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**Cindy Morris** is a plant pathologist and microbial ecologist and currently the Director of the Plant Pathology Research Unit at Institut National de la Recherche Agronomique (INRA) centre in Avignon, France. She obtained her PhD in 1985 at the University of Wisconsin-Madison, USA. There she worked on the adaptation of *Pseudomonas syringae* to the leaf surface habitat in a team which had discovered ice nucleation activity of this bacterium a few years previously. Dr. Morris spent four years as a post-doctoral researcher at Beijing Agricultural University in China working on aerial dissemination of bacterial plant pathogens and on the role of ice-nucleation-active bacteria in post-harvest decay of Chinese cabbage. Since 1989, her work as a research scientist at INRA in Avignon has focused on the ecology of plant-pathogenic bacteria, and in particular on *P. syringae* that causes a devastating disease in cantaloupe across France.

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## Microbiological Meteorology: investigating atmospheric processes at the cross-roads of biological and physical sciences

The abundance of micro-organisms in the atmosphere has been known since the insightful experiments of Spallanzani in the 18<sup>th</sup> century and Pasteur in the 19<sup>th</sup> century. These first observations of atmospheric microflora were crucial for refuting the theory of spontaneous generation and in establishing microbiology as a veritable scientific discipline. Today, studies of the microbiology of the atmosphere are on the verge of contributing to another paradigm shift: that airborne micro-organisms contribute to processes influencing atmospheric chemistry, planetary albedo, and precipitation in similar and even more varied ways as aerosol particles.

These potential roles of micro-organisms have been inferred from properties of their cells that can take part in radiative forcing, in the formation of cloud droplets and ice crystals, or in metabolism of chemical components of aerosols. During the past few years, we have been working to bring together different fields of scientific expertise—micro-

biology, atmospheric physics and chemistry, environmental modelling, and agronomy—to create a field that we have christened “Microbiological Meteorology” [1].

The research contributing to this paradigm shift has four main branches, analogous to research on atmospheric aerosols:

- 1) identification of potential atmospheric influence of micro-organisms based on their behaviour in laboratory studies;
- 2) quantification of the abundance of these micro-organisms in air, clouds, and precipitation;
- 3) estimation of the influence of airborne micro-organisms on atmospheric processes based on laboratory and field observations coupled to modelling and
- 4) characterisation of the sources of microbial aerosols and elucidation of processes involved in their formation; and transport in the atmosphere.

The goals of these studies are particularly pertinent to contemporary changes in

the environment of our planet. One of these goals is to understand the effects of anthropogenic sources of airborne micro-organisms on atmospheric processes and to determine how they could buffer or mitigate climate change.

Classical microbiology is at the heart of the idea that micro-organisms could have an influence on the atmosphere. A century of studies on microbial behaviour has revealed properties that can be crucial in atmospheric processes. The most striking example is that certain bacteria and fungi can catalyse freezing of super-cooled water at temperatures near 0°C.

For bacteria, this property is conferred by an unusual, highly folded protein produced on the cell surface. This property was discovered in the search for the cause of plant frost damage and of ice crystal formation leading to snow [2].

Lively speculation still takes place about the ability of these biological ice nucleators to influence precipitation [3,4].

The continuing discussion on this subject can be followed at:

<http://bio-ice.forumotion.com/forum.htm>.

Bacterial cells also seem capable of acting as cloud condensation nuclei (particles around which cloud droplets form by condensation of atmospheric water vapour) [5, 6], most probably because of the hygroscopic (moisture-absorbing) polysaccharides on their cell surface. Some bacteria also produce strong surfactants (wetting agents that lower the surface tension of a liquid allowing easier spreading) (bio-surfactants). This can be an advantage on waxy hydrophobic (water-repellent) plant surfaces where they help these bacteria to degrade plant tissue (and hence to access food resources) [7].

Furthermore, by enhancing the condensation of atmospheric water across a large number of airborne particles that might otherwise be hydrophobic, bio-surfactants of airborne bacteria could favour the persistence of fog. In other words, surfactants would enhance the formation of numerous very small water drops that could remain suspended in air (fog) rather than formation of large droplets apt to precipitate [8].

Studies of microbial metabolism for diverse purposes such as industrial processes, bioremediation (detoxifying pollutants by micro-organisms), and deciphering plant-microbe interactions have revealed the capacity of micro-organisms to metabolise (break down organic material to obtain energy and form cell material), for example, dicarboxylic acids, methane, isoprene, and longer chained alkanes and phenols that constitute the bulk of dissolved organic carbon or major pollutants in atmospheric aerosols. The significance of these capacities for atmospheric chemistry is being explored actively [9].

In addition to the direct roles that micro-organisms could play in atmospheric processes, there are exciting questions to consider about feedbacks. Micro-organisms are metabolically active with dynamic biological properties, many of which are likely maintained also in the atmosphere.

Hence, the microbial traits that lead to their potential effects on the atmosphere are due to capacities that vary with metabolism, gene expression, the distribution of charges across the cell wall, and with other cellular characteristics. These capacities wax and wane as a function of the local environment and as cells mature and senesce.

The study of aerobiology, with regard to

micro-organisms, has overwhelmingly been the realm of plant pathologists attempting to follow the flight of drying, UV-stressed propagules (bacterial cells or parts of fungi or yeasts) of plant pathogens such as fungal spores, single cells of bacteria, or yeasts on their way to distant cropped fields. The decisive work of Stackman [10] and of Gregory [11] on aerial dissemination of spores that spread rusts and other plant diseases set the stage for decades of similar pursuit. This research has nourished the literature with data on the occurrence and abundance of fungi, bacteria, yeasts, and viruses in the air.

In the early 1900s, microbiologists accompanied Charles Lindberg on flights to assess the abundance of micro-organisms in the upper atmosphere [11].

In the late 1970s scientists in the Soviet Union used meteorological rockets to assess the presence of micro-organisms at farther reaches of Earth's atmosphere to define the limits of the biosphere [12].

But only recently has there been an attempt to characterise the microflora of clouds *per se*, where they are set to play important roles. Micro-organisms that can be cultured are present at about  $10^3$ – $10^5$  propagules  $\text{ml}^{-1}$  of cloud water [13–16] and include dozens of species of bacteria and fungi and several yeasts among which are strains capable of metabolising atmospheric organic compounds under laboratory conditions [14].

Overall, data on names and numbers of micro-organisms is accumulating. That oceanic sources – as well as plants – also contribute to the microflora of clouds is also becoming clear [17].

However, there is a great need for assessing the *in situ* state of these micro-organisms to better evaluate whether they are indeed in a physiological condition necessary for influencing atmospheric processes. A significant step in this direction is the recently developed technique to quantify biological ice nucleators directly from environmental samples without culturing the microbial components of the sample [18, 19].

This has revealed that up to 69–100% of the ice nuclei in fresh snowfall can be of biological origin. In over 45% of these samples, biological ice nuclei were sensitive to lysozyme (an enzyme that specifically degrades components of bacterial cell walls) suggesting that they were associated with bacteria [18].

Evidence that micro-organisms indeed

have effects on atmospheric processes is currently circumstantial or indirect. The coming decades will see great progress in obtaining more solid evidence. Biological ice nucleators have been found in clouds [14,20], and, compared to other substrates, freshly fallen snow has been observed to contain an enriched concentration of ice-nucleation-active strains of certain bacteria [21]. Under simulated cloud conditions, these bacteria can induce ice crystal formation [22,23] and, under certain conditions in simulated numerical models, they can have an effect on precipitation [3,24].

Likewise, the roles of micro-organisms in atmospheric chemistry have been studied mostly in laboratory reactors [13,14] to estimate potential kinetics for subsequent use in atmospheric models. Attempts have been made to evaluate the overall metabolic activity of the whole complex of micro-organisms in cloud water directly in fresh samples via incorporation of radioactive isotopes under super-cooled conditions [16] or by assessing growth without nutrient supplementation [25].

Similarly, by determining the concentration of adenosine triphosphate (ATP) in cloud samples, we have revealed that the vast majority of micro-organisms in these samples has maintained metabolic activity [26].

The major gap in knowledge about the interaction of micro-organisms and atmospheric processes concerns microbial emissions. The few published measurements of microbial flux into the atmosphere were reported 15 to 20 years ago [27–30]. Plants are considered to be one of the major sources of micro-organisms in the atmosphere. Many of the micro-organisms cited as potential actors in atmospheric processes are typical inhabitants of plant surfaces and some of these are *bona fide* plant pathogens. As a source, leaf surfaces represent over  $10^9$   $\text{km}^2$  of microbial habitat and likely harbour  $10^{24}$ – $10^{26}$  total bacteria [31] and so far unestimated numbers of fungi and yeasts.

At present, we do not know what fraction of these micro-organisms take off into the atmosphere and in what state – single cells and spores, or clumps of micro-organisms and debris. Factors determining the source strength of plant canopies are likely to be complicated by the plant species involved – different species and cultivars harbour widely different quantities of micro-organisms that potentially can influence the atmosphere [32, 33] – and by the local land

scape through its influence on micro-climate.

Renewed efforts to install field platforms for assessment of microbial flux into the atmosphere [34] are necessary to achieve the long term goals of Microbiological Meteorology. If in fact microbes are involved in atmospheric processes, there are exciting questions to address about the leverage of agronomy and land use practices – via, for example, grazing, crop varietal selection, rotations, and intercropping – on these processes. ■

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