*Review*

# Atmosphere: A Source of Pathogenic or Beneficial Microbes?

**Paraskevi N. Polymenakou**

Hellenic Centre for Marine Research, Institute of Marine Biology and Genetics, Gournes Pediados, P.O. Box 2214, 71500 Heraklion, Crete, Greece; E-Mail: polymen@her.hcmr.gr; Tel.: +30-2810-337855; Fax: +30-2810-337870

**Abstract:** The atmosphere has been described as one of the last frontiers of biological exploration on Earth. The composition of microbial communities in the atmosphere is still not well-defined, and taxonomic studies of bacterial diversity in the outdoor air have just started to emerge, whereas our knowledge about the functional potential of air microbiota is scant. When in the air, microorganisms can be attached to ambient particles and/or incorporated into water droplets of clouds, fog, and precipitation (*i.e.*, rain, snow, hail). Further, they can be deposited back to earth's surfaces via dry and wet deposition processes and they can possibly induce an effect on the diversity and function of aquatic and terrestrial ecosystems or impose impacts to human health through microbial pathogens dispersion. In addition to their impact on ecosystem and public health, there are strong indications that air microbes are metabolically active and well adapted to the harsh atmospheric conditions. Furthermore they can affect atmospheric chemistry and physics, with important implications in meteorology and global climate. This review summarizes current knowledge about the ubiquitous presence of microbes in the atmosphere and discusses their ability to survive in the atmospheric environment. The purpose is to evaluate the atmospheric environment as a source of pathogenic or beneficial microbes and to assess the biotechnological opportunities that may offer.

## 1. Introduction/Motivation

Microbes, which make up most of the Earth's biomass, have been found in virtually every environment, surviving and thriving in extremes of heat, cold, radiation, pressure, salinity, acidity, and

darkness. The presence of these life forms has predominantly been studied on land and in water, but his focus is challenging. A variety of sites including geysers and oceanic thermal vents, deep-sea, hypersaline basins, Antarctic sea ice and oxygen-depleted rivers and lakes have been increasingly and extensively explored for their biology within the past few years [1]. However, the atmospheric environment has been not appreciated as a biological entity so far, even if it is as alive as soil or water [2].

The air that we breathe not only comprises nitrogen, oxygen and carbon dioxide but also traces of other gases, inorganic particles and particles of biological origin [3]. The latter are termed bioaerosols and a large part of them are microorganisms that colonize soil, water bodies, plant surfaces, rocks and buildings and readily released into the air by wind erosion and splashing water [4,5]. When in the air, microorganisms can be attached to ambient particles and/or incorporated into water droplets of clouds, fog, and precipitation (*i.e.*, rain, snow, hail) [6–11], while they can be transported to long distances by advection processes. In turn, air microbiota can be further deposited back to earth's surfaces via dry and wet deposition processes and they can possibly induce an effect on the diversity and function of aquatic and terrestrial ecosystems [12] or impose impacts to human health through microbial pathogens dispersion [13]. Bioaerosol particles can include viruses, bacteria, fungi and their spores, lichen fragments, protists (e.g., protozoa, algae and diatoms), spores and fragments of plants, pollen, small seeds, invertebrates (e.g., nematodes, mites, spiders and insects) and their fragments as well as fecal material [3].

In addition to their impact on ecosystem and public health, there are strong indications that air microbes are metabolically active and well adapted to the harsh atmospheric conditions [1]. Furthermore they can affect atmospheric chemistry and physics, with important implications in meteorology and global climate [14]. This review summarizes current knowledge about the ubiquitous presence of microbes in the atmosphere and discusses their ability to survive in the atmospheric environment. Further, it is evaluated whether the atmospheric environment acts as a conveyor of pathogenic or beneficial microbes with potential biotechnological applications.

## 2. The Ubiquitous Presence of Microbes in the Atmosphere

Bioaerosols are considered ubiquitous constituents of the atmosphere, as a large number of these particles are small-sized microorganisms. Estimates of the biomass content in atmospheric particulate having an aerodynamic diameter of less than 2.5 μm (PM 2.5) range from 3% to 11% by weight [15,16], whereas other studies have estimated that up to 25% of the insoluble fraction of aerosols is made of biological material [16,17]. At remote sites representing background atmospheric conditions, airborne bacterial and fungal cells have been found to reach concentrations of ~$10^4$ and ~$10^3$ cells m$^{-3}$, respectively [18,19]. Many of the identified microbes in outdoor air are similar or identical to known soil bacteria or fungi as well as to isolates previously characterized from aquatic environments [20].

Interestingly, bacteria and fungi have been detected in various atmospheric layers, such as the boundary layer (up to 1.5 km altitude), the upper troposphere (up to 12 km altitude) and even the stratosphere at altitudes of 20 km and 41 km above the sea surface [21,22]. In addition, isolated cultures of the common mould, *Penicillium notatum*, have been collected at an altitude of 77 km, and

the bacteria *Micrococcus albus* and *Mycobacterium luteum* at an altitude of 70 km [23]. Due to their small size, microbes can be transported by upper air currents over long distances within or between continents, and thus are able to travel/deposited to the most distant areas of the world. In these terms, the movement of air masses serves as the primary mechanism for the rapid conveyance of microorganisms among widely dispersed habitats [24,25].

More recently, desert dust storms have been shown to be an important source and the most efficient transportation mechanism of bioaerosols, enabling the spread of microbes for over 5000 km away from their sources [25–27]. The largest sources of dust to Earth's atmosphere are the Sahara and Sahel regions of North Africa and the Gobi, Taklamakan, and Badain Juran deserts of Asia [27]. The current estimate for the quantity of arid soil that moves some distance in Earth's atmosphere is 2 billion metric tons per year [28], whereas 50% to 75% of this quantity is believed to originate from the Sahara and Sahel [29–32]. These regions serve as a source of dust to Earth's atmosphere throughout the year affecting the air quality in the Middle East, Europe, the Caribbean, and the Americas [29,33]. On the other hand, the desert dust events of Asia are seasonal and it has been shown that they can impact the atmosphere of remote areas including the French Alps, the Arctic, and the North Pacific [34–36].

In addition to inorganic particles, the clouds of desert dust can carry a sizable inoculum of microorganisms and microbiological materials [13,37]. Though, very few publications have investigated desert dust-associated microbes after long-distance transport at both the African and Asian dust systems [27]. Different analytical approaches have been applied to answer different questions, as the focus of the previous investigations varied from allergens [38–40], to coral pathogens [41] and the phylogenetic characterization of the dust-associated microbial community [20,25,26,42–44]. As a rough approximation, Griffin and Kellogg [45] adopted a conservative estimate of $10^4$ bacteria per gram of soil and calculated that $10^{16}$ dustborne bacteria are moving around the atmosphere for every 1 million tons of emitted soil particles (this estimate does not include the prevalent populations of fungi and viruses [45].

With the rise of genetic techniques, a great degree of automation was achieved, which hold particular promise for exploring the vast amounts of biological material in the air. In addition to culture-independent techniques such as the qualitative 16S rRNA gene clone library analysis, the quantitative polymerase chain reaction (Q-PCR) allows the genetic identification of bioaerosol particles to at least the genus level, whereas it is possible the simultaneous counting of the number of cells of each microorganisms [46]. This quantitative molecular technique is finding increased use in indoor and outdoor aerosol science. However, Hospodsky, *et al.* [47] has shown that several variables can influence accuracy, precision and method detection limits of qPCR for bioaerosol research. Despite the weakness of these methods, the use of genetic techniques is gaining popularity in environmental microbiology and several studies focus on using genetic methods for detailed analysis of the biological aerosols.

Only a handful of studies have attempted to use culture-independent techniques in outdoor aeromicrobiology, but such studies can help identify ubiquitous species that are likely to have large geographical range sizes. Spore-forming organisms, such as *Bacillus* species (phylum of Firmicutes) and other Gram-positives such as Actinobacteria, tend to dominate culture-dependent surveys of airborne microbial diversity [2]. Firstly, Maron, *et al.* [48] applied culture-independent methods, such as

automated-ribosomal intergenic spacer analysis (ARISA) and clone libraries of 16S rDNA, in air samples collected north of France in order to analyze community composition and diversity. Most of the identified bacteria, which were affiliated within Proteobacteria, Firmicutes and Actinobacteria, were found to be closely associated with soil or plant communities. De Santis, *et al.* [49] performed a comparative analysis between 16S rRNA gene clone libraries and microarrays using samples from air, soil, and water. In 2007, Brodie, *et al.* [50] used clone libraries and high-density DNA microarrays to detect a diverse bacterial community in the urban aerosols of two large US cities. Using these highly selective techniques, they were able to identify pathogenic members including environmental relatives of bioterrorism significance. However, they did not attempt to investigate the particle size distribution of the detected microorganisms, as was the case in our recent study [44]. In this study, we investigated the bacterial community composition in the atmosphere of the Eastern Mediterranean during an intense African dust event. During this strong event, we used a five-stage cascade impactor for bioaerosol collection and the bacterial communities associated with aerosol particles of six different size ranges were characterized following clone library analysis of 16S rRNA genes. Spore-forming bacteria, such as Firmicutes, dominated large particle sizes (>3.3 μm in diameter), whereas clones affiliated with Actinobacteria (commonly found in soil) and Bacteroidetes (widely distributed in the environment) gradually increased their abundance in aerosol particles of reduced size (<3.3 μm). A large portion of the clones detected at respiratory particle sizes (<3.3 μm) were phylogenetic neighbors to human pathogens that have been linked to several diseases. Our results demonstrated the potential of aerosolized bacteria for long-range transboundary transportation and foreshadowed their negative impact on human, agricultural and ecosystem health [44].

Very few studies have attempted also to approach the biogeography of the atmosphere by investigating the spatial-temporal variability of microbial community composition in the air using culture-independent methods. These studies have mostly demonstrated significant differences on community structure in short-term (daily) and seasonal cycles and a relatively static community structure across time [2]. In 2008, a short-term temporal variability in the airborne microbial assemblages was investigated at a single site using 16S rRNA gene analysis [51]. At this site, the air samples were dominated by ascomycete fungi of the *Hypocreales* order and a diverse array of bacteria, including members of the proteobacterial and *Cytophaga-Flavobacterium-Bacteroidetes* groups that are commonly found in comparable culture-independent surveys of airborne bacteria. Later, Fröhlich-Nowoisky, *et al.* [52] investigated the seasonal cycles of various groups of fungi in coarse and fine particulate matter in aerosol samples collected for over 1 year in Mainz, Germany. The same year, the atmospheric microbial abundance, community composition, and ice nucleation was investigated at a high-elevation site in northwestern Colorado for a 2-week collection period [53]. The diversity and composition of the airborne microbial communities were found to be relatively static dominated by members of the proterobacterial groups *Burkholderiales* and *Moraxellaceae*. Similar observations (static communities) were made by Pearce, *et al.* [54] who investigated airborne microbial diversity over an isolated scientific research station on an ice-shelf in continental Antarctica. In this study no significant patterns were detected in aerial biodiversity between the austral summer and the austral winter. Recently, Fahlgren, *et al.* [55] monitored the airborne bacterial community during an annual survey using samples collected from a 25-m tower near the Baltic Sea coast. 16S rRNA gene clone

library analysis revealed a highly diverse community with a few abundant operational taxonomic units which most of them belonged to the genera *Sphingomonas* sp. and *Pseudomonas* sp. Potentially pathogenic strains as well as sequences closely resembling bacteria known to act as ice nuclei were also found whereas the origin of the sampled air mass indicating a predominant marine source [55].

## 3. The Atmosphere as a Source of Pathogenic Microbes

To date, most of the studies dealing with the presence of microbes in the atmosphere were devoted to the investigation of possible links between dust-related aerosol dispersal with the transportation of potential pathogens. These studies represent significant starting points in efforts to understand the composition and viability of potential pathogens in outdoor air and to assess their negative impact on ecosystem and public health on a global scale. Recently, this issue raised special concern due to the close link between climate change and bacterial aerial dispersal. More specifically, the increase of desertification during the last decade has resulted in a concomitant increase of atmospheric particulates, whereas a connection between El Nino weather events and increased flux of Saharan dust across the Atlantic has also been observed [32,50,56]. These effects from climate change may also induce an increase in the dispersal of airborne pathogens.

In the last decades, there have been increasing numbers of reports of marine diseases and epidemics, affecting a wide range of organisms, such as plants, invertebrates and mammals [45,57]. Several attempts have been made to connect these diseases with climate change, whereas special attention has been given on identifying possible connections among dust, iron, microbes, and climate change. Recent studies have assumed that the iron carried with desert dust, in addition to triggering harmful algal blooms can also trigger growth of opportunistic marine microbial pathogens that may be present in the transported dust or may already exist in the ecosystem [58]. The first substantial evidence of a microbial link was provided when the terrestrial fungus *Aspergillus sydowii*, which is unable to be reproduced in seawater but can be carried with dust storms, was identified as the causative agent of sea fan disease [59]. Furthermore, a strong connection between African desert dust and Caribbean coral reef decline was also demonstrated when Shinn, *et al.* [60] noticed a relation between two decades of coral reef decline and the coincident increase in African dust being monitored by Prospero [61] for more than 40 years in Barbados. Because of these observations, scientists have started to connect several diseases of marine organisms, such as the disease outbreak in Caribbean sea urchins *Meoma ventricosa*, and the reported septicemia in sea turtle *Caretta caretta* from the Canary Islands, to microbial pathogens that have been identified in dust storms [45].

In addition to their impact on ecosystem health, airborne microorganisms carried by dust clouds can also directly impact human health via pathogenesis, the exposure of sensitive individuals to cellular components (e.g., pollen and fungal allergens and lipopolysaccharide), and the development of sensitivities (*i.e.*, asthma) through prolonged exposure [13]. It has been demonstrated that exposure to airborne fungal and bacterial spores can cause a series of allergic reactions. In addition to intact microbial cells, numerous microorganism-derived molecules, such as endotoxins (membrane lipopolysaccharides shed by Gram-negative bacteria) and fungal mycotoxins can also trigger respiratory problems [62,63].

The closest known associations of human disease of microbial origin and dust storms are the outbreaks of meningitis (primarily due to *Neisseria meningitidis* infection) that occur within the "meningitis belt" of North Africa [64]. These outbreaks occur frequently in the Sahel region of North Africa between the months of February and May and affect as many as 200,000 individuals annually [64,65]. Isolates of *Neisseria meningitidis* have also been retrieved from settled-dust samples from Kuwait together with other pathogens such as *Staphylococcus aureus* (wide range of infections) and *Ralstonia paucula* (e.g., septicemia, peritonitis). Bacterial species that are known human pathogens have also been collected in the air of Bamako, Mali during strong dust events [20] as well as in the African dust corridor over the mid-Atlantic ridge [66]. In the atmosphere over the USA Virgin Islands, the opportunistic pathogen *Pseudomonas aeruginosa*, which can cause fatal infections in burn patients, has been isolated [26], whereas several pathogens have also been identified in the atmosphere of Erdemli, Turkey [13] and Crete, Greece [44], during dust events. A detailed overview of studies associated with desert dust transportation of microorganisms and their implication for human health has been presented by Griffin, *et al.* [13]. According to this review the microbiological research conducted to date has identified a wide range of airborne pathogenic microorganisms that move great distances through the atmosphere and thus more risk-oriented studies need to be conducted.

## 4. The Atmosphere as a Source of Beneficial Microbes in the View of Their Metabolic Activities

During the last decade scientists have discovered novel "extremophiles", microorganisms capable of living in seemingly intolerable environments, such as those with extreme acidity or alkalinity, high salt concentrations, low oxygen levels, extreme temperatures (as low as −20 °C and as high as 300 °C), high concentrations of heavy metals, and high pressure [67–70]. Considering the extremes of temperature (both low and high), solar irradiation (especially the ultraviolet-UV portion of the spectrum) and desiccation (drying in ambient air), as well as the presence of strong chemical oxidants (e.g., ozone, hydroxyl and nitrate radicals), the earth's atmosphere can be considered as an extreme environment where microbes can reside [71]. However, according to Womack, *et al.* [2], the atmosphere is not the most extreme microbial habitat. By several measures (pH, temperature, ultraviolet-UV radiation, resource and water availability), the atmosphere appears to be less extreme than many other microbial habitats that permits microbial residence and growth. Despite these conditions, biologists and microbial ecologists have not yet conceived the atmosphere as a source of beneficial microbes by exploring their metabolic properties either have assessed the biotechnological opportunities that may offer.

Although microorganisms can be found in the ambient air, it is still unclear whether these are metabolically active or simply use the atmosphere as a path for the transportation of their live but largely inactive cells and spores [1]. For microbial cells, the atmosphere is a very stressful environment [17]. Low temperature is considered to be a limiting factor for cell activity in the air, although some studies have demonstrated that bacterial activity can occur at subzero temperatures [7,8,72]. Microbes are capable of adopting several survival mechanisms with their genomes containing the directions for countless biochemical transformations. For instance, in cold temperatures they can reduce their cell size and the thickness of their capsular polysaccharide by changing their phospholipids composition or by creating microenvironments of ice crystals through catalytic redox reactions [73]. In addition to temperature, other limiting factors constraining the

survival of microbes in the atmosphere are the presence of oxidants and solar radiation. However, microbial cells can reduce or trap radicals through the expression of specific enzymes (e.g., superoxidase or peroxydases; [74]), whereas they can produce a diverse range of pigments (e.g., carotenoids) that absorb from UV-B to red wavelengths, avoiding DNA damage [75]. The majority of isolates from the atmosphere are usually bacteria of high guanine (G) and cytosine (C) content DNA, which is more resistant to UV damage compared to low G/C content, and/or with pigmented cell walls, which enhance their UV blocking capacity [76,77]. Air microbiota can also suffer from dehydration and desiccation, but their incorporation into clouds and fog droplets can protect them and sustain their viability and activity [78–80]. Viable bacteria, fungi and yeasts have been observed in fogs, super-cooled cloud droplets, and snow samples [10,11,81]. For example, Fuzzi, *et al.* [9] observed the presence of viable bacteria in fog droplets, whereas Sattler, *et al.* [11] collected bacteria from cloud droplets and demonstrated their growth ability at low temperatures. Since cloud droplets are a liquid solution which contains carbon and nitrogen sources, it could be a privileged medium for microbial activity in the atmosphere [81].

In addition to their ability to survive in the extremes of cold, oxidants, irradiation and desiccation, the presence of microorganisms in the atmosphere has many meteorological and climatic implications through their impact on atmospheric chemistry and physics [14]. Bioaerosols in the troposphere may act as efficient cloud condensation nuclei or ice nuclei thus affecting cloud formation and evolution [82]. Knowledge of the nature and sources of ice nucleation in the atmosphere is important for understanding the meteorological processes for precipitation [7,8]. The most widespread and well-studied biological aerosols with ice-nucleating activity are comprised of certain species of plant-associated bacteria (*Pseudomonas syringae*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Pantoea agglomerans*, and *Xanthomonas campestris*), but also fungi (e.g., *Fusarium avenaceum*) [7,8,83]. Air microorganisms are also suspected to play a role in the chemical composition of the atmosphere by contributing to the degradation of organic compounds [84–86]. Very few research groups have investigated so far the enzymatic potential of microorganisms isolated from the atmosphere to biotransform organic compounds [81]. Both carboxylic acids and aldehydes exhibit high concentrations in cloud waters that can reach values of more than 1 mg·L$^{-1}$ [87–89]. The chemistry of these soluble organic compounds play a crucial role in the budget of volatile organic compounds in the troposphere and in the budget of secondary organic aerosol particles, which is a major uncertainty in the assessment of the role of aerosol particles in climate change [90]. The first study providing evidence for the degradation capabilities of microorganisms in the atmosphere was the degradation of formic and acetic acids by bacteria isolated from rainwater [91]. In 1990, Kawamura and Kaplan, suggested that the observed losses of carboxylic acids in rainwater samples during storage experiments, was likely due to microbial degradation processes [92]. In 2002, Ariya, *et al.* [85] provided strong evidence that an important chemical group of organic aerosols, dicarboxylic acids, can be efficiently transformed by airborne microbes existing in the boundary layer. In 2004, Ariya and Amyot [14] investigated the potential role of microbes in controlling the physical and chemical properties of atmospheric water forms via chemical transformation of organics. In latter studies, the structure of the microbial community present in atmospheric water samples from clouds at the Puy de Dôme (altitude of 1465 m, France) was described and the metabolic potential of some bacteria was

investigated by Amato, *et al.* [78,93]. In 2006, Durand, *et al.* [94] isolated from a cloud water sample the first pure strain capable of rapidly degrading the herbicide mesotrione, while Amato, *et al.* [84] isolated a large number of strains of different taxonomic groups from cloud water samples and demonstrated their ability to degrade some of the main atmospheric carboxylic compounds: formate, acetate, lactate, succinate, as well as formaldehyde and methanol. Recently, the biodegradation of the most abundant atmospheric organic $C_1$ to $C_4$ compounds (formate, acetate, lactate, succinate) by five selected representative microbial strains (three *Pseudomonas* strains, one *Sphingomonas* strain, and one yeast strain) isolated from cloud water at the Puy de Dôme was investigated [90,95]. According to this study, the biological activity drives the oxidation of carbonaceous compounds during the night (90% to 99%), while its contribution account for 2% to 37% of the reactivity during the day, competing with photochemistry.

Overall, microorganisms are suspected to play multiple roles on atmospheric chemistry and physics but the full extent of their impacts remains largely unknown. Moreover, microorganisms being able to survive in the harsh conditions of the atmosphere may be equipped with special genetic features, which under targeted research could be exploited for the benefit of our society.

## 5. Benefits of Air Microbiota to Biotechnology—A Metagenomic Approach

Metagenomics can be used to address the challenge of studying prokaryotes in the environment that are, as yet, unculturable and which represent more than 99% of the organisms in some environments, thus sidestepping the need for culturing or isolation [96]. This discipline builds on the successes of culture-independent 16S rRNA gene surveys of environmental samples [97]. Direct cloning of DNA from environmental samples makes it possible to avoid some of the biases of cultivation and polymerase chain reaction (PCR). In addition, genomic fragments that are >100 kb long can be obtained, and provide significant functional and taxonomic information about the organisms from which they were derived. Until now, such metagenomic libraries have been used to identify novel genes from uncultivated microbial species that are responsible for significant ecosystem processes such as proteorhodopsin-based phototrophy [98,99], and viral activity [100] in the ocean. More importantly, the resultant wealth of genes and molecular structures that can be deciphered from uncultured microorganisms has tremendous potential in the development of novel biocatalysts for industrial and medical applications [101,102].

In principle, any environment is amenable to metagenomic analysis provided that nucleic acids can be extracted from sample matrix. To date, the largest metagenomic study is the Global Ocean Sampling Expedition, which follows the voyage of Darwin's ship HMS Beagle. Metagenomics is now also being adopted in medicine. Of particular note is the Human Microbiome Project, which aims to map human-associated microbial communities (including those of the gut, mouth, skin and vagina) [103]. New powerful natural enzymes can be rapidly found through metagenomics, which is the approach not only to analyze the phylogenetic and functional diversity of environmental samples, but also to locate genes and operons encoding properties of biotechnological interests. Until now, metagenomics has led to the discovery and characterization of a wide range and a remarkable number of biocatalysts. This has been made possible because of the unlimited possibilities of prokaryotes to survive, thrive and populate any extreme environment on earth [104]. By far, too many different

biocatalysts have been detected such as a series of esterase or lipases, polysaccharide degrading enzymes (e.g., amylases, cellulases), novel genes encoding the synthesis of vitamins such as biotin and vitamin C, nitrilases, nitrile hydratases and amidases, oxidoreductases/dehydrogenases, proteases (in detergens and food industry), antibiotics and pharmaceuticals [104]. Elegant examples of the biotech applications of the metagenomic tool are the functional analysis of hindgut microbiota of a wood-feeding higher termite [105] and worms [106], which provide potential sources of biochemical catalysts for converting wood into biofuels.

To date, a total of 1057 metagenome projects [107] are currently prospecting microbial communities for new genes but most of them are taking place in a wide range of extreme environments, such as hydrothermal vents and hot springs as well as in other aquatic and soil ecosystems. Although, the atmosphere is another environment, where diverse microbial populations can dwell, the ecologists and biotechnologists have not sufficiently considered that as a microbiological habitat with potential biotechnological applications. Relatively little is known about how microbes survive the stress of becoming airborne, and whether are genetic contributors to aerosolization of airborne dissemination. For this reason, it constitutes a novel, open arena of research and the application of metagenomics to air microbial population offers an unconventional approach to unravel the magnitude of the microbial metabolic capabilities of the atmospheric biomes. The first airborne metagenome was constructed in 2008 by Tringe, *et al.* [108] in one sample of urban indoor air, identifying genes potentially involved in resistance to desiccation and oxidative damage, and demonstrating that air harbors a unique and dynamic microbial community that may originate from a variety of niches. With the rapid progression of technology, new and emerging tools, such as the metagenomic analysis of air microbial habitats should greatly enhance our understanding of airborne microbial ecology and assess their biotechnological opportunities.

## 6. Conclusions

The composition of microbial communities in the atmosphere is still not well-defined, and taxonomic studies of bacterial diversity in the outdoor air have just started to emerge. More importantly, our knowledge about the functional potential of air microbiota is scant [44,50]. This knowledge gap is due to the poor integration of biotechnology with aerosol science and the traditional connection of atmospheric sciences with chemical pollution problems [16]. In the present review we have shown that air microbiota may be more important than previously recognized since their ubiquitous presence in the atmosphere gives them the potential to spread and colonize ecosystems all over the world inducing impacts to ecosystem and public health through pathogens dispersion. In addition, air microbiota possessing the ability to survive in the extreme conditions of the atmospheric environment may be equipped with special genetic features with potential biotechnological applications. Furthermore, they can have many meteorological and climatic implications through their impact on atmospheric chemistry and physics (e.g., degradation of organic compounds). For these reasons, new and advanced genomic tools must be introduced in the field of Atmospheric Microbiology that will allow us to address the fundamental questions "who is there?", "what are they doing?", and "how are they doing it?". A series of powerful molecular approaches can be profitably used to describe air microbial diversity and clarify

whether air microorganisms are metabolically active or simply use the atmosphere as a route for the transportation of their live but inactive cells.

## Acknowledgments

## References

1. Rothschild, L.J.; Mancinelli, R.C. Life in extreme environments. *Nature* **2001**, *409*, 1092–1101.
2. Womack, A.M.; Bohannan, B.J.M.; Green, J.L. Biodiversity and biogeography of the atmosphere. *Phil. Trans. R. Soc. B* **2010**, *365*, 3645–3653.
3. Lacey, M.E.; West, J.S. *The Air Spora: A Manual for Catching and Identifying Airborne Biological Particles*; Springer: Dordrecht, The Netherlands, 2006.
4. Kuske, C.R. Current and emerging technologies for the study of bacteria in the outdoor air. *Curr. Opin. Biotech.* **2006**, *17*, 291–296.
5. Stetzenbach, L.D.; Buttner, M.P.; Cruz, P. Detection and enumeration of airborne biocontaminants. *Curr. Opin. Biotech.* **2004**, *15*, 170–174.
6. Bauer, H.; Kasper-Giebl, A.; Löflund, M.; Giebl, H.; Hitzenberger, R.; Zibuschka, F.; Puxbaum, H. The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. *Atmos. Res.* **2002**, *64*, 109–119.
7. Christner, B.C.; Cai, R.; Morris, C.E.; McCarter, K.S.; Foreman, C.M.; Skidmore, M.L.; Montross, S.N.; Sands, D.C. Geographic, seasonal, and precipitation chemistry influence on the abundance and activity of biological ice nucleators in rain and snow. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 18854–18859.
8. Christner, B.C.; Morris, C.E.; Foreman, C.M.; Cai, R.; Sands, D.C. Ubiquity of biological ice nucleators in snowfall. *Science* **2008**, *319*, doi:10.1126/science.1149757.
9. Fuzzi, S.; Mandrioli, P.; Perfetto, A. Fog droplets—An atmospheric source of secondary biological aerosol particles. *Atmos. Environ.* **1997**, *31*, 287–290.
10. Möhler, O.; DeMott, P.J.; Vali, G.; Levin, Z. Microbiology and atmospheric processes: The role of biological particles in cloud physics. *Biogeosci. Discuss.* **2007**, *4*, 2559–2591.
11. Sattler, B.; Puxbaum, H.; Psenner, R. Bacterial growth in supercooled cloud droplets. *Geophys. Res. Lett.* **2001**, *28*, 239–242.
12. Jones, S.E.; Newton, R.J.; McMahon, K.D. Potential for atmospheric deposition of bacteria to influence bacterioplankton communities. *FEMS Microbiol. Ecol.* **2008**, *64*, 388–394.
13. Griffin, D.W.; Kubilay, N.; Kocak, M.; Gray, M.A.; Borden, T.C.; Shinn, E.A. Airborne desert dust and aeromicrobiology over the Turkish Mediterranean coastline. *Atmos. Environ.* **2007**, *41*, 4050–4062.
14. Ariya, P.A.; Amyot, M. New directions: The role of bioaerosols in atmospheric chemistry and physics. *Atmos. Environ.* **2004**, *38*, 1231–1232.

15. Boreson, J.; Dillner, A.M.; Peccia, J. Correlation bioaerosol load with PM2.5 and PM10cf concentrations: A comparison between natural desert and urban fringe aerosols. *Atmos. Environ.* **2004**, *38*, 6029–6041.

16. Peccia, J.; Hernandez, M. Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. *Atmos. Environ.* **2006**, *40*, 3941–3961.

17. Jones, A.M.; Harrison, R.M. The effects of meteorological factors on atmospheric bioaerosol concentrations—A review. *Sci. Total Environ.* **2004**, *326*, 151–180.

18. Harrison, R.M.; Jones, A.M.; Biggins, P.D.E.; Pomeroy, N.; Cox, C.S.; Kidd, S.P.; Hobman, J.L.; Brown, N.L.; Beswick, A. Climate factors influencing bacterial count in background air samples. *Int. J. Biometeorol.* **2004**, *49*, 167–178.

19. Katial, R.K.; Zhang, Y.; Jones, H.J.; Dyer, P.D. Atmospheric mold spore counts in relation to meteorological parameters. *Int. J. Biometeorol.* **1997**, *41*, 17–22.

20. Kellogg, C.A.; Griffin, D.W.; Garrison, V.H.; Peak, K.K.; Royall, N.; Smith, R.R.; Shinn, E.A. Characterization of aerosolized bacteria and fungi from desert dust events in Mali, West Africa. *Aerobiologia* **2004**, *20*, 99–110.

21. Griffin, D.W. Terrestrial microorganisms at an altitude of 20,000 m in Earth's atmosphere. *Aerobiologia* **2004**, *20*, 135–140.

22. Wainwright, M.; Wickramasinghe, N.C.; Narlikar, J.V.; Rajaratnam, P. Microorganisms cultured from stratospheric air samples obtained at 41 km. *FEMS Microbiol. Lett.* **2003**, *218*, 161–165.

23. Imshenetsky, A.A.; Lysenko, S.V.; Kasakov, G.A. Upper boundary of the biosphere. *Appl. Environ. Microbiol.* **1978**, *35*, 1–5.

24. Isard, S.A.; Gage, S.H. *Flow of Life in the Atmosphere: An Airscape Approach to Understanding Invasive Organisms*; Michigan State University Press: Detroit, MI, USA, 2001; p. 304.

25. Prospero, J.M.; Blades, E.; Mathison, G.; Naidu, R. Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. *Aerobiologia* **2005**, *21*, 1–19.

26. Griffin, D.W.; Kellogg, C.A.; Garrison, V.H.; Lisle, J.T.; Borden, T.C.; Shinn, E.A. Atmospheric microbiology in the northern Caribbean during African dust events. *Aerobiologia* **2003**, *19*, 143–157.

27. Kellogg, C.A.; Griffin, D.W. Aerobiology and the global transport of desert dust. *Trends Ecol. Evol.* **2006**, *21*, 638–644.

28. Perkins, S. Dust, the thermostat. *Sci. News* **2001**, *160*, 200–201.

29. Goudie, A.S.; Middleton, N.J. Saharan dust storms: Nature and consequences. *Earth Sci. Rev.* **2001**, *56*, 179–204.

30. Moulin, C.; Lambert, C.E.; Dulac, F.; Dayan, U. Control of atmospheric export of dust from North Africa by the North Atlantic oscillation. *Nature* **1997**, *387*, 691–694.

31. Perry, K.D.; Cahill, T.A.; Eldred, R.; Dutcher, D.D. Long-range transport of North African dust to the eastern United States. *J. Geophys. Res.* **1997**, *102*, 11225–11238.

32. Prospero, J.M.; Lamb, P.J. African droughts and dust transport to the Caribbean: Climate change implications. *Science* **2003**, *302*, 1024–1027.

33. Middleton, N.J.; Goudie, A.S. Saharan dust: Sources and trajectories. *Trans. Inst. Br. Geogr. NS* **2001**, *26*, 165–181.

34. Betzer, P.R.; Carder, K.L.; Duce, R.A.; Merrill, J.T.; Tindale, N.W.; Uematsu, M.; Costello, D.K.; Young, R.W.; Feely, R.A.; Breland, J.A.; *et al.* Long-range transport of giant mineral aerosol particles. *Nature* **1988**, *336*, 568–571.

35. Grousset, F.E.; Ginoux, P.; Bory, A.; Biscaye, P.E. Case study of a Chinese dust plume reaching the French Alps. *Geophys. Res. Lett.* **2003**, *30*, doi:10.1029/2002GL016833.

36. Rahn, K.A.; Boyrs, R.D.; Shaw, G.E.; Schutz, L.; Jaenicke, R. Long-Range Impact of Desert Aerosol on Atmospheric Chemistry: Two Examples. In *Saharan Dust*; Fenner, F., Ed.; John Wiley and Sons: Chichester, UK, 1977; pp. 243–266.

37. Griffin, D.W. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clin. Microbiol. Rev.* **2007**, *20*, 459–477.

38. Ho, H.-M.; Rao, C.Y.; Hsu, H.-H.; Chiu, Y.-H.; Liu, C.-M.; Chao, H.J. Characteristics and determinants of ambient fungal spores in Hualien, Taiwan. *Atmos. Environ.* **2005**, *39*, 5839–5850.

39. Wu, P.-C.; Tsai, J.-C.; Li, F.-C.; Lung, S.-C.; Su, H.-J. Increased levels of ambient fungal spores in Taiwan are associated with dust events from China. *Atmos. Environ.* **2004**, *38*, 4879–4886.

40. Yeo, H.-G.; Kim, J.-H. SPM and fungal spores in the ambient air of west Korea during the Asian dust (Yellow sand) period. *Atmos. Environ.* **2002**, *36*, 5437–5442.

41. Weir-Brush, J.R.; Garisson, V.H.; Smith, G.W.; Shinn, E.A. The relationship between gorgonian coral (Cnidaria: Gorgonacea) diseases and African dust storms. *Aerobiologia* **2004**, *20*, 119–126.

42. Choi, D.-S.; Park, Y.-K.; Oh, S.-K.; Yoon, H.-J.; Kim, J.-C.; Seo, W.-J.; Cha, S.-H. Distribution of airborne microorganisms in yellow sands of Korea. *J. Microbiol.* **1997**, *35*, 1–9.

43. Griffin, D.W.; Garrison, V.H.; Herman, J.R.; Shin, E.A. African desert dust in the Caribbean atmosphere: Microbiology and public health. *Aerobiologia* **2001**, *17*, 203–213.

44. Polymenakou, P.N.; Mandalakis, M.; Tselepides, A.; Stephanou, E.G. Particle size distribution of airborne microorganisms and pathogens during an intense African dust event in the Eastern Mediterranean. *Environ. Health Persp.* **2008**, *116*, 292–296.

45. Griffin, D.W.; Kellogg, C.A. Dust storms and their impact on ocean and human health: Dust in Earth's atmosphere. *EcoHealth* **2004**, *1*, 284–295.

46. Burrows, S.M.; Elbert, W.; Lawrence, M.G.; Pöschl, U. Bacteria in the global atmosphere—Part 1: Review and synthesis of literature data for different ecosystems. *Atmos. Chem. Phys.* **2009**, *9*, 9263–9280.

47. Hospodsky, D.; Yamamoto, N.; Peccia, J. Accuracy, precision, and method detection limits of quantitative PCR for airborne bacteria and fungi. *Appl. Environ. Microbiol.* **2010**, *76*, 7004–7012.

48. Maron, P.-A.; Lejon, D.P.H.; Carvalho, E.; Bizet, K.; Lemanceau, P.; Ranjard, L.; Mougel, C. Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16S rDNA clone library. *Atmos. Environ.* **2005**, *39*, 3687–3695.

49. DeSantis, T.Z.; Brodie, E.L.; Moberg, J.P.; Zubieta, I.X.; Piceno, Y.M.; Andersen, G.L. High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. *Microb. Ecol.* **2007**, *53*, 371–383.

50. Brodie, E.L.; DeSantis, T.Z.; Parker. J.P.M.; Zubietta, I.X.; Piceno, Y.M.; Andersen, G.L. Urban aerosols harbor diverse and dynamic bacterial populations. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 299–304.

51. Fierer, N.; Liu, Z.; Rodríguez-Hernández, M.; Knight, R.; Henn, M.; Hernandez, M.T. Short-term temporal variability in airborne bacterial and fungal populations. *Appl. Environ. Microbiol.* **2008**, *74*, 200–207.

52. Fröhlich-Nowoisky, J.; Pickersgill, D.A.; Després, V.R.; Pöschl, U. High diversity of fungi in air particulate matter. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 12814–12819.

53. Bowers, R.M.; Lauber, C.L.; Wiedinmyer, C.; Hamady, M.; Hallar, A.G.; Fall, R.; Knight, R.; Fierer, N. Characterization of airborne microbial communities at a high elevation site and their potential to act as atmospheric ice nuclei. *Appl. Environ. Microbiol.* **2009**, *75*, 5121–5130.

54. Pearce, D.A.; Hughes, K.A.; Lachlan-Cope, T.; Harangozo, S.A.; Jones, A.E. Biodiversity of air-borne microorganisms at Halley station, Antarctica. *Extremophiles* **2010**, *14*, 145–159.

55. Fahlgren, C.; Hagström, A.; Nilsson, D.; Zweifel, U.L. Annual variations in the diversity, viability, and origin of airborne bacteria. *Appl. Environ. Microbiol.* **2010**, *76*, 3015–3025.

56. Moulin, C.; Chiapello, I. Impact of human-induced desertification on the intensification of Sahel dust emission and export over the last decades. *Geophys. Res. Lett.* **2006**, *33*, doi:10.1029/2006GL025923.

57. Harvell, C.D.; Kim, K.; Burkholder, J.M.; Colwell, R.R.; Epstein, P.R.; Grimes, D.J.; Hofmann, E.E.; Lipp, E.K.; Osterhaus, A.D.M.E.; Overstreet, R.M.; *et al*. Emerging marine diseases—Climate links and anthropogenic factors. *Science* **1999**, *285*, 1505–1510.

58. Hayes, M.L.; Bonaventura, J.; Mitchell, T.P.; Prospero, J.M.; Shinn, E.A.; van Dolan, F.; Barber, R.T. How are climate and marine biological outbreaks functionally linked? *Hydrobiologia* **2001**, *460*, 213–220.

59. Smith, G.W.; Ives, L.D.; Nagelkerken, I.A.; Ritchie, K.B. Caribbean sea-fan mortalities. *Nature* **1996**, *383*, doi:10.1038/383487a0.

60. Shinn, E.A.; Smith, G.W.; Prospero, J.M.; Betzer, P.; Hayes, M.L.; Garrison, V.; Barber, R.T. African dust and the demise of Caribbean coral reefs. *Geophys. Res. Lett.* **2000**, *27*, 3029–3032.

61. Prospero, J.M. Saharan dust impacts and climate change. *Oceanography* **2006**, *19*, 60–61.

62. Braun-Fahrlander, C.; Riedler, J.; Herz, U.; Eder, W.; Waser, M.; Grize, L.; Maisch, S.; Carr. D.; Gerlach, F.; Bufe, A.; *et al*. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N. Engl. J. Med.* **2002**, *347*, 869–877.

63. Jie, Y.; Houjin, H.; Feng, J.; Jie, X. The role of airborne microbes in school and its impact on asthma, allergy, and respiratory symptoms among school children. *Rev. Med. Microbiol.* **2011**, *22*, 84–89.

64. Sultan, B.; Labadi, K.; Guegan, J.F.; Janicot, S. Climate drives the meningitis epidemics onset in West Africa. *PLoS Med.* **2005**, *2*, doi:10.1371/journal.pmed.0020006.

65. Molesworth, A.M.; Cuevas, L.E.; Conner, S.J.; Morse, A.P.; Thomson, M.C. Environmental risk and meningitis epidemics in Africa. *Emerg. Infect. Dis.* **2003**, *9*, 1287–1293.

66. Griffin, D.W.; Westphal, D.L.; Gray, M.A. Airborne microorganisms in the African desert dust corridor over the mid-Atlantic ridge, Ocean Drilling Program, leg 209. *Aerobiologia* **2006**, *22*, 211–226.

67. Brock, T.D.; Freeze, H. *Thermus aquaticus* gen. n. and sp. n., a non-sporulating extreme thermophile. *J. Bacteriol.* **1969**, *98*, 289–297.

68. Demirjian, D.C.; Morís-Varas, F.; Cassidy, C.S. Enzymes from extremophiles. *Curr. Opin. Chem. Biol.* **2001**, *5*, 144–151.

69. Kato, C.; Takai, K. Microbial diversity of deep-sea extremophiles—Piezophiles, Hyperthermophiles, and subsurface microorganisms. *Biol. Sci. Space* **2000**, *14*, 341–352.

70. Pakchung, A.A.H.; Philippa, S.; Simpson, J.L.; Codd, R. Life on earth. Extremophiles continue to move the goal posts. *Environ. Chem.* **2006**, *3*, 77–93.

71. Gregory, P.H. Airborne microbes: Their significance and distribution. *Proc. R. Soc. B Biol. Sci.* **1971**, *177*, 469–483.

72. Junge, K.; Eicken, H.; Deming, J.W. Bacterial activity at −2 to −20 °C in Arctic wintertime sea ice. *Appl. Environ. Microbiol.* **2004**, *70*, 550–557.

73. Deguillaume, L.; Leriche, M.; Amato, P.; Ariya, P.A.; Delort, A.-M.; Pöschl, U.; Chaumerliac, N.; Bauer, H.; Flossmann, A.I.; Morris, C.E. Microbiology and atmospheric processes: Chemical interactions of primary biological aerosols. *Biogeosciences* **2008**, *5*, 1073–1084.

74. Ochner, U.A.; Vasil, M.L.; Alsabbagh, E.; Parvatiyar, K.; Hassett, J. Role of the *Pseudomonas aeruginosa oxyR-oxyG* operon in oxidative stress defense and DNA repair: oxyR-dependent regulation of *katB-ankB*, *ahpB*, and *ahpC-ahpF*. *J. Bacteriol.* **2000**, *182*, 4533–4544.

75. Mueller, D.R.; Vincent, W.F.; Bonilla, S.; Laurion, I. Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. *FEMS Microbiol. Ecol.* **2005**, *53*, 73–87.

76. Singer, C.E.; Ames, B.N. Sunlight ultraviolet and bacterial DNA base ratios. *Science* **1970**, *170*, 822–825.

77. Sundin, G.W.; Jacobs, J.L. Ultraviolet radiation (UVR) sensitivity analysis and UVR survival strategies of a bacterial community from the phyllosphere of field-grown peanut (*Arachis hypogeae* L.). *Microb. Ecol.* **1999**, *38*, 27–38.

78. Amato, P.; Parazols, M.; Sancelme, M.; Laj, P.; Mailhot, G.; Delort, A.-M. Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: Major groups and growth abilities at low temperature. *FEMS Microbiol. Ecol.* **2007b**, *59*, 242–254.

79. Amato, P.; Parazols, M.; Sancelme, M.; Mailhot, G.; Laj, P.; Delort, A.-M. An important oceanic source of microorganisms for cloud water at the Puy de Dôme (France). *Atmos. Environ.* **2007c**, *41*, 8253–8263.

80. Dimmick, R.L.; Wolochow, H.; Chatigny, M.A. Evidence that bacteria can form new cells in airborne particles. *Appl. Environ. Microbiol.* **1979**, *37*, 924–927.

81. Delort, A.-M.; Vaïtilingom, M.; Amato, P.; Sancelme, M.; Parazols, M.; Mailhot, G.; Laj, P.; Deguillaume, L. A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. *Atmos. Res.* **2010**, *98*, 249–260.

82. Sun, J.; Ariya, P.A. Atmospheric organic and bio-aerosols as cloud condensation nuclei (CCN): A review. *Atmos. Environ.* **2006**, *40*, 795–820.

83. Lundheim, R. Physiological and ecological significance of biological ice nucleators. *Philos. Trans. R. Soc. Lond.* **2002**, *357*, 937–943.

84. Amato, P.; Demeer, F.; Melaouhi, A.; Fontanella, S.; Martin-Biesse, A.-S.; Sancelme, M.; Laj, P.; Delort, A.-M. A fate for organic acids, formaldehyde and methanol in cloud water: Their biotransformation by microorganisms. *Atmos. Chem. Phys.* **2007a**, *7*, 4159–4169.

85. Ariya, P.A.; Nepotchatykh, O.; Ignatova, O.; Amyot, M. Microbiological degradation of atmospheric organic compounds. *Geophys. Res. Lett.* **2002**, *29*, 2077–2080.

86. Côté, V.; Kos, G.; Mortazavi, R.; Ariya, P.A. Microbial and "de novo" transformation of dicarboxylic acids by three airborne fungi. *Sci. Total Environ.* **2008**, *390*, 530–537.

87. Kawamura, K.; Steinberg, S.; Kaplan, I.R. Homologous series of $C_1$-$C_{10}$ monocarboxylic acids and $C_1$-$C_6$ carbonyls in Los Angeles air and motor vehicle exhausts. *Atmos. Environ.* **2000**, *34*, 4175–4191.

88. Kawamura, K.; Steinberg, S.; Ng, L.; Kaplan, I.R. Wet deposition of low molecular weight mono- and di-carboxylic acids, aldehydes and inorganic species in Los Angeles. *Atmos. Environ.* **2001**, *35*, 3917–3926.

89. Marinoni, A.; Laj, P.; Sellegri, K.; Mailhot, G. Cloud chemistry at puy de Dôme: Variability and relationships with environmental factors. *Atmos. Chem. Phys.* **2004**, *4*, 715–728.

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

91. Herlihy, L.J.; Galloway, J.N.; Mills, A.L. Bacterial utilization of formic and acetic acid in the rainwater. *Atmos. Environ.* **1987**, *21*, 2397–2402.

92. Kawamura, K.; Kaplan, I.R. Stabilities of carboxylic acids and phenols in Los Angeles rainwaters during storage. *Water Res.* **1990**, *24*, 1419–1423.

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

94. Durand, S.; Amato, P.; Sancelme, M.; Delort, A.-M.; Combourieu, B.; Besse-Hoggan, P. First isolation and characterization of a bacterial strain that biotransforms the herbicide mesotrione. *Lett. Appl. Microbiol.* **2006**, *43*, 222–228.

95. Fuzzi, S.; Andreae, M.O.; Huebert, B.J.; Kulmala, M.; Bond, T.C.; Boy, M.; Doherty, S.J.; Guenther, A.; Kanakidou, M.; Kawamura, K.; *et al.* Critical assessment of the current state of scientific knowledge, terminology, and research needs concerning the role of organic aerosols in the atmosphere, climate, and global change. *Atmos. Chem. Phys.* **2006**, *6*, 2017–2038.

96. Amann, R.I.; Ludwig, W.; Schleifer, K.H. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Rev.* **1995**, *59*, 143–169.

97.  Pace, N.R.; Stahl, D.A.; Lane, D.J.; Olsen, G.J. Analyzing natural microbial populations by rRNA sequences. *ASM News* **1985**, *51*, 4–12.

98.  Béjà, O.; Aravind, L.; Koonin, E.V.; Suzuki, M.T.; Hadd, A.; Nguyen, L.P.; Jovanovich, S.B.; Gates, C.M.; Feldman, R.A.; Spudich, J.L.; *et al.* Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science* **2000**, *289*, 1902–1906.

99.  Béjà, O.; Spudich, E.N.; Spudich, J.L.; Leclerc, M.; DeLong, E. Proteorhodopsin phototrophy in the ocean. *Nature* **2001**, *411*, 786–789.

100. Breitbart, M.; Salamon, P.; Andresen, B.; Mahaffy, J.M.; Segall, A.M.; Mead, D. Genomic analysis of uncultured marine viral communities. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14250–14255.

101. Cottrell, M.T.; Moore, J.A.; Kirchman, D.L. Chitinases from uncultured marine microorganisms. *Appl. Environ. Microbiol.* **1999**, *65*, 2553–2557.

102. Gillespie, D.E.; Brady, S.F.; Bettermann, A.D.; Cianciotto, N.P.; Liles, M.R.; Rondon, M.R.; Clardy, J.; Goodman, R.M.; Handelsman, J. Isolation of antibiotics turbomycin A and B from a metagenomic library of soil microbial DNA. *Appl. Environ. Microbiol.* **2002**, *68*, 4301–4306.

103. Hugenholtz, P.; Tyson, G.W. Microbiology: Metagenomics. *Nature* **2008**, *455*, 481–483.

104. Schmeisser, C.; Steele, H.; Streit, W.R. Metagenomics, biotechnology with non-culturable microbes. *Appl. Microbiol. Biotechnol.* **2007**, *75*, 955–962.

105. Warnecke, F.; Luginbühl, P.; Ivanova, N.; Ghassemian, M.; Richardson, T.H.; Stege, J.T.; Cayouette, M.; McHardy, A.C.; Djordjevic, G.; Aboushadi, N.; *et al.* Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* **2007**, *450*, 560–565.

106. Woyke, T.; Teeling, H.; Ivanova, N.N.; Huntemann, M.; Richter, M.; Gloeckner, F.O.; Boffelli, D.; Anderson, I.J.; Barry, K.W.; Shapiro, H.J.; *et al.* Symbiosis insights through metagenomic analysis of a microbial consortium. *Nature* **2006**, *443*, 950–955.

107. Joint Genome Institute, Metagenomics Program, Exploration of Microbial Communities. Available online: http://genome.jgi.doe.gov/ (accessed on 12 January 2012).

108. Tringe, S.G.; Zhang, T.; Liu, X.; Yu, Y.; Lee, W.H.; Yap, J.; Yao, F.; Suan, S.T.; Ing, S.K.; Haynes, M.; *et al.* The airborne metagenome in an indoor urban environment. *PLoS One* **2008**, *3*, doi:10.1371/journal.pone.0001862.