



Survival and ice nucleation activity of bacteria as aerosols

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This discussion paper is/has been under review for the journal Atmospheric Chemistry and Physics (ACP). Please refer to the corresponding final paper in ACP if available.

Survival and ice nucleation activity of bacteria as aerosols in a cloud simulation chamber

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Received: 23 December 2014 – Accepted: 27 January 2015 – Published: 12 February 2015

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Published by Copernicus Publications on behalf of the European Geosciences Union.

ACPD

15, 4055–4082, 2015

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Abstract

The residence time of bacterial cells in the atmosphere is predictable by numerical models. However, estimations of their aerial dispersion as living entities are limited by lacks of information concerning survival rates and behavior in relation to atmospheric water. Here we investigate the viability and ice nucleation (IN) activity of typical atmospheric ice nucleation active bacteria (*Pseudomonas syringae* and *P. fluorescens*) when airborne in a cloud simulation chamber (AIDA, Karlsruhe, Germany). Cell suspensions were sprayed into the chamber and aerosol samples were collected by impingement at designated times over a total duration of up to 18 h, and at some occasions after dissipation of a cloud formed by depressurization. Aerosol concentration was monitored simultaneously by online instruments. The cultivability of airborne cells decreased exponentially over time with a half-life time of 250 ± 30 min (about 3.5 to 4.5 h). In contrast, IN activity remained unchanged for several hours after aerosolization, demonstrating that IN activity was maintained after cell death. Interestingly, the relative abundance of IN active cells still airborne in the chamber was strongly decreased after cloud formation and dissipation. This illustrates the preferential precipitation of IN active cells by wet processes. Our results indicate that from 10^6 cells aerosolized from a surface, one would survive the average duration of its atmospheric journey estimated at 3.4 days. Statistically, this corresponds to the emission of 1 cell that achieves dissemination every ~ 33 min perm² of cultivated crops fields, a strong source of airborne bacteria. Based on the observed survival rates, depending on wind speed, the trajectory endpoint could be situated several hundreds to thousands of kilometers from the emission source. These results should improve the representation of the aerial dissemination of bacteria in numeric models.

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1 Introduction

Microorganisms are known to be dispersed into the atmosphere and disseminated over long distances (e.g., Bovallius et al., 1978; Brodie et al., 2007; Griffin et al., 2001; Smith et al., 2013, and review by Morris et al., 2014b). This has obvious implications for Human, animal and plant epidemiology as well as microbial ecology (Monteil et al., 2014; Morris et al., 2007, 2008; Šantl-Temkiv et al., 2013). Moreover, some particular bacteria notably found in the atmosphere and clouds can induce heterogeneous ice formation (Cochet and Widehem, 2000; Joly et al., 2013; Lindemann et al., 1982), which probably affects cloud physics and potentially triggers precipitation (Möhler et al., 2007). All these aspects motivated the development of numerical models intended to describe and predict the aerial dispersion of microorganisms. For instance, Burrows et al. (2009a, b) constrained a general atmospheric circulation model using data from the literature and estimates of concentrations and vertical fluxes of airborne microorganisms. They estimated that $\sim 10^{24}$ bacteria are emitted into the atmosphere each year at the global scale, with a residence time aloft between 2 and 10 days (~ 3 days on average) depending on emission sources and on meteorological conditions. Such a time span should allow microbial cells (i.e. particles of $\sim 1 \mu\text{m}$) to travel over hundreds or thousands of kilometers. However, it is not clear what fraction of the microorganisms aerosolized survive over this time scale, and if they maintain properties pertinent with interactions with atmospheric water.

Most studies aiming at predicting the death rate of airborne bacteria were carried out in the late 1960's and early 70's, with particular emphasis on the influence of temperature and relative humidity (Cox and Goldberg, 1972; Ehrlich et al., 1970; Lighthart, 1973; Wright et al., 1969). The ability of bacteria to survive as aerosols and the influence of abiotic parameters on survival were shown to strongly depend on the microorganism (Marthi et al., 1990). In experiments at constant temperature ranging from -18 to 49°C , the survival rate of *Mycoplasma pneumoniae*, *Serratia marcescens* and *Escherichia coli* decreased with increasing temperature, while this had no or little effect

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on the survival of *Bacillus subtilis* (Ehrlich et al., 1970; Wright et al., 1969). The highest survival rates were invariably observed at extreme low and high levels of humidity (Cox and Goldberg, 1972; Wright et al., 1969). Finally, carbon monoxide concentration was shown to have variable impacts on the viability of airborne bacteria, with protective or deleterious effects depending on humidity and on the species (Lighthart, 1973, p. 73). Lighthart (1989) compiled these data and others to build statistical models describing the death rate of airborne bacteria based on aerosol age, temperature, Gram reaction and humidity. Survival rate was resolved by aerosol age, i.e. time after aerosolization, at more than 90 %.

In a scientific context motivated by interrogations about cloud-microbes interactions, we studied bacteria originating from atmospheric samples and selected for their relevance to atmospheric questions, *Pseudomonas syringae* and *P. fluorescens*. Indeed, these bacteria are among the most frequent species recovered from natural clouds (Vähtilingom et al., 2012), some strains are known plant pathogens (Berge et al., 2014) and some, including those investigated here, are ice nucleation (IN) active and have potential impacts on cloud microphysics and precipitation (e.g., Attard et al., 2012; Cochet and Widehem, 2000; Joly et al., 2013; Möhler et al., 2007; Sands et al., 1982). IN active bacteria were shown earlier to induce the formation of ice crystals within simulated clouds (Maki and Willoughby, 1978; Möhler et al., 2008). Here we aimed at examining the survival and IN activity of such typical bacterial aerosols in the atmosphere, using the AIDA cloud chamber (Aerosol Interactions and Dynamics in the Atmosphere). Cell suspensions were sprayed in the chamber and the concentrations of airborne micron-sized particles, total and cultivable cells and IN particles were measured over time for up to several hours after aerosolization. The influence of cloud formation and the presence of sulfates were briefly approached and seemed to deeply alter cell survival and IN activity. The data presented could be used for improving the parameterization of numeric models describing the atmospheric dispersion of bacteria.

2 Material and methods

2.1 Experimental setup and particle concentration measurements

The AIDA (Aerosol Interaction and Dynamics in the Atmosphere) 84 m³ chamber at the Karlsruhe Institute of Technology was used in this study both as a static aerosol chamber in order to store and age the bacterial cell aerosols for up to 18 h, and as an expansion cloud chamber in order to simulate cloud activation events and its effect on the viability of bacterial cells. The experiments were conducted during the BIO06 campaign in May 2011. For the ageing experiments at constant atmospheric pressure, the cell suspensions were sprayed into the aerosol and cloud chamber at the beginning of the experiment. Inside the chamber, the spray droplet water quickly evaporated and the bacterial cells were thus released into a dry aerosol state where they just floated around for up to 18 h at the given pressure, temperature and relative humidity, which was around 90 to 95 % with respect to ice in all ageing experiments. Three ageing experiments were performed at constant temperature, in one experiment the temperature was slightly reduced with time (see Table 1).

Aerosol concentration and size in the chamber were monitored using the combination of a Scanning Mobility Particle Spectrometer (SMPS) and an Aerodynamic Particle Sizer (APS), both from TSI Incorporated, USA. The concentration of particles in the size mode around 0.6 µm to about 5 µm is referred to here as Cells_{APS}; it corresponds to single intact bacterial cells and small agglomerates of cells.

Several aerosol samples were taken during these experiments in order to measure the number concentrations of total cells, and the cultivable cell number fraction. During most experiments, only one sample was taken 30 min after spray generation of the aerosol. During three experiments, a second sample was taken after a cloud activation and evaporation cycle in the AIDA chamber. Such a cycle is initiated by reducing the chamber pressure within a few minutes from about 1000 to 800 hPa by strong pumping. This pressure change simulates the conditions of an air parcel rising in the atmosphere at a vertical updraft velocity of up to a few m s⁻¹, which induces a respective cooling of

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the air and an increase of the relative humidity. The expansion run starts at a relative humidity of about 90 to 95 % with respect to ice, so that at start temperatures below 0 °C the air in the cloud chamber first exceeds saturation with respect to the ice phase and then saturation with respect to the supercooled liquid droplet phase. Depending on the temperature and the ice nucleation activity of the bacterial cells, some ice particles may already be formed in the regime between ice and water saturation. In all experiments discussed here, water saturation was exceeded so that all bacterial cells acted cloud condensation nuclei and by that were immersed in supercooled cloud droplets for a few minutes. After the pumping stopped at a pressure of about 800 hPa, the temperature started to increase due to heat flow from the warmer chamber walls, and the cloud droplets started to evaporate. After full evaporation of the cloud droplets, the chamber was filled with particle free synthetic air to atmospheric pressure and the second cell sample was taken from the chamber.

2.2 Bacterial strains and preparation of cell suspensions

The following bacterial strains were used: *Pseudomonas syringae* 13b-2 and *P. syringae* 32b-74, both isolated from cloud water samples collected from the puy de Dôme Mountain in France (GenBank accession numbers of the 16S rRNA gene sequences: DQ512785 and HQ256872, respectively, Amato et al., 2007; Vařilingom et al., 2012), and *P. fluorescens* CGina-01 isolated from Cotton Glacier in Antarctica (GenBank accession number FJ152549, Foreman et al., 2013). These were all previously demonstrated to be IN active by droplet-freezing assays (Attard et al., 2012; Joly et al., 2013). *P. syringae* 32b-74 in suspension in deionized water at the concentration of $\sim 10^9$ cells mL⁻¹ nucleated ice at -3 °C; the frequency of ice nucleating particle (INP) per cell was > 2 % at -4 °C and > 4 % at -6 °C, which ranks this strain among the most efficient IN active bacteria described so far. The onset freezing temperature of *P. fluorescens* CGina-01 at similar cell concentration was -4 °C, with an INP per cell frequency 3 to 4 orders of magnitude lower than that of 32b-74. *P. syringae* 13b-2 nucleated ice at -4 to -5 °C, with a much lower activity ($\sim 10^{-7}$ INP per cell at -6 °C).

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Bacteria from stock suspensions were grown on King's medium B agar (King et al., 1954) for two days at ambient room temperature (i.e. 22–25 °C). Cells were then scrapped off agar using sterile plastic loops, suspended in sterile deionized water at a concentration of approximately $\sim 10^9 \text{ mL}^{-1}$, and incubated overnight at 4 °C. In each experiment, a volume of $\sim 50 \text{ mL}$ of the cell suspension was sprayed into the cloud simulation chamber (for details see Möhler et al., 2008). The actual cell concentration in the initial suspensions was determined later by flow cytometry (total cells) and standard dilution plating (colony forming units; CFU), as described. These were used for inferring the initial concentrations of total and cultivable cells airborne in the AIDA chamber, considering a volume of 84 m^3 ; these are referred to as $\text{Cells}_{\text{SUSP}}$ and CFU_{SUSP} , respectively.

In one of the experiments, cell suspension was prepared in a solution of $(\text{NH}_4)_2\text{SO}_4$ (50 g L^{-1} , or 0.38 M) in order to examine the influence of sulfate coating.

2.3 Sampling from the cloud simulation chamber for microbiological analyses

Sampling for microbiological analyses was performed using an ethanol-washed impinger (SKC Biosampler; Lin et al., 1999) rinsed several times with sterile deionized water and filled with $\sim 20 \text{ mL}$ of sterile deionized water just prior to use. The water used as the impingement liquid served as a negative control for ice nucleation assays and cell counts. In those controls, no ice nucleation event was detected within the temperature range investigated, and cell count was $< 0.005\%$ of the cell counts in samples. Sampling operations were performed at a constant air flow of 12.5 L min^{-1} for 10 min periods using a membrane vacuum pump (KFC), with the inlet of the impinger connected to the inside of the chamber via a stainless steel sampling tube of 4 mm inner diameter. The exact volume of water contained in the sampler ($\sim 20 \text{ mL}$) before and after sampling was determined by weighting. It was used to relate the total and cultivable cell concentrations in the impingement liquid to their respective concentrations in the air in the AIDA chamber when equilibrated with atmospheric pressure, considering the volume of the impingement liquid and the sampling rate and time, and

assuming 100% collection efficiency. These are referred to as Cells_{IMP} and CFU_{IMP} throughout the manuscript.

Aerosols from the chamber were systematically collected 30 min after spraying bacterial suspensions. In the course of aerosol ageing experiments, samples were also taken 120 min (2 h), 300 min (5 h), 420 min (7 h), 1020 min (17 h), and 1080 min (18 h) after spraying. In all cases, from both fresh and aged bacterial aerosols, a cloud was generated afterwards by expansion cooling (i.e. depressurization) for microphysical characterization; at some occasions, aerosol samples were also collected once the cloud had dissipated and the pressure inside the chamber was returned to ambient pressure.

2.4 Total cells and colony counts

The concentrations of cultivable and total cells in the impingement liquid were determined by 2 complementary methods. Cultivable cells were counted as colony forming units (CFU). Twenty μL of 10-fold serial dilutions of the impingement liquid were spread on R2A medium (Reasoner and Geldreich, 1985) and incubated at 22–25 °C for 2 to 3 days before counting the colonies formed. Total cells were counted by flow cytometry, on triplicate samples of 450 μL of the impingement liquid mixed with 50 μL of 5% glutaraldehyde (Sigma) (0.5% final concentration) and stored at –20 °C. These were then mixed with one volume (500 μL) of Tris-EDTA buffer at pH 8.0 (10 mM Tris; 1 mM EDTA, final concentrations) and diluted in deionized water to a range of cell concentrations compatible with the analysis. Finally, 10 μL of the DNA specific fluorochrome SYBR-Green (100 \times concentration; Invitrogen) were added to the samples before incubation in the dark for at least 20 min then injection into the flow cytometer (Becton–Dickinson FACScalibur). Particles fluorescing at 530 nm when excited at 488 nm, i.e. labeled with SYBR-Green, were detected and counted by the cytometer. Counts were performed for 2 min or 100 000 events at a flow rate of about 90 $\mu\text{L min}^{-1}$. The exact flow rate was then measured for each series of measurements by weighting a water sample before and after a 20 to 30 min run in the instrument. All solutions used for flow cy-

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ometry analyses were freshly filtered through polycarbonate syringe filters (0.22 μm porosity, Whatman) before use in order to prevent the presence of contaminating particles. In each sample, a population of particles unambiguously attributed to bacterial cells based on their intensity of fluorescence and side-scattering was detected. Finally, cultivability was calculated as the ratio between CFU and total cells counts.

2.5 IN assays

The concentration of ice nucleating particles (C_{INP}) in the collection liquid was assayed by the drop-freezing method described previously (Vali, 1971). Series of sixteen 0.2 mL microtubes containing 20 μL of the impingement liquid, undiluted or diluted 10-fold in distilled water, were placed in a cooling bath (Ecoline Staredition Lauda E200) and exposed to decreasing temperatures from -2 to -10°C with 1°C steps. The number of tubes containing aliquots still in the liquid phase was counted after exposition for 8 min at each temperature step, and C_{INP} was calculated as:

$$C_{\text{INP}} = [\ln(N_{\text{total}}) - \ln(N_{\text{liquid}})]_T / V \times (1/D_f)$$

with N_{total} is the total number of tubes tested in a given dilution series (16), N_{liquid} the corresponding number of tubes still liquid after 8 min at temperature T , V the volume of liquid in each tube (0.02 mL) and D_f the dilution factor (1 or 10). C_{INP} were finally normalized to the corresponding total cell concentrations measured by flow cytometry.

2.6 Data analyses

Exponential regression curves of the type $y = a \times e^{(-bt)}$ were fitted to the data. As all the data were normalized to the first time point measured in the corresponding experiment (i.e. 30 min after spraying, time set as the “time zero” for data analysis), a was equal to 1 and the concentration had its maximum value at $t = 30$ (time t being expressed in minutes). The time constant of this first-order decay equation is $\tau = 1/b$, b being

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the decay rate constant, and the half-life time $t_{1/2}$, at which the concentration has decreased to half the start value, can be calculated as $t_{1/2} = \ln(2)/b$.

All statistical analyses were performed using PAST version 2.04 (Hammer et al., 2001).

3 Results and discussion

3.1 Total and cultivable airborne cell concentrations and survival

A total of 9, 3 and 2 experiments were carried out in the cloud simulation chamber with the strains *Pseudomonas syringae* 32b-74, *P. fluorescens* CGina-01 and *P. syringae* 13b-2, respectively. The initial airborne total and cultivable cell concentrations inferred from in the initial cell suspensions (SUSP subscript), and the concentrations measured with the APS (APS subscript) and from impinger samples (IMP subscript) 30 min and up to 1080 min (18 h) after aerosolization are presented in Table 1. 50 mL of cell suspensions at concentrations ranging from 3.65×10^8 to 1.15×10^9 cells mL⁻¹ were sprayed in the chamber, corresponding to theoretical initial total airborne cell concentrations (Cells_{SUSP}) of 217 to 684 cells cm⁻³ in the 84 m³-chamber. The concentrations actually measured 30 min later by the APS (Cells_{APS}) and from impinger samples (Cells_{IMP}) were both significantly lower (t test; $p < 10^{-6}$ and $p = 0.02$, respectively; $n = 13$) and ranged in average between 138 and 289 cells cm⁻³ and between 258 and 451 cells cm⁻³, respectively. At this time point, Cells_{IMP} was significantly higher than Cells_{APS} by a factor of 1.82 ± 0.40 in average (t test; $p < 0.01$; $n = 13$), indicating the presence of cell aggregates in the $\sim 1 \mu\text{m}$ aerosol population (it extended to about $5 \mu\text{m}$ at the beginning of the experiments). These were disrupted in the impinger during sampling and counted later as individual cells by flow cytometry. The presence of aggregates was also evidenced in the suspensions sprayed by the fact that the concentration cultivable cell (CFU_{SUSP}) exceeded that of total cells (Cells_{SUSP}) (t test; $p < 0.01$; $n = 13$), with particularly large deviations on CFU counts between technical

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replicates, and resulting in cultivability $> 100\%$, and at some occasions $> 1000\%$ (see Table 1). Cell suspensions were prepared by scratching colonies from the surface of agar plates. Even though care was taken for homogenizing them, some heterogeneity probably persisted and resulted in the presence of cell clusters. However, it unintentionally mimicked bacterial aerosols in natural context, as most cultivable bacteria in the atmosphere were found associated with particles (Shaffer and Lighthart, 1997).

With the intention to take only into account cells already airborne and avoid any eventual impact of the spraying process on cultivability, data analysis was restricted to $t \geq 30$ min after aerosolization and data were normalized to the values measured at this experimental time point. This normalization also allowed cleaning the data by avoiding the large deviations on cultivable cell concentration and on cultivability rate in the initial suspensions (CFU_{SUSP}). Each individual absolute value of cultivability (i.e. not normalized to the cultivability measured at this time point) is plotted in Fig. S1. The normalized temporal decay of airborne micron-sized particles ($\text{Cells}_{\text{APS}}$), total cells ($\text{Cells}_{\text{IMP}}$) and cultivable cells (CFU_{IMP}) concentrations was determined from experiments #7, #8, #10 and #11 (Fig. 1). The concentration of particles in the $1\ \mu\text{m}$ -mode ($\text{Cells}_{\text{APS}}$) decreased exponentially over time with a time constant $\tau = 1260 \pm 170$ min (Pearson's $r = 0.992$; $n = 7$). The concentration of airborne cells ($\text{Cells}_{\text{IMP}}$) decreased faster with a time constant $\tau = 500 \pm 120$ min (Pearson's $r = 0.937$; $n = 9$). The upper bound diameter of the $\text{Cells}_{\text{APS}}$ size mode, extending to approximately $5\ \mu\text{m}$ at the beginning of the experiments, decreased to around $3\ \mu\text{m}$ after 7 h, and the cell-to-particle ratio ($\text{Cells}_{\text{IMP}}/\text{Cells}_{\text{APS}}$) decreased from 1.82 ± 0.40 ($n = 13$) to 1.06 ± 0.06 ($n = 2$). These indicated that the cell clusters were progressively removed from the aerosol population by sedimentation. Cultivable cell concentration (CFU_{IMP}) decreased with a time constant $\tau = 230 \pm 10$ min (Pearson's $r = 0.990$; $n = 9$). This concentration therefore decreased about twice as fast as that of the concentration of total cells $\text{Cells}_{\text{IMP}}$ due to additional temporal loss of cultivability. The decay rate constant b for cultivability was $\sim 0.28\ \%\ \text{min}^{-1}$, corresponding to a time constant $\tau = 360 \pm 40$ min and a half-life $t_{1/2} = 250 \pm 30$ min (3.5 to 4.5 h) (Pearson's $r = 0.911$; $n = 9$) (Fig. 2). This has to be

regarded as the most conservative estimate (lower bound) for viability, as viable but non cultivable (VBNC) state is common in aerosolized cells (Heidelberg et al., 1997).

Despite the fact that the bacteria investigated here are non-spore-formers, they lost cultivability only 1.5 to 3 times faster than spores of *Bacillus subtilis* within the same temperature range, which decayed at rates of 0.19 and 0.10 % min⁻¹ at -29 and 4 °C, respectively (Ehrlich et al., 1970). Lighthart (1989) proposed a general time-dependent model of biological decay (decrease of survival rate) for airborne bacteria by mixing experimental data from several bacterial strains, including *Pseudomonas* species (Fig. 2). This fits our data with a Pearson's *r* of only 0.517 (*n* = 9), and we observed a much higher cultivability than what would have been expected from this model, at least for the first 10 h following aerosolization. This implies that the *Pseudomonas* strains investigated here, which were originally isolated from atmospheric samples, are more resistant as airborne than the "average" bacterium considered in this model; it could indicate that these strains are to some extent adapted to atmospheric transport (e.g., Šantl-Temkiv et al., 2012).

Assuming that bacteria have an aerodynamic diameter of about 1 μm, they have a low sedimentation velocity on the order of 10⁻⁴ ms⁻¹ (Malcolm and Raupach, 1991). In addition, such particles fall into the so-called "scavenging gap", and they have a particularly long residence time in the atmosphere (Hobbs, 1993, p. 57). Indeed, residence time was estimated to be 2.3 to 9.6 days in the case of single bacterial cells depending on the source ecosystem, with a global mean of 3.4 days (Burrows et al., 2009a). Under our conditions, after 1 day airborne, 1.7% of the cells would still be cultivable, and based on these extreme and mean residence times, between 0.009% and 1.22 × 10⁻¹⁵ % of aerosolized cells (0.0001 % in average, i.e. 1/10⁶) would survive the duration of their atmospheric journey until deposition. Statistically, this implies that the emission of at least 11 000 cells is necessary, 10⁶ in average, to assure that one survives the residence time and arrives at its endpoint by atmospheric dissemination. Plants are among the strongest natural sources of airborne bacteria identified, with emission fluxes around 500 CFU m⁻² s⁻¹ measured above bean and alfalfa fields (Lin-

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demann et al., 1982). At such a rate, each m^2 of crop field would emit 1 cell capable of surviving its atmospheric transport every 33 min. In other words, 1 cell capable of disseminating alive would be emitted every second by a field of $\sim 2000 \text{m}^2$. Once airborne, as a first approximation bacteria are passively transported horizontally at the speed of horizontal wind. So, for typical horizontal winds in the troposphere, i.e. ~ 2 to $\sim 30 \text{ms}^{-1}$ (not considering extreme events such as storms or cyclones), at the survival rate measured here, 50 % of the cells emitted alive from a source would be transported about 30 to 600 km away, and 1 % would reach the ground up to 4000 km away (Fig. 3). The conditions investigated here (temperature between -20 and 0°C and absence of light) can be considered relatively close to the conditions encountered in the high atmosphere during the night. It is probable that in nature, during the day, UV light have deleterious effect and increases mortality rates (Tong and Lighthart, 1997). In addition, cloud formation can alter viability, as shown in samples collected after expansion cooling (i.e. depressurization) experiments (experiments #6 and #9). Even though it is not statistically testable here, we noticed a strong decrease in the cultivability of *P. s. 32b-74* and *CGina-01* cells exposed to a cloud (see Table 1, Figs. 2 and S1). Fractions of only about ~ 12 and $\sim 40\%$ of the cells cultivable before expansion cooling remained cultivable after cloud dissipation for *32b-74* and *CGina-01*, respectively, compared to $\sim 70\%$ when the pressure was maintained constant. For cloud formation in the AIDA chamber, pressure was typically decreased at rates of 30 to 50hPa min^{-1} during expansion, and the associated cooling rates were typically 2K min^{-1} at the beginning of an expansion and below 0.5K min^{-1} towards the end of the expansion. Considering pressure and temperature changes with altitude of 10hPa and 1K every 100m , these roughly correspond to uplifts of air masses of around 100 to 500m min^{-1} (1.7 to 8.3ms^{-1}), which falls within the range of observations for convective precipitating clouds (Balsley et al., 1988). Our results suggest that the shifts in environmental conditions encountered by living cells transported upward, along with the osmotic shock and free radicals generated by water condensation and freezing (e.g., Stead and Park,

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2000; Tanghe et al., 2003) in clouds probably alter airborne cell survival to a larger extent compared with non convective situations.

3.2 Ice nucleation activity

Figure 4 shows freezing profiles of air samples collected by impingement from the cloud chamber at different times after injection of *P. syringae* 32b-74 suspensions. Thirty minutes after aerosolization, there were $\sim 2 \times 10^{-5}$ INP cell⁻¹ at -3°C (1 INP every $\sim 50\,000$ cells) and $\sim 3 \times 10^{-3}$ INP cell⁻¹ at -5°C (1 INP every ~ 333 cells) on a per-total-cells (Cells_{IMP}) basis. This is about one thenth the IN activity of cells in suspension for this strain (Joly et al., 2013). Decreased IN activity in airborne bacteria compared with suspension was expected from previous observations in cloud simulation chamber involving *P. syringae* (Maki and Willoughby, 1978). No further significant loss of activity over time was observed at temperatures $\leq -4^{\circ}\text{C}$ in aerosolized cells (ANOVA, 5% confidence level), i.e. the frequency of INP cell⁻¹ did not vary with time after aerosolization. This confirmed that non-viable cells retained IN activity, as previously reported (Kozloff et al., 1991).

In the natural atmosphere, phenomena such as coating may affect bacterial IN activity. For example coating with sulfate was reported to decrease the IN activity of soot or Arizona Test Dust particles, a material widely used in laboratory ice nucleation studies as a surrogate for natural mineral aerosols (Cziczo et al., 2009; Möhler et al., 2005; Niedermeier et al., 2011; Pinti et al., 2012). However, sulfate coating had no detectable impact on the IN activity of the commercial powder of lyophilized IN active *P. syringae* cells Snomax (Chernoff and Bertram, 2010). In order to further investigate the influence of sulfate coating, cells were suspended in a solution of ammonium sulfate instead of water before being sprayed into the chamber (experiment #12, Table 1). Thirty minutes after spraying, we found that the frequency of INP per cell had decreased markedly compared to cells sprayed from water suspensions, especially at the warmest temperatures of activity: the frequency of IN active cells was decreased by about 98.5, 91 and 34% at -4 , -5 , and -7°C , respectively (Fig. 4). In this particular bacterial strain, pH

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at values typical for cloud water influenced by anthropogenic emissions (pH ~ 4) were also shown to be responsible for a significant decrease of INA (Attard et al., 2012). Such observations show that the IN activity of bacteria is clearly modulated by abiotic factors, and this must be kept in mind when replacing experimentations into environmental context.

The capacity of cells of nucleating ice in the atmosphere is particularly relevant where condensed water is present, i.e. in clouds. Using the AIDA chamber, it was shown previously that some strains of *P. viridiflava* and *P. syringae* can act as INP in clouds at temperatures around -10°C in the immersion-freezing mode (Möhler et al., 2008). Here, clouds were formed in the chamber by expansion cooling in two experiments (experiments #6 and #9), and aerosol samples were collected by impingement after cloud dissipation, when the pressure inside the chamber was back to ambient pressure (Table 1). The onset ice formation temperature of the impingement liquid was -6°C , compared to -3°C in samples not exposed to cloud, and the frequency of IN per cell was decreased by three orders of magnitude (Fig. 4). A possible “deactivation effect” of the IN activity of bacteria was already suggested from equivalent experiments (Möhler et al., 2008). However, our results expressed on a per-cell basis suggest that, more likely, IN active cells among a population of airborne bacteria were more efficiently precipitated than others. This could explain the observed distribution of IN active bacteria in natural air, clouds and precipitation: Stephanie and Waturangi (2011) observed that the proportion of IN active bacterial strains was higher in falling rain water than in the air at the same location. In addition, whereas only 50% of the *P. syringae* strains isolated from non-precipitating cloud water were IN active (8 strains) (Joly et al., 2013), those isolated from freshly fallen snow by Morris et al. (2008) all had this capacity (47 strains).

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4 Conclusions

In this work, we observed that the concentration and cultivability of cells aerosolized in the AIDA cloud chamber decreased exponentially over time at constant rates. Aggregation seemed to favor cell survival, but this was of course at the cost of the time span as airborne and so, in nature, of the potential distance of dispersion. Hence, in bacteria, aerial dissemination is clearly a compromise between the distance travelled (decreasing for large aggregates) and the chances of dissemination (increasing for large aggregates).

The survival rate determined should provide basis to the existing numerical models describing the aerial dispersion of bacteria (Burrows et al., 2009a; Sesartic et al., 2012) to better predict their atmospheric transport as living entities. By focusing on time as the only explicative variable, we were able to explain quite well (Pearson's $r = 0.911$) the decrease of cultivability observed for *Pseudomonas syringae* and *P. fluorescens* in the AIDA chamber, though adjustments of the predictions in an environmental context could be made by integrating viability parameters as needed, like temperature, humidity, UV, or phenotypic traits. Some work in this direction has already been carried out (Attard et al., 2012; Lighthart, 1973; Lighthart et al., 1971; Smith et al., 2011; Tong and Lighthart, 1997), but more experiments would help building a more mechanistic viability model. In addition, these models are still weakened by the large uncertainties that remain concerning the input to be used, as there are still very few data available about the sources of airborne bacteria and the associated emission fluxes (e.g. Lindemann et al., 1982). These need to be documented for different surface types and meteorological situations.

Numerical simulations demonstrated that the impact of IN active bacteria on precipitation is probably negligible at the scale of the planet (Hoose et al., 2010; Sesartic et al., 2012). However precipitation patterns at regional scales have important socio-economic impacts and the underlying processes still need to be elucidated. We observed that the IN activity of airborne bacteria did not change over time for at least

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several hours after aerosolization. In nature, this is enough time for an IN active cell to be transported to high altitudes and get incorporated into a cloud. Then, as suggested by others (Constantinidou et al., 1990; Möhler et al., 2008; Morris et al., 2008, 2014a), they can induce freezing of supercooled droplets, trigger precipitation and thus selectively prime their own redeposition. For a complete and accurate description of the transport of bacteria in the atmosphere, the partitioning of cells, in particular of IN active cells, between air, clouds and precipitation should be determined.

The Supplement related to this article is available online at doi:10.5194/acpd-15-4055-2015-supplement.

Acknowledgements. This research was funded by the joint DFG-CNRS project “BIOCLOUDS” (DFG contract MO 668/2-1) and by the international program EUROCHAMP (Integration of European Simulation Chambers for Investigating Atmospheric Processes). We particularly thank Martine Sancelme, Catherine Glaux, and Jonathan Colombet for technical assistance with microbiology and flow cytometry. E. Attard acknowledges Blaise Pascal University and CNRS for postdoctoral fellowships. M. Joly is grateful to the Auvergne Region for PhD scholarship. Continuous support by the AIDA technical team is gratefully acknowledged. We also thank Thomas Schwartz from the KIT Institute for Functional Surfaces for kind material support to microbiological manipulations. The AIDA part is also funded by the Helmholtz Association through its program “Atmosphere and Climate (ATMO)”.

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**Table 1.** Detailed cell concentrations and cultivability for the different experiments carried out in the chamber, expressed as the mean of triplicate analyses \pm standard error from the mean when available (*P.s.*: *Pseudomonas syringae*; *P.f.*: *Pseudomonas fluorescens*).

Exp #	AIDA-06 Exp #	Strain	Initial characteristics ^a			Time after spraying (min)	Initial temperature and conditions of experiment	Airborne in the cloud chamber ^b			
			Cells _{SUSP} cm ⁻³	CFU _{SUSP} cm ⁻³	% cultivable cells _{SUSP}			Cells _{APS} cm ⁻³	Cells _{IMP} cm ⁻³	CFU _{IMP} cm ⁻³	% cultivable cells _{IMP}
1 ^c	2	<i>P.s.</i> 32b-74	684 \pm 64	3522 \pm 2138	515 \pm 316 %	30	-5.2 °C, no cloud	138	293 \pm 5	123 \pm 63	42 \pm 21 %
2 ^c	4	<i>P.s.</i> 32b-74	530 \pm 59	1091 \pm 169	206 \pm 39 %	30	0.1 °C, no cloud	173	279 \pm 232	328 \pm 34	118 \pm 99 %
3 ^c	6	<i>P.s.</i> 13b-2	474 \pm 19	694 \pm 172	147 \pm 37 %	30	-2.3 °C, no cloud	216	382 \pm 8	314 \pm 10	82 \pm 3 %
4 ^c	8	<i>P.s.</i> 13b-2	474 \pm 19	694 \pm 172	147 \pm 37 %	30	-17.8 °C, no cloud	204	448 \pm 5	217 \pm 61	49 \pm 14 %
5 ^c	10	<i>P.f.</i> CGina-01	217 \pm 34	1339 \pm 107	616 \pm 108 %	30	-2.3 °C, no cloud	269	361 \pm 49	249 \pm 24	69 \pm 11 %
13 ^c	29	<i>P.s.</i> 32b-74	491 \pm 80	13 591 \pm 13 980	2770 \pm 2884 %	30	-7.2 °C, no cloud	180	387 \pm 22	227 \pm 26	59 \pm 8 %
14 ^c	32	<i>P.s.</i> 32b-74	491 \pm 80	13 591 \pm 13 980	2770 \pm 2884 %	30	-13.7 °C, no cloud	178	306 \pm 19	195 \pm 37	64 \pm 13 %
7 ^d	15	<i>P.s.</i> 32b-74	437 \pm 82	754 \pm 96	173 \pm 39 %	30	-1.4 °C, no cloud	214	436 \pm 74	128 \pm 21	29 \pm 7 %
						120		184	291 \pm 35	79 \pm 11	27 \pm 5 %
						300		155	171 \pm 27	42 \pm 4	25 \pm 4 %
						420		141	143 \pm 19	26 \pm 3	18 \pm 3 %
8 ^d	17	<i>P.s.</i> 32b-74	525 \pm 48	1349 \pm 326	257 \pm 67 %	30	-2 to -20 °C, no cloud	185	349 \pm 24	237 \pm 69	68 \pm 20 %
						120		ND	261 \pm 11	128 \pm 15	49 \pm 6 %
						300		ND	185 \pm 13	69 \pm 6	37 \pm 4 %
						420		139	154 \pm 15	46 \pm 2	30 \pm 3 %
10 ^d	22	<i>P.s.</i> 32b-74	473 \pm 90	1567 \pm 757	332 \pm 172 %	30	-1.3 °C, no cloud	172	451 \pm 59	276 \pm 31	61 \pm 11 %
						1020		92	101 \pm 11	6 \pm 1	6 \pm 1 %
11 ^d	24	<i>P.f.</i> CGina-01	303 \pm 22	10 700 \pm 12 584	3529 \pm 4158 %	30	-1.3 °C, no cloud	289	321 \pm 36	242 \pm 43	75 \pm 16 %
						1080		121	82 \pm 2	2 \pm 0	3 \pm 0 %
6 ^e	12	<i>P.f.</i> CGina-01	217 \pm 34	1339 \pm 107	616 \pm 108 %	30	-16.7 °C, before cloud formed	186	282 \pm 36	264 \pm 92	94 \pm 35 %
9 ^e	19	<i>P.s.</i> 32b-74	529 \pm 103	13 026 \pm 12 101	2464 \pm 2339 %	150	After cloud dissipation	ND	221 \pm 19	83 \pm 29	38 \pm 13 %
						30	-19 °C, before cloud formed	158	258 \pm 24	124 \pm 7	48 \pm 5 %
12 ^e	26	<i>P.s.</i> 32b-74	340 \pm 107	11 161 \pm 10 312	3285 \pm 3207 %	150	After cloud dissipation	ND	139 \pm 19	8 \pm 4	6 \pm 3 %
						30	-16.7 °C, in the presence of ammonium sulfate	ND	318 \pm 24	10 \pm 3	3 \pm 1 %

^a As inferred from the cell suspensions sprayed.^b As measured by aerosol particle sizer and from impinger samples.^c Experiments intended to investigate the impact of fresh IN active bacterial aerosols on the microphysics of clouds generated in the AIDA chamber by expansion cooling; no sample for microbiological analyses was collected after cloud dissipation.^d Experiments intended to investigate the impact of ageing on the survival and IN activity of bacteria as aerosols; clouds were generated afterward for investigating their impacts on microphysics; no sample for microbiological analyses was collected after cloud dissipation.^e Experiments intended to investigate the impact of clouds or sulfate coating on the survival and IN activity of bacterial aerosols.

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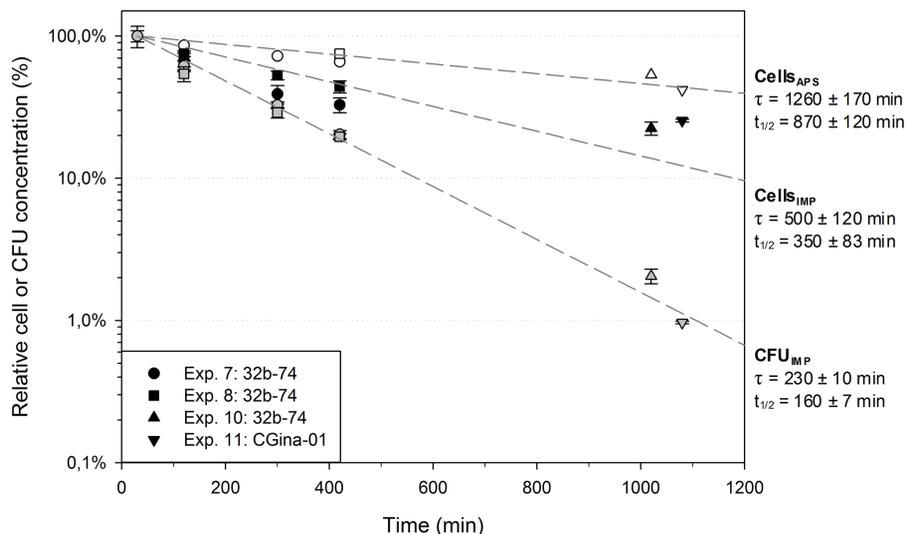


Figure 1. Temporal evolution of total airborne cell concentration measured with the APS ($\text{Cells}_{\text{APS}}$, open symbols) and total and cultivable cells concentrations measured from impinger samples ($\text{Cells}_{\text{IMP}}$, black symbols, and CFU_{IMP} , grey symbols, respectively) of *P. syringae* 32b-74 and *P. fluorescens* CGina-01 in the chamber, relative to the concentrations measured 30 min after spraying cell suspensions. Error bars are SDs from the mean of triplicate samples. The curves show fitted exponential temporal decays. For total particles: Pearson's $r = 0.992$. $n = 7$; for total cells: Pearson's $r = 0.937$. $n = 9$; for cultivable cells: Pearson's $r = 0.990$. $n = 9$. Corresponding calculated time constants (τ) and half-life times ($t_{1/2}$) are indicated on the right of the figure (mean \pm SD).

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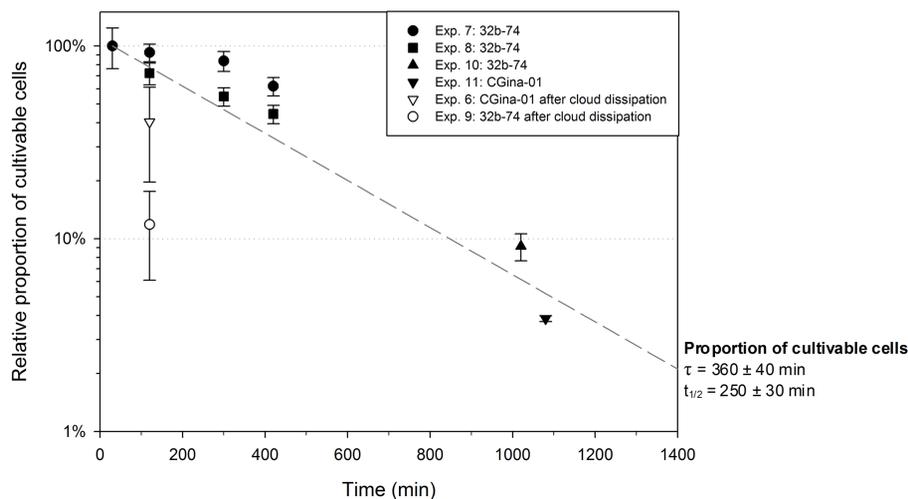


Figure 2. Temporal evolution after aerosolization of the proportion of cultivable cells (cultivability) in impinger samples in *P. syringae* 32b-74 and *P. fluorescens* CGina-01, relative to the cultivability measured 30 min after spraying cell suspensions, in the absence of cloud (black symbols) or after cloud formation and dissipation (open symbols). Error bars are SDs from the mean of triplicate samples. The black dashed curve shows fitted exponential temporal decay of cultivability in the absence of a cloud (Pearson's $r = 0.911$, $n = 9$). The corresponding calculated time constant (τ) and half-life time ($t_{1/2}$) are indicated on the right of the figure (mean \pm SD). Data using the Eq. (5) from Lighthart (1989) are also plotted for comparison (dashed grey line); this model valid for an "average" bacterial strain fits our data with a Pearson's r of 0.517 ($n = 9$).

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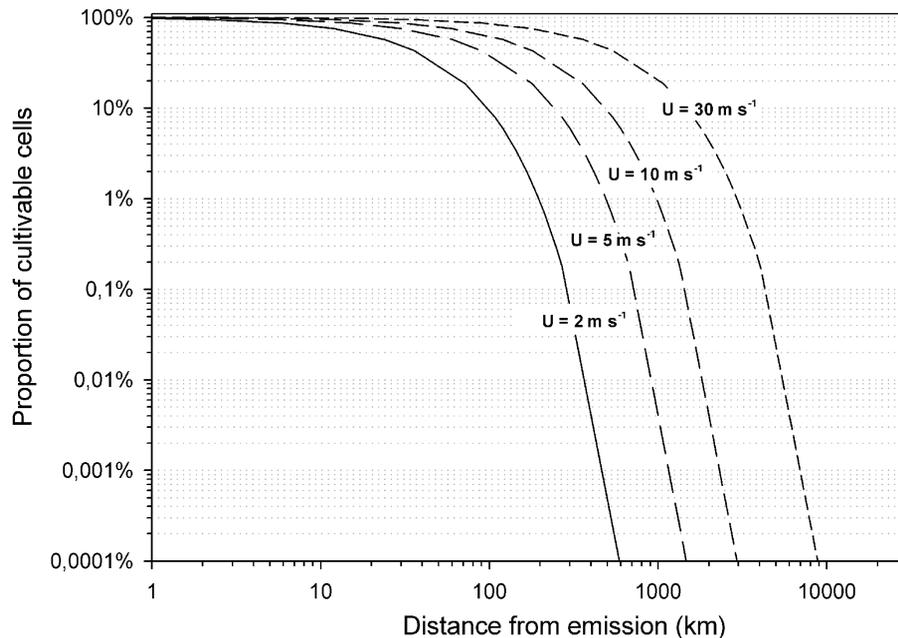


Figure 3. Proportion of cultivable airborne cells associated with the distance reached from their emission source for typical horizontal wind velocities (2, 5, 10 and 30 m s^{-1} , i.e. 7.2, 18, 36 and 108 km h^{-1} , respectively), relative to the respective initial cultivability, as inferred from the data presented in Fig. 2. The proportion of 0.0001% is reached in 3.4 days, the mean residence time of bacteria in the atmosphere estimated by Burrows et al. (2009a).

Survival and ice nucleation activity of bacteria as aerosols

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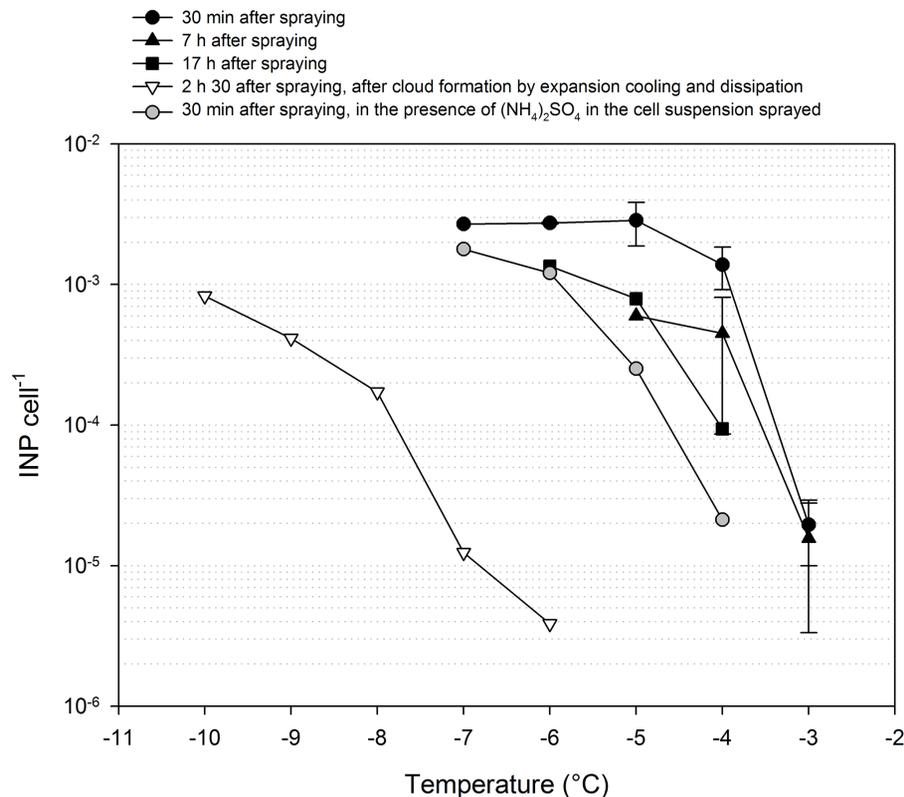


Figure 4. Cumulative frequencies of INP per total airborne cell in *P. syringae* 32b-74 within the AIDA chamber 30 min, 7 and 17 h after aerosolization in the absence of cloud (black symbols), 30 min after aerosolization in the presence of ammonium sulfate (grey symbols), and when the pressure inside the chamber was returned to ambient after cloud formation by expansion cooling (open triangles). Error bars are SDs from the mean of independent experiments, when available.