetime. Cloud medium thus allows microorganisms to maintain a metabolic activity and develop by sustaining organic substrates to the cells, as suggested by Fuzzi et al. (1997). This metabolic activity was largely confirmed by assaying the ATP (Adenosine triphosphate) content directly in cloud water samples. The theoretical concentration of ATP in bacteria and fungi while taking into account their total number, was compared to those measured in clouds. The similar results suggest that most of the microorganisms were active (Amato et al. 2005). A long term survey of these in situ measurements at the puy de Dôme station showed that the ATP content is rather stable, independently from the season or the geographical origin of the air mass (Vaïtilingom et al. 2012). Hill et al. (2007) also demonstrated that 76% of the bacteria were alive in cloud waters as they were able to uptake the dye CTC (5-cyano-2,3-ditolyl tetrazolium chloride). The existence of metabolic activity in clouds implies the uptake of molecular compounds by cells as nutrients, and so of their contribution to cloud chemical reactivity.

A few studied have focused on the biodegradation of some of the cloud carbon sources by cloud microorganisms. The first type of compounds investigated were carboxylic acids which constitute one of the major classes of organic compounds in cloud waters (Deguillaume et al. 2014). The group of Ariya studied the degradation of series of di-carboxylic acids (malonic, oxalic, succinic, glutaric, adipic, pimelic and pinic) by airborne fungi (Ariya et al. 2002; Côté et al. 2008). They showed that these were all good substrates except oxalate. Other studies confirmed that formic, acetate, succinate and malonic were degraded by cloud bacteria but not oxalate (Amato et al. 2007a; Vaïtilingom 2011; Vaïtilingom et al. 2013). Experimental data concerning the non-degradability of oxalate are not consistent with the description of the oxalate-degrading bacteria (*Oxalobacteraceae*) described by DNA-based methods (DeLeon-Rodriguez et al. 2013). This could result from the fact that this genus is not active in real atmospheric conditions or that it is not present in cloud samples collected at the puy de Dôme contrarily to sample collected at high altitude over the Atlantic Ocean. Mono-carboxylic acids such as formate, acetate and lactate are also easily degraded by cloud microorganisms (Amato et al. 2005, 2007a; Vaïtilingom et al. 2010, 2011, 2013). Herlily et al. (1987) also showed some years ago that acetate and formate were biodegraded in rainwater. Between 2 and 9 carboxylic acids (lactate, acetate, glycolate, propionate, formate, glyoxylate, α-keto-glutarate, succinate, tartrate) could be also used by seven strains of

*Methylobacterium* and one strain of *Bradyrhizobium* isolated from hailstones (Šantl-Temkiv et al. 2013a).

C1 compounds such as methanol and formaldehyde also represent valuable carbon sources for cloud microorganisms; the metabolic routes can be variable according to the strains. Among the theoretical potential pathways the transformations of formaldehyde into methanol (reduction) and/or into formate and $CO_2$ have been identified in 60 cloud strains using $^{1}$H-NMR (Amato et al. 2007b). A more detailed study on a *Bacillus* strain by $^{13}$C NMR showed that $^{13}$C-formaldehyde was both reduced to methanol, oxidized to formate and $CO_2$ and also integrated in the serine metabolism leading to the production of glycerol, 1,2- and 1,3-propanediol (Husarova et al. 2011). The presence of facultative methyloptroph strains, belonging to the genus *Methylobacterium*, have been described as important in hailstones (Šantl-Temkiv et al. 2012). These strains were actively using methane under simulated cloud conditions, as demonstrated by enrichment techniques, and could thus represent a sink for atmospheric methane.

Finally, a recent paper reports the efficient degradation of sugars present in aerosols by a *Bacillus* strain isolated in cloud waters, as a model of cloud microorganisms (Matulová et al. 2014). These sugars have a biogenic origin; they include alditols (manitol, glucitol, arabitol), monosaccharides (arbinose, xylose, ribose, fructose, glucose, galactose, mannose, rhamnose), disaccharides (lactose, sucrose, maltose, tetrahose, cellobiose), oligosaccharides and polysaccharides (cellotetraose, cellulose, arabinogalactan, glucuronoxylan, inulin, starch). The degradation rates of theses sugars measured by $^{1}$H-NMR was in the same range of order than those measured for C1 compounds by the same strain.

Most of these experiments have been performed on simplified microcosms, starting from a single strain incubated with a single substrate (Ariya et al. 2002; Côté et al. 2008; Husarova et al. 2011; Amato et al. 2007a; Vaïtilingom et al. 2010; Matulová et al. 2014; Šantl-Temkiv et al. 2012), but more complex systems closer to cloud environment have been used including artificial cloud water tested with 17 representative strains incubated at temperatures relevant for clouds (5° and 17 °C) (Vaïtilingom et al. 2011) or real cloud waters containing the whole endogenous microflora (with or without solar light) (Vaïtilingom et al. 2013). To conclude cloud waters provide numbers of carbon sources to maintain a high microbial metabolic activity.

### *10.3.2 Microorganisms Can Resist to Atmospheric Stresses*

Cloud medium is clearly a harsh environment as fully explained in Sect. 1 both because it is constantly in evolution and thus under different micro-physical status and because cloud is a very active chemical reactor. However the fact that metabolically active microorganisms are present in clouds suggests that they have elaborated strategies to survive in clouds and resist to these stressing conditions.

## 10.4 Anti-stress General Features: Pigments, Spores, ExoPolymeric Substances (EPS)

First some general features characterize these microorganisms; they are related to stress protection. For instance about 50% of the cloud isolates are pigmented (yellow, red, orange) (Amato et al. 2005, 2007d; Vaïtilingom et al. 2012), as those recovered from permanently cold regions (Fong et al. 2001; Mueller et al. 2005; Dieser et al. 2010). These pigments are probably carotenoïds that could be incorporated in the microbial membrane and might have a double role: (i) they help maintaining membrane fluidity in the cold; (ii) they could also act as free radical scavengers (Gourmelon et al. 1994). Interestingly, these pigments are produced by bacteria exposed to UV light on plant leave surfaces, so these are "primed" for cloud conditions before being aerosolized. Some microorganisms resist against stresses being in the form of spores, it can be the case of fungal spores but also of some specific bacteria such as *Bacillus* species. Spore forming microorganisms have been found in large number in the atmosphere. *Bacillus* are widely present in the air (Maki et al. 2013; Šantl-Temkiv et al. 2012). Previous studies (Elbert et al. 2007; Heald and Spracklen 2009) estimated the fungal spore concentrations from $10^4$ to $10^6$ $m^{-3}$ of air with a huge spatial and temporal variability. Elbert et al. (2007) report an average of 35% of the total aerosol mass to be fungal spores in the Amazon region. Fungal spores contribute to 0.9% of the total OC (Organic Carbon) mass in the Austrian Alps and could reach up to 14% of the OC mass concentration in summer at a suburban site (Bauer et al. 2002, 2008). More recently, different groups report on-line detection of bio-aerosols and particularly of fungal spores. This detection is based on the analysis of the fluorescent properties of biological compounds (including NADH and tryptophan) [see Pan (2015) for review]. Two main types of instruments are used: Ultraviolet Aerosol Particle Sizer (UV-APS) and Waveband Integrated Bioaerosol Sensor (WIBS). Although the global trends measured by these techniques are in accordance with the already published values of bioaerosol concentrations, these approaches are still under development to be able to discriminate between fungal spores, bacteria and yeasts. Interesting papers have precisely compared these real-time fluorescent techniques with classical microscopy: Gabey et al. (2013) described some disparity between bacteria numbers and fluorescent particles concentration, while Healy et al. (2014) reported a good correlation between spore numbers and their fluorescent measurements; however they pointed the difficulty to detect *Cladosporium* spores because of their dark, highly absorptive cell-wall. Unfortunately these on-line techniques cannot be applied to analyse atmospheric water contents. Microscopy techniques have shown that yeast, fungi as well as spore forming bacteria such as *Bacillus* strains are present in cloud and fog waters (Fuzzi et al. 1997; Amato et al. 2007d; Vaïtilingom et al. 2012). Their concentration was higher when the water pHs were lower suggesting an adaption to this polluted media (Fuzzi et al. 1997; Amato et al. 2007d).

Finally another global protective strategy against numerous stresses relies on the formation of a biofilm or aggregates using the synthesis of ExoPolymeric Substances (EPSs). Actually the formation of biofilms seems to be the typical way for bacterial cells to grow in nature as it confers many ecological advantages (Davey and O'toole 2000; Flemming and Wingender 2010). This highly hydrated layer surrounding the cells can protect them against desiccation and UV exposure (Davey and O'toole 2000) or protect them in extreme marine habitats (Poli et al. 2010). Monier and Lindow (2003) showed a differential survival of solitary and aggregated bacterial cells on leaf surfaces, aggregates were increasing drastically bacterial survival when exposed to desiccation. This study was performed using *Pseudomonas syringae* as a model strain. It is worth noting that this bacterial species is one of the most abundant cultivable species found in clouds, these bacteria might be aerosolized in the atmosphere under aggregated forms and therefore particularly well adapted to survive to atmospheric stresses (Vaïtilingom et al. 2012). It is also likely that microorganisms which are transported on dust storms are under the form of biofilms. Tong and Lighthart (1998) reported that cell aggregation or association with particles increased survival during the day due to a shielding effect. Although bacterial EPS synthesis has been studied in many environments, only one paper is related to the atmospheric environment. Matulova et al. (2014) showed that a cloud bacterium (*Bacillus* sp. 3B6) could produce two types of EPSs from various saccharides (L-arabitol, D-fructose, sucrose, D-glucose, cellotetraose, cellulose, starch) present in the atmosphere. Their structures were identified as 1,6-α-galactan and partially acetylated polyethylene glycol (AcPEG) (Matulová et al. 2014).

## 10.5 Facing Specific Stresses: Oxidative, Osmotic and Temperature Stresses

Apart from these general strategies, microorganisms can modify specifically part of their metabolism to face specific stresses encountered in clouds.

One major problem for microorganisms is the presence of oxidants or their sources, including $^{\bullet}OH$ radicals, $H_2O_2$, iron, and solar light. Joly et al. (2015) have investigated the survival of 5 strains isolated from cloud waters towards 100 μM $H_2O_2$ in artificial cloud water; this concentration is realistic for $H_2O_2$ in cloud waters as it was previously detected at concentrations ranging from 0 to 247 μM (Table 10.1). The strains were chosen as models of strains collected at the puy de Dôme station representative of the cloud cultivable microbiome: 2 *Pseudomonas*, 1 *Sphingomonas* and 1 *Arthrobacter* among bacteria and 1 Basidiomycota yeast related to the genus *Dioszegia* (Vaïtilingom et al. 2012). The results showed that under these conditions close (or slightly overestimating) to what is found in clouds, i.e. 100 μM $H_2O_2$, the five strains were not affected (Table 10.3). Joly et al. (2015)

**Table 10.3** Survival rates observed for the experimental conditions the most relevant for clouds and proportion of cloud samples from which representatives of the corresponding genus were detected by culture

| | Survival rates to stresses | | | | Occurrence in clouds[a] (%) |
|---|---|---|---|---|---|
| Taxonomic affiliation and type of microorganism | $H_2O_2$ (90 min, 100 µM) (%) | Solar light (10 h) (%) | Osmotic shock (1 M NaCl) (%) | Freeze-thaw ($10^6$ cells $mL^{-1}$, per cycle) (%) | |
| *Dioszegia hungarica* | 84 | 37 | 56 | 80 | 71 |
| Yeast | | | | | |
| *Sphingomonas* sp. | 70 | 101 | 15 | 71 | 46 |
| Gram negative bacterium | | | | | |
| *Pseudomonas syringae* 32b-74 | 77 | 104 | 78 | 51 | 58 |
| Gram negative bacterium | | | | | |
| *Pseudomonas syringae* 13b-2 | 94 | 104 | 63 | 45 | |
| Gram negative bacterium | | | | | |
| *Arthrobacter* sp. | 129 | 96 | 101 | 16 | 13 |
| Gram positive bacterium | | | | | |

Adapted from Joly et al. (2015)

[a]Proportion of clouds samples from which representatives of the corresponding genus were detected by culture (data from Vaïtilingom et al. 2012)

also exposed the same strains to artificial solar light mimicking the natural light inside a cloud at the top of the puy de Dôme station (Joly et al. 2015) and demonstrated that the light available inside a cloud does not significantly impact bacterial viability (Table 10.3).

These previous experiments were performed under simplified conditions and using model strains. The results were confirmed using real cloud water samples incubated in a photo-bioreactor, specifically designed to reproduce cloud environment (Vaïtilingom et al. 2013). 3 cloud samples from independent cloud events were collected at the puy de Dôme station where they contained a very complex mixture of organic compounds and a whole biodiversity of the cloud microbiome. Each sample was divided into four parts: two parts were filtered through 0.22 µm porosity and while the two other remained intact, generating sterile or non-sterile samples, respectively. A sterile and a non-sterile subsamples were exposed to artificial solar light. At the same time, the other sterile and non-sterile subsamples were incubated in darkness. This experimental design allowed us to demonstrate:

- *abiotic reactions*: typically Fenton reactions (iron + $H_2O_2$) and photochemical reactions (iron + $H_2O_2$ + light); these reactions generate hydroxyl radicals from $H_2O_2$ source.
- *biotic + abiotic reactions:* iron + $H_2O_2$ + microorganisms with or without light. These conditions allow studying the interactions between these oxidants (including radicals) and cloud microorganisms. Biotic reactions could be inferred from the difference between the reactions observed in the 2 conditions.

ADP/ATP ratio which reflects the metabolic status of cloud microorganisms was monitored over time; no change was measured in the presence or the absence of light. This clearly means that microorganisms were not impacted by the generation of radicals and by solar light. In parallel it was shown that the endogenous cloud microflora was able to degrade $H_2O_2$ present in the cloud samples, suggesting the presence of very active catalases. In addition the degradation rates of $H_2O_2$ were not decreased in the presence of light, showing no inhibition of the biological process by light and radicals.

Solar light is actually composed of two types of highly energetic wavelengths (UV-C, ~190–290 nm, and UV-B, ~290–320 nm) that can potentially affect microorganisms mainly by causing DNA damage ($\lambda$ = 260 nm; see the review by Witkin (1976)). However UV-C radiations are mostly absorbed by stratospheric ozone, i.e. above the highest altitudes and thus have no effect on tropospheric clouds where microorganisms live. If microorganisms present in the air reach this stratospheric zone, they can be indeed damaged. This was shown by Smith et al. (2011) who exposed spores of *Bacillus subtilis* to a series of stratospheric simulations combining temperature, desiccation, pressure and UV light. UV light was the only stress to have an impact on *Bacillus* survival. For UV-B radiations, the deleterious effects are limited and can be balanced by repair mechanisms. Solar light is also composed of longer UV wavelengths (UV-A, ~320–400 nm) and visible light (~400–800 nm) which can indirectly alter cell viability by producing Reactive Oxygen Species (ROS) that include $^{\bullet}OH$ and $O_2^{\bullet -}$ radicals (Fig. 10.3). These extracellular radicals are produced mainly by direct photolysis of $H_2O_2$ or by photo-Fenton reactions (Fe + $H_2O_2$) and can diffuse into the cell across the cytoplasmic membrane. The same type of radicals can also be produced intracellularly when $O_2$ diffuses inside the cell during the respiration process of aerobic microorganisms. These radicals are extremely deleterious for cells as they can damage the major cellular components (Proteins, DNA, lipids, etc.) and induce cell death. Hopefully microorganisms are protected from these ROS with the assistance of various mechanisms involved in the oxidative stress metabolism [see Davey and O'toole (2000) for review]. First radicals can be scavenged by antioxidant molecules such as vitamins (ascorbic acid, α-tocopherol.), gluthatione, and pigments (carotenoïds) (Tong and Lighthart 1998; Dieser et al. 2010). As already stated the majority of atmospheric microorganisms is pigmented: 50% in clouds and 80% in the air (Amato et al. 2005; Fahlgren et al. 2010; Vaïtilingom et al. 2012; Zweifel et al. 2012). Another protection mechanism to fight the presence of ROS is based on the activity of specific enzymes of the oxidative metabolism (Fig. 10.3): Super

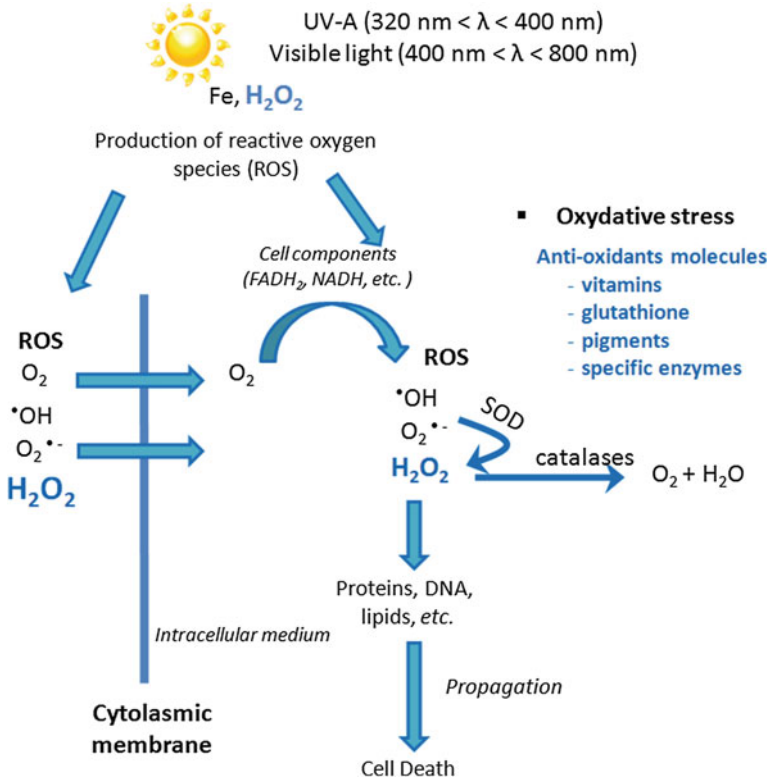


**Fig. 10.3** In clouds, microbial cells are exposed to reactive oxygen species (ROS) produced by the uptake of $O_2$ during respiration or by chemical reactions (photolysis of $H_2O_2$, Fenton or photo-Fenton reactions) occurring in cloud water. Cells react to this oxidative stress thanks to the production of vitamins, glutathione, pigments or specific enzymes

oxide dismutase (SOD) is able to transform $O_2^{•-}$ into $H_2O_2$ which again can be transformed in $O_2$ and $H_2O$ as non-toxic compounds by catalases (Vorob'eva 2004; Davies 2000). Catalases can also act directly on $H_2O_2$ which has been transferred in the cell from the extracellular medium (cloud water).

A second important stress is osmotic stress. Once in clouds, microorganisms are protected against desiccation by the presence of condensed water; however they are then exposed to rapid variations of osmolarity due to repeated condensation-evaporation cycles. In clouds, microorganisms are subject to osmotic variations when water condensates or evaporates (Fig. 10.4). Evaporation will induce an increase of the extracellular concentration and thus a hyper-osmotic shock, while the reverse happens during the condensation phase and creates a hyper-osmotic shock. To mimic this evaporation-condensation phenomenon, Joly et al. (2015) have exposed the 5 cloud microbial strains to a hyper-osmotic shock (1 M NaCl, realistic concentration for cloud conditions), followed by a hypo-osmotic shock, and they measured viability by culture (Table 10.3). The survival rates for the 2 *Pseudomonas*

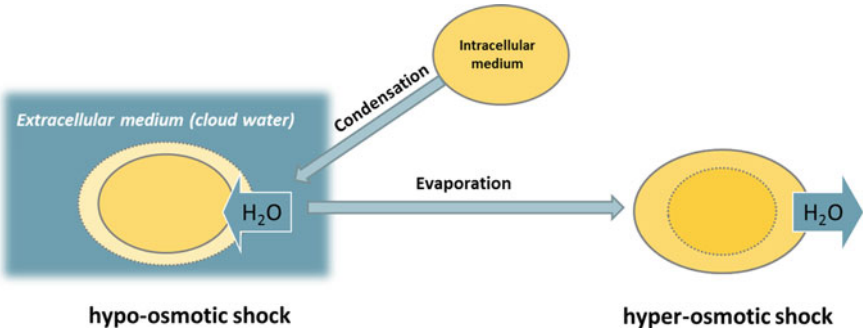


**Fig. 10.4** During the evaporation-condensation cycles of cloud droplets, microbial cells experience hyper-osmotic and hypo-osmotic shocks due to the change of solute concentrations surrounding the cells. Cells answer by water efflux or influx modifying the cellular volume

and the *Arthrobacte*r strains was quite high, that of the yeast *Dioszegia* was also important, showing that these microorganisms can resist to osmotic stress. However, the *Sphingomonas* strain was very impacted by such osmotic shock. Although these model strains might give a limited view of the whole cloud microbiome, this work suggests that the adaptation to osmotic stress is not a common feature to microbial cells living in clouds, and it is more likely strain dependent.

Microorganisms have developed different processes to fight against osmotic shocks (see *Csonka*, 1989 for review). When the extracellular concentration is increased (hyperosmotic shock), microorganisms have to equilibrate internal and external osmolarities by immediate efflux of water molecules (Fig. 10.4). This response leads to increased intracellular concentration of metabolites, decreased metabolic activity, and shrinkage of the cell. To reestablish influx of water, cells increase cytoplasm osmolarity by synthesizing or up-taking compounds known as "compatible solutes" or "osmoprotectants": potassium ions, amino acids (proline, glutamate…), sugars (trehalose, sucrose…) or peptides. A recent publication demonstrated the production of compatible solutes including betaine, ectoine, *N*-acetylglutaminylglutamine amide (NAGGN), and trehalose by a strain of *Pseudomopnas syringae* under osmotic stress conditions (Kurz et al. 2010). This is of interest as this species is one of the major ones encountered in clouds.

Further stress to be encountered by cloud microorganisms is temperature; this includes living in the cold, and enduring cold shocks as well as freeze-thaw cycles. Cloud temperature varies with the altitude, the latitude and the season. Although some microorganisms have been collected at 77 km of altitude where the temperature is extremely low, this review focuses on microorganisms in low altitude clouds called "warm clouds", which have been examined in more detail than the ones in high atmosphere. In such clouds collected at the puy de Dôme station in France at 1465 m a.s.l., the annual average temperature is around 5 °C but can reach 17 °C or more during the summer. It has been shown that a great part of the cloud microbial isolates are psychrophile or psychrotolerant and are clearly adapted for growth at the temperatures existing in warm clouds (Amato et al. 2007d; Vaïtilingom et al. 2012).

In addition, as explained earlier, more than 50% of these isolates are pigmented, they can also be embedded in biofilms or aggregates, all these factors protect them against the cold. This ability to grow and survive at low temperatures is actually not surprising as microorganisms have been found in many cold environments including snow and ice, particularly in polar environments (Carpenter et al. 2000; Foght et al. 2004; Dieser et al. 2010; Amato et al. 2007b; Junge et al. 2006; Groudieva et al. 2004; Christner et al. 2001, 2003; Price 2000). The specificity of the atmospheric medium is that, because it is a transient habitat, microorganisms have to adapt from a terrestrial environment to an atmospheric one, they have to cope with cold shocks. In our group, we have studied this cold shock impact on a model strain, *Pseudomonas syringae* 13b2 using metabolomics (unpublished data). Incubations of the strain at 17 and 5 °C were compared; key biomarkers were identified by Nuclear Magnetic Resonance and Mass Spectrometry. These markers indicate that at 5 °C, many metabolic changes occur to compensate the effects of low temperature. The first important feature is to re-establish membrane fluidity by changing membrane composition which allows the membrane functionality (Shivaji and Prakash 2010). Also we observed that cryo-protectants, in particular trehalose, carnitine, glutamate, glycine, and glycerol were produced, and the energetic state was boosted (increase of ATP) as well as the sulfur metabolism (including glutathione over production). This global answer of the strain shows that a single stress such as a cold shock can also induce responses common with other stresses like osmotic shocks (osmo-protectants are often similar to cryo-protectants) and oxidative stress (gluthatione). These crossed answers have been often reported in the literature (Mikami et al. 2002; Tanghe et al. 2003). The regulation systems to cold and osmotic stresses present many similarities (Suzuki et al. 2001; Mikami et al. 2002), and osmoprotectant compatible solutes such as trehalose, glycerol and saccharose also serve as cryoprotectants (De Antoni et al. 1989; Panoff et al. 2000). Tanghe et al. (2006) highlighted the importance of aquaporins, channel proteins devoted to the transport of water through the cell membrane, in the resistance to freezing. Many studies have reported a close relationship between cold stress and oxidative stress in bacteria. Indeed it was shown that the production of free radicals ($O_2^{\circ -}$ and °OH) and $H_2O_2$ is increased when the temperature is decreased (Zhang et al. 2012).

In addition to cold shocks, microorganisms can experience repeated freeze-thaw cycles due to the temperature gradient existing in the troposphere associated with rapid vertical winds. To test the resistance of cloud microorganisms to such a stress, Joly et al. (2015) have subjected the 5 model strains mentioned above to 6 consecutive freeze-thaw cycles (−40 °C/+5 °C) and measured their survival (Table 10.3). The resistance was highly strain dependent, ranging from high to very low survival rates (1–80% per freeze-thaw cycle). Interestingly when cell concentration was increased the survival rate was improved, this is consistent with the observed improved resistance of aggregated cells (Monier and Lindow 2003). Also the ice nucleation activity of the *Pseudomonas* strains did not seem to be a protection.

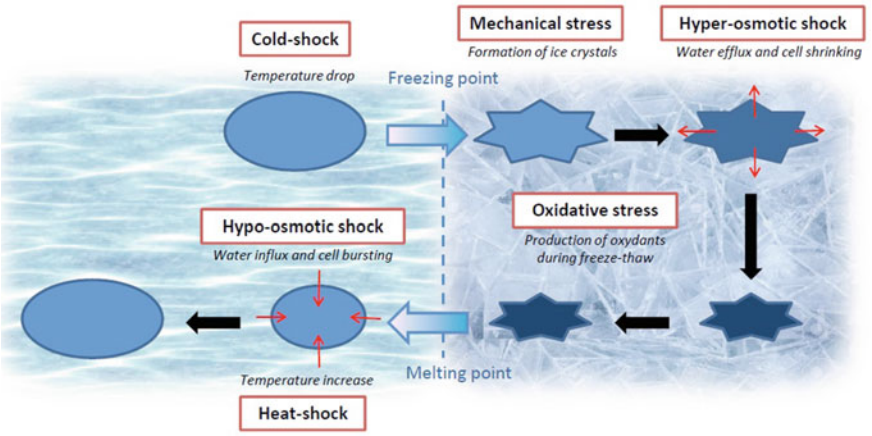


**Fig. 10.5** Freeze-thaw cycles: a combination of multiple stresses

Freeze-thaw cycle is actually a complex mechanism involving a combination of different stresses (Fig. 10.5). The first step is directly related to a cold shock, as the temperature decreases very quickly, leading to the formation of ice. The formation of ice crystals represents then a mechanical stress for the cells which is combined with an osmotic stress as the osmolites are concentrated in the extracellular medium (hyper-osmotic shock). Indeed the formation of ice results in a supersaline liquid network of microveins where microbial cells are trapped (Price 2000; Mader et al. 2006; Amato et al. 2009). It is also combined with a change of the cell shape and possible mechanical damages. When ice is thawing, again a temperature shock (heat shock) is experienced by the cells in addition to another osmotic stress as the solutes are diluted in the extracellular medium (hypo-osmotic shock). During freezing-thawing, ROS are produced, inducing thus an additional oxidative stress in cells (Stead and Park 2000). We can thus hypothesize that the cells will adapt by modifying their metabolism to face these different stresses. For counteracting the mechanical damages caused by freezing, microorganisms can produce antifreeze proteins (Duman and Olsen 1993). For the other stresses (temperature, osmotic, and oxidative), as shown above, common response pathways can be induced (Mikami et al. 2002; Tanghe et al. 2003). In some cases it has been shown that pre-exposition to one stress can eventually help the cell to resist to other stresses; for instance oxidative stress resistance was shown to be improved by pre-exposition to low doses of oxidants as the result of the induction of oxidative (Storz et al. 1990) and general stress response (Tanghe et al. 2003).

Because the mechanisms of stress resistance are so complex, it is no possible to just sum the impacts of the different individual stresses for estimating the chances of survival in clouds. However it can be noted that the survival rate of the tested microorganisms facing freeze-thaw cycles roughly matches, perhaps coincidentally, the frequency of cloud events collected at the puy de Dôme station in which the different genera of bacteria or yeasts were detected viable by culture (Table 10.3).

For instance the yeast *Dioszegia hungarica* which was almost insensitive to freeze-thaw cycles (survival rate of 80%) belongs to the yeast genus the most represented in cloud water samples (71%). The Actinobacterium *Arthrobacter* which highly suffered from repeated freeze-thaw (survival rate of 16%) was detected as alive much less frequently (13% of the clouds). The Proteobacteria *Pseudomonas syringae* and *Sphingomonas* sp. survived freeze-thaw cycles with a rate of 45–71% were the most represented bacterial genera, present in about 40–60% of the samples.

## 10.6 Conclusions and Perspectives

In conclusion we know now that metabolically active microorganisms are present in clouds which represent a transient habitat. They can maintain viability to different major stresses present in this harsh environment thanks to various general or specific strategies. The recurring presence of some groups of microorganisms in clouds can possibly result from two mechanisms: (i) these microorganisms are aerosolized from major specific sources on Earth's surface (ii) these microorganisms have elaborated strategies to improve their survival in clouds. Previous studies suggest that stress response is very often strain dependent; therefore it is difficult to make a general assessment correlating the occurrence of specific microorganisms in clouds with their stress resistance. However our work, although it is restricted to a limited number of model strains (Table 10.3) and thus is still speculative, tends to give some tendencies: the resistance to freeze-thaw cycles seems to be a major factor driving the final frequency of microbial strains in clouds.

Future work is needed to better describe and understand this unexplored world which is the cloud microbiome. Thanks to the development of new technologies such as devices allowing high sampling volumes and sensitive molecular tools, it is hoped that "omics" studies will be available soon. Metagenomics and metatranscriptomics could give new insights into the structure and function of cloud communities. metabolomics could also bring valuable information about the response and adaptation of cloud microorganisms to atmospheric stresses. Finally, more sampling site should be considered as it is to date mainly restricted to a few sites over the world. For that new collaborations and interdisciplinary teams between biologists and physicists of the atmosphere should be established.

## References

Ahern HE, Walsh KA, Hill TCJ, Moffett BF (2007) Fluorescent pseudomonads isolated from Hebridean cloud and rain water produce biosurfactants but do not cause ice nucleation. Biogeosciences 4(1):115–124

Aleksic N, Roy K, Sistla G, Dukett J, Houck N, Casson P (2009) Analysis of cloud and precipitation chemistry at whiteface mountain, NY. Atmos Environ 43(17):2709–2716. doi:10.1016/j.atmosenv.2009.02.053

Aller JY, Kuznetsova MR, Jahns CJ, Kemp PF (2005) The sea surface microlayer as a source of viral and bacterial enrichment in marine aerosols. J Aerosol Sci 36(5–6):801–812. doi:10.1016/j.jaerosci.2004.10.012

Amato P, Ménager M, Sancelme M, Laj P, Mailhot G, Delort A-M (2005) Microbial population in cloud water at the Puy de Dôme: implications for the chemistry of clouds. Atmos Environ 39 (22):4143–4153

Amato P, Demeer F, Melaouhi A, Fontanella S, Martin-Biesse AS, Sancelme M, Laj P, Delort AM (2007a) A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by micro-organisms. Atmos Chem Phys 7:4159–4169

Amato P, Hennebelle R, Magand O, Sancelme M, Delort AM, Barbante C, Boutron C, Ferrari C (2007b) Bacterial characterization of the snow cover at Spitzberg, Svalbard. FEMS Microbiol Ecol 59(2):255–264

Amato P, Parazols M, Sancelme M, Laj P, Mailhot G, Delort A-M (2007c) Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: major groups and growth abilities at low temperatures. FEMS Microbiol Ecol 59(2):242–254

Amato P, Parazols M, Sancelme M, Mailhot G, Laj P, Delort AM (2007d) An important oceanic source of micro-organisms for cloud water at the Puy de Dôme (France). Atmos Environ 41 (37):8253–8263

Amato P, Doyle S, Christner BC (2009) Macromolecular synthesis by yeasts under frozen conditions. Environ Microbiol 11(3):589–596. doi:10.1111/j.1462-2920.2008.01829.x

Amato P, Joly M, Schaupp C, Attard E, Möhler O, Morris CE, Brunet Y, Delort A-M (2015) Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber. Atmos Chem Phys 15(11):6455–6465. doi:10.5194/acp-15-6455-2015

Anastasio C, Faust BC, Allen JM (1994) Aqueous phase photochemical formation of hydrogen peroxide in authentic cloud waters. J Geophys Res Atmos 99(D4):8231–8248. doi:10.1029/94JD00085

Ariya PA, Nepotchatykh O, Ignatova O, Amyot M (2002) Microbiological degradation of atmospheric organic compounds. Geophys Res Lett 29(22):2077–2081

Ariya PA, Sun J, Eltouny NA, Hudson ED, Hayes CT, Kos G (2009) Physical and chemical characterization of bioaerosols—implications for nucleation processes. Int Rev Phys Chem 28 (1):1–32. doi:10.1080/01442350802597438

Bauer H, Kasper-Giebl A, Löflund M, Giebl H, Hitzenberger R, Zibuschka F, Puxbaum H (2002) The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. Atmos Res 64(1–4):109–119

Bauer H, Schueller E, Weinke G, Berger A, Hitzenberger R, Marr IL, Puxbaum H (2008) Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol. Atmos Environ 42(22):5542–5549. doi:10.1016/j.atmosenv.2008.03.019

Bianco A, Passananti M, Perroux H, Voyard G, Mouchel-Vallon C, Chaumerliac N, Mailhot G, Deguillaume L, Brigante M (2015) A better understanding of hydroxyl radical photochemical sources in cloud waters collected at the Puy de Dôme station—experimental versus modelled formation rates. Atmos Chem Phys 15(16):9191–9202. doi:10.5194/acp-15-9191-2015

Blanchard DC (1989) The ejection of drops from the sea and their enrichment with bacteria and other materials: a review. Estuaries Coasts 12(3):127–137

Bottos EM, Woo AC, Zawar-Reza P, Pointing SB, Cary SC (2014) Airborne bacterial populations above desert soils of the McMurdo Dry Valleys, Antarctica. Microb Ecol 67(1):120–128. doi:10.1007/s00248-013-0296-y

Bowers RM, Lauber CL, Wiedinmyer C, Hamady M, Hallar AG, Fall R, Knight R, Fierer N (2009) Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. Appl Environ Microbiol 75(15):5121–5130

Brodie EL, DeSantis TZ, Parker JPM, Zubietta IX, Piceno YM, Andersen GL (2007) Urban aerosols harbor diverse and dynamic bacterial populations. Proc Natl Acad Sci 104(1):299–304

Burrows SM, Butler T, Jöckel P, Tost H, Kerkweg A, Pöschl U, Lawrence MG (2009a) Bacteria in the global atmosphere—part 2: modelling of emissions and transport between different ecosystems. Atmos Chem Phys 9(3):10829–10881

Burrows SM, Elbert W, Lawrence MG, Pöschl U (2009b) Bacteria in the global atmosphere—part 1: review and synthesis of literature data for different ecosystems. Atmos Chem Phys 9(3):10777–10827

Carpenter EJ, Lin S, Capone DG (2000) Bacterial activity in South Pole snow. Appl Environ Microbiol 66(10):4514–4517

Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2001) Isolation of bacteria and 16S rDNAs from Lake Vostok accretion ice. Environ Microbiol 3(9):570–577

Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2003) Bacterial recovery from ancient glacial ice. Environ Microbiol 5(5):433–436. doi:10.1046/j.1462-2920.2003.00422.x

Christner BC, Morris CE, Foreman CM, Cai R, Sands DC (2008a) Ubiquity of biological ice nucleators in snowfall. Science 319(5867):1214

Christner BC, Cai R, Morris CE, McCarter KS, Foreman CM, Skidmore ML, Montross SN, Sands DC (2008b) Geographic, seasonal, and precipitation chemistry influence on the abundance and activity of biological ice nucleators in rain and snow. Proc Natl Acad Sci 105 (48):18854–18859

Collett JL Jr, Aaron Bator D, Sherman E, Moore KF, Hoag KJ, Demoz BB, Rao X, Reilly JE (2002) The chemical composition of fogs and intercepted clouds in the United States. Atmos Res 64(1–4):29–40

Côté V, Kos G, Mortazavi R, Ariya PA (2008) Microbial and 'de Novo' transformation of dicarboxylic acids by three airborne fungi. Sci Total Environ 390(2–3):530–537

Davey ME, O'toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64(4):847–67. doi:10.1128/MMBR.64.4.847-867.2000

Davies KJ (2000) Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50(4–5):279–289. doi:10.1080/713803728

De Antoni GL, Pérez P, Abraham A, Añón MC (1989) Trehalose, a cryoprotectant for Lactobacillus bulgaricus. Cryobiology 26(2):149–153. doi:10.1016/0011-2240(89)90045-X

Decesari S, Facchini MC, Matta E, Lettini F, Mircea M, Fuzzi S, Tagliavini E, Putaud JP (2001) Chemical features and seasonal variation of fine aerosol water-soluble organic compounds in the Po Valley, Italy. Atmos Environ 35(21):3691–3699

Deguillaume L, Charbouillot T, Joly M, Vaïtilingom M, Parazols M, Marinoni A, Amato P et al (2014) Classification of clouds sampled at the Puy de Dôme (France) based on 10 Yr of monitoring of their physicochemical properties. Atmos Chem Phys 14(3):1485–1506. doi:10.5194/acp-14-1485-2014

DeLeon-Rodriguez N, Lathem TL, Rodriguez-R LM, Barazesh JM, Anderson BE, Beyersdorf AJ, Ziemba LD, Bergin M, Nenes A, Konstantinidis KT (2013) Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. Proc Natl Acad Sci 110(7):2575–2580. doi:10.1073/pnas.1212089110

DeMott PJ, Prenni AJ (2010) New directions: need for defining the numbers and sources of biological aerosols acting as ice nuclei. Atmos Environ 44(15):1944–1945

Després VR, Nowoisky JF, Klose M, Conrad R, Andreae MO, Pöschl U (2007) Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. Biogeosciences 4(6):1127–1141

Deutsch F, Hoffmann P, Ortner HM (2001) Field experimental investigations on the Fe(II)- and Fe (III)-content in cloudwater samples. J Atmos Chem 40(1):87–105

Dieser M, Greenwood M, Foreman CM (2010) Carotenoid pigmentation in Antarctic heterotrophic bacteria as a strategy to withstand environmental stresses. Arct Antarct Alp Res 42(4):396–405. doi:10.1657/1938-4246-42.4.396

Duman JG, Olsen TM (1993) Thermal hysteresis protein activity in bacteria, fungi, and phylogenetically diverse plants. Cryobiology 30(3):322–328. doi:10.1006/cryo.1993.1031

Ekström S, Nozière B, Hultberg M, Alsberg T, Magnér J, Nilsson ED, Artaxo P (2010) A possible role of ground-based microorganisms on cloud formation in the atmosphere. Biogeosciences 7 (1):387–394. doi:10.5194/bg-7-387-2010

Elbert W, Taylor PE, Andreae MO, Pöschl U (2007) Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. Atmos Chem Phys 7(17):4569–4588

Erel Y, Pehkonen SO, Hoffmann MR (1993) Redox chemistry of iron in fog and stratus clouds. J Geophys Res 98(D10):18423–18434

Ervens B, George C, Williams JE, Buxton GV, Salmon GA, Bydder M, Wilkinson F et al (2003) CAPRAM 2.4 (MODAC mechanism): an extended and condensed tropospheric aqueous phase mechanism and its application. J Geophys Res 108(D14):4426. doi:10.1029/2002jd002202

Ervens B, Wang Y, Eagar J, Leaitch WR, Macdonald AM, Valsaraj KT, Herckes P (2013) Dissolved Organic Carbon (DOC) and select aldehydes in cloud and fog water: the role of the aqueous phase in impacting trace gas budgets. Atmos Chem Phys 13(10):5117–5135. doi:10.5194/acp-13-5117-2013

Ervens B, Renard P, Tlili S, Ravier S, Clément J-L, Monod A (2015) Aqueous-phase oligomerization of methyl vinyl ketone through photooxidation—part 2: development of the chemical mechanism and atmospheric implications. Atmos Chem Phys 15(16):9109–9127. doi:10.5194/acp-15-9109-2015

Fahlgren C, Hagström Å, Nilsson D, Zweifel UL (2010) Annual variations in the diversity, viability, and origin of airborne bacteria. Appl Environ Microbiol 76(9):3015–3025. doi:10.1128/AEM.02092-09

Fierer N, Liu Z, Rodriguez-Hernandez M, Knight R, Henn M, Hernandez MT (2008) Short-term temporal variability in airborne bacterial and fungal populations. Appl Environ Microbiol 74 (1):200–207

Flemming H-C, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8(9):623–633. doi:10.1038/nrmicro2415

Foght J, Aislabie J, Turner S, Brown CE, Ryburn J, Saul DJ, Lawson W (2004) Culturable bacteria in subglacial sediments and ice from two southern hemisphere glaciers. Microb Ecol 47 (4):329–340

Fong N, Burgess M, Barrow K, Glenn D (2001) carotenoid accumulation in the psychrotrophic bacterium 'Arthrobacter Agilis' in response to thermal and salt stress. Appl Microbiol Biotechnol 56(5–6):750–756. doi:10.1007/s002530100739

Fröhlich-Nowoisky J, Pickersgill DA, Després VR, Pöschl U (2009) High diversity of fungi in air particulate matter. Proc Natl Acad Sci 106(31):12814–12819. doi:10.1073/pnas.0811003106

Fuzzi S, Mandrioli P, Perfetto A (1997) Fog droplets—an atmospheric source of secondary biological aerosol particles. Atmos Environ 31(2):287–290

Gabey AM, Vaitilingom M, Freney E, Boulon J, Sellegri K, Gallagher MW, Crawford IP, Robinson NH, Stanley WR, Kaye PH (2013) Observations of fluorescent and biological aerosol at a high-altitude site in Central France. Atmos Chem Phys 13(15):7415–7428. doi:10.5194/acp-13-7415-2013

Gandolfi I, Bertolini V, Ambrosini R, Bestetti G, Franzetti A (2013) Unravelling the bacterial diversity in the atmosphere. Appl Microbiol Biotechnol 97(11):4727–4736. doi:10.1007/s00253-013-4901-2

Garcia E, Hill TCJ, Prenni AJ, DeMott PJ, Franc GD, Kreidenweis SM (2012) Biogenic ice nuclei in boundary layer air over two U.S. High Plains agricultural regions. J Geophys Res Atmos 117:D018209. doi:10.1029/2012JD018343

Gourmelon M, Cillard J, Pommepuy M (1994) Visible light damage to Escherichia Coli in seawater: oxidative stress hypothesis. J Appl Bacteriol 77(1):105–112

Griffin DW (2007) Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clin Microbiol Rev 20(3):459–477. doi:10.1128/CMR.00039-06

Groudieva T, Kambourova M, Yusef H, Royter M, Grote R, Trinks H, Antranikian G (2004) Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic Sea Ice, Spitzbergen. Extremophiles Life Under Extreme Conditions 8(6):475–88. doi:10.1007/s00792-004-0409-0

Heald, CL, Spracklen DV (2009) Atmospheric budget of primary biological aerosol particles from fungal spores. Geophys Res Lett 36(9). doi:10.1029/2009GL037493

Healy DA, Huffman JA, O'Connor DJ, Pöhlker C, Pöschl U, Sodeau JR (2014) Ambient measurements of biological aerosol particles near Killarney, Ireland: a comparison between real-time fluorescence and microscopy techniques. Atmos Chem Phys 14(15):8055–8069. doi:10.5194/acp-14-8055-2014

Herckes P, Valsaraj KT, Collett JL Jr (2013) A review of observations of organic matter in fogs and clouds: origin, processing and fate. Atmos Res 132–133:434–449. doi:10.1016/j.atmosres.2013.06.005

Herlihy LJ, Galloway JN, Mills AL (1987) Bacterial utilization of formic and acetic acid in rainwater. Atmos Environ 21(11):2397–2402

Herrmann H, Schaefer T, Tilgner A, Styler SA, Weller C, Teich M, Otto T (2015) Tropospheric aqueous-phase chemistry: kinetics, mechanisms, and its coupling to a changing gas phase. Chem Rev 115(10):4259–4334. doi:10.1021/cr500447k

Hill KA, Shepson PB, Galbavy ES, Anastasio C, Kourtev PS, Konopka A, Stirm BH (2007) Processing of atmospheric nitrogen by clouds above a forest environment. J Geophys Res Atmos 112(D11):D11301. doi:10.1029/2006JD008002

Hirano SS, Upper CD (2000) Bacteria in the Leaf Ecosystem with Emphasis on Pseudomonas syringae—a Pathogen, Ice Nucleus, and Epiphyte. Microbiol Mol Biol Rev 64(3):624–653. doi:10.1128/MMBR.64.3.624-653.2000

Hoose C, Möhler O (2012) Heterogeneous ice nucleation on atmospheric aerosols: a review of results from laboratory experiments. Atmos Chem Phys 12:9817–9854

Hoose C, Kristjánsson JE, Burrows SM (2010) How important is biological ice nucleation in clouds on a global scale? Environ Res Lett 5(2):024009

Houdier S, Barret M, Dominé F, Charbouillot T, Deguillaume L, Voisin D (2011) Sensitive determination of glyoxal, methylglyoxal and hydroxyacetaldehyde in environmental water samples by using dansylacetamidooxyamine derivatization and liquid chromatography/fluorescence. Anal Chim Acta 704(1–2):162–173

Husarova S, Vaitilingom M, Deguillaume L, Traikia M, Vinatier V, Sancelme M, Amato P, Matulova M, Delort A-M (2011) Biotransformation of methanol and formaldehyde by bacteria isolated from clouds. Comparison with radical chemistry. Atmos Environ 45(33):6093–6102. doi:10.1016/j.atmosenv.2011.06.035

Hutchings JW, Robinson MS, McIlwraith H, Kingston JT, Herckes P (2009) The chemistry of intercepted clouds in Northern Arizona during the North American Monsoon Season. Water Air Soil Pollut 199(1–4):191–202. doi:10.1007/s11270-008-9871-0

Igawa M, William Munger J, Hoffmann MR (1989) Analysis of aldehydes in cloud- and fogwater samples by HPLC with a postcolumn reaction detector. Environ Sci Technol 23(5):556–561

Imshenetsky AA, Lysenko SV, Kazakov GA (1978) Upper boundary of the biosphere. Appl Environ Microbiol 35(1):1–5

Jacob DJ, Waldman JM, William Munger J, Hoffmann MR (1984) A field investigation of physical and chemical mechanisms affecting pollutant concentrations in fog droplets. Tellus B 36B(4):272–285

Jeon EM, Kim HJ, Jung K, Kim JH, Kim MY, Kim YP, Ka J-O (2011) Impact of Asian dust events on airborne bacterial community assessed by molecular analyses. Atmos Environ 45(25):4313–4321. doi:10.1016/j.atmosenv.2010.11.054

Joly M, Attard E, Sancelme M, Deguillaume L, Guilbaud C, Morris CE, Amato P, Delort A-M (2013) Ice nucleation activity of bacteria isolated from cloud water. Atmos Environ 70:392–400

Joly M, Amato P, Deguillaume L, Monier M, Hoose C, Delort AM (2014) Quantification of ice nuclei active at near 0 °C temperatures in low-altitude clouds at the Puy de Dôme atmospheric station. Atmos Chem Phys 14(15):8185–8195. doi:10.5194/acp-14-8185-2014

Joly M, Amato P, Sancelme M, Vinatier V, Abrantes M, Deguillaume L, Delort A-M (2015) Survival of microbial isolates from clouds toward simulated atmospheric stress factors. Atmos Environ 117:92–98. doi:10.1016/j.atmosenv.2015.07.009 (September)

Jones AM, Harrison RM (2004) The effects of meteorological factors on atmospheric bioaerosol concentrations: a review. Sci Total Environ 326(1–3):151–180

Jr C, Jeffrey L, Daube BC, Jr DG, Hoffmann MR (1990) Intensive studies of Sierra Nevada cloudwater chemistry and its relationship to precursor aerosol and gas concentrations. Atmos Environ Part A Gen Top 24(7):1741–1757

Junge K, Eicken H, Swanson BD, Deming JW (2006) Bacterial incorporation of leucine into protein down to −20 °C with evidence for potential activity in sub-eutectic saline ice formations. Cryobiology 52(3):417–429

Kellogg CA, Griffin DW (2006) Aerobiology and the global transport of desert dust. Trends Ecol Evol 21(11):638–644

Köhler H (1936) The nucleus in and the growth of hygroscopic droplets. Trans Faraday Soc 32:1152–1161. doi:10.1039/TF9363201152

Kourtev PS, Hill KA, Shepson PB, Konopka A (2011) Atmospheric cloud water contains a diverse bacterial community. Atmos Environ 45(30):5399–5405

Kurz M, Burch AY, Seip B, Lindow SE, Gross H (2010) Genome-driven investigation of compatible solute biosynthesis pathways of pseudomonas syringae pv. syringae and their contribution to water stress tolerance. Appl Environ Microbiol 76(16):5452–5462. doi:10.1128/AEM.00686-10

LeClair JP, Collett JL, Mazzoleni LR (2012) Fragmentation analysis of water-soluble atmospheric organic matter using ultrahigh-resolution FT-ICR mass spectrometry. Environ Sci Technol 46 (8):4312–4322. doi:10.1021/es203509b

Lighthart B (1997) The ecology of bacteria in the alfresco atmosphere. FEMS Microbiol Ecol 23 (4):263–274

Lindemann J, Constantinidou HA, Barchet WR, Upper CD (1982) Plants as sources of airborne bacteria, including ice nucleation-active bacteria. Appl Environ Microbiol 44(5):1059–1063

Lindow SE, Arny DC, Upper CD (1978) Distribution of ice nucleation-active bacteria on plants in nature. Appl Environ Microbiol 36(6):831–838

Löflund M, Kasper-Giebl A, Schuster B, Giebl H, Hitzenberger R, Puxbaum H (2002) Formic, acetic, oxalic, malonic and succinic acid concentrations and their contribution to organic carbon in cloud water. Atmos Environ 36(9):1553–1558

Mader HM, Pettitt ME, Wadham JL, Wolff EW, John Parkes R (2006) Subsurface ice as a microbial habitat. Geology 34(3):169–172. doi:10.1130/G22096.1

Maki T, Susuki S, Kobayashi F, Kakikawa M, Tobo Y, Yamada M, Higashi T et al (2010) Phylogenetic analysis of atmospheric halotolerant bacterial communities at high altitude in an Asian Dust (KOSA) arrival region, Suzu City. Sci Total Environ 408(20):4556–4562. doi:10.1016/j.scitotenv.2010.04.002

Maki T, Kakikawa M, Kobayashi F, Yamada M, Matsuki A, Hasegawa H, Iwasaka Y (2013) Assessment of composition and origin of airborne bacteria in the free troposphere over Japan. Atmos Environ 74:73–82. doi:10.1016/j.atmosenv.2013.03.029

Marinoni A, Laj P, Sellegri K, Mailhot G (2004) Cloud chemistry at the Puy de Dôme: variability and relationships with environmental factors. Atmos Chem Phys 4(3):715–728

Marinoni A, Parazols M, Brigante M, Deguillaume L, Amato P, Delort A-M, Laj P, Mailhot G (2011) Hydrogen peroxide in natural cloud water: sources and photoreactivity. Atmos Res (In Press), Corrected Proof. http://www.sciencedirect.com/science/article/B6V95-528YX2G-1/2/b7d2b89d5b5564997e0d6a96a1c84635

Marks R, Kruczalak K, Jankowska K, Michalska M (2001) Bacteria and fungi in air over the Gulf of Gdansk and Baltic sea. J Aerosol Sci 32(2):237–250

Maron P-A, Lejon David PH, Carvalho E, Bizet K, Lemanceau P, Ranjard L, Mougel C (2005) Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16S rDNA clone library. Atmos Environ 39(20):3687–3695. doi:10.1016/j.atmosenv.2005.03.002

Matsumoto K, Kawai S, Igawa M (2005) Dominant factors controlling concentrations of aldehydes in rain, fog, dew water, and in the gas phase. Atmos Environ 39(38):7321–7329. doi:10.1016/j.atmosenv.2005.09.009

Matulová M, Husárová S, Capek P, Sancelme M, Delort A-M (2014) Biotransformation of various saccharides and production of exopolymeric substances by cloud-borne Bacillus sp. 3B6. Environ Sci Technol 48(24):14238–14247. doi:10.1021/es501350s

Mayol E, Jiménez MA, Herndl GJ, Duarte CM, Arrieta JM (2014) Resolving the abundance and air-sea fluxes of airborne microorganisms in the North Atlantic Ocean. Front Microbiol 5. doi:10.3389/fmicb.2014.00557

Mikami K, Kanesaki Yu, Suzuki I, Murata N (2002) The histidine kinase Hik33 perceives osmotic stress and cold stress in Synechocystis sp. PCC 6803. Mol Microbiol 46(4):905–915

Möhler O, DeMott PJ, Vali G, Levin Z (2007) Microbiology and atmospheric processes: The role of biological particles in cloud physics. Biogeosciences 4(6):1059–1071

Monier J-M, Lindow SE (2003) Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. Proc Natl Acad Sci 100(26):15977–15982. doi:10.1073/pnas.2436560100

Morris CE, Georgakopoulos DG, Sands DC (2004) Ice nucleation active bacteria and their potential role in precipitation. J Phys IV France 121:87–103

Morris CE, Sands DC, Vinatzer BA, Glaux C, Guilbaud C, Buffière A, Yan S, Dominguez H, Thompson BM (2008) The life history of the plant pathogen Pseudomonas syringae is linked to the water cycle. ISME J 2(3):321–334

Mueller DR, Vincent WF, Bonilla S, Laurion I (2005) Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. FEMS Microbiol Ecol 53(1):73–87. doi:10.1016/j.femsec.2004.11.001

Olszyna KJ, Meagher JF, Bailey EM (1988) Gas-phase, cloud and rain-water measurements of hydrogen peroxide at a high-elevation site. Atmos Environ (1967) 22(8):1699–1706

Padan E, Bibi E, Ito M, Krulwich TA (2005) Alkaline pH homeostasis in bacteria: new insights. Biochim Biophys Acta (BBA) Biomembr 1717(2):67–88. doi:10.1016/j.bbamem.2005.09.010

Pan Y-L (2015) Detection and characterization of biological and other organic-carbon aerosol particles in atmosphere using fluorescence. J Quant Spectrosc Radiat Transfer Topical Issue Opt Part Charact Remote Sens Atmos Part I 150:12–35. doi:10.1016/j.jqsrt.2014.06.007 (January)

Panoff J-M, Thammavongs B, Guéguen M (2000) Cryoprotectants lead to phenotypic adaptation to freeze–thaw stress in Lactobacillus delbrueckii ssp. bulgaricus CIP 101027T. Cryobiology 40(3):264–269. doi:10.1006/cryo.2000.2240

Parazols M, Marinoni A, Amato P, Abida O, Laj P, Mailhot G, Delort A-M, Sergio Z (2007) Speciation and role of iron in cloud droplets at the Puy de Dôme Station. J Atmos Chem 57 (3):299–300

Pehkonen SO, Erel Y, Hoffmann MR (1992) Simultaneous spectrophotometric measurement of iron(II) and iron(III) in atmospheric water. Environ Sci Technol 26(9):1731–1736

Poli A, Anzelmo G, Nicolaus B (2010) Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. Mar Drugs 8(6):1779–1802. doi:10.3390/md8061779

Price P Buford (2000) A habitat for psychrophiles in deep Antarctic ice. Proc Natl Acad Sci 97 (3):1247–1251. doi:10.1073/pnas.97.3.1247

Prospero JM, Blades E, Mathison G, Naidu R (2005) Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. Aerobiologia 21(1):1–19. doi:10.1007/s10453-004-5872-7

Pruppacher HR, Jaenicke R (1995) The processing of water vapor and aerosols by atmospheric clouds, a global estimate. Atmos Res 38(1–4):283–295

Renoux A, Boulaud D (1998) Les Aérosols: Physique et Métrologie. Lavoisier Technique & Documentation

Richards LW (1995) Airborne chemical measurements in nighttime stratus clouds in the Los Angeles Basin. Atmos Environ 29(1):27–46

Šantl-Temkiv T, Finster K, Hansen BM, Nielsen NW, Karlson UG (2012) The microbial diversity of a storm cloud as assessed by hailstones. FEMS Microbiol Ecol 81(3):684–695. doi:10.1111/j.1574-6941.2012.01402.x

Šantl-Temkiv T, Finster K, Dittmar T, Hansen BM, Thyrhaug R, Nielsen NW, Karlson UG (2013a) Hailstones: a window into the microbial and chemical inventory of a storm cloud. PLoS ONE 8(1):e53550. doi:10.1371/journal.pone.0053550

Šantl-Temkiv T, Finster K, Hansen BM, Pašić L, Karlson UG (2013b) Viable methanotrophic bacteria enriched from air and rain can oxidize methane at cloud-like conditions. Aerobiologia 29(3):373–384. doi:10.1007/s10453-013-9287-1

Sattler B, Puxbaum H, Psenner R (2001) Bacterial growth in supercooled cloud droplets. Geophys Res Lett 28(2):239–242

Sauer F, Schuster G, Schäfer C, Moortgat GK (1996) Determination of $H_2O_2$ and organic peroxides in cloud and rain water on the Kleiner Feldberg during FELDEX. Geophys Res Lett 23(19):2605–2608

Schleper C, Puehler G, Holz I, Gambacorta A, Janekovic D, Santarius U, Klenk HP, Zillig W (1995) Picrophilus Gen. Nov., Fam. Nov.: a novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. J Bacteriol 177 (24):7050–7059

Shivaji S, Prakash Jogadhenu S S (2010) How do bacteria sense and respond to low temperature? Arch Microbiol 192(2):85–95. doi:10.1007/s00203-009-0539-y

Smith DJ, Griffin DW, McPeters RD, Ward PD, Schuerger AC (2011) Microbial survival in the stratosphere and implications for global dispersal. Aerobiologia 27(4):319–332. doi:10.1007/s10453-011-9203-5

Smith DJ, Timonen HJ, Jaffe DA, Griffin DW, Birmele MN, Perry KD, Ward PD, Roberts MS (2013) Intercontinental dispersal of bacteria and archaea by transpacific winds. Appl Environ Microbiol 79(4):1134–1139. doi:10.1128/AEM.03029-12

Stead D, Park SF (2000) Roles of Fe superoxide dismutase and catalase in resistance of Campylobacter Coli to freeze-thaw stress. Appl Environ Microbiol 66(7):3110–3112

Stephanie, Waturangi DE (2011) Distribution of Ice Nucleation-Active (INA) bacteria from rain-water and air. HAYATI J Biosci 18(3):108–112

Storz G, Tartaglia LA, Farr SB, Ames BN (1990) Bacterial defenses against oxidative stress. Trends Genet 6:363–368. doi:10.1016/0168-9525(90)90278-E

Sun J, Ariya PA (2006) Atmospheric organic and bio-aerosols as Cloud Condensation Nuclei (CCN): a review. Atmos Environ 40(5):795–820

Suzuki I, Kanesaki Yu, Mikami K, Kanehisa M, Murata N (2001) Cold-regulated genes under control of the cold sensor Hik33 in Synechocystis. Mol Microbiol 40(1):235–244. doi:10.1046/j.1365-2958.2001.02379.x

Tanghe A, Van Dijck P, Thevelein JM (2003) Determinants of freeze tolerance in microorganisms, physiological importance, and biotechnological applications. Adv Appl Microbiol 53:129–176

Tanghe A, Van Dijck P, Thevelein JM (2006) Why do microorganisms have aquaporins? Trends Microbiol 14(2):78–85. doi:10.1016/j.tim.2005.12.001

Tilgner A, Herrmann H (2010) Radical-driven carbonyl-to-acid conversion and acid degradation in tropospheric aqueous systems studied by CAPRAM. Atmos Environ 44:5415–5422. doi:10.1016/j.atmosenv.2010.07.050

Tong Y, Lighthart B (1998) Effect of simulated solar radiation on mixed outdoor atmospheric bacterial populations. FEMS Microbiol Ecol 26(4):311–316. doi:10.1111/j.1574-6941.1998.tb00515.x

Vaïtilingom M (2011) Rôle Des Microorganismes Des Nuages Dans La Chimie Atmosphérique. Comparaison Avec La Chimie Radicalaire. Blaise Pascal, Clermont-Ferrand, France. http://tel.archives-ouvertes.fr/tel-00783928

Vaïtilingom M, Amato P, Sancelme M, Laj P, Leriche M, Delort A-M (2010) Contribution of microbial activity to carbon chemistry in clouds. Appl Environ Microbiol 76(1):23–29

Vaïtilingom M, Charbouillot T, Deguillaume L, Maisonobe R, Parazols M, Amato P, Sancelme M, Delort A-M (2011) Atmospheric chemistry of carboxylic acids: microbial implication versus photochemistry. Atmos Chem Phys 11:8721–8733

Vaïtilingom M, Attard E, Gaiani N, Sancelme M, Deguillaume L, Flossmann AI, Amato P, Delort A-M (2012) Long-term features of cloud microbiology at the Puy de Dôme (France). Atmos Environ 56:88–100. doi:10.1016/j.atmosenv.2012.03.072

Vaïtilingom M, Deguillaume L, Vinatier V, Sancelme M, Amato P, Chaumerliac N, Delort A-M (2013) Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. Proc Natl Acad Sci 110(2):559–564. doi:10.1073/pnas.1205743110

Valverde-Canossa J, Wieprecht W, Acker K, Moortgat GK (2005) $H_2O_2$ and organic peroxide measurements in an orographic cloud: the FEBUKO experiment. Atmos Environ 39(23–24):4279–4290

van Pinxteren D, Plewka A, Hofmann D, Müller K, Kramberger H, Svrcina B, Bächmann K et al (2005) Schmücke hill cap cloud and valley stations aerosol characterisation during FEBUKO (II): organic compounds. Atmos Environ 39(23–24):4305–4320

Vorob'eva LI (2004) Stressors, stress reactions, and survival of bacteria: a review. Appl Biochem Microbiol 40(3):217–224. doi:10.1023/B:ABIM.0000025941.11643.19

Wang Y, Guo J, Wang T, Ding A, Gao J, Zhou Y, Collett JL Jr, Wang W (2011) Influence of regional pollution and sandstorms on the chemical composition of cloud/fog at the summit of Mt. Taishan in Northern China. Atmos Res 99(3–4):434–442. doi:10.1016/j.atmosres.2010.11.010

Wang Y, Chiu C-A, Westerhoff P, Valsaraj KT, Herckes P (2013) Characterization of atmospheric organic matter using size-exclusion chromatography with inline organic carbon detection. Atmos Environ 68:326–332. doi:10.1016/j.atmosenv.2012.11.049

Watanabe K, Ishizaka Y, Takenaka C (2001) Chemical characteristics of cloud water over the Japan Sea and the Northwestern Pacific Ocean near the central part of Japan: airborne measurements. Atmos Environ 35(4):645–655

Weller C, Tilgner A, Bräuer P, Herrmann H (2014) Modeling the impact of iron-carboxylate photochemistry on radical budget and carboxylate degradation in cloud droplets and particles. Environ Sci Technol 48(10):5652–5659. doi:10.1021/es4056643

Witkin EM (1976) Ultraviolet mutagenesis and inducible DNA repair in *Escherichia Coli*. Bacteriol Rev 40(4):869–907

Womack AM, Bohannan Brendan J M, Green JL (2010) Biodiversity and biogeography of the atmosphere. Philos Trans R Soc B Biol Sci 365(1558):3645–3653. doi:10.1098/rstb.2010.0283

Zhang J, Li Y, Chen W, Guo-Cheng D, Chen J (2012) Glutathione improves the cold resistance of Lactobacillus Sanfranciscensis by physiological regulation. Food Microbiol 31(2):285–292. doi:10.1016/j.fm.2012.04.006

Zweifel UL, Hagström Å, Holmfeldt K, Thyrhaug R, Geels C, Frohn LM, Skjøth CA, Karlson UG (2012) High bacterial 16S rRNA gene diversity above the atmospheric boundary layer. Aerobiologia 28(4):481–498. doi:10.1007/s10453-012-9250-6