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Chapter

Microbial Ecology in the Atmosphere: The Last Extreme Environment

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Abstract

The atmosphere is an extreme environment where organisms are subject to low temperatures and high radiation. Many of the microorganisms detected there appear in resistant forms or show mechanisms of adaptation designed to withstand these extreme conditions. Airborne microorganisms may play an important role in the global climate system, biogeochemical cycling, and health. Dust storms are the atmospheric phenomenon that move more topsoil through the Earth's atmosphere, and numerous microorganisms attached to dust particles are thus transported. The Iberian Peninsula is periodically affected by this phenomenon as African dust frequently reaches southern Europe and the Mediterranean basin. There are numerous methods for sampling airborne microbes, but factors such as low biomass and high variability of the atmosphere render them not yet sufficiently efficient. Very few studies have been conducted directly in the atmosphere via sampling using airborne platforms. The National Institute for Aerospace Technology has two CASA C-212-200 aircraft that have been suitably modified to operate as airborne research platforms. These aircraft are a unique tool for the study of atmospheric microbial diversity and the different environments where they can be found. A study of the airborne microbial diversity in a Saharan dust event from four aerobiology sampling flights is provided in advance.

Keywords: extremophiles, aerobiology, airborne, aerial platforms, aircraft, dust storm, Saharan dust

1. Introduction

Extremophile organisms capable of growing in extreme conditions draw considerable attention since they show that life is robust and adaptable and help us understand its limits. In addition, they show a high biotechnological potential [1, 2]. Most of the best-characterized extreme environments on Earth are geophysical constraints (temperature, pressure, ionic strength, radiation, etc.) in which opportunistic microorganisms have developed various adaptation strategies. Deep-sea environments, hot springs and geysers, extreme acid waters, hypersaline environments, deserts, and permafrost or ice are some or the most recurrent examples of extreme environments [3]. However, the atmosphere is rarely thought of as an extreme habitat. In the
atmosphere, the dynamics of chemical and biological interactions are very complex, and the organisms that survive in this environment must tolerate high levels of UV radiation, desiccation (wind drying), temperature (extremely low and high temperatures), and atmospheric chemistry (humidity, oxygen radicals, etc.) [4]. These factors turn the atmosphere (especially its higher layers) into one of the most extreme environments described to date and the airborne microorganisms into extremophiles or, at least, multiresistant ones [5].

It is known that airborne cells can maintain viability during their atmospheric residence and can exist in the air as spores or as vegetative cells thanks to diverse molecular mechanisms of resistance and adaptation [2, 6]. The big question is whether some of them can be metabolically active and divide. Bacterial residence times can be several days, which facilitate transport over long distances. This fact, together with the extreme conditions of the atmosphere, has led researchers to think for years that they do not remain active during their dispersion. However, recent studies strongly suggest that atmospheric microorganisms are metabolically active and were aerosolized organic matter and water in clouds would provide the right environment for metabolic activity to take place. Thus, the role played by microorganisms in the air would not only be passive but could also influence the chemistry of the atmosphere. In any case, only a certain fraction of bacteria in the atmosphere would be metabolically active [2, 7].

Despite recognizing its ecological importance, the diversity of airborne microorganisms remains largely unknown as well as the factors influencing diversity levels. Recent studies on airborne microbial biodiversity have reported a diverse assemblage of bacteria and fungi [4, 8–12], including taxa also commonly found on leaf surfaces [13, 14] and in soil habitats [15]. The abundance and composition of airborne microbial communities are variable across time and space [11, 16–19]. However, the atmospheric conditions responsible for driving the observed changes in microbial abundances have not been thoroughly established. One reason for these limitations in the knowledge of aerobiology is that until recently, microbiological methods based on culture have been the standard, and it is known that such methods capture only a small portion of the total microbial diversity [20]. In addition, because pure cultures of microorganisms contain a unique type of microbes, culture-based approaches miss the opportunity to study the interactions between different microbes and their environment.

Another limitation for the study of aerial microbial ecology at higher altitudes or in open ocean areas is the difficulty of repeated and dedicated use of airborne platforms (i.e., aircraft or balloons) to sample the air. Most studies to date on the atmospheric microbiome are restricted to samples collected near the Earth’s surface (e.g., top of mountains or buildings). Aircraft, unmanned aerial systems (UASs), balloons or even rockets, and satellites could represent the future in aerobiology knowledge [5, 21, 22]. These platforms could open the door to conducting microbial studies in the stratosphere and troposphere at high altitudes and in open-air masses, where long-range atmospheric transport is more efficient, something that is still poorly characterized today. The main challenge in conducting these kinds of studies stems from the fact that microbial collection systems are not sufficiently developed. There is a need for improvement and implementation of suitable sampling systems for platforms capable of sampling large volumes of air for subsequent analyses using multiple techniques, as this would provide a wide range of applications in the atmospheric, environmental, and health sciences.

In aerobiology, dust storms deserve special mention. Most of them originate in the world’s deserts and semideserts and play an integral role in the Earth system [23, 24]. They are the result of turbulent winds, including convective haboobs [25]. This dust reaches concentrations in excess of 6000 μg m⁻³ in severe events [26]. Dust and dust-associated bacteria, fungal spores, and pollen can be transported thousands of kilometers in the presence of dust [9].
In this chapter, we approach the atmosphere as an extreme environment and make use of some advanced data from an example of an in situ study of the atmosphere: the analysis of bacterial diversity of the low troposphere of the Iberian Peninsula during an intrusion of Saharan dust using a C-212 aircraft adequately improved for aerobiological sampling.

2. Atmosphere, an extremophile environment

It is well known that there is a biota in the atmospheric air. The first study dates back to the nineteenth century, which speak about the presence and dispersion of microorganisms and spores in the atmosphere [27, 28]. Although the atmosphere represents a large part of the biosphere, the density of airborne microorganisms is very low. Estimates suggest that from the ground surface up to about 18 km above sea level (troposphere), there is less than a billionth of the number of cells...
found in the oceans, soils, and subsurface. Between approximately 18 and 50 km above sea level (stratosphere), temperature, oxygen, and humidity decrease and with them the number of cells. Above the ozone layer (between 18 and 35 km into stratosphere), ultraviolet (UV) and cosmic radiation become lethal factors. Once in the mesosphere (above 50 km), life is difficult to imagine; however microorganisms of terrestrial origin could arrive to the stratosphere from lower layers via different phenomena (human activity, thunderstorms, dust storms, or volcanic activity), and bacteria have been found isolated up to 41 km or in dust samples from the International Space Station (Figure 1) [6, 29]. Therefore, airborne microbes are always present in the atmosphere [11, 30, 31], and their permanence is dynamic, resulting in an environment with enormous variability. Estimates calculate that over 1021 cells are lifted into the atmosphere every year, leading to considerable transport and dispersal around the atmosphere, with a large portion of these cells returning to the surface due to different atmospheric events as part of a feedback cycle. Undoubtedly, airborne microbes play an important role in meteorological processes. They have been linked to the nucleation phenomena that lead to the formation of clouds, rain, and snow and to the alteration of precipitation events [32–34]. Their presence is essential to understand long-range dispersal of plant and potential pathogens [7, 35, 36] and maintain diversity in ground systems and could interfere with the productivity of natural ecosystems [17, 18]. On the other hand, airborne bacteria can have important effects on human health, being responsible for different phenomena such as seasonal allergies and respiratory diseases. Based on data from terrestrial environments, the global abundance of airborne bacteria has been estimated to range between 104 and 106 m⁻³ [37]. However, more recent studies incorporating direct counting by microscopy or quantitative PCR have provided more accurate estimates of the number of airborne microbes, which apparently point to a higher number of cells present in the atmosphere [38–41].

3. Microbial sampling

There is a great variety of airborne microorganism sampling systems, allowing us to select the most suitable one depending on our objectives [42]. On the other hand, no standardized protocols exist, which is a major pitfall when developing our objectives. This fact has led some authors to propose the creation of consortiums of interested parties for establishing standardized protocol reproducibility [20], as well as the need to establish global networks of aerobiological studies [11]. Two approaches are proposed: particles or cells can be collected passively or directly from the atmosphere. Passive media usually involves decanting [43] and collecting particles over snow [44] or through the collection of atmospheric water [45]. On the other hand, active methodologies entail three major approaches: filtration, impaction, and liquid impingement. All three approaches are very efficient when developing culture-dependent techniques. In contrast, culture-independent approaches produce some serious problems that make the work difficult: the high variability of the system and the low biomass mean that sampling campaigns are, in many cases, extremely inefficient [20]. Lastly, the use of airborne platforms is not very extended, but they represent a good opportunity to conduct a more direct study of the atmosphere [5, 19, 31].

3.1 Filtration

Filtration is a simple and cheap method that is often efficient. It involves pumping air through a filter where the mineral and biological particles are trapped. Filters of different materials and porosity are available made of cellulose, nylon, polycarbonate
or fiberglass, or quartz. Sizes used range from 0.2 to 8 $\mu$m, depending on the size of the particles to be captured and the capacity of the pump. In many cases, a PM10 filter can give better results when collecting smaller bacteria, as it allows greater airflow. Airflow filtration rates generally range between 300 and 1000 L/minute [4, 46]. Microorganisms trapped in the filter can be cultured, or the filters can be directly used for DNA extraction. In addition, filters are a very suitable support for microscopy, and countless holders for filters are available (an example is shown in Figure 2A).

3.2 Impingement

In impingement, particles are collected in a liquid matrix [20]. Normally a buffer is used such as phosphate buffer saline (PBS) that helps maintain the viability of the cells. One of the more widely used liquid impingers is BioSampler SKC (Figure 2B). In this case, the tangential movement of the particles inside the flow impinger retains the particles in the collecting liquid. The suspension obtained could be used for culturing or for molecular ecology assays [20]. One of the advantages of impingement collection is that it facilitates quantitative techniques such as flow cytometry or in situ hybridization [48].

3.3 Impaction

In this system, the particles generally impact into a petri dish with an enrichment medium. It is, possibly, the most efficient and most used method to conduct studies based on culture. Airflow impacting onto the plates is controlled by slots that allow the homogeneous distribution of the air. The system can be single stage or several stages in cascade, causing the particles to be distributed by size in the different petri dishes [20]. Some variants replace petri dishes with agarose filters or Vaseline strips, in order to carry out independent culture methodologies, but efficiency is very low. The original and more popular impactor is the Andersen cascade impactor (Figure 2C) [47].

3.4. Airborne platforms

Several studies explain and compare sampling methodologies in aerobiology, but most of them focus on the surface of the Earth (e.g., on top of mountains or buildings) or indoors [42, 49–54]. However, small studies have been conducted at higher altitudes or in open sea areas. The use of airborne platforms (balloons, aircraft, rockets, etc.) for aerobiology sampling would allow conducting a direct study of the microbial ecology of the atmosphere. Another advantage of airborne platforms is the possibility of studying the vertical distribution of airborne microbial communities. In addition, some aircraft allow us to develop studies in the upper troposphere or in the stratosphere. Unfortunately, atmospheric microbial collection instruments have not been developed enough for airborne platforms.

Among the different airborne platforms, aircraft, due to their versatility and access, are particularly interesting. Some studies have been conducted, but not enough samples have been developed yet, and efficiency is still very low. As already mentioned, the efficiency of samplers in soil-level aerobiology faces a series of problems (low biomass, high variability of populations, lack of standardized protocols). In the case of airplanes, in addition to these intrinsic problems associated with atmospheric microbial ecology, other additional ones exist: (1) the high velocity of the aircraft in relation to the relative quiescent air mass. This makes it difficult to obtain an isokinetic sampler and, therefore, one that is sufficiently efficient that would allow us to obtain a correct quantification of the incoming air [55]; (2) the sampler...
must be in a location on the airplane that avoids chemical contamination from the operation of the device. Previous studies have used wing-mounted air samplers or the roof of the aircraft to reduce the possibility of in-flight contamination [21, 22, 56–58]. Similarly, it should allow the aseptic collection of samples, avoiding microbiological contamination during the process. This operation, which can be very simple in the laboratory or at ground level, becomes tremendously complicated on an airplane, since air intakes that are part of the fuselage of the aircraft are often difficult to sterilize. It is therefore necessary to develop robust sterilization protocols. The spectacular work of DeLeon-Rodriguez of 2013 has been criticized in this aspect [40, 59]; (3) sampling time. A possible solution to the low biomass of the atmosphere is to increase sampling time, but in the case of flights, we are limited to the flight autonomy of the aircraft. Although scarce, some studies from airplanes have been conducted. The first studies that were conducted in airplanes were carried out by impaction on a petri plate with enrichment means, which allowed isolating microorganisms from the upper troposphere and even from the stratosphere [21, 57, 60]. However, advances in molecular ecology have caused the most recent studies to favor filtration [40, 58].

The European Facility for Airborne Research (EUFAR) program brings together infrastructure operators of both instrumented research aircraft and remote sensing instruments with the scientific user community. However, it lacked aircraft
Microbial Ecology in the Atmosphere: The Last Extreme Environment
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Prepared for microbiological sampling. The National Institute for Aerospace Technology (INTA) belonging to the Spanish Ministry of Defence has two CASA C-212-200 aircraft that were suitably modified to be used as flying research platforms. Now, these two aircraft are a unique tool for the study of atmospheric microbial diversity and the different environments of the EUFAR program. Our
research group has a CASA-212 aircraft with an air intake located on the roof of the aircraft. A metal tube fits the entrance and is fitted inside the aircraft to a filter holder, a flowmeter, and a pump (Figure 3). This simple system is easy to sterilize, and both the metal tube and the filter holder can be replaced in flight by other sterile ones if we want to take different samples. Using PM10 fiberglass filters, we can obtain isokinetic conditions and pass 1800 L of air per hour through the filter, as indicated by the flowmeter.

In a series of recent experiments, we tried to install a multi-sampler system in our aircraft, where we had five systems in parallel and connected to the same intake of the plane: one filter holder, two impingement systems, and two impactors (Figure 4). The results clearly showed that in the case of our aircraft, filtration was more efficient (data not shown).

4. Microbial characterization

Aerobiology studies have traditionally focused on the collection of bacterial cells and the analysis of samples by total counting and culture-based techniques. It is known that such methods capture only a small portion of the total microbial diversity [61]. The almost exclusive use, for years, of these methodologies is one of the reasons for these limitations in the knowledge of aerobiology. In addition, culture-dependent methods do not allow us to study the interactions between different species of microorganisms. Culture-independent methods have been used to assess microbial diversity, increasing the specificity of microbial identification and the sensitivity of environmental studies, especially in extreme environments. These methods have recently been applied to various areas of airborne microbiology [62–65] revealing a greater diversity of airborne microorganisms when compared to culture-dependent methods. Some good studies approach the challenges and opportunities of using molecular methodologies to address airborne microbiology [20, 66]. Although molecular ecology methods allow the rapid characterization of the diversity of complex ecosystems, the isolation of the different components is essential for the study of their phenotypic properties in order to evaluate their role in the system and their biotechnological potential. A combination of culture-dependent and culture-independent methods is ideal to address the complete study of the system.

Modern culture-independent approaches to community analysis, for example, metagenomics and individual cell genomics, have the potential to provide a much deeper understanding of the atmospheric microbiome. However, molecular ecology techniques face several particular challenges in the case of the atmospheric microbiome: (1) very low biomass [20]; (2) inefficient sampling methods [20]; (3) lack of standard protocols [9, 20]; (4) the composition of airborne microbes continuously changes due to meteorological, spatial, and temporal patterns [7, 62, 67–70]; and (5) avoidance of the presence of foreign DNA in the system [59]. Because these issues are not yet resolved, most of the non-culturing approaches focus on microbial diversity, where they are highly efficient.

The most recurrent techniques are those based on DNA extraction, gene amplification of 16S/18S rRNA, and next-generation sequencing (NGS) technologies. Often, this approach is more efficient due to the greater efficiency and sensitivity of this process, as opposed to gene cloning and Sanger sequencing; thus some authors are inclined toward metagenomics instead of amplification. This provides more information and avoids an intermediate step, but bioinformatic processing is tedious and often only provides data in relation to diversity, making the annotation of the rest of the information very complicated [20]. These approaches can be complemented with quantitative methods such as qPCR, flow cytometry, or fluorescence in
situ hybridization (FISH) [41, 48, 66, 71]. FISH is surely the best and most specific cell quantification methodology that exists. However, in the case of aerobiology, it cannot always be used. A minimum number of cells must exist so that we can observe and count them under a fluorescence microscope. Due to the variability of microbial populations in the air, this is not always achieved. In our research group, we have obtained very good results in this regard, optimizing cell concentration. **Figure 5** shows epifluorescence micrographs of bacteria from an air sample. On this occasion, sampling was performed using a biological air sampler (Coriolis μ, Bertin Technologies SAS), where biological particles are collected and concentrated in a liquid (PBS). Sampling was conducted for 2 hours at ground level, pumping a total of 36,000 L of air. After this time, the sample was paraformaldehyde fixed and filtered through a 0.2 μm pore size, hydrophilic polycarbonate membrane, 13 mm diameter (GTTP, Millipore). A half sample was hybridized with the universal Bacteria domain probe, EUB338I-III [72], following a conventional protocol [73]. The second half was hybridized with the probe NON338 [74] as negative control. In this case, an average of 140 cells per liter of air was counted. Occasionally, FISH also allows to observe bacteria attached to mineral particles (**Figure 5C–D**).

DNA gives us much information about the diversity of the system, but if we wish to obtain information about the metabolic activity that is taking place in the ecosystem, metabolomic and metatranscriptomic approaches are needed [50, 66]. In the case of the atmosphere, this is crucial, since we are not fully certain if the cells present are active. Some studies indicate that a part of the microorganisms in the atmosphere are developing an activity [6], but until we conduct RNA-based and metabolite-based studies, we will not have the certainty that this is the case. The big problem is that it is very difficult to carry out these studies using the current microbial capture systems.

**Figure 5.**
Epifluorescence micrographs of bacteria from an air sample. (A and C) DAPI-stained cells; (B and D) same fields as A and C, respectively, showing cells hybridized with probes EUB338I-III (Cy3 labeled), specific for Bacteria domain. All micrographs correspond to the same hybridization process, performed with a sample obtained after 4 hours sampling at ground. C and D show microorganisms attaches to a mineral particles (arrow sign). Bars, 5 μm.
Scanning electron microscopy (SEM) also provides much information of the aerobiology [7]. Specifically, it allows the characterization of eukaryotic cells (e.g., diatoms) and, above all, pollens and fungal spores, from which we can obtain great information with good images alone. Figure 6A shows pine tree pollen observed via SEM in a sample obtained after a 30 minutes flight of the C-212 aircraft.

5. Mechanisms of microbial survival of airborne bacteria

As mentioned above, factors, such as the shortage of nutrients and substrates, high UV radiation, drying, changes in temperature and pH, or the presence of...
reactive oxygen species, make the atmosphere an extreme environment. However, it is possible that the high variability of its conditions is the one characteristic that makes this environment more extreme [1, 20]. Among the cells present in the atmosphere, a considerable portion appears in the resistance forms capable of withstanding low-temperature and high-radiation conditions. This is what probably happens with fungi and gram-positive bacteria. Bacillus strains recurrently isolated from the atmosphere have characteristics and a capacity to sporulate very similar to strains isolated from the soil. Undoubtedly, another part of the cells will be in the form of latency and may even suffer modifications of the cell wall and slow down or stop their metabolic activity [75, 76]. These transformations can improve resistance to physical stresses, such as UV radiation [58]. On the other hand, some of the bacteria present in the atmosphere, such as Geodermatophilus, show pigmentation that undoubtedly protects it from excessive radiation. The microorganisms that are usually detected in the atmosphere originate mainly from the soil, which means they will share similar mechanisms of resistance. In some strains, metabolic adaptations have been observed to lack nutrients such as cytochrome bd biosynthesis to survive iron deprivation [77]. Deinococcus is also a recurrent genus in the atmosphere, which, like those in soil, has multiresistance mechanisms based on high DNA-repair efficiency. Bacteria that do not form spores and certain archaea, in contrast, often have genomes rich in G + C, which may increase tolerance to UV rays and overall survival [78].

Another strategy of resistance could be cell clustering and adhesion to particles. Several studies have confirmed the loss of viability and shielding or the reflective properties of the mineral particles as an important role for the protection of UV radiation [19, 31]. In that sense, it is very possible that many cells have mechanisms that promote aggregation. In our samples, we often find the cells adhered to each other or to minerals, which undoubtedly makes them more resistant (Figure 6).

6. Emission sources

Global and regional models have been used to explain bioaerosol emission, transport, and atmospheric impact [17, 18, 79–84]. Even so, it is not an easy phenomenon to explain, since it depends on a large number of factors. On the one hand, there are numerous sources of tropospheric aerosols, which include sea salt, volcanic dust, cosmic dust, industrial pollutants, and desert and semidesert areas [6, 85]. We must also consider the factors that make the transfer of particles possible, for example, meteorological phenomena, solar radiation, temperature, tides, erosion, etc. [85]. On the other hand, anthropogenic activities can also affect dust emissions indirectly, by changing the climate and the hydrological cycle. In these aerosols, microorganisms will be included in a greater or lesser number. The degree of richness in cells of tropospheric aerosols will depend largely on the source of emission. Thus, the large wooded masses or fields of crops provide the atmosphere with a good number of microorganisms due to the effect of air or the aerosols produced by rain. Similarly, anthropogenic activity contributes large amounts of bacteria to the environment, treatment plants, and composting areas being sources of airborne microorganisms [85].

Desert dust storms play a major role in particle emissions and with them that of microorganisms. In this way, most of the material reaching the atmosphere from the surface comes from desert and semidesert areas, which is known as desert dust. The Sahara-Sahel desert, the Middle East, central and eastern Asia, and Australia are the major sources of desert dust, although all the arid zones of the world are emission sources [9, 86]. Dust storms are atmospheric events typically associated with dry lands due to the preponderance of dried and unconsolidated substrates with
little vegetation cover. The strong and turbulent winds that blow on these surfaces raise fine-grained material, a large part of which consists of particles the size of silt (4–62.5 μm) and clay (<4 μm), reducing visibility to less than 1 km. The atmospheric concentrations of PM10 dust exceed 15,000 μg/m³ in severe events [87], although the concentrations naturally decrease with the distance from the areas of origin, extending hundreds of kilometers. The dust particles and cells associated with them are transported in this manner and will be deposited finally, by the effect of rain, snow, or other meteorological phenomena. Therefore, there is a continuous transfer of mineral and biological matter through the atmosphere that moves from the air to the terrestrial environment and changes its geographical area [7, 24].

7. Saharan dust

The Sahara-Sahel desert located in northwestern Africa is one of the major sources of windblown dust in the world [9]. This phenomenon has an impact on the Mediterranean coastline, but Saharan dust has been transported toward the north of Europe and has been found on numerous occasions in the Alps [88, 89] or blown toward the Atlantic and Caribbean [8, 90]. It has been estimated that 80–120 tons of dust are transported annually through the Mediterranean toward Europe [23, 91, 92]. In particular, dust transported by the winds can reach an elevation of up to 8 km in the atmosphere over the Mediterranean basin [93]. Because of its geographic position, the Iberian Peninsula is often affected by these dust events. Specifically, the Sahara-Bodele depression, located at the southern edge of the Sahara desert, has been described as the richest dust source reaching the Iberian Peninsula. Southern Spain is the main area affected, but dust can reach the Pyrenees and even France [43]. Different researchers have studied the mineralogical and chemical composition of Saharan dust, which has been observed to contain calcite, dolomite, quartz, different clay minerals, and feldspars as the main mineral components [94]. The intrusion of big amounts of these components is an important influence on nutrient dynamics and biogeochemical cycling in the atmosphere of the Iberian Peninsula.

Despite the large number of studies on dispersion, geochemistry, and mineralogy of African dust, few are focused on microbiology. All these studies conclude that there are microbes associated with dust because there are higher concentrations of aerosolized microorganisms during dust events [43, 90, 93–96]. However, the magnitude of the concentrations and the specific microbes associated with dust events remain the subject of debate. On the other hand, the viability of these microorganisms is another big question. The United States Geological Survey (USGS) develops the Global Dust Program to investigate the viability of microorganisms transported in dust masses. USGS authors using DNA sequencing of the ribosomal gene were able to isolate and identify more than 200 viable bacteria and fungi in St. John’s samples in the USA [8, 36, 90]. Fungi and bacteria associated with atmospheric dust can be recovered and cultivated, but they must be gram-positive bacteria and many spore formers, which makes them resistant to the extreme conditions of the atmosphere.

Therefore, fungi and bacteria associated with dust may have been isolated from dust intrusions, but a percentage of the viable ones already remains an unanswered question. Another big question is the activity of these cells in the atmosphere. It is clear that they are resistant to extremophile conditions, but the question is whether they are developing their life cycle in this particular environment. This question could be answered by molecular ecology methodologies based on the isolation and sequencing of mRNA, but low atmospheric biomass and high variability are, once again, the great problem when developing this type of
RNA-based methodologies. On the other hand, clinical records point to many of the viable microorganisms identified in the Saharan dust as the cause of respiratory diseases (asthma and lung infections or allergic reactions), cardiovascular diseases, and skin infections [7, 90, 97, 98]. It is known that other microbes associated with dust in the air are pathogenic to humans, including those that cause anthrax and tuberculosis, or to livestock (such as foot and mouth disease) or plants [7, 90, 97, 98]. Characterization, quantification, and feasibility studies are vital to address these problems.

It is common to find fungal spores belonging to the genus *Aspergillus*, *Nigrospora*, *Arthrinium*, and *Curvularia* associated with Saharan dust. Bacterial taxa comprised a wide range of phyla, including *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*. Generators of genus spores such as *Clostridium* and *Bacillus* are very common, along with other gram-positive ones such as *Geodermatophilus* or *Streptococcus*. Also, *Alphaproteobacteria*, a very common bacterium class in soils (e.g., the family *Sphingomonadaceae*), are associated with dust [4, 9]. As regards *Archaea*, there are few studies of the atmosphere, in general, and of dust, in particular, that focus on this domain. Surely, reduced cases of pathogenic archaea have been studied to a lesser extent. *Aeropyrum* is the most detected genus of airborne archaea, but it is related to marine aerosols [11]. On the other hand, studies of pollen associated with dust are widespread. An interesting study investigated pollen transported from North Africa to Spain through Saharan dust and found that pollen from five non-native plant species was detected exclusively during dust events [99]. Lastly, viruses and virus-like particles have a great interest in the emission of dust. One study mentions virus-like particles associated with a transoceanic dust event. This report is based on epifluorescent microscopy of filters stained with a specific nucleic acid stain. An increase in the order of magnitude of virus-like particles was observed, from 104 to 2105 m−3 between the baseline condition and dust conditions in the Caribbean [41]. It is speculated that free airborne viruses show worse resistance to high ultraviolet radiation and dry air associated with long-distance transport in dust events resist worse than others [9].

8. Microbial diversity study in the atmosphere of the Iberian Peninsula after a Saharan dust intrusion

Four aerobiology sampling flights took place during February and March 2017 using the CASA C-212-200 aircraft from INTA. The study focused on microbial diversity in the atmosphere of the Iberian Peninsula during and after a Saharan dust intrusion. Flights took place under four different conditions: (1) during a strong Sahara dust storm that reached the north of the Iberian Peninsula, from February 22 to 24, 2017 (February 23, 2017) (Figure 7); (2) following precipitation (February 28, 2017); (3) following a dry period (March 8, 2017); and (4) along the northern coast of Spain (March 9, 2017). In each flight, samples were collected at different altitudes, and air samples were obtained simultaneously at ground level. A total of 20 samples were collected and are being analyzed. Cell presence was observed by scanning electron microscopy (SEM), and bacterial diversity is being studied by DNA extraction, 16S rRNA gene amplification, and Illumina MiSeq sequencing. Results are being analyzed via bioinformatics and biostatistical software (MOTHUR, SPSS, STAMP, CANOCO, and PAST) which will allow us to compare the results between the different flows and scenarios.

Although this study is not yet finished, some data can be advanced in this chapter. Figure 6 shows SEM microphotographs obtained from samples in different scenarios. In general, the samples obtained during the days of dust intrusion (flight
of February 23) appear completely covered with mineral particles. In these cases, more biological cells were detected than in the rest of the days. In the particular case of samples from the marine coast flight, more diatoms were observed (Figure 6E).

The analysis of diversity using the Shannon index showed that, in all cases, diversity was greater on days of Saharan dust intrusion, both in the samples taken from the ground and those taken at higher altitudes with the aircraft. This indicates that Saharan dust contributes microorganisms that are not present in the atmosphere on a daily basis. Diversity analysis showed phylum characteristics of soils, being Alpha- and Betaproteobacteria the most abundant classes. All of the analyses performed showed that bacterial diversity detected at ground level and in-flight samples during the dust intrusion event were similar among one another. The genus taxonomic levels of Sphingomonas, Geodermatophilus, Methylobacter, Rhizobiales, Bacillus, or Clostridium were present in every sample, but their sequences were more abundant in the case of ground samples and dust intrusion samples collected during the day flight. However, sequences of the genus Flavobacterium, Streptococcus, or Cupriavidus were most abundant in the case of samples collected during flight.

Preliminary conclusions show that bacterial diversity of airborne bacteria during days of dust intrusion is higher and similar to bacterial diversity commonly detected in soil samples. Further analyses are being conducted with these samples to obtain a complete description of the evolution of bacterial diversity during those days.

9. Conclusions

Intense UV radiation, low pressure, lack of water and nutrients, and freezing temperatures turn the atmosphere into an extreme environment, especially its upper layers. However, it is widely known that airborne bacteria, fungal spores, pollen, and other bioparticles exist. Numerous bacteria and fungi have been isolated and can survive even at stratospheric altitudes. Microbial survival in the atmosphere requires extremophilic characteristics, and therefore airborne microbiota is potentially useful for biotechnological applications. The role of airborne microbial communities is vital in the Earth, including interactions among the atmosphere, biosphere, climate, and public health. Airborne microorganisms are involved in meteorological processes and can serve as nuclei for cloud drops and ice crystals that precede precipitation, which influences the hydrological cycle and climate. Furthermore, their knowledge is essential in understanding the reproduction and propagation of organisms through various ecosystems. Furthermore, they can cause or improve human, animal, and plant diseases.
Airborne platforms that allow conducting a direct study of microorganisms in the atmosphere and molecular methodologies (e.g., “omics”) could represent a major opportunity for approaching this question. Nevertheless, some challenges must yet be solved, such as low biomass, efficiency of sampling methods, the absence of standard protocols, or the high variability of the atmospheric environment.

Deserts and arid lands are one of the most important sources of aerosol emissions. Clouds of dust generated by storms mobilize tons of mineral particles, and it is known that microorganisms remain attached to the particles being transported over long distances. The large number of mineral particles and microorganisms thus placed into the atmosphere has global implications for climate, biochemical cycling, and health. North African soils, primarily the Sahara Desert, are one of the major sources of airborne dust on Earth. Saharan dust is often transported to southern Europe and could even reach high altitudes over the Atlantic Ocean and the European continent. Again, airborne platforms could be a perfect opportunity for conducting a direct study of the microbiology of this kind of events.

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