Biodiversity: are microbial species threatened?
Commentary

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Introduction

A well known microbiologist and I were talking recently about microbial diversity. He asked me, "Microbiologists are not concerned about endangered species, are we?" His attitude reflects the view of many microbiologists. Whereas botanists and zoologists, as well as the public at large, know that many plant and animal species are threatened with extinction, the question as it pertains to microorganisms is not so readily answered. In this article I will attempt to explain the current views of microbiologists on this subject, which touches on a number of areas that are poorly understood and in need of further research.

At the outset, the diversity of microbial life deserves some comment. Molecular phylogenic studies based on ribosomal RNA sequences indicate that microorganisms are members of all three Domains of life [1]. The Domain Bacteria (or Eubacteria) includes most of the bacteria typically found in soil and aquatic habitats and the animal and plant pathogens and symbionts. The Domain Archaea contains the methane-producing bacteria, the extreme halophiles, and the hyperthermophilic bacteria that grow in hot springs and marine hydrothermal vents. Most of the diversity within the third Domain (Eucarya) is also microbial, consisting of the various protistan groups, the fungi, and the algae. Indeed, the tree of life is largely a tree of microorganisms, illustrating that much of the diversity on Earth is microbial with the plants and animals appearing as small, terminal branches.

Species extinction can be either a natural process, whereby one species is replaced by another through natural selection pressures, or it can be the result of extreme pressures, such as loss of habitat due to anthropogenic practices such as deforestation, overharvesting, and excessive hunting. Species extinction can also be caused by unusual natural phenomena such as hurricanes, volcanic eruptions, or asteroid impacts that have a cataclysmic affect on some area containing endemic species.

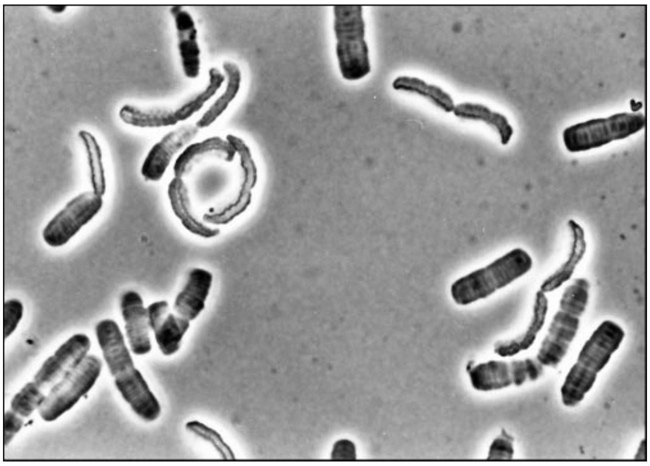
There is a simple answer to this question of whether microorganisms are threatened, and that answer is "Yes, some microbial species are threatened." The best evidence for endangered microorganisms comes from studies of symbionts or pathogens of plants and animals that are themselves threatened. For example, several fungal species are associated with each species of plant. Some species grow on the roots, others on the bark, others on the leaves and so forth. The ratio mycologists use is 1:6; that is, on average, six fungal species are found on each species of plant (see [2]). Thus, if a plant species is endangered, then its microbial symbionts are threatened as well. One example of this effect is the fungus *Bridgeoporus nobilissimus*, which characteristically grows on old growth noble fir in the Pacific Northwest of the United States [3]. This polypore, commonly called the 'Fuzzy Sandozi' is the largest fungus in North America, with some fruiting bodies weighing more than 130 kg. The old growth forests in which this species occurs have been drastically reduced, so the existence of this species is threatened. Fortunately, some research has been funded to examine this species, which is listed as an endangered species by the state of Oregon. Similarly endangered fungi are on national Red Lists of threatened and endangered species in several countries in Europe [4,5] as well as in the United States. Although loss of habitat is the culprit in most instances, some mycorrhizal fungi are threatened by excessive fertilization in countries such as The Netherlands [6].

The situation is very similar for lichens, many of which are endangered due to habitat loss. Some species, such as the Rock Gnome Lichen (*Gymnoderma lineare*) in Tennessee and North Carolina, are threatened by air pollution [7]. Likewise, microbial symbionts and pathogens of endangered animals are also threatened, although specific information on protistan and bacterial species is difficult to find.

Humans constitute a special group of the animal kingdom. Through human medicine and science it is possible to effect the extinction of microbial species that cause disease. Even though *Homo sapiens* does not appear to be threatened, some of its pathogens certainly are, such as the smallpox virus currently held only in American and Russian virus collections. The attitude of most microbiologists and the general public, too, regarding pathogens is "good riddance". Indeed, it is considered a victory of human science and technology that microbial species that pose a threat to humans can be exterminated. The success of driving microbial pathogens to extinction has become a cause of great celebration and other pathogenic microorganisms, such as *Mycobacterium leprae*, the causative agent of leprosy, are on the World Health Organization blacklist for future eradication.

Even some benign human microbial symbionts are also endangered, if not on the brink of extinction. One example is a harmless symbiont of humans, *Simonsiella muelleri* (Figure 1). The genus *Simonsiella* is a normal inhabitant of the oral cavities of humans, cats, dogs, sheep and other mammals [8,9]. Each animal studied has its own distinct species; however, the human species, *S. muelleri*, is rarely encountered in people from Western countries at this time (D Kuhn, personal communication). Apparently, *S. muelleri* has been largely eliminated from humans by modern dental hygiene practices or diet, or a combination of the two. The species is found only in humans from remote areas, such as native Americans from Alaska. Thus, it appears that *S. muelleri* is threatened with extinction. Does anyone care that this has happened? Will humans lose anything from the extinction of this species? One possibility is that we may become more susceptible to oral infections such as periodontitis and tooth decay. The importance of this may never be realized unless or until some social upheaval or other disruption places Western civilization in jeopardy.

Figure 1



A phase contrast photomicrograph showing several multicellular filaments of the gliding bacterium *Simonsiella muelleri*. This strain, obtained from an Alaskan native, was isolated on blood agar plates. Unlike other bacteria, this watchband-shaped organism exhibits dorsal-ventral asymmetry. The thinner, curved filaments are on their sides (ventral, gliding surface on concave side) whereas the wider filaments show their natural appearance when viewed from above. Filament diameter is about 5 μm.

Most microorganisms are not symbionts of plants and animals, so in a sense the positive answer given above applies only to a select group of them. Nonetheless, one could argue that the phenomenon of endangered symbiotic species is illustrative of a more general phenomenon: microorganisms, both symbiotic and free-living, are endangered when their niche is threatened.

Because microorganisms existed on Earth for two to three billion years before plants and animals evolved, there are many free-living microorganisms (such as lichens) or microorganisms that live in close-knit consortial associations with other microorganisms. Furthermore, these free-living organisms are a very important component of the biosphere. Their ancestors produced the original biosphere of Earth. Not only have they made conditions suitable for the evolution and existence of macroscopic life forms, but they also continue to drive and profoundly influence many of the essential biogeochemical cycles. The initial question should be rephrased, therefore: “Are free-living microbial species threatened with extinction?” This is one of the most provocative questions in microbiology, and apart from known examples of threatened saprophytic fungi from soil, dung, and wood habitats [4], we have few answers.

Differences between microorganisms and plants and animals

Before treating this subject in greater detail, it is worthwhile considering some of the differences between plants and animals on the one hand and microorganisms, particularly bacteria, on the other. As these differences have a strong bearing on the topic of endangered species, understanding them will help explain the views of microbiologists on species extinction. The differences discussed below include the issues of species definition and the arbitrary species concept, population size, biogeography, and kinship.

Species definition and the arbitrary species concept

A major distinction between microorganisms and plants and animals concerns the definition of a species. For example, the currently accepted species definition of bacteria is based on DNA–DNA reassociation. Strains that exhibit at least 70% reassociation by this procedure are regarded as members of the same species [10]. This is a much broader definition of a species than that used for primates, which, like that for most plants and animals, has been based on phenotypic features and ability to interbreed. The DNA–DNA hybridization value between *Homo sapiens* and the closely related species, the chimpanzee (*Pan troglodytes*) is 98.4% (1.6% divergent) [11,12]. For comparison, here are the DNA–DNA hybridization similarities between *Homo sapiens* and other primates: Gorilla 97.7%, Orangutan 96.5%, Siamang gibbon 95.5, Hamadras baboon 92.7%; Lemur 78% [11,12].

The implications that these differing species definitions have on our views of endangered species is dramatic. If we apply the bacterial species definition to primates, then all primates listed above would comprise a single species — in short, there would be only one cosmopolitan species. Furthermore, with the large population size of humans on Earth, one would conclude that none of Earth’s primates are currently endangered, which is clearly absurd.

The major reason bacteriologists have chosen such a broad definition of a bacterial species is that it is often difficult to find suitable phenotypic markers to distinguish strains from one another when they are closely related. Because of the need for rapid identification in clinical laboratories, readily determinable tests are required. Since DNA–DNA reassociation tests cannot be performed quickly, they are not used for routine identification in clinical labs, which relies on phenotypic tests. This may change as molecular probes for pathogenic strains and species become more widely available. In the meantime, bacteriologists continue to use a very broad definition for a species.

Many microbiologists view the species concept in a way that is quite different from that of botanists and zoologists. Whereas plant and animal species are discrete entities, some microbiologists view bacteria as an almost endless continuum of varieties. By this admittedly arguable viewpoint, which I refer to as the arbitrary species concept, a single strain is selected from the continuum in nature. This strain is identified as the type strain of the species and those strains that are sufficiently similar to it, on the basis of DNA–DNA reassociation, are considered members of this same arbitrary species. As a result, there is no species entity *per se*, but rather one that has been chosen artificially from this continuous spectrum of varieties.

This arbitrary species concept is derived, in part, from the differences in genetic makeup of bacteria when compared to eucaryotic organisms. In contrast to those of eucaryotes, bacterial genomes are smaller and they are also haploid. Genetic features can be transferred among quite distantly related bacteria via various genetic exchange mechanisms such as transformation and conjugation. Genetic features may reside in the cell on a plasmid or become incorporated into the bacterial chromosome. Genetically exchanged features can be rather remarkable and have a major impact on the characteristics of the bacteria that acquire them. For example, some pathogenic species, such as *Bacillus anthracis* and *Corynebacterium diphtheriae*, are differentiated from nonpathogenic species and strains only by virtue of their plasmid-borne virulence factors.

Furthermore, speciation processes in bacteria may differ in significant ways from that of plants, animals, and even eucaryotic microorganisms. For example, genetic exchange in bacteria is regarded to play a different role in evolution from that in plants and animals [13]. This is because genetic exchange between procaryotes in nature is estimated to be very rare (10^{-7} to 10^{-8} per gene per genome per generation) and the amount of genetic information exchanged is small. Moreover, when gene exchange does occur in bacteria, it can occur across much greater evolutionary distances (i.e. a higher percent divergent) than in plants and animals. Mutation also plays a significant and somewhat different role in bacterial evolution. Mutational events that favor a strain in a niche will be immediately expressed in haploid bacterial genomes, and with the short generation times found in bacteria can result in the rapid displacement of a parental genotype [14]. Thus, in bacteria, this adaptive mutation has resulted in a purging of the parental genotype diversity, a phenomenon that does not occur in plants and animals.

In accordance with the arbitrary species concept and the genetic exchange considerations discussed above, the loss of a bacterial species is of little concern, because in the continuum of related species the genes and functions of a ‘threatened species’ still exist and are not endangered at all. What with the rapid growth rates of bacteria, the ‘species’ that was lost could be quickly regenerated, providing a niche was available.

Population size and the paucity of species

It is estimated that each human carries in his or her intestinal tract 1–10 billion single-celled *Escherichia coli* organisms. This population of *E. coli* in a single individual animal is comparable to the entire population of humans on the planet. Furthermore, *E. coli* is found in many warm-blooded animals, so there are staggering numbers of this species on Earth. *E. coli* is not even the most numerous species of bacteria found in the intestinal tract of humans and other animals.

Similar population densities of free-living species resident in soils, muds, and aquatic habitats on Earth can be calculated. Soil bacterial species may occur in concentrations of 10^6 to 10^7 per gram of soil. When this is multiplied by the metric tons of soil in a biome, the numbers of individuals of a single species can be gigantic. These large population sizes provide a disquieting argument for those who might initially express anxiety over endangered microbial species.

Compounding the problem of population sizes and the species definition is our poor understanding of the current diversity of species that exist on Earth. For example, only 4000 species of bacteria have been described, whereas minimum estimates of numbers range into the millions [15]. Low numbers are also reported for other microbial groups. These low figures indicate that microbiologists have described only a minor fraction of the microbial world.

Biogeography

Because of the broad species definition, many free-living bacteria are considered cosmopolitan in distribution, a view that is consistent with that of LMG Baas-Becking [16]. If most bacteria are cosmopolitan, then the gigantic numbers of soil or aquatic bacteria found in similar ecosystems of one continent or ocean can be multiplied by the number of continents or oceans that contain similar ecosystems. The conclusion is that these cosmopolitan bacteria and other microorganisms are widespread and therefore not considered to be threatened. It should be noted, however, that Baas-Becking's hypothesis has not been challenged intensively by microbial taxonomists using molecular biological approaches; therefore, it is premature to conclude that endemic species do not occur on Earth.

Kinship

One of the major differences between microorganisms and plants and animals is the emotive issue of kinship. Humans share a strong kinship with many animals and, to a lesser extent, most plants. This attitude is reasonable in that these living organisms are part of our everyday experience. Our fondness for another species is dependent on a number of factors. In part it depends on how closely the other species is related to us phylogenetically. We share a special kinship with other primates, who are our closest relatives, and we are also particularly fond of other mammals, the 'warm and fuzzy' ones, many of which are threatened.

The relationship to humans in the food chain is another factor. We have strong affection for grazing mammals such as cattle and whales for they, as herbivores, are nonthreatening to us. Smaller nonthreatening carnivorous mammals, such as domestic cats and dogs, are more intelligent than large grazing mammals and are therefore considered excellent pets. In contrast, animals such as fish and invertebrates, which are quite different phylogenetically and occupy a different position in the food chain, do not evoke such strong feelings of kinship.

Although most may not be 'tree huggers', humans share a strong kinship with plants. Plants, as primary producers, are our ultimate source of food either directly (as foodstuff) or indirectly (through being eaten by herbivores and carnivores). For many millenia humans have cultivated plants to better ensure our survival.

Microorganisms are generally so small they are unnoticed by humans. Throughout our lives we are not acquainted with them in the same manner we are with plants and animals. Indeed, even those symbionts such as our intestinal microbiota, which are critical to our digestion of foods and nutrition, are given only token acknowledgement by most humans until the community has been eradicated by unwise antibiotic therapy leading to infections by *Clostridium difficile*. Yet, these unheralded microorganisms live more closely with us physically than any other organisms.

Procaryotic and eucaryotic microorganisms are not, and never will be, the subjects of concern in the same sense that animal rights advocates demonstrate for mammals and environmentalists march for endangered animal and plant species. Who cares that our phylogenetic ancestors were microorganisms and our eucaryotic cells, mitochondria, and genes are derived in large part from Eubacterial and Archaeal lineages? Microorganisms evoke many negative views because they cause disease and food spoilage. This is not to say that microorganisms are not beneficial to us and that our society would not miss their activities. Indeed, we depend on them to carry out important processes in our everyday lives, such as dairy and plant fermentations, leavening bread, antibiotic production, and agricultural processes to name a few. Most humans, however, are not aware these are microbial activities. Because microorganisms rank so low on the kinship scale, the demise of a microbial species is not an emotional issue for humans.

The differences cited above between microorganisms and macroorganisms regarding species definitions, population size, biogeography, and kinship all tend to minimize concerns that microbiologists might have about the extinction of free-living microbial species. Indeed, most microbiologists believe that the extinction of microbial species is a process that is occurring continually as a natural course of events during evolution, natural selection, and speciation. There is a cautionary note to this: the appalling reality is that we have described so few species; many species may be threatened whose existence are still unknown.

Threatened habitats versus threatened species

Most microbiologists believe it is important to protect unusual or unique habitats. One prime example of this is the thermal habitats on Earth, areas such as Yellowstone National Park in the United States. These areas, which harbor some of the most fascinating Archaea [17], are threatened by industrial developments of steam and electrical power generation. Likewise, sea ice microbial communities harbor the most psychrophilic (cold-loving) microorganisms known [18–20]. Because many of the sea ice bacteria are obligate psychrophiles, their existence is threatened by global warming.

The loss of these and other habitats could result in the loss of many species, especially if endemic species exist. Although proposals have been made to preserve samples from such habitats, the most satisfactory manner in which to preserve the organisms is through protection of the environment and thereby the natural community itself, if at all possible. So, even though many microbiologists express little concern about protecting individual free-living species, most would be alarmed if entire communities of microorganisms were threatened.

Principle of fecundity of function

One of the more remarkable aspects of microorganisms is the apparent persistence of functional attributes over evolutionary time. It seems that, no matter how inefficient a process is, as long as energy can be generated from a transformation, microorganisms will persevere and refine their ability to occupy a niche. Anoxygenic photosynthesis is an example of this phenomenon.

Photosynthesis apparently evolved in the Eubacteria at least 3.2 billion years ago. Photosynthesis is currently found in several of the major phylogenetic groups of Eubacteria, including the Green Filamentous bacteria, the Green Sulfur bacteria, the Proteobacteria, the Gram-positive bacteria, and the Cyanobacteria. It is not clear which photosynthetic bacterial group evolved first, but most bacteriologists consider that anoxygenic photosynthesis occurred initially. Then later on, perhaps 2.5 billion years ago, the oxygenic photosynthetic bacterial group, the Cyanobacteria, evolved, resulting in the build-up of oxygen in the atmosphere [21]. Algal and plant photosynthesis is also oxygenic by virtue of the chloroplast which evolved from endosymbiotic cyanobacteria.

Oxygenic photosynthesis now dominates photosynthesis on Earth. It is a very efficient process compared with anoxygenic photosynthesis. Nonetheless, several groups of anoxygenic photosynthetic bacteria and their less efficient photosynthetic processes persist, despite the fact that they have been relegated to low light conditions and depend on other microbial groups to produce hydrogen sulfide or hydrogen gas, which serve as reductants for carbon dioxide fixation. Of course, they must continue to refine their adaptation to the ever changing nuances of their niche or they may be overtaken by a competitor. Even so, it is remarkable how diverse and fecund these anoxygenic photosynthetic processes are. Anoxygenic photosynthetic bacteria are but one example illustrating the fecundity of function, or the persistence of physiological abilities through evolution, that is found in bacteria. Some of the best examples involve anaerobic species that are involved in processes that can only yield energy when two or more species work together in a consortium.

The implication of the fecundity of function principle is that, if some catastrophic event threatened life on Earth, a variety of energy generating mechanisms that still persist in some obscure procaryotic group may again come to the fore. It is highly likely that events such as the collision of Earth with a large asteroid would not imperil all life. Even if aerobic photosynthetic processes were totally annihilated for millenia, anaerobic bacterial activities would likely persist, especially those that reside in deep subterranean environments in which hydrogen gas generated abiotically is used as an energy source [22]. In such a scenario, the anaerobes would continue to evolve to refine their functionality in a newly evolving biosphere. In this sense, biological activity on Earth would be protected despite the fact that Earth's biosphere was dramatically altered.

The fecundity of function principle probably applies equally well to all organisms, including plants and animals. Macroscopic organisms that are entirely aerobic, however, would not be as able to survive such cataclysmic events as the bacteria which occupy such a physiologically diverse and ancient lineage. Thus, it does not seem possible that plants and animals could survive an asteroid impact so severe as to eliminate oxygenic photosynthesis for extended periods of time. Nevertheless, the conditions of the severely affected biosphere with its residual procaryotic communities would once again favor the evolution of eucaryotic organisms leading again to the evolution of land plants and animals. The nature of these new organisms might differ dramatically, however, from present-day plant and animal species.

It is noteworthy that the extinction of critical members of the biogeochemical cycles could pose a serious threat to Earth's biosphere and biota. A hypothetical example of this is the loss of the nitrifying bacteria that are responsible for oxidation of nitrite to nitrate. These few species obtain their energy from the oxidation of nitrite and, as chemoautotrophs, use carbon dioxide as their principal source of carbon. The loss of this functional group would result in the build-up of nitrite in the biosphere. Nitrite is toxic to many organisms and could pose a threat to many existing species of microorganisms, plants and animals. This is but one example of a microbial extinction event that could threaten human existence. Because of the principle of fecundity of function, it appears unlikely that this sort of event would ever occur.

Conclusion

Our knowledge of microbial diversity, particularly bacterial diversity, is so meagre that we do not yet know if and when most species are threatened. We do not know this, in part, because we still do not have even a reasonable estimate of the total number of species that exist. Only about 4000 bacterial species have been described. Some of my colleagues believe about 100 000 bacterial species actually exist, others a million, and some more than a billion. Until microbiologists can provide meaningful estimates of global diversity from thorough studies of selected habitats and a better understanding of the importance of biogeography, it will be fruitless to estimate the degree to which microbial species on Earth are threatened. Our very inability to answer the question of threatened microbial species cries loudly for the need for microbial systematists and ecologists to begin to address the exciting challenges regarding our knowledge of the extent of microbial diversity on Earth. Fortunately, using molecular tools such as *in situ* PCR technology [23] and improved cultivation procedures [24], we can now approach this important issue in more meaningful ways.

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