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Survival of microbial isolates from clouds toward simulated atmospheric stress factors

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HIGHLIGHTS

- The viability of microorganisms in clouds could be altered by environmental factors.
- Exposition to H_2O_2, solar light, osmotic shocks and freeze-thaw cycles were tested.
- Survival depended on the microorganism and on the stress.
- Oxidants and light had limited impacts on survival at levels relevant for clouds.
- Freeze-thaw appeared to be the most stringent factor.

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ABSTRACT

In the atmosphere, airborne microbial cells are exposed to conditions that are thought to affect their survival. Here, we investigated the survival of 5 microorganisms among the most represented in the cultivable community of clouds (4 bacteria affiliated to *Pseudomonas*, *Sphingomonas* and *Arthrobacter* and 1 yeast of *Dioszegia*) after exposition to different atmospheric factors generally considered stressful for cells: artificial solar light (10 h), oxidant (hydrogen peroxide: 0–1 mM for 90 min), osmotic shocks (0.1–2.5 M NaCl) and freeze-thaw cycles (6 cycles of 5 °C/-40 °C). Each condition was applied separately to cell suspensions, and survival rates were examined by culture. Survival was highly strain and stress dependent, with no relationship with pigmentation or ice nucleation activity. In all strains, solar light had no or mitigated influence, and exposition to H_2O_2 at the concentration measured in cloud water only slightly impacted viability (>70% of the cells survived). The strain *Sphingomonas* sp. was particularly impacted by osmotic shocks while repeated freeze-thaw was particularly damaging for *Arthrobacter* and *Pseudomonas* species. Overall, our results tend to indicate that in the atmosphere, the most stringent selection factors on living organisms are probably freeze-thaw and condensation/evaporation (osmotic shocks) cycles, whereas the impacts of oxidants and of solar light are limited.

1. Introduction

Airborne microorganisms are suspected to participate to numerous atmospheric physico-chemical processes: cloud droplet formation, ice nucleation, precipitation initiation and chemical compounds degradation in cloud water (*e.g.* see the reviews by Ariya and Amyot, 2004; Deguillaume et al., 2008; Delort et al., 2010; Després et al., 2012; Möhler et al., 2007). Yet, from the time when living bacteria and fungi are aerosolized from their source ecosystem, they have to cope with numerous stresses inflicted by brutal shifts of environmental conditions. In dry air, microbial cells endure solar radiation (ultraviolets (UVs) and visible light), oxidants and relatively low access to nutrient. In clouds, the presence

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of condensed water protects cells against desiccation, but subjects them to rapid variations of osmolarity due to repeated condensation–evaporation cycles: Pruppacher and Jaenicke (1995) estimated that atmospheric water condensates and evaporates in average 10 times before being removed by precipitation. Furthermore, cloud water contains a mixture of chemical compounds including strong acids, potentially toxic molecules (formaldehyde for example), and strong oxidants like hydrogen peroxide or radicals (Deguillaume et al., 2014; Hill et al., 2007). Microorganisms also have to respond to drops in temperature associated with vertical movements, and ultimately to freezing and thawing.

Despite these challenging conditions, a significant fraction of the microorganisms in clouds remains alive and metabolically active (Amato et al., 2007b; Bauer et al., 2002; Hill et al., 2007; Vaïtilingom et al., 2013). Hence, it is probable that cells have efficient defense mechanisms to resist in the atmospheric environment.

Long-term microbiological studies of cloud water at the puy de Dôme station (France, 1465 a.s.l.) revealed the presence of recurring microorganisms among the cultivable microflora: bacteria belonging to *Pseudomonas* and *Sphingomonas* genera were detected in 58% and 46%, respectively, of the samples collected between 2007 and 2010, and the yeast community was dominated by *Dioszegia*, *Udeniomyces* and *Cryptococcus* genera (71%, 65% and 47% of the samples, respectively) (Vaïtilingom et al., 2012). Interestingly, phylogenetic analyses of the housekeeping genes in *Pseudomonas syringae* strains highlighted the under-representation in clouds of clades common elsewhere in the environment, to the benefit of rarer groups (Joly et al., 2013). Such unusual prevalence could result from a selection process occurring in the atmosphere.

Here, we investigated the survival rates of selected microbial strains isolated from cloud water to stress factors likely encountered in the atmosphere: presence of oxidants (H_2O_2), exposition to solar light, osmotic shocks and freeze-thaw cycles. The levels of stress applied to cells ranged from realistic to unrealistically high values for the atmospheric environment in order to determine the tolerance limits of these microorganisms. The results are discussed in regard to the possible means of resistance deployed by cells, and to current knowledge about the microorganisms living in the atmosphere.

2. Material and methods

2.1. Microbial strains

Five microbial strains were selected as model microorganisms from our bank of cloud water isolates collected at the puy de Dôme atmospheric station (1465 m a.s.l.): one yellow-pigmented Actinobacterium (*Arthrobacter* sp. 5b-11), two ice nucleation (IN) active Gamma-Proteobacteria (*Pseudomonas syringae* 13b-2 and *P. syringae* 32b-74), one orange-pigmented Alpha-Proteobacterium (*Sphingomonas* sp. 32b-11) and one pink-pigmented Basidiomycota yeast (*Dioszegia hungarica* 24b-2) (Amato et al., 2007a; Joly et al., 2013; Vaïtilingom et al., 2012) (Table 1). These strains were selected for their belonging to genera frequently cultivated from clouds (Vaïtilingom et al., 2012), for their taxonomic diversity (Gram-positive and Gram-negative bacteria and a yeast) and for having phenotypes potentially involved in environmental stress tolerance (pigmentation and IN activity) (Table 1). The 5 strains are able to sustain growth from 5 °C to 27 °C (data not shown). Since less than ca. 1% of the microorganisms present in clouds are cultivable (e.g. Vaïtilingom et al., 2012), it is possible that these strains reflect only the viable fraction but not the community in its entirety.

2.2. Culture conditions

For all experiments, pure cultures were grown aerobically in liquid R2A medium (Reasoner and Geldreich, 1985) at 17 °C, 200 rpm shaking, until exponential growth phase was reached. Cells were then harvested by centrifugation (3 min; 12 000 × g) and pellets were rinsed three times with the incubation medium of the corresponding experiment. Survival rates were determined by plate counts on R2A agar, after incubation for 2–4 days at 17 °C. Three independent replicates were conducted for each condition and strain.

2.3. Oxidative stress

Cells were suspended at a concentration of ~10^6 cells mL^{-1} based on OD_{600nm} in 5 mL of artificial continental cloud water (see Vaïtilingom et al., 2011, Table 1, for chemical composition) supplemented with 0, 0.1, 0.25, 0.5 or 1 mM hydrogen peroxide (final concentration). Cultivable cell concentrations were determined at the beginning of the experiment and after a 90 min-exposure at 20 °C in the dark.

2.4. Solar radiation

Cells were suspended at a concentration of ~10^6 cells mL^{-1} in 30 mL of sterile artificial continental cloud water and placed in self-designed photobioreactors (Vaïtilingom et al., 2013). These suspensions were incubated at 5 °C under agitation (200 rpm) and exposed to artificial solar light (Repti Glo 5.0, EXOTERRA®). The lamp spectrum was measured using an optical fiber coupled with a CCD spectrophotometer (Ocean Optics USB2000 þ UVeVIS) previously calibrated with a reference lamp (DH-2000-CAL, Ocean Optics). Lamp spectrum is presented in Figure S1, compared to a typical solar light spectrum measured in a cloud at the puy de Dôme summit in autumn. UV and visible lights were emitted by the lamp, at precise wavelengths with total light intensities in the UV-B and UV-C closely matching real situation (~1 W m^{-2}), whereas UV-A and visible light intensities were lower by about an order of magnitude (~25 W m^{-2}; versus ~150 W m^{-2}). Experimental controls consisted of cell suspensions incubated in the dark under similar conditions. The survival rates were determined every 2 h for a total duration of 10 h.

2.5. Osmotic shocks

Cells were exposed to consecutive osmotic shocks to simulate condensation–evaporation cycles of droplets in clouds. Cells from cultures were harvested by centrifugation and washed three consecutive times in 0.1 M NaCl. Cells were finally suspended at concentrations of ~10^6 cells mL^{-1} in 0.1 mL of 0.1 M, 0.5 M, 1 M or 2.5 M NaCl solution to cause a range of hyper-osmotic shocks, and incubated at 5 °C for 30 min. The concentration of NaCl was then adjusted back to 0.1 M by addition of sterile deionized water (hypo-osmotic shock), and cell suspensions were incubated for another 30 min at 5 °C. Survival rates to these treatments were determined by plating the cell suspensions on R2A before and after the cycle of hyper-hypo-osmotic shock.

In parallel, the minimum growth inhibitory concentration (MIC) of NaCl was measured for each strain in 5 mL of liquid R2A medium supplemented with NaCl at concentrations ranging from 0 to 2.5 M, with 0.1 M intervals. The presence or absence of growth was controlled visually after three weeks of incubation at 17 °C.