Xerotolerant bacteria: surviving through a dry spell

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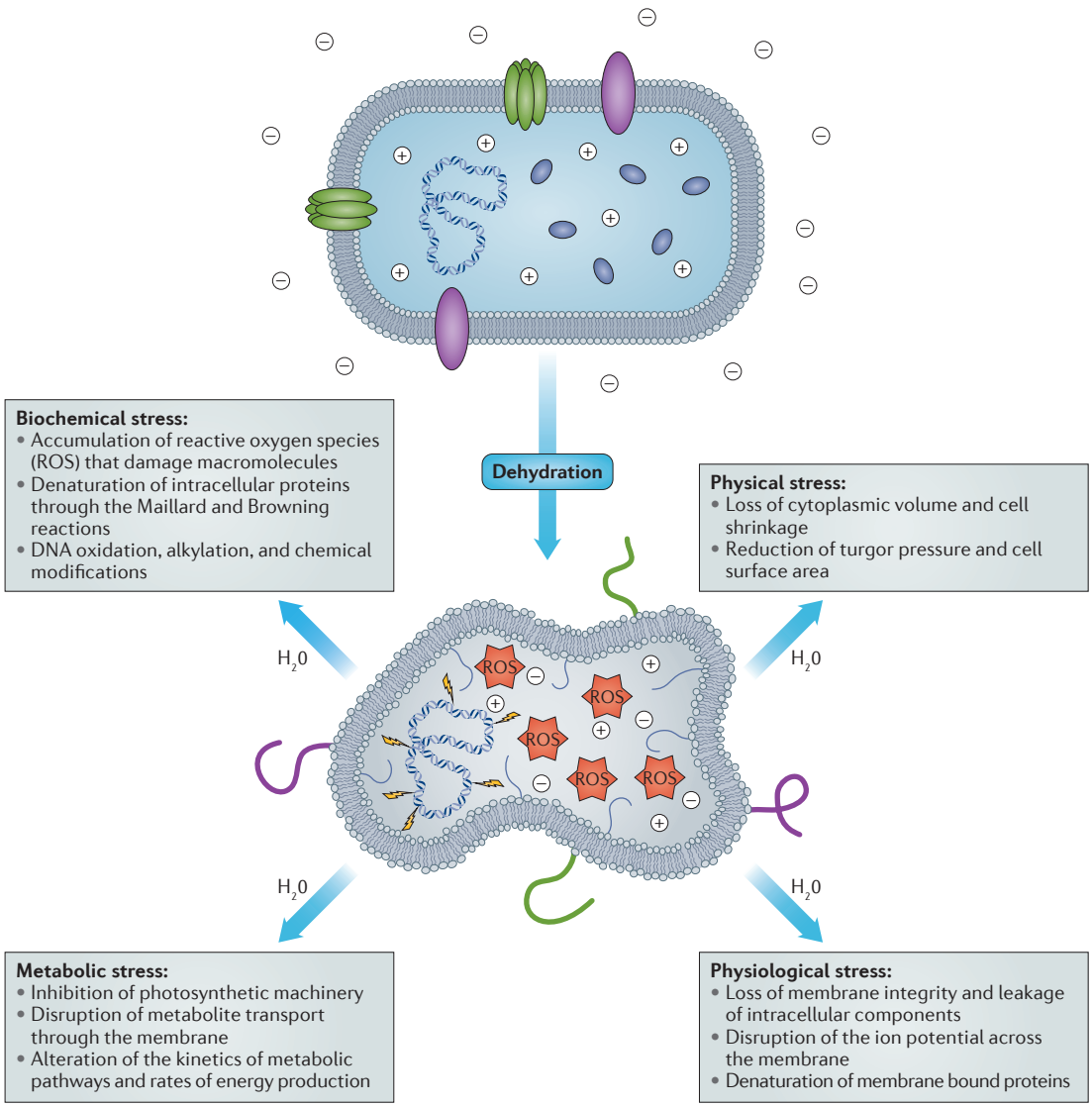
Abstract

Water is vital for many biological processes and is essential for all living organisms. However, numerous macroorganisms and microorganisms have adapted to survive in environments in which water is scarce; such organisms are collectively termed xerotolerant. With increasing global desertification due to climate change and human-driven desertification processes, it is becoming ever more important to understand how xerotolerant organisms cope with a lack of water. In this Review, we discuss the environmental, physiological and molecular adaptations that enable xerotolerant bacteria to survive in environments in which water is scarce and highlight insights from modern 'omics' technologies. Understanding xerotolerance will inform and hopefully aid efforts to regulate and even reverse desertification.

Water is essential for all living organisms as it is crucial for many biomolecular processes, including protein folding and stability, enzyme-substrate interactions and maintenance of the cell structure[1,2]. Therefore, exposure to xeric conditions can profoundly impede functioning and survival of the cell (FIG 1). Briefly, xeric stress, which is caused either by a lack of water (desiccation) or excessive solute concentrations (hypertonicity) in the surrounding environment, removes water from the cell and thereby causes biochemical, metabolic, physical and physiological stress. Yet, microorganisms living in hyper-arid deserts and in desiccated foods and macroorganisms, such as desert-dwelling plants, have developed intricate means to survive when only trace amounts of water are available. Most prokaryotes cannot actively divide at water activity (a_w) values below 0.91, and most fungi are no

Figure 1 | Effects of desiccation on the physiology and biochemistry of bacterial cells. Desiccating prokaryotic cells experience a decrease in surface area and shrinking of the capsular layer, which is linked to the rapid loss of cytoplasmic volume[71]. This loss of intracellular water reduces the turgor pressure, which in turn results in an increase in intracellular metabolites and ions, crowding of macromolecules and decreasing fluidity[2,78]. Membrane integrity is compromised due to an increase in van der Waals interactions between phospholipids, which are normally attenuated by the presence of water molecules attached to the phosphate headgroups. The resulting non-homogenous variations in gel-to-liquid transition temperature cause membrane fusion and disruption, changes in lamellar architecture of the membrane, protein aggregation, and macromolecular leakage upon rehydration[114]. Protein conformation and integrity are also disrupted by the removal of the water layers that normally interact with the surface of macromolecules, and through Maillard reactions, in which proteins become cross-linked and eventually irreversibly polymerised[115]. The resulting disruption of protein function impairs major biosynthesis, transport, repair pathways and results in the accumulation of free radicals. The sudden intracellular accumulation of reactive oxygen species (ROS) causes oxidative stress and consequently cell death[2]. The production of ROS is a direct result of dehydration, as the loss of membrane integrity leads to the disruption of the respiratory chain, the accumulation of superoxide ions (O_2-) and pH and ion imbalance[116]. Furthermore, the malfunction of transport proteins causes the intracellular accumulation of iron (Fe^{2+}), which in turn results in the production of hydroxyl radicals through the Fenton reaction[116]. ROS accumulation leads to biochemical cascades that re-enforce their detrimental effects, such as lipid peroxidation and production of reactive aldehydes that damage proteins through the Maillard reaction, direct protein modification through metal-catalysed oxidations, and DNA damage through chemical modifications, cross-linking and other lesions [116,117]. In this way, dehydration damages the DNA and disrupts DNA protection and DNA repair pathways (for example, Dps, H-NS and RecA)[77].



Box 1 | Current xerotolerance record holders

Traditionally, extremophilic eukaroytes were thought to be better at surviving under water stress than prokaryotes, with some fungal species being capable of cell division and germination at a far lower water activity values (a_w <0.7)[24] than the most xerotolerant haloarchaea (a_w = 0.75)[6]. However, this perspective is changing, as several archaeal and bacterial species have recently been shown to grow at a_w values comparable to the most extreme xerotolerant eukaryotes[3] (see table). Examples of extremely xerotolerant prokaryotes include the unclassified haloarchaeal strains GN-2 and GN-5, which can undergo cell division at a_w = 0.635, which is comparable to the levels empirically determined for the current xerotolerant eukaryote records holders, *Xeromyces bisporus* (a_w = 0.637)[104] and *Aspergillus penicillioides* (a_w = 0.585)[105]. Theoretical survival limits of ~0.61 a_w are suggested for bacteria, archaea and eukaryotes, which suggests that the same physicochemical constraints and water-activity limits apply to all three domains of life[3].

	Species	Lowest water activity (a_w) for cell division	Environmental source	References
Bacteria and Archaea	Haloarchaea GN-2	0.635	Solar salterns, Mexico	3
	Haloarchaea GN-5	0.635	Solar salterns, Mexico	3
	Halorhabdus utahensis DSM 12940	0.647	Salt Lake, USA	3
	Halobacterium strain 004.1	0.658	brine pool, UK	3
	Halorhodospira halophila DSM 244	0.66	Salt lake	3
	Salinibacter ruber DSM13855	0.725	Solar salterns, Spain	3
	Salisaeta longa DSM 21114	0.747	Dead Sea, Israel	3
Fungi	*Aspergillus penicillioides*	0.585	Raisins, Australia	7,107
	Xeromyces bisporus	0.637	Antique Wood, Thailand	6,7,3, 106
	Eurotium amstelodami FRR2792	0.656	Dates, Australia	7
	Chrysosporium xerophilium FRR 0530	0.686	High-moisture prunes, Australia	7
	Eurotium chevalieri PIL 119	0.71	Soiled prunes, Australia	7

metabolically active at a_w < 0.7, but in many of the more extreme environments studied, microorganisms have been found to cope with a_w levels well below these limits[3] (BOX 1). These organisms are collectively termed xerophiles, from the Greek *xēros* or 'dry', and *philos* meaning 'loving', due to the fact that they survive under stringent xeric conditions. However, in practical terms obligatory xerophiles do not exist, as these organisms invariably display optimal physiological and biochemical activities at much higher a_w Hence the term

xerotolerant might be more appropriate to describe organisms that are capable of withstanding xeric stress but do not require low a_w to thrive.

The arid zones where xerotolerant organisms survive constitute approximately 10% of the Earth's land surface and this area is projected to increase substantially as a result of climate change and human activity-driven desertification processes[4,5] (BOX 2). Xerotolerant microorganisms are not restricted to desert soils; they also occur in hypersaline aquatic environments in which high salt concentrations limit water availability, and in preserved foods[6]. Therefore, understanding the physiological, biochemical and molecular adaptation mechanisms that xerotolerant microorganisms use is relevant for environmental, food and medical sciences. Recent advances in sequencing technologies and metagenomics have allowed us to explore and compare the microbial diversity associated with these extreme environments and gain insights into how microorganisms survive in these harsh ecological niches (Table 1). For example, metagenomic surveys of hyper-arid deserts such as the Atacama and Antarctic deserts have revealed a high diversity of xerotolerant and mesophilic bacterial phyla, many of which cannot be detected through conventional culture-based methods[7-10]. Many of these bacteria are found in specialised niches, such as fissures, pores and cracks of rocky surfaces that provide shelter from abiotic stressors while creating a hydrated environment by trapping dew and fog that stimulates photoautotrophic and heterotrophic activity[11-16](FIG. 2). The biodiversity, spatial and temporal ecology, and niche specialization of these microbial communities have been extensively covered in recent literature[11-13,17] and therefore we will not discuss them in detail here.

Similarly, some preserved foods represent xeric environments in which a high osmotic pressure is caused by high concentration of salt and other solutes and low water content. Many preservation processes, such as drying and salt-curing, reduce water activity to levels that compromise microbial viability and prevent the growth of food-borne pathogens[18]. Nevertheless, some pathogenic bacteria, such as *Salmonella enterica*, *Cronobacter sakazakii* and *Listeria monocytogenes*, have been found to survive and even thrive in preserved foods, such as fruit conserves and peanut butter, for several years post-inoculation[18-21]. Similarly, pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae* can survive for weeks to years on dry surfaces including skin and clothing[22,23]. Therefore, understanding how bacteria survive xeric stress is key to the development of control strategies for these pathogens[20].

Box 2 | **The problem of desertification**

Desertification is defined as the process of land degradation of arid, semiarid, and dry sub-humid areas. It results from a combination of climate and human factors, many of which share synergistic relationships and vary demographically. Human factors such as unsustainable farming practices, deforestation for settlement and agricultural expansion and creation of artificial irrigation systems have been identified as major drivers of desertification. Desertification leads to soil salinization, exhaustion of soil nutrient banks, soil erosion through disturbance of the vegetation mesh that maintains surface cohesion, and ultimately to loss of fertility and biodiversity[106,107]. Climate change, which is intrinsically linked to human activity, is also seen as a major driver of desertification[106]. Global warming and the resulting temperature fluctuations, shifting rainfall patterns and increased periods of drought ultimately lead to loss of vegetation and the formation of sand seas, such as the Kalahari or the Sahara desert[106,108].

Desertification not only negatively affects biodiversity and ecology, but also has a severe effect on the socio-economic stability of human populations[109]. An estimated 52% of the global agricultural dryland, which directly supports 2.6 billion people, is moderately or severely affected by soil degradation. Prolonged desertification of this land will lead to a substantial decrease in global food production and consequently mass population displacement[109].

The microbial biodiversity in drylands is an important factor for limiting the process of desertification. Biocrusts, which are comprised of surface layers of cyanobacteria, lichens and mosses, are important for the stability and water retaining properties of the soil, and increase soil fertility through carbon and nitrogen cycling[110,111] (see figure). However, biocrusts are vulnerable to the effects of climate change, particularly shifts in patterns of precipitation and warming. Such changes can alter the life cycles, distribution, and functions of biocrust biota, and consequentlydisturb the carbon and nitrogen balance of the soil[112]. Efforts to maintain and restore the native soil biocrusts of drylands in risk of desertification can therefore be an effective tool for the ecological restoration of soils that have been degraded, as well as a for the prevention of desertification[113].

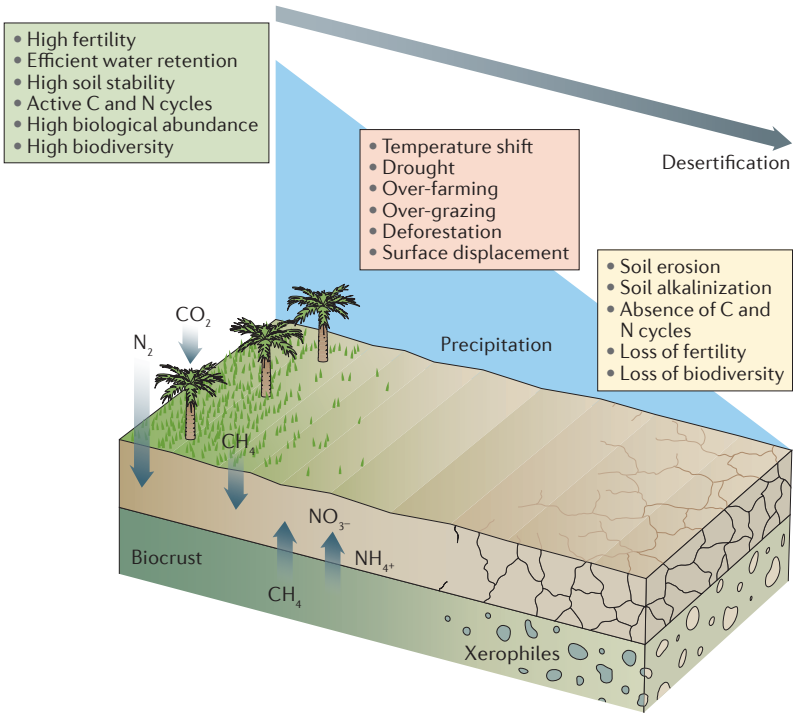


Figure 2 | Niches of xerotolerant communities. Xerotolerant microorganisms can be found in distinct niches along the surface and in the sub-layers of rocky landscapes of semi-arid and hyper-arid environments. 2A) Epiliths (A1) are found on the exposed surfaces of the rock and are dominated by lichens and mosses[53,55]. These communities are commonly found as multi-layered mats in which the more UV-sensitive photosynthetic cells are shielded by melanin- and mycosporine-producing biomass that operates as a sunscreen[55]. Chasmoendoliths (A2) and cryptoendoliths (A3) occupy the sheltered crevices and pores of porous rocks, respectively[52]. The physical buffering of the rock surface and the entrapment of moisture from precipitation and fog allow for a greater biodiversity in these communities, which include cyanobacteria, heterotrophic bacteria, algae, lichens, and free-living fungi[53,55]. Hypoliths (A4) are found on the ventral surfaces of translucent stones and are dominated by cyanobacteria and several heterotrophic bacteria, including acidobacteria and proteobacteria[11,53,55]. Hypolithic communities represent the dominant carbon and nitrogen fixers and biomass producers in many hyper-arid desert environments[55]. The access to sunlight is a determining factor to the establishment of prokaryotic communities. For example, colonies that are dominated by photo-autotrophic cyanobacteria preferentially reside in substrates in which sunlight can be accessed, whereas epiliths require mechanisms for radiation protection[53,55]. 2B) Hypolithic communities can be dominated by moss. 2C) Cyanobacteria can also dominate hypolithic communities. 2D) Electron microscopy imaging shows a cyanobacteria dominated biofilm community adherent to the rock, in which filamentous cyanobacteria (arrows) are predominant. 2E) Electron microscopy imaging shows an EPS matrix of a hypolithic community that contains cyanobacteria (arrows) and heterotrophic bacteria (arrowheads). Figures 2B, 2C, 2D, and 2E reproduced from Ref. 9.

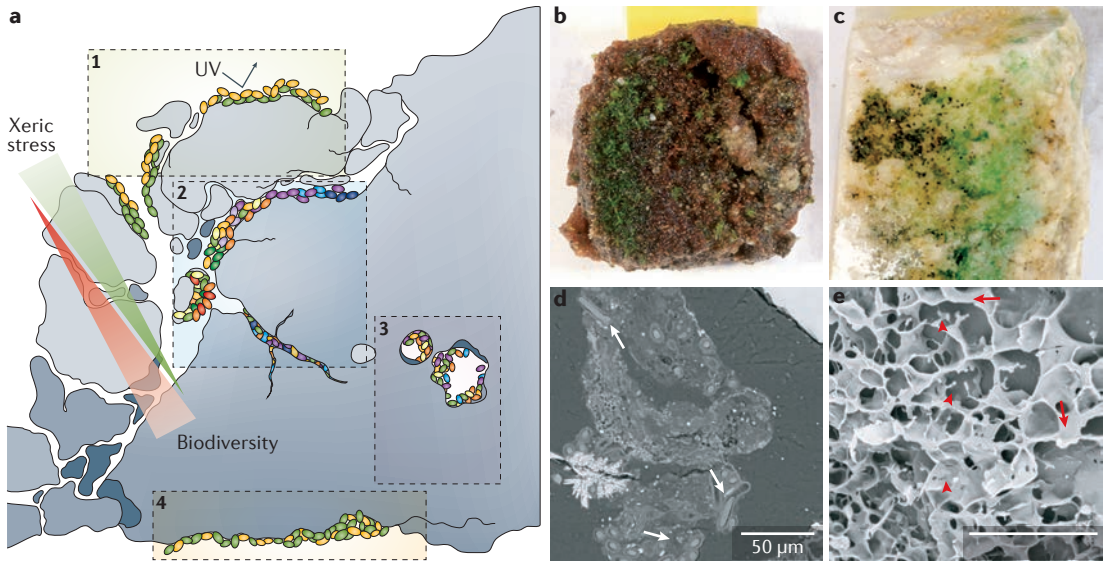


Figure 3| Adaptive mechanisms of xerotolerant bacteria. Generally, cells resist to desiccation by reducing energy consumption, preventing water loss and increasing water retention, and by protecting DNA and protein damage through the accumulation and expression of osmoprotectants. Metabolic activity shifts towards energy preservation, alternative carbon sources like fatty acids are used to produce higher ATP yields, and energy consuming processes such as flagellar motility are repressed[72,88]. Photosynthesis is also down-regulated to prevent the accumulation of oxygen and subsequent formation of reactive oxygen species (ROS), which are the main source of DNA damage during desiccation. Synergistically, ROS scavengers like superoxide dismutase (SOD) and catalases are up-regulated. DNA is also protected by the expression of DNA-binding proteins that act as a physical shield, and DNA repair proteins[18,77]. On the other hand, water loss triggers the accumulation of compatible solutes and salts that not only replace water as macromolecule and membrane stabilisers, but also prevent the formation of hydroxyl radicals by lowering intracellular diffusion rates [69,70]. Furthermore, phospholipid modifications make the cell membrane more capable of retaining water intracellularly[69]. Secreted EPS forms a protective biofilm that shields the cells from abiotic stresses and absorbs water[62,63]. Although the detailed molecular switches that activate these mechanisms are unknown, it has been suggested that alternative sigma factors, which are involved in general stress responses, are crucial for co-ordinating cellular adaptation to xeric stress[18,72].

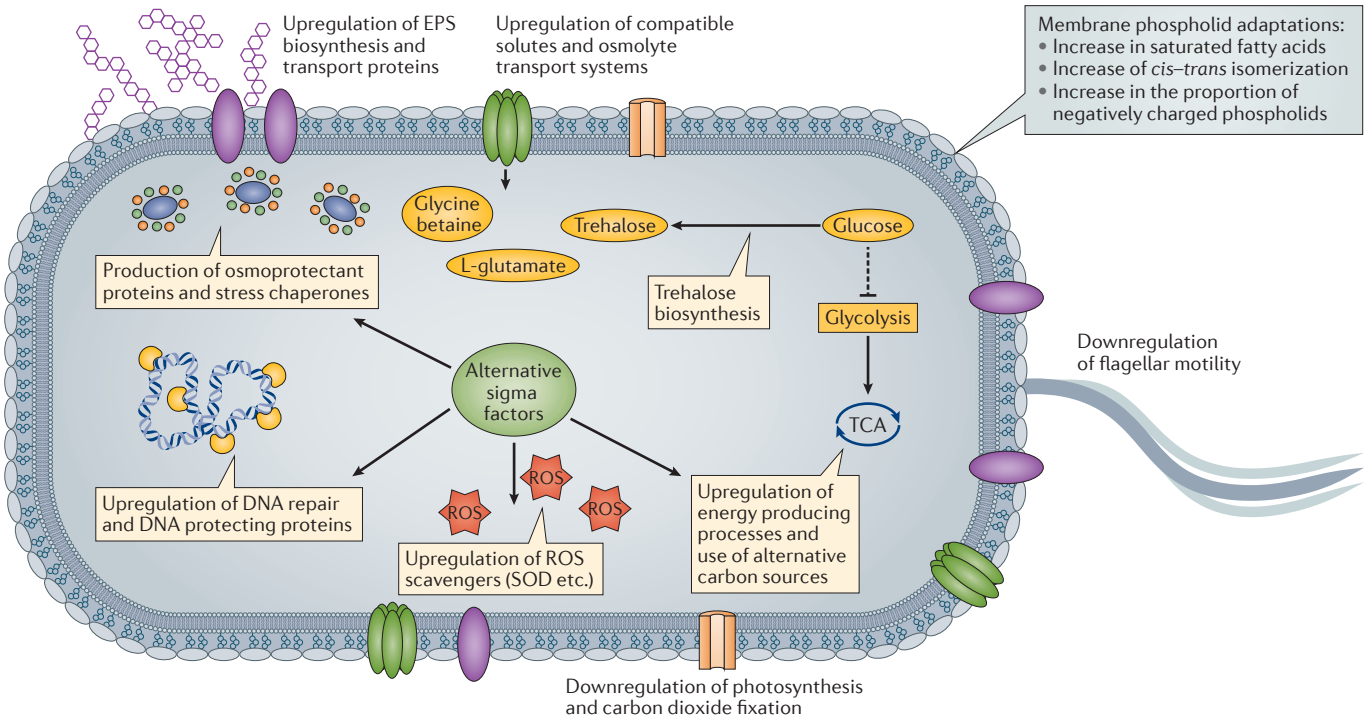


Table 1 | Metagenomic studies of prokaryotic communities living in xeric environments.

Xeric biome	Number of metagenomes	Analyses undertaken	Habitat	Location	Material	Dominant phylum
Desert	19	WMS	Cold-winter desert	Atacama (Valle de la Luna)	Rock	Cyanobacteria
		WMS	Cold-winter desert	Atacama (Monturaqui)	Rock	Cyanobacteria, Actinobacteria
		WMS	Cold-winter desert	Atacama	Rock	Unclassified
		WMS	Tundra	Canada (Axel Heiberg Island)	Soil	Firmicutes, Proteobacteria
		WMS	Hot desert	Chad Desert	Dust	Actinobacteria, Proteobacteria
		WMS, MTX	Cold desert	Wright Valley, Antarctica	Soil	Actinobacteria/Unclassified
		WMS, MTX	Cold desert	Lake Hoare Valley, Antarctica	Soil	Actinobacteria/Unclassified
		WMS, MTX	Cold desert	Lake Fryxell Valley, Antarctica	Soil	Proteobacteria/Unclassified
		WMS, MTX	Cold desert	Lake Bonney Valley,Antarctica	Soil	Actinobacteria/Actinobacteria
		WMS, MTX	Cold desert	Antarctica (Garwood Valley)	Soil	Proteobacteria/Unclassified
		WMS, MTX	Hot desert	Chihuahuan Desert, USA	Soil	Proteobacteria/Unclassified
		WMS, MTX	Hot desert	Mojave Desert, USA	Soil	Proteobacteria/Cyanobacteria
		WMS, MTX	Hot desert	Kutch Saline Desert, India	Soil	Proteobacteria, Euryarchaeota
		MTX	Cold-winter desert	Moab Utah	Soil, biocrust	Firmicutes, Actinobacteria, Cyanobacteria

		MTX	**Tundra**	**Arctic circle**	**Soil**	**Actinobacteria**
		MTX	**Hot desert**	**Kalahari, Botswana**	**Soil**	**Actinobacteria, Proteobacteria**
		MTX	**Hot desert**	**Mount Crawford, Australia**	**Soil**	**Actinobacteria, Proteobacteria**
		MTX	**Hot desert**	**North Tamborine, Australia**	**Soil**	**Firmicutes, Actinobacteria**
		MTX	**Hot desert**	**Beuadesert, Australia**	**Soil**	**Firmicutes, Actinobacteria**
Hypersaline	**7**	**WMS**	**Hypersaline lake**	**Salton sea, USA**	**Hypersaline water**	**Proteobacteria**
		WMS	**Saltern pond**	**Santa Pola, Spain**	**Hypersaline water**	**Euryarchaeota**
		WMS/MTX	**Brine pool**	**Pittsburgh, USA**	**Brine pool**	**Proteobacteria**
		WMS/MTX	**Saltern pond**	**Guerrero Negro, Mexico**	**Microbial mat**	**Proteobacteria**
		MTX	**Hypersaline lagoon**	**Floreana island, Ecuador**	**Hypersaline water**	**Proteobacteria**
		MTX	**Hypersaline lagoon**	**Mar Menor, Spain**	**Hypersaline water**	**Proteobacteria**
		MTX	**Hypersaline lake**	**Great Salt Lake, USA**	**Hypersaline water**	**Proteobacteria**

[1]Xeric environment genomes were identified using metadata searches with the key words "desert", "hypersaline" and "hyper-arid" in the MG-RAST metagenomics repository[118] (http://metagenomics.anl.gov)

Abbreviations: A- Actinobacteria; C- Cyanobacteria; E- Euryarchaeota; F- Firmicutes; P- Proteobacteria; U- Unclassified; WMS – whole metagenome sequencing; MTX – metataxonomic sequencing.

To counteract the negative morphological, physiological and biochemical consequences of desiccation, xerotolerant microorganisms have developed a broad range of adaptive strategies. Despite the broad spectrum of habitats in which these microorganisms occur, much of the literature has focused on fungi associated with preserved food products, as they are frequently associated with food spoilage[24]. By contrast, there are relatively few data on xerotolerant bacteria. Here we provide an overview of the various behavioural, physiological and molecular mechanisms used by xerotolerant bacteria to cope with desiccation. In particular, we focus on recent insights from 'omics' technologies.

Xerotolerance in the 'omics' era

Bacteria respond to xeric stress through several distinct but inter-related physiological and molecular mechanisms, which are temporally coordinated and result in a global shift in the transcriptional and translational patterns of the cell[25-27] (FIG. 3). Modern 'omics' approaches have provided a more complete picture of the spatial and temporal response of bacteria to xeric stress.

Metagenomic technologies have revolutionized the study of the compositions and functions of complex microbial communities[28-30] and they enable analysis of both the culturable minority and the uncultured 'microbial dark matter' majority of any microbial community[28-31]. In particular, functional metagenomic approaches have been instrumental in showing how different xerotolerance mechanisms are employed by bacteria in the context of the extreme environments they inhabit[32-34]. Pioneering metagenomics studies have highlighted the tolerance pathways, such as trehalose biosynthesis and extracellular polysaccharides (EPS) secretion (discussed in more detail below), that are of ecological importance to communities living in xeric environments[32,33]. Next generation sequencing technologies, which allow inexpensive and massively parallel whole metagenome sequencing (WMS) of microbial communities[28,30], have given the field a further boost. For instance, metataxonomic (MTX) approaches, which give detailed insights into the relative abundance and diversity of the members in these communities, have been essential in highlighting the high level of biodiversity and complexity of microbial communities that persist in hyper-arid deserts[28,34]. However, to date, relatively few metagenomes from xeric environment have been sequenced (Table 1). The majority of these are derived from natural environments, such as hot and cold desert and tundra soils, no metagenomes are available yet from low a_w foods. Increasing desertification (BOX 2) and increasing awareness of xerotolerant pathogens in desiccated food will undoubtedly drive future sequencing of metagenomes from xeric environments and these datasets will have a pivotal role in deciphering how microbial communities survive in these environments.

On the other hand, transcriptomic and proteomic approaches provide information on the timeline and magnitude of prokaryotic tolerance to xeric stress. Comprehensive quantitative PCR (qPCR) and transcriptome studies have broadened our understanding of how specific species react to deprivation of water[35-37]. However, these studies are limited to the laboratory

setting. More recent *in situ* applications of 'omics' technologies are providing key insights into the responses of xerotolerant microorganisms in their natural xeric environments. For example GeoChip technology, which encompasses an array of functional genes involved in metabolism and stress response, has been used together with MTX to directly measure xeric stress responses at both the phylum and ecological niche levels in Antarctic desert soil, and has shown that niche localization determines both the abundance and diversity of xeric tolerance pathways amongst bacterial communities in these soils [38]. Further studies that integrate metagenomic, transcriptomic, and proteomic approaches will provide a holistic view on how prokaryotic communities react to changes in the environment, and more specifically to abiotic stressors such as water scarcity.

Behavioural adaptations

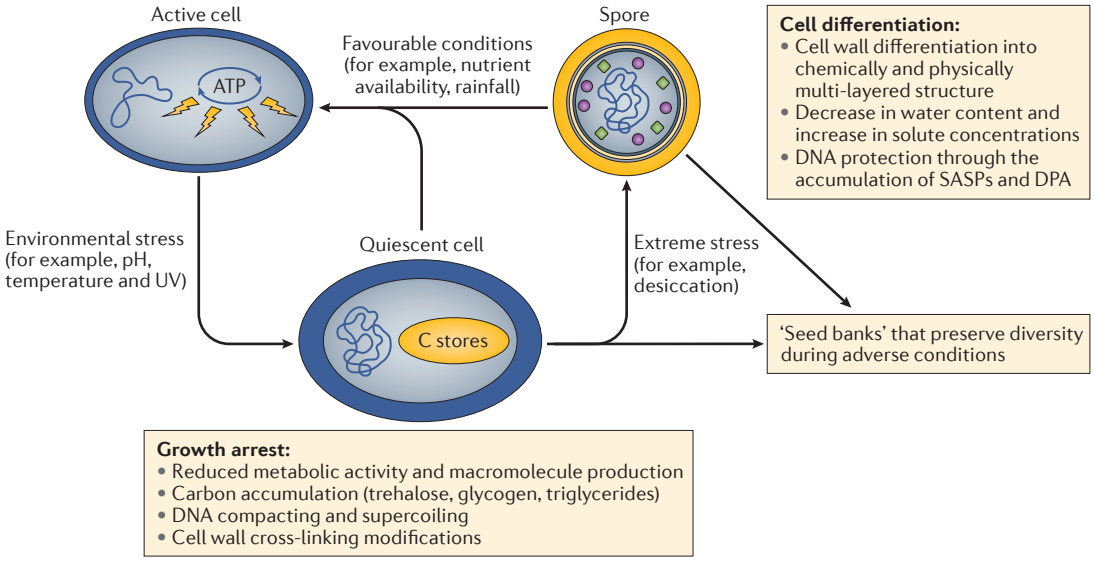
Dormancy and sporulation

A common response of bacteria to many abiotic stresses, including water deprivation, is to enter a reversible form of dormancy, in which the cell shifts into a reduced or inert state of metabolic activity and resumes normal metabolism when favourable conditions are restored[39-42] (FIG. 4).

In bacteria found in desert soils and preserved meats, such as members of the genera *Bacillus* and *Clostridium*, dormancy involves differentiation into highly resistant spores, which have been shown to survive a broad range of biotic and abiotic extremes, including UV irradiation, desiccation, high pressures, high and low temperatures and chemical treatments for long periods of time[42, 43]. Whereas the specific molecular mechanisms of spore resistance to xeric stress are still unknown, desiccation tolerance of spores has been largely attributed to the accumulation of dipicolinic acid (DPA) and alpha/beta-type small, acid-soluble spore proteins (SASPs) in the intracellular spore space, both of which are believed to physically and chemically protect DNA from oxidative damage[43-46].

Members of some cyanobacterial orders found in dry environments, specifically *Nostocales* and *Stigonematales*, can differentiate into spore-like structures called akinetes under conditions of nutrient starvation and desiccation[47]. The low cytoplasmic water content of

Figure 4 | Dormancy and evasion of environmental stress. To survive environmental stressors such as desiccation, many bacteria enter a state of non-replicative viability, which is also referred to as quiescence, or genuine dormancy through spore formation[40,43,44]. Quiescent cells maintain the membrane potential and energy homeostasis by reducing carbon consumption and shifting metabolic activity toward the storage of carbon, commonly as glycogen, trehalose, and triglycerides[43]. These cells also express histone-like proteins that compact DNA and protect it from physical and chemical damage[41]. Because these cells maintain some metabolic activity, quiescence can lead to sporulation under more limiting conditions. For example, quiescent cells modify the peptidoglycan cross-linking of the cell wall to create a denser structure that is conducive to the formation of the spore's compact shell[43]. Sporulation is characterized by asymmetric cell division into specialised cells that are protected by a multi-layered shell[44]. The spore core has a low water content due to the accumulation of minerals such as Mn(II), dipicolinic acid (DPA) and small acid soluble proteins (SASPs), which provide desiccation tolerance through DNA shielding and decreasing rates of ROS production[44,47,117]. It is important to note that dormancy is not only a crucial survival strategy under extreme conditions, but also maintains the microbial diversity by generating 'seed banks' that preserve the microbial populations during extreme conditions[40,41].



akinetes, together with the accumulation of high quantities of storage compounds such as cyanophycin and glycogen, might also have a role in xeric tolerance[47,48]. Akinetes of *Anabaena cylindrica* were able to germinate after five years in a dark and desiccated environment[49].

Another form of dormancy is metabolic dormancy, which may also represent a key strategy of desiccation tolerance in many non-sporulating prokaryotes. This shift to a viable but non-culturable (VBNC) state has been reported for many bacteria, including pathogens such as *Salmonella enterica* and *Legionella pneumophila*[50], as well as soil bacteria such as *Sinorhizobium meliloti*[51]. Recent transcriptomic data have shown that less than 5% of the genome of *S. enterica* cultured in peanut oil (a_w = 0.3) is transcribed, compared with 78% of the genome for the same organism grown in complex aqueous media, and that this low level of activity was essential for the persistence of *S. enterica* in spoilt foods[52].

Dormancy is not without risk, as it requires many resources to drive the shift into and out of dormant states[44]. However, dormancy is thought to be an important strategy for prokaryotic communities in xeric environments, not only by allowing for long periods of survival but also by preserving biodiversity through the creation of 'seed' banks', in which dormant cells become protected reservoirs of genetic diversity [40,53].

Extracellular polysaccharides and biofilm formation

Biofilms are found in various xeric environments as dominant community structures in which algae, fungi, archaea and bacteria co-exist[53-56], and have thus been strongly correlated with the survival of microorganisms in soils with low moisture content[57]. Most of the biomass of biofilms is composed of extracellular polysaccharides (EPS), which are complex polymers that either form a capsule that is covalently attached to the cell surface or are secreted into an external sticky mesh that determines the physical and physiological properties of the biofilm[58].

EPS are crucial for biofilm-mediated desiccation tolerance. EPS biosynthesis mutants of *Escherichia coli*, *Pantoea stewartii* and *Acinetobacter calcoaceticus* showed a six-fold reduction in their survival rates under desiccating conditions[59]. Similarly, EPS produced by the methanogenic archaeon *Methanosarcina barkeri* contributes to the long-term viability of

desiccated cells[60]. The role of EPS in resistance to desiccation can be linked to its hygroscopic nature[2,61]. When *P. putida* is subjected to water stress, it over-produces the anionic EPS alginate, which can hold several times its weight in water[61]. The hydrophilic properties of EPS also contribute to the rapid water absorption rates and restoration of photosynthetic activity observed in *Nostoc commune* upon rehydration after prolonged desiccation[26]. Alternatively, many cyanobacteria secrete EPS containing ester-linked deoxysugars, peptidic moieties and acetyl groups that increase the hydrophobic nature. The hydrophobicity of these EPS layers is thought to stabilise soil communities by clogging sand particles and enhancing surface water run-off, thus preventing soil biocrust communities from being washed away[62]. Considering that biofilms are complex architectural structures of multi-species communities, it is likely that EPS layers are not homogeneous, but rather a diverse palette of different polysaccharides with a broad range of physical and functional properties[58].

Transcriptome studies have shown that biofilm formation is directly involved in desiccation tolerance in *L. monocytogenes*, *S. enterica* and *Bradyrhizobium japonicum*, in which genes associated with biofilm formation are up-regulated in response to desiccation[36,63,64]. Biofilm formation also affects survival of microbial communities under xeric stress; for example, EPS production by the cryptoendolithic *Nostoc commune* increased the desiccation tolerance of other organisms occupying the same xeric niches, including *Chlorella* spp. and *Chroococcidiopsis* spp.[65]. Similarly, tolerance of the drought-tolerant *Nostoc* sp. HK-01 to increased NaCl levels was attributed to increased EPS secretion, which accounted for 65% of the total dry weight of the cells[66]. In fact, EPS producing microorganisms such as the cyanobacteria *Microcoleus vaginatus* are thought of as pioneer colonizers of deserts and dry lands owing to their ability to produce biofilms that retain water and form the supporting substrate for other prokaryotes to colonise[67]. Functional metagenomic analysis of microbial mats from the cold deserts of the Arctic and Antarctica have shown a high abundance of genes for EPS biosynthesis, mainly in cyanobacteria[33]. These include genes for synthesis of the monosaccharides xylose, mannose, and rhamnose, which are EPS components that are produced by many bacterial species[33]. Considering the importance of biofilms in sustaining the life of microbial communities through nutrient retention and re-cycling, modulating cell-to-cell interactions and stratification of functional properties[58], it is plausible to think of biofilms as the first line of defence against xeric stress.

Physiological adaptations to water stress

Cell membrane adaptations

As the main barrier between the intracellular space and the external environment, the cellular membrane of bacteria is severely affected by changes in the external water availability (FIG. 1). Bacteria exposed to extreme fluctuations in water status typically have modified membrane phospholipid and fatty acid compositions, including increases in the ratio of saturated to unsaturated fatty acids and *trans*- to *cis*-mono-unsaturated fatty acids, as well as increased conversion of monoenoic to cyclopropane fatty acids[68,69]. The increased ratio of fatty acids with tighter lipid packing order is thought to preserve the membrane in a liquid crystalline phase during moderate desiccation and to increase the temperature at which the lipid membrane transitions from the liquid phase to the more disordered hexagonal II phase during extreme desiccation[70,71]. Increased cyclopropane fatty acid content also reduces membrane permeability to protons and thereby balances the intracellular pH[72], which is crucial for the stability and activity of many intracellular proteins. In *S. enterica*, the lipid A palmitoleoyl acyltransferase gene *ddg*, which replaces lipid A laurate with palmitoleate, is up-regulated in response to desiccation, increasing the fluidity of the outer membrane[25].

Xerotolerant bacteria also adapt to low a_w by increasing the proportion of negatively charged phospholipids, including phosphatidylglycerol lipids and cardiolipin, at the expense of zwitterionic lipids like phosphatidylethanolamine[68,72,73]. This facilitates preservation of membrane bilayer structural integrity, as the increase in the phosphatidylglycerol to cardiolipin ratio may inhibit the tendency of phosphatidylethanolamine to form non-bilayer structures when exposed to osmotic stress[68,73]. Increases in anionic phospholipids also positively affect membrane osmosensing proteins, which have a role in osmolyte accumulation. For instance, cardiolipin was shown to control the localization and range of osmolarity at which the osmolyte-H^+ symporter ProP is active[73]. The production of pigments may also positively affect desiccation tolerance[2]. Carotenoid mutants of *C. sakazakii* showed reduced long-term desiccation survival[74]. This may be linked to the protective role of these pigments against oxidative damage through the quenching the oxygen radicals that build up inside and outside the cell at lower a_w[74].

Accumulation of compatible solutes

In desiccating conditions, the intracellular accumulation of small molecules and salts restores the osmotic equilibrium and permits continued protein function at low a_w, which allows cells to adapt to xeric stress[2,75-77]. Extreme halophiles use a 'salting-in' strategy in which they import inorganic ions, referred to as osmolytes, to match the extracellular osmotic pressure[78]. On the other hand, xerotolerant bacteria use a bi-phasic 'salting-out' process. Initially, they rapidly accumulate charged solutes (such as potassium and glutamate) in response to osmotic stress; then the charged solutes are replaced by neutral organic solutes that are more compatible with organic reactions, maintain a long-term osmotic balance and function as stabilizers by limiting the unfolding and denaturation of proteins and other macromolecules[76,79,80]. More in-depth comparisons between the two osmotic acclimation strategies can be found in recent reviews on the subject[76,78,81].

Compatible solutes such as trehalose and glycine betaine are part of the general adaptive strategy in most xerotolerant microorganisms[77,79,81]. These low molecular weight compounds are either accumulated from the environment or biosynthesised. They inhibit the membrane disruption associated with water removal and decrease intracellular fluidity through vitrification[82,83], a property that has also been observed for the monosaccharides that form EPS layers in biofilms[56]. Bacteria use a wide range of compatible solutes, which include carbohydrates (such as trehalose and sucrose) and amino acids and their derivatives (such as proline, ectoine and glycine betaine)[75,77,79]. Trehalose, ectoine and proline can be synthesized *de novo* and therefore are widespread across xerotolerant bacteria[35,81,84]. *B. japonicum* and *S. enterica* have three distinct systems for trehalose biosynthesis — trehalose synthase, maltooligosaccharyl trehalose synthase and trehalose-6-phosphate synthase — and the transcription of genes in two of these pathways is up-regulated upon desiccation[36,85]. The biosynthetic pathways for trehalose and 2-sulfotrehalose are also widely distributed among members of the order Halobacteriales, which are common in salt-saturated habitats and therefore subjected to extreme osmotic pressures[86]. In the soil-abundant actinomycete *Rhodococcus jostii* RHA1, genes for ectoine biosynthesis, including *ect*A and *ect*C, are up-regulated under desiccation stress[35]. On the other hand, the halotolerant cyanobacterium *Aphanothece halophytica* uses glycine betaine as the dominant compatible solute during salt acclimatization, although sucrose, trehalose and the amino acid proline are also accumulated

at lower concentrations[87]. Glycine betaine is mostly taken up from the extracellular medium rather than synthesised, as few prokaryotes contain the pathways required for synthesis of this solute[81]. For many bacteria, importation across the membrane is the major means of accumulating intracellular reserves of compatible solutes. Transcriptome and proteome studies have demonstrated the up-regulation of solute transporters such as the glycine betaine/choline transporter encoded by *opuC*, the glycine betaine/proline uptake system encoded by *proU*, *proV*, *proW* or *proX*, glutamate transporters and outer membrane porins in response to desiccation[25,37,88,89].

Functional metagenomic studies have been fundamental for elucidating compatible solute strategies in the context of natural xeric environments, as opposed to the laboratory conditions in which many desiccation and osmotic shock experiments are performed. Two metagenomic studies showed that microbial mat colonies growing on Antarctic rocks contained genes for the proline symporter *opuE* and the *proU* glycine betaine/proline ABC transporter system, mainly in members of the phyla Actinobacteria and Proteobacteria[32,33]. Similarly, another study showed the presence of trehalose synthesis pathway genes (*ost*A and *ost*B) in microbial mats from cold hyper-arid deserts[38].

Metabolic adaptations

Desiccation places a severe metabolic burden on cells by altering the turnover rates of molecules, the kinetics of metabolic pathways, the rate of energy generation and ultimately cellular growth rates. To cope with such changes in metabolic flux and to compensate for changing energy demands, bacteria differentially up- and down-regulate various metabolic pathways, which typically leads to a shift from anabolic to catabolic metabolism[27]. Photosynthesis and carbon dioxide fixation are down-regulated in response to desiccation in *Anabaena* sp. PCC7120[37], which might also protect the cell from oxidative stress by preventing ROS formation[37,52] . To generate the required energy, pathways involved in aerobic respiration — glycolysis, the tricarboxylic acid (TCA) cycle, the glyoxylate shunt and oxidative phosphorylation — are generally induced in desiccated cells[25,27,79]. Fermentative enzymes and those of the oxidative pentose phosphate pathway are induced in *C. sakazakii* and *B. japonicum* under desiccating conditions, as are fatty acid catabolic genes[36,79] . In air-dried *S. enterica*, the most up-regulated gene was *fadA*, which encodes 3-ketoacyl-CoA thiolase and is involved in the degradation of long chain fatty acids into acetyl-coA, which is

transferred to the TCA cycle[85]. The TreBCR and TreA/F pathways, which are involved in the transport of trehalose into the cell and its degradation to glucose, are also up-regulated in desiccated *S. enterica* cells[85].

The balance between anabolic and catabolic metabolism is complex; for example, fatty acid oxidation yields more ATP molecules per carbon atom than glucose. Thus, under desiccating conditions glucose is diverted to trehalose synthesis, and fatty acid oxidation becomes a highly efficient alternative energy source for the cell[18,85]. Genes involved in glycogen metabolism are also expressed at higher levels in desiccated rhizobial cells, and intracellular glycogen may aid in restoring cell volume[36]. Metabolic versatility may also contribute to the ability of bacteria to adapt to xeric environments. For example, *Pontibacter* sp. X14-1, which was isolated from desert soil, encodes proteins for the utilisation of a wide array of carbon substrates, as well as key enzymes for CO_2 fixation that are absent from *Pontibacter* species isolated from non-xeric environments[4].

Many energy-consuming processes, such as flagellar motility and chemotactic machinery[26,27,78,85], are down-regulated in cells under desiccating conditions. Similarly, flagellar motility mutants of the food-borne pathogen *L. monocytogenes* tolerated desiccation better than wild-type cells[90]. Given the energy requirements of flagellar motility, it may become a dispensable function under desiccating conditions, allowing ATP to be redirected to more important metabolic processes [90].

Molecular adaptations

Xerotolerant prokaryotes express a range of proteins to counteract the effects of low water activity. Accumulation of late embryogenesis abundant (LEA) proteins occurs during the late stages of seed maturation under water-stress conditions in plants, but these proteins are also found in other organisms such as *E. coli* and *Saccharomyces cerevisiae*[91]. Of the six families of LEAs described so far, family 3 proteins and their homologues occur in a wide range of organisms that are well adapted to desiccation, including *Deinococcus radiodurans* and *Haemophilus* influenza, and have been strongly linked to survival of these bacteria under xeric conditions[92,93]. For example, *D. radiodurans* mutants that lack family 3 LEA-like proteins were more sensitive to dehydration than wild-type cells[94]. All six families of LEA

proteins share a common glycine-rich and highly hydrophilic backbone that remains in an unstructured, coiled state during normal hydrated conditions, and transitions into an ordered structure under desiccating conditions. This ordered structure interacts non-specifically with several classes of macromolecules, including proteins and DNA[93]. Aav-LEA1, a family 3 LEA found in the nematode *Aphalencus avenea*, inhibits aggregation of housekeeping proteins such as malate dehydrogenase, citrate synthase and fumarase upon partial dehydration[95]. This has led to the hypothesis that Aav-LEA1 and similar proteins act as osmoprotectants by providing a hydrophilic environment around macromolecules and other cellular structures that compensates for the loss of water[93,95].

Several other proteins have been associated with a xerotolerance in bacteria (FIG. 3). The cyanobacterium *M. vaginatu*s up-regulates several shock-response genes upon dehydration, including those coding for the molecular chaperones GroES , GroEL, DnaJ and DnaK, as well as oxidative stress proteins such as Mn-containing catalases and thioredoxins, and DNA-protective proteins such as the DNA-binding ferritin Dps[67]. The ROS scavenger SodF, which is an Fe-containing superoxide dismutase (SOD), is abundantly synthesised in *N. commune* upon exposure to desiccation [96]. In addition, *N. commune* also up-regulates the acidic water stress protein (WspA) under desiccating conditions, which is a chemotaxis-like protein that normally associates with the glycan extracellular matrix secreted by this cyanobacterium and co-ordinates biofilm formation[2,97]. Furthermore, WspA increased the survival of transformed *E. coli* cells upon slow rehydration[98]. Several transcriptomic and proteomic analyses that have been conducted on a wide range of microorganisms exposed to desiccation stress have shown the up-regulation of transcriptional regulators in response to low a_w. Signal transduction systems sense low a_w and other environmental stresses and activate regulatory cascades that control the expression of genes and the production of proteins necessary for various alterations in cellular physiology to cope with the stresses[27,99]. In particular, the induction of alternative sigma factors is key to the global response to low a_w stress. *S. enterica* and *E. coli* up-regulate the master stress regulator *rpoS* under desiccating conditions, as the regulon controlled by RpoS includes the DNA protection protein Dps, trehalose biosynthesis genes *otsAB* and several oxidative damage response genes[18,26]. RpoE, which controls exopolysaccharide production and has a role in the control of misfolding of secreted proteins, is also up-regulated under xeric stress[18,26,77,100].

Several additional phenotypes have been shown to be stimulated in microbial cells under desiccating conditions. In *E. coli*, genes encoding virulence factors are upregulated, including

esc, which encodes a type III secretion system, and *eae*, which encodes the outer membrane adhesin intimin[26]. This is consistent with the observation that bacterial pathogens show increased virulence after exposure to environmental stresses[101,102]. Finally, *B. japonicum* up-regulated twelve transposases in response to desiccation, which may explain the adaptive capacity of microbial populations when exposed to harsh environmental conditions[36].

Concluding Remarks

Bacteria use diverse mechanisms to survive the many biotic and abiotic challenges of extreme environments. Many of the adaptive mechanisms that are used by xerotolerant bacteria are universal responses that are observed regardless of the type of xeric stress. Examples include accumulation and biosynthesis of compatible solutes such as proline and trehalose and expression of ROS scavengers such as peroxide dismutase. This universality reflects the common features of xeric stress — that is, the loss of cytoplasmic water, the resulting loss of protein and membrane integrity and accumulation of ROS through oxidative stress — and explains the reliance on universal stress response transcription regulators such as *rpoB* and *rpo*S across different species[21,] . On the other hand, specific adaptive mechanisms such as membrane modifications and sporulation require a considerable commitment of energy and resources, and therefore are part of a broader pre-emptive strategy that relies on quorum sensing to detect partial desiccation before water activity reaches levels that are too low for meaningful biological activity[72,73].

Much of the mechanistic data discussed above were produced in tightly controlled laboratory settings. In natural extreme xeric environments however, selection pressure is imposed by several interacting abiotic stressors such as temperature fluctuations, extreme solar irradiation (UV and ionizing radiation), water deprivation and nutrient scarcity. Not surprisingly, some redundancy has been observed in the adaptive responses of bacteria to different extreme conditions, as many of the mechanisms discussed above can be triggered by and provide resistance to multiple stressors. For example, both xeric stress and solar radiation cause oxidative stress through the accumulation of ROS, and therefore similar mechanisms are activated by both stressors. Indeed, *D. radiodurans* accumulates small organic and inorganic Mn^{2+} complexes with antioxidant properties in response to ionizing radiation and these complexes also provide resistance to desiccation[103]. Furthermore, both UV radiation and

desiccation cause the up-regulation of *wsp*A and *sod*F in *N. commune*, which highlights the overlap that exists between tolerance pathways to different stressors [98]. The synergistic effects of specific adaptive mechanisms to multiple stressors in not limited to desiccation and extreme radiation. In *S. enterica*, both starvation and desiccation trigger the accumulation and biosynthesis of trehalose , which acts as both an compatible solute that stabilizes proteins and the cell membrane in the absence of water and an alternative carbon source that compensates for nutrient deprivation[85]. Therefore, the adaptive mechanisms that xerotolerant bacteria trigger in response to xeric stress cannot be seen as unique to this stressor, but rather as part of a broader response that allows bacteria to survive in extreme biomes. As such, metagenomics and other 'omics' approaches have a key role in deciphering the complexity of this adaptive response and in providing an holistic overview of bacterial survival under extreme conditions.

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Glossary

Hypertonicity: A solution that contains a greater concentration of solutes than another solution.

Hyper-arid: Oligotrophic environment with severe water shortage, low precipitation and soil erosion that poses extreme challenges to the survival of living organisms. Technically, hyper-arid environment have an aridity index (AI) of less than 0.05.

Water activity (a_w): A measurement of water available to an organism in the environment. It is calculated as the ratio of the vapour pressure in an environment relative to pure water under identical conditions.

Metataxonomic (MTX) approaches: The high-throughput sequencing of taxonomic markers (such as 16S rRNA genes) from metagenomic DNA from an environmental sample and subsequent phylogenetic and taxonomic analyses of the microbial community composition and structure.

Poikilohydric: The inability to regulate or maintain water content and achieve cellular homeostasis.

Akinetes: A thick-walled dormant cell that is formed through enlargement of a vegetative cell in non-sporulating cyanobacteria and green algae.

Hexagonal II phase: A membrane lipid polymorphism in which lipids aggregate into cone shapes with the polar headgroups on the inside and the hydrophobic hydrocarbons tails on the outside. The creation of these aggregates increases the packing disorder of lipid membranes under xeric stress.

Monoenoic …. fatty acids: An unsaturated fatty acid with only one double bond.

Cyclopropane fatty acids: Rare fatty acids that are produced by cyclopropanation of unsaturated fatty acids.

Cardiolipin: A negatively charged diphosphatidylglycerol lipid that consists of a glycerol backbone linked to two phosphatidic acid moieties.

Phosphatidylglycerol lipids: Glycerophospholipids consisting of a L-glycerol 3-phosphate backbone ester-linked to saturated on unsatured fatty acids at carbon 1 and 2.

Halophiles: Organisms that are adapted to thrive in environments with saturated salt content and do not grow optimally in more mesophilic environments.

Vitrification: The formation of glasses by disaccharides (such as trehalose and sucrose), which is induced by removal of water from the intracellular environment. The reduction of diffusion rates inside the cell caused by vitrification is thought to be crucial for the resistance to water stress, as it prevents the accumulation of harmful reactive oxygen species (ROS).

Glyoxylate shunt: A variant of the tricarboxylic acid (TCA) cycle that involves the conversion of acetyl-CoA to succinate for the biosynthesis of carbohydrates.

Chemotactic machinery: The system of membrane chemoreceptors and signal transducing pathways that govern the ability of prokaryotes to move by means of flagella towards attractants and away from repellents.

Housekeeping proteins: Proteins that are necessary for normal cellular functions, typically encoded by constitutively expressed genes.

Catalases: Enzyme common to all domains of life that catalyses the degradation of hydrogen peroxide to water and oxygen.

Thioredoxins: A class of small proteins involved mainly in redox signalling.

Type III secretion system: A protein machinery found in the Gram-negative bacteria that secretes effector proteins that assist in infection of eukaryotic hosts.

Alternative sigma factors: Specialised sigma factors react to external environmental triggers and regulate specific cell functions, such as stress tolerance, flagellar motility, and virulence.

Maillard reaction: A non-enzymatic reaction in which the reactive carbonyl group of a sugar reacts with primary amines of nucleic acids and amino groups of proteins, forming covalent bonds that cause cross-links between proteins and DNA. This reaction is also referred to as the Browning reaction.

Hydroxyl radicals: The neutral form of the hydroxide ion (OH^-)

Key points

- Xerotolerant microorganisms are extremophiles that survive in environments with extremely limited water availability. Despite their importance to these ecosystem, xerotolerant bacteria have been largely overlooked.
- A high diversity of xerotolerant bacteria can be found in many different extreme environments, including hot and cold environments such as the Atacama and Antarctic deserts. In these biomes, xerotolerant microorganisms survive in sheltered geological niches that allow for biological activity.
- Dormancy and sporulation are common behavioural responses to desiccation that allow xerotolerant microorganisms to react to the sporadic cycles of rainfall and drought by remaining in an inert metabolic state.
- Xerotolerant bacteria use several physiological mechanisms to prevent cell disruption and water loss, including phospholipid modifications to maintain membrane fluidity, secretion of water-retaining extracellular polysaccharides (EPS), and the accumulation of compatible solutes that preserve the osmotic potential across the membrane.
- For xerotolerant microorganisms, DNA and protein stability are crucial to ensure that cellular activity is resumed under favourable conditions. Consequently, most molecular adaptations to xeric stress revolve around the up-regulation of proteins that are stable under low water activity and preserve the integrity of DNA through physical protection and repair.
- Although xerotolerant bacteria are unique in their capacity to survive in water-scarce environments, many of the adaptive mechanisms that they use are also triggered by other abiotic stresses present in these environments. Therefore, these mechanisms are part of broader adaptive response that allows prokaryotic survival in extreme biomes.

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ToC blurb

Dry-adapted communities that inhabit natural arid and hyper-arid play a crucial role in the ecology of these niches, and therefore are important for understanding and preventing desertification. In this Review, Lebre, De Maayer and Cowan discuss the adaptations that allow xerotolerant bacteria to survive extreme dryness and highlight insights from recent metagenomic and transcriptomic studies.