The camels of the prokaryotic world

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ABSTRACT

Hyper-arid deserts can be considered extreme environments with respect to their conditions of low humidity and low water activity. Traditionally we associate camels and cacti with the ability to survive in arid environments for extended periods without replenishing their water supply. Little is known about the diversity and ecology of prokaryotes in arid or hyper-arid regions. Using culturing and non-culturing molecular approaches, the presence of prokaryotes in the soils of such arid environments has been demonstrated. The numbers of culturable microorganisms in environments of varying water activity has been determined and representatives identified. The majority of organisms cultured are spore-forming Gram positive bacteria. In some cases similar organisms have been detected at the molecular level allowing the taxonomic status of such environtaxa to be predicted. A proportion of the organisms isolated were ionizing radiation resistant enforcing the link between desiccation resistance and ionizing radiation resistance.

Introduction

Extreme environments

The description of certain environments as “extreme” results from the perception of environmental conditions that are considered normal to be those that are capable of supporting life. This definition is influenced by our view that higher eukaryotes could not survive or reproduce in extreme environments. These environments contain conditions at the limits either side of the physiological normal. Environments exhibiting extremes of temperature, pH, and salinity, or a combination of these extremes, fall into the extreme environment category. Numerous reports of the isolation or detection of prokaryotes from environments considered extreme indicate the presence of life in these environments. The microorganisms living in extreme environments have been put under the banner of “extremophiles”. Water activity, or the lack of it, is one physical parameter that has received little study with respect to the survival of microorganisms under extreme conditions. In order for cells to carry out metabolism that allows growth and reproduction, water is required, optimally at a water content of around 80% [5]. Dose [5] has pointed out the difference between the growth of an organism in an aqueous solution in which an equilibrium can develop, and growth of an organism exposed to air at 80% relative humidity, with the subsequent decrease in the cellular water content to 10-20% of the normal. The survival of microorganisms under conditions of desiccation can be attributed to survival strategies such as synthesis of desiccation resistant proteins, accumulation of non-reducing sugars or the formation of dormant life stages such as endospores [5]. The Earth’s atmosphere in moderate climates has a relative humidity of 50–90%, while in

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desert environments this drops to 5-30% [5]. In the laboratory, a silica gel desiccator will have a relative humidity of 5% which can be reduced to ~0% under high vacuum. Environmental levels of ~0% relative humidity will be found on the Martian surface or in free space [29]. The extreme conditions of desiccation in free space or on the Martian surface can be considered obstacles to the existence or survival of microorganisms on Mars. Traditionally we associate the camel or cacti with the ability to survive in arid environments for extended periods without replenishing their water supply.

Arid environments on Earth

The world's dry or arid lands are grouped in the general term "deserts". Although the word "desert" means "solitary" (from the Latin *desertus*), it is applied to barren landscapes in which life forms can only rarely subsist. The definition of a desert has been refined over time, but the underlying components are the lack of water, and impracticality of agriculture and population support.

Examination of the literature shows that the two criteria taken into consideration in the definition or classification of arid regions are climate and vegetation. In 1953, Meigs [15] developed a classification for arid lands in response to the UNESCO Arid Zone Research Programme. This classification balances water input in the form of rainfall with water loss from evaporation from the environment and plants. Three categories of arid lands fall within the classification: semi-arid, arid and hyper-arid. Semi-arid regions receive less than 600mm (24 inches) of rainfall annually. Less than 200mm (8 inches) of precipitation occurs in arid lands, while in hyper-arid lands less than 25mm (1 inch) is received. A classification scheme based on the type of vegetation cover was proposed by Shantz [24]. Extreme arid lands, under this definition, are devoid of vegetation. The presence of a desert grass savannah indicates an arid region, while semi-arid lands contain sclerophyll bushland, thorn forest and short grass. Both of these classification systems indicate that arid lands make up around one-third of the Earth's land surface. These arid lands, especially the hyper-arid regions, provide the most extreme natural environments on earth with respect to the degree of desiccation.

Microbiology of arid environments

The majority of microbiological studies of arid environments have been limited to basic isolation and enumeration studies. The studies presented in the literature fall into four categories: (*i*) description of the cultured bacterial components of a given sample; (*ii*) selective isolation and identification of actinomycetes [2, 3, 7, 8, 10, 26]; (*iii*) examination of the role of microorganisms in the formation of desert varnish [4, 11, 13, 19, 20, 27]; and (*iv*) detection of anaerobic bacteria by measurement of their physiological activity [21]. These studies were carried out on material from arid environments around the world, but none of the studies involved a comprehensive approach using both culturing and culture independent techniques.

The link between desiccation and DNA damage

The *Deinococcus* species represent a deep branching lineage of the domain Bacteria [23]. These species are noted for their capacity to resist the lethal effects of ionizing radiation [16, 17, 25], surviving 0.5 MRad γ radiation without loss of viability [18], with survivors routinely recovered from cultures exposed to as much as 2.0 MRad [1, 12]. Mattimore and Battista [14] demonstrated that ionizing radiation sensitive strains of *D. radiodurans* are

sensitive to desiccation and proposed that ionizing radiation resistance and desiccation resistance are functionally interrelated phenomena and that by losing the ability to repair ionizing radiation-induced DNA damage, *D. radiodurans* is sensitized to the lethal effects of desiccation.

In a previously described study the phylogenetic diversity of the 16S rDNAs amplified by PCR from DNA extracted from the desert soil was determined [22]. The majority of the clone sequences were found to fall into three different groups. Two of these clusters were within the actinomycetes (*Geodermatophilus* and *Rubrobacter* lineages) and the other within the beta subclass of the Proteobacteria. Since these sequences can be placed in a taxonomic context and they are known to originate from the environmental sample under investigation, we will refer to the source organisms of the sequences as "environtaxa". Of the 221 clone sequences analyzed 44% grouped within the radiation of the genus *Rubrobacter*, a genus presently comprised of two species both of which have been demonstrated to be ionizing radiation resistant [28]. This led us to devise an isolation and enrichment strategy to attempt to recover these environtaxa as pure cultures. In order to obtain additional insight into the link between ionizing radiation resistance and desiccation resistance we used a selective enrichment strategy to isolate ionizing radiation resistant organisms from a naturally occurring desiccated environment. By using various doses of ionizing and ultraviolet radiation as a selective strategy, we aimed to recover the components of the prokaryotic community that are radiation resistant.

Methods

Soil samples were obtained from two arid regions, the Atacama Desert, Chile and the Sonoran Desert, USA. The Atacama Desert is considered to be one of the driest places on Earth, receiving essentially no rainfall [9]. Only a few such desert areas, where no precipitation has occurred for decades, are known to exist. The relative humidity in the Atacama Desert varies between 5% and 30% [6]. This region falls into the category of an extreme or hyper-arid environment. The Sonoran desert represents an arid region, receiving on average less than 200mm of precipitation annually. The samples were removed from the surface layer with a sterile trowel and placed in sterile containers. Samples were stored at ambient temperature until further investigation.

Samples were dilution plated on nutrient agar to determine the number of cfu/g in each sample. Selected colonies were picked from the dilution plates and the isolates maintained for further analysis. Identity of the isolates was determined by partial 16S rRNA gene sequence determination and analysis. In order to select for radiation resistant organisms and to determine the proportion of the colony forming units that are ionizing radiation resistant, soil samples were irradiated with doses ranging from 0.1MRad to 2.5MRad of ionizing radiation (^{60}Co).

Results

Numbers and identity of isolates

The results of this preliminary study indicate that microorganisms are present and that many are in a viable and culturable state within the desert soil samples studied. The total number of culturable organisms recovered from the Atacama sample on nutrient agar incubated aerobically at 28°C over a period of 2-14 days was in the range 2.2×10^4 to 1.8×10^5 per gram soil. The majority of the isolates from the Atacama soil for which a

phylogenetic affiliation was determined fell within the radiation of the Gram positive phylum, either low G+C *Bacillus* species or high G+C actinomycetes. From the Sonoran Desert sample, the number of culturable organisms was around 10^6 per gram soil. The majority of isolates that were further characterized from the Sonoran sample were those that showed some degree of ionizing radiation resistance. These fell into four main groups, *Geodermatophilus*, *Deinococcus*, *Cytophaga* and the alpha subclass of the Proteobacteria. Many of the *Geodermatophilus* sequences were highly similar to those previously recovered by non-culturing molecular analyses [22].

Effect of radiation dose on the types of organisms recovered

Atacama soil samples were exposed to 0, 0.1, 0.3 and 0.5 MRad of ionizing radiation, plated, and the identity of the surviving organisms determined. Increasing radiation dose was found to significantly affect the type of isolate recovered after plating of the irradiated sample (Table 1). The proportion of isolates belonging to the *Geodermatophilus* group increased from 32-33% in the lower exposures to 77% for 0.3 MRad, and this taxon constituted 100% of isolates obtained after 0.5 MRad exposure. The members of the *Micrococcineae* decreased on exposure to 0.1 MRad, while the other Actinobacteria, the *Bacillus* group and the Gram-negative Bacteria increased as a percentage of the organisms cultivated, with increasing exposure.

Table 1. Identity of the strains isolated after irradiation of an Atacama Desert soil sample as a percentage of total organisms identified.

Taxon	γ - radiation dose (MRad)			
	untreated	0.1 MRad	0.3 MRad	0.5 MRad
Geodermatophilus group	33	32	77	100
Micrococcineae	40	16	0	0
Other Actinobacteria	16	30	3	0
Bacillus species	5	7	0	0
Deinococcus species	0	2	0	0
Gram negative bacteria	6	13	20	0

Effect of radiation dose on the proportion of surviving organisms

Sonoran Desert soil samples were exposed to radiation doses ranging between 0.1 and 2.5 MRad, and increasing in 0.2 MRad increments. The proportion of organisms surviving these treatments was determined by plating exposed samples on nutrient agar and comparing the number of colonies obtained with the number obtained from plating an untreated control. After 0.1 MRad exposure, 61% of the original number of colony-forming units had survived. Higher exposures resulted in a more dramatic decrease in percentage survivors, with only 18% survival after 0.5 MRad exposure. Less than 1% survival was observed after exposure at doses greater than 1.3 MRad. However, it is interesting to note that even at doses greater than 2.0 MRad as many as 0.05% of the original population survived. The majority of the survivors at doses greater than 1 MRad were members of four taxonomic groups, namely novel *Deinococcus* species, novel *Geodermatophilus* species, *Cytophaga* species, and alpha-Proteobacteria.

Conclusions

The results obtained in this study on the microbiology of these arid environments demonstrate that microorganisms are present and can be detected by culturing approaches. The numbers of organisms present have been shown to be low when compared to the values determined for soils from environments considered not to be extreme (e.g. agricultural or forest soils). Strain identity based on 16S rRNA gene sequence comparison indicates that the degree of diversity is limited in terms of its range across the bacterial tree, but within the groups recovered, a high degree of diversity exists, including numerous novel species of the genera *Deinococcus* and *Geodermatophilus*. When the results of this study are compared with the results of the non-culturing approach [22], it can be demonstrated that a given group of highly related prokaryotes can be recovered both as culturable entities and as environtaxa. The use of ionizing radiation as a selective agent has been shown in this study to result in the isolation of novel ionizing-radiation resistant organisms which would be overgrown under normal plating conditions. For the first time, we have demonstrated that members of the genus *Geodermatophilus* can survive ionizing radiation doses as high as those to which the classic radiation-resistant prokaryote *Deionococcus radiodurans* is resistant.

To conclude on a lighter note, and in reference to the title of this paper, we present the results of this study as evidence for the existence of prokaryotes in the same extreme environments where the thick-skinned camel and cacti flourish. The resistance of these prokaryotic organisms to ionizing radiation suggests them to be similarly thick-skinned.

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