Microbial anhydrobiosis

Jason Bosch[1*], Gilda Varliero[1*], John E. Hallsworth[2], Tiffany D. Dallas[2], David Hopkins[3], Beat Frey[4], Weidong Kong[5], Pedro Lebre[1], Thulani P. Makhalanyane[1], Don A. Cowan[1]

[1] Centre for Microbial Ecology and Genomics, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, 0002, South Africa

[2] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, BT9 5DL, Northern Ireland, United Kingdom

[3] Scotland's Rural College, Edinburgh, EH9 3JG, United Kingdom

[4] Rhizosphere Processes Group, Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland

[5] State Key Laboratory of Tibetan Plateau Earth System Science (LATPES), Institute of Tibetan Plateau Research, Chinese Academy of Sciences (CAS), Beijing, 100101, China

*These authors contributed equally to this work.

Correspondence: don.cowan@up.ac.za

Abstract

The loss of cellular water (desiccation) and the resulting low cytosolic water activity are major stress factors for life. Numerous prokaryotic and eukaryotic taxa have evolved molecular and physiological adaptions to periods of low water availability or water-limited environments that occur across the terrestrial Earth. The changes within cells during the processes of desiccation and rehydration, from the activation (and inactivation) of biosynthetic pathways to the accumulation of compatible solutes, have been studied in considerable detail. However, relatively little is known on the metabolic status of organisms in the desiccated state; that is, in the sometimes extended periods between the drying and rewetting phases. During these periods, which can extend beyond decades and which we term 'anhydrobiosis', organismal

survival could be dependent on a continued supply of energy to maintain the basal metabolic processes necessary for critical functions such as macromolecular repair. Here, we review the state of knowledge relating to the function of microorganisms during the anhydrobiotic state, highlighting substantial gaps in our understanding of qualitative and quantitative aspects of molecular and biochemical processes in desiccated cells.

1. Introduction

Although most of the Earth is covered by water, more than 35% of its land surface exists in a state of near-permanent aridity or hyper-aridity (Pointing and Belnap, 2012; Mirzabaev *et al.*, 2019), with hot deserts being the most common dryland type. In some of these areas, liquid water inputs may be restricted to only a few centimetres of precipitation over decades, centuries or even longer (Cherlet *et al.*, 2018). Other drylands, which form part of the Earth's cryosphere (such as polar, high alpine and high plateau areas), are 'biologically arid' because water is in the form of ice and therefore unavailable for metabolism (Quinton *et al.*, 2011; Larose *et al.*, 2013). In all types of drylands (hot, cold or temperate), water availability is the dominant driver of biological community structure and function (Maestre *et al.*, 2015; Naidoo *et al.*, 2021).

Conditions of periodic or permanent low water availability are unquestionably challenging to organisms and can be incompatible with life. This is often evidenced by the near-complete absence of macrofauna and plants in the more extreme desert habitats (Hufnagel *et al.*, 2018; Berdugo *et al.*, 2021). It might be expected that microbial populations would also be either scarce or absent in drylands (Dragone *et al.*, 2021). In general, however, this is not the case: these environments harbour diverse microbial taxa which exhibit multiple adaptations to extreme environments, arising from an evolutionary history spanning long periods when extreme conditions were the norm (Torsvik and Øvreås, 2008; Singh *et al.,* 2019). Hot hyper-arid desert soils are populated by complex microbial communities (Cowan *et al.*, 2020) and Antarctic Dry Valley soils contain a high diversity of microbial taxa (Cary *et al.*, 2010; Chong *et al.*, 2015). To exist in such habitats, microorganisms must be capable of surviving extended periods of low water availability (i.e., they must exhibit extreme xerotolerance) and be able to successfully

respond to sometimes sudden, short-lived and unpredictable changes in water availability due to precipitation or flooding. While the generation of highly stable and resistant resting bodies (e.g., spores) is one such option, this capacity is restricted to a limited range of taxa. Other xerotolerant microbes must subsist in hydrated microniches, such as rock cracks and fissures (Lebre *et al.*, 2017), or enter a desiccation-induced state known as anhydrobiosis, also termed as anabiosis or cryptobiosis, to escape these harsh conditions (Crowe and Cooper, 1971).

The most intensively studied groups of anhydrobiotic organisms are tardigrades, diminutive eukaryotes members of the phylum Tardigrada (Wang *et al.*, 2014; Kamilari *et al.*, 2019; Pedersen *et al.*, 2021); the dipteran insect *Polypedilum vanderplanki* (Ryabova *et al.*, 2020; Voronina *et al.*, 2020); brine shrimps, crustacea in the *Artemia* genus adapted to high-salt conditions (Clegg, 2005); angiosperm resurrection plants (Farrant, 2000; Farrant *et al.*, 2015) and domesticated strains of the ascomycete yeast *Saccharomyces cerevisiae* (Dupont *et al.*, 2014; Rapoport *et al.*, 2019). Desiccated cells (as opposed to those which are partially-dehydrated) have traditionally been assumed to be metabolically inactive. This assumption has been based on commonly-used means of assessment, and is largely based on well-studied macroscopic organisms where survival in a desiccated state has been observed over decade-long time scales (Clegg, 1967; Guidetti and Jönsson, 2002). This view is supported by various studies: a reduction in the size of tardigrade storage cells during starvation, but not during desiccation, over the same length of time (Reuner *et al.*, 2010); the processing of $^{14}CO_2$ in different fractions of brine shrimp embryos only until complete desiccation, after which it remained stable (Clegg, 1967); the lack of detectable change in stored carbohydrate content in desiccated brine shrimp over a period of 28 years (Clegg, 1967); and the observation that, during prolonged desiccation conditions, certain plants enter a cellular state that mimics death (Singh *et al.*, 2015). While these observations point to a lack of metabolic activity during anhydrobiosis in higher organisms, it is unclear whether this applies to microorganisms, particularly those adapted to low water-activity lifestyles.

Large and complex microbial communities occur in low water activity habitats such as brines (Lee *et al.*, 2018; Benison *et al.*, 2021) and the soils of some of the driest environments in the world

(Cowan *et al.*, 2002; Uritskiy *et al.*, 2019). This suggests that many microorganisms might be particularly adept at entering, surviving and then recovering from anhydrobiosis. We speculate that microscopic, unicellular organisms are less metabolically constrained than their multicellular counterparts under anhydrobiotic conditions, due to the lack of a dependence on inter-cellular transport or signalling. Several studies suggest that, whereas most of cellular metabolism shuts down during the anhydrobiotic phase, metabolic activity related to microbial cellular maintenance and repair is still present in low water-activity conditions (Sun and Leopold, 1997; Deng *et al.*, 2012; Pazos-Rojas *et al.*, 2019).

The published anhydrobiosis literature includes numerous studies on metabolic changes as eukaryotes enter or leave the anhydrobiotic state, with most focusing on the responses to mechanical, structural and oxidative constraints that must be overcome during dehydration/rehydration cycles (Crowe *et al.*, 2002; Dupont *et al.*, 2014; Farrant *et al.*, 2015; Leprince and Buitink, 2015; Koshland and Tapia, 2019; Oliver *et al.*, 2020; Câmara and Sant'Ana, 2021). By comparison, there are few studies focused on metabolic processes during the anhydrobiotic state (e.g., Wang *et al.*, 2014; Farrant *et al.*, 2015). Even fewer studies focus on the most common taxa in desiccated environments: prokaryotes and unicellular eukaryotes such as fungi (e.g., Cytryn *et al.*, 2007; Deng *et al.*, 2012; Pazos-Rojas *et al.*, 2019).

In this review, we re-evaluate key aspects of the ecophysiology of microbial anhydrobiosis; specifically, we define the concept of cellular anhydrobiosis; identify metabolic processes that can occur in anhydrobiotic microorganisms; and explore microbial community functions under conditions of near-complete aridity.

2. Definition of anhydrobiosis

The word "anhydrobiosis" is formed by combining the prefix *an-* (from Ancient Greek, meaning "not"), *hydro* (from Ancient Greek, meaning "water") and the suffix *-biosis* (meaning "a way of living"); i.e., life without water (Wharton, 2015). The classical view of anhydrobiosis is a desiccation-induced state of an organism when it "shows no visible signs of life and when its

metabolic activity becomes hardly measurable, or comes reversibly to a standstill" (Keilin, 1959). This definition asserts that anhydrobiosis has the following criteria: it is induced by desiccation; has no observable metabolism; and is reversible. However, we argue that this definition does not reflect the complex and subtle realities of microbial cell biology under arid conditions/in arid habitats.

3. Desiccation versus osmotic stress

While anhydrobiosis has been the target of much research, it is important to acknowledge methodological inconsistencies of many studies. These inconsistencies are partly due to a confusion between desiccation, (partial) dehydration, and osmotic stress. Whereas there are physical similarities between desiccation and osmotic stress, these two cellular stresses are mechanistically distinct (Billi and Potts, 2002; Kieft, 2003; Vriezen *et al.*, 2007; Gunde-Cimerman *et al.*, 2018). The hybrid condition induced by high salt concentrations, osmobiosis, is characterised by cellular changes and responses beyond those imposed by hyperosmotic processes (Gade *et al.*, 2020). Many experimental 'desiccation' studies are performed by the addition of highly concentrated salt or solute solutions as a mean of reducing water activity (Stevenson *et al.*, 2015; Stevenson *et al.*, 2017; Bremer and Krämer, 2019). These represent examples of systems where water is physically present, but where much of the water is not biologically available.

"Complete desiccation" has been quantitatively defined as the condition in which the water content is less than 0.1 g H_2O g^{-1} dry mass (Alpert 2005). This is roughly equivalent to drying in air at 50% relative humidity and 20 ^{o}C; corresponding to a water activity of 0.50 and to a water potential of -100 MPa (Gaff, 1997; Haranczyk *et al*., 1998; Proctor, 2003). The presence of water, even if it contains high solute concentrations, still allows for the movement of microorganisms and facilitates the diffusion of nutrients. In contrast, bulk water is absent from desiccated cells and bacterial motility is highly restricted (Dechesne *et al.*, 2010). Similarly, the absence of a liquid continuum limits the diffusion of nutrients and is likely to be a major limiting factor of microbial activity in soils with low water contents (Manzoni *et al.*, 2012).

Experimental data indicate that high concentrations of salts and other solutes affect cellular processes beyond the changes imposed by osmotic stress (Gervais *et al.*, 1988; de Lima Alves *et al.*, 2015). For example, the activity of the *Synechococcus* photosystem is impaired by the osmotic effects of 1 M sorbitol, but by both ionic and osmotic effects of 0.5 M NaCl (Allakhverdiev *et al.*, 2000). High concentrations of salts such as $MgCl_2$ simultaneously impose multiple stresses on cells including osmotic stress, reduced water activity and chaotropicity (Hallsworth *et al.*, 2007; de Lima Alves *et al.*, 2015).

4. Transitions to and from the anhydrobiotic state

The assertion that anhydrobiosis is characterised by a total lack of metabolic activity (Keilin, 1959) is debatable, given that a lack of "signs of life" (e.g., movement in multicellular organisms, motility in unicellular organisms, and cell division) does not indicate the full extent of possible metabolic functions (Walters *et al.*, 2005; Hallsworth, 2018). A summary of the functional changes in organisms entering and leaving the anhydrobiotic state is presented in Figure 1.

In all organisms, higher-order functions such as cell division and motility are rapidly inhibited, or even lost altogether, as intracellular water is removed (Trevors, 2011; Leprince and Buitink, 2015). For example, incubation of cyanobacterium *Synechococcus* cells in 0.5 M aqueous NaCl (water activity 0.98) reduced photosynthetic activity by 80% within 2 hours (Allakhverdiev *et al.*, 2000). Gram-negative bacteria such as *Salmonella*, *Vibrio* and *Escherichia,* require a water activity greater than 0.95 for cell division (Fontana and Carter, 2020). However, the water-activity limits of biological functioning are highly organism-specific: for example, some Haloarchaea continue to divide in concentrated saline media at 0.635 water activity (Stevenson *et al.*, 2015) and *Aspergillus penicillioides* cells are capable of cell division at water activities down to 0.585 (Stevenson *et al.*, 2017). During desiccation, multiple cellular responses to water depletion are also activated. The expression of genes upregulated during the first few hours from the onset of desiccation include those coding for heat shock proteins and chaperones, and those involved in oxidative stress, DNA repair, DNA synthesis, compatible solute metabolism/transport, modification of the cell envelope, and fatty acid metabolism (Katoh *et al.*, 2004; Cytryn *et al.*,

2007; LeBlanc *et al.*, 2008; Li *et al.*, 2012; Rajeev *et al.*, 2013; Garcia-Fontana *et al.*, 2016; Palud *et al.*, 2020). As desiccation progresses, cells eventually enter a state of anhydrobiosis, where cellular water may exist only in the bound (Type I) state (Figure 1).
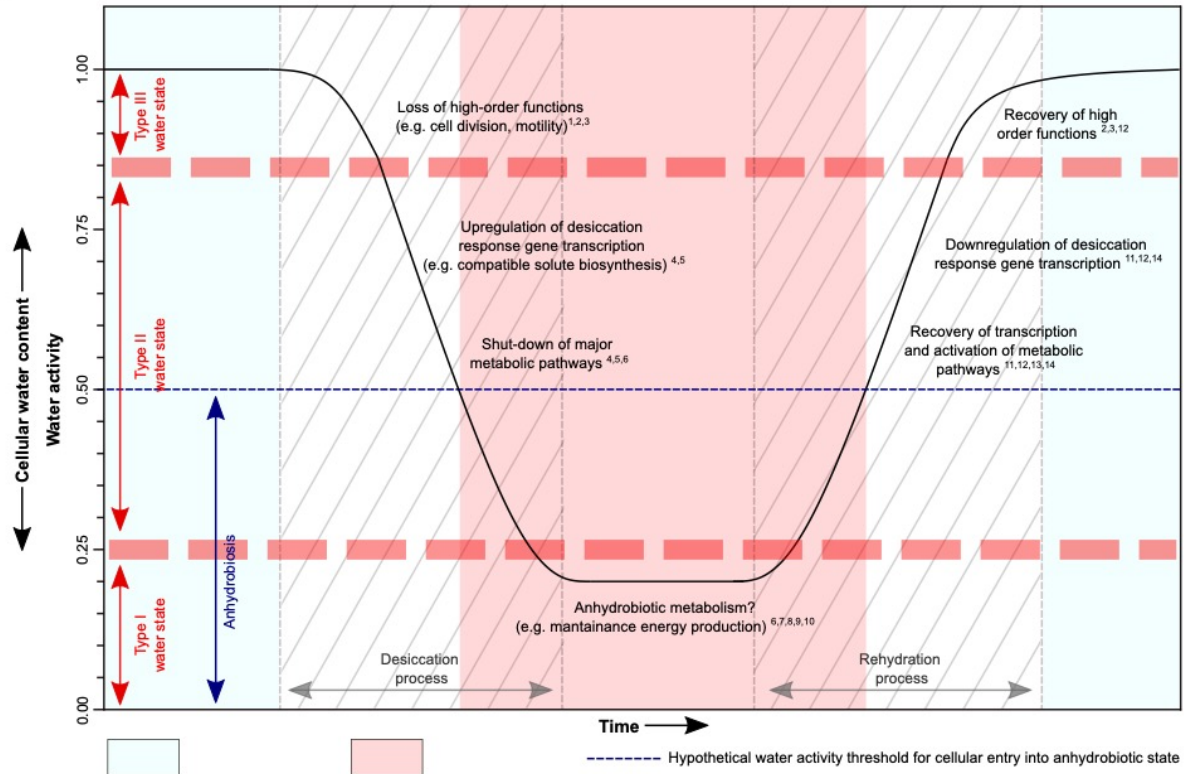

Figure 1. Conceptual view of microbial cells (black line, cellular water content) undergoing a desiccation/hydration cycle. Following entry into the desiccation phase, the fully hydrated cells lose high-order functions. With the progression of the desiccation phase the cells go from Type III water condition (0.85 to 1 water activity; Box 1) to the Type II state (0.25 to 0.85 water activity; Box 1), the transcription of genes involved in desiccation responses is upregulated (e.g. biosynthesis of trehalose and/or other stress protectants) and major metabolic pathways become inactive. With the progressive decrease of water activity, cells enter the anhydrobiotic phase (water activity = 0.50), where cells retain basal metabolism. At this point, water may be in the Type I state (0.00 to 0.25 water activity; Box 1). The induction of the rehydration phase is triggered by the increase of water activity. Once water activity is back to 0.50, the cells exit the anhydrobiotic state. Transcriptional pathways that were shut down during the desiccation phase are reactivated, followed by a downregulation of genes involved in the desiccation response, and the recovery of higher-order functions. The proposed boundaries for the various functional transitions are broadly based on published studies: Trevors, 2011 (1); Vriezen *et al.*, 2012 (2); Pazos-Rojas *et al.*, 2019 (3); Katoh *et al.*, 2004 (4); LeBlanc *et al.*, 2008 (5); Deng *et al.*, 2012 (6); Cytryn *et al.*, 2007 (7); Greening *et al.*, 2016 (8); Ortiz *et al.*, 2020 (9); Candotto Carniel *et al.*, 2021 (10); Rajeev *et al.*, 2013 (11); López-Lara *et al.*, 2020 (12); Kuliešienė *et al.*, 2021 (13); León-Sobrino *et al.*, 2021 (14).

During the rehydration process, desiccated microorganisms show rapid changes in gene expression (Finn *et al.*, 2013; Rajeev *et al.*, 2013; López-Lara *et al.*, 2020). For example, López-Lara *et al.* (2020) reported that within 20 minutes of water addition, the expression of genes involved in phenylalanine/tyrosine catabolism in the Gram-negative bacterium *Pseudomonas putida* were strongly upregulated, providing carbon substrates for energy generation. In a recent metatranscriptomic study of desiccated and wetted soil microbiomes, statistically significant changes in gene upregulation (e.g., motility genes, transporter genes) and downregulation (e.g., stress-response genes, autotrophic carbon-acquisition genes) were observed only 10 minutes after the addition of water to desiccated soils (León-Sobrino *et al.*, 2021).

5. The anhydrobiotic state

How microorganisms survive during the anhydrobiotic state has been hotly debated. It has been generally recognised that vitrification of the cytoplasm, with accumulation of compatible solutes, contributes to survival (Sun and Leopold, 1997; Crowe et al., 1998; Boothby and Pielak, 2017; Crowe, 2014). The most widely studied compatible solute, trehalose, creates a glassy matrix which protects cellular structures, such as vesicles, during desiccation (Cesàro *et al.*, 2008). As for all compatible solutes, trehalose stabilised macromolecules by replacing water in H-bond networks (water-replacement hypothesis) (Crowe *et al.*, 1998), but may also contribute to the retention of residual water (Kilburn *et al.*, 2006; Kumar Jain *et al*., 2008) and serve as an energy source (Tapia and Koshland, 2014).

Following desiccation, processes such as the Maillard reaction and the Haber-Weiss and Fenton reactions can cause harmful effects due to the accumulation of reactive oxygen species and damage to protein and DNA (Kranner and Birtic, 2005; Potts *et al*., 2005). The presence of trehalose or other intracellular xero(desiccation) protectants reduces desiccation damage (França *et al.*, 2007; Narváez-Reinaldo *et al.*, 2010; Kosar *et al.*, 2019). There is ~~some~~ also evidence that anhydrobiotic organisms accumulate DNA damage during desiccation, triggering repair processes on cellular rehydration (Mosca *et al.*, 2019), although active DNA repair processes have been observed in desiccated cells (Greffe and Michiels, 2020).

It is commonly assumed that total vitrification of the cytoplasm would inactivate all metabolic pathways (Evangelista *et al.*, 2017; Hibshman *et al.*, 2020) but it is not known whether the cytoplasm is indeed fully vitrified during anhydrobiosis (Ratnakumar and Tunnacliffe, 2006). A recent study by Candotto Carniel *et al.* (2021) demonstrated that a state of vitrification in lichens was only reached at 0.12–0.08 g H_2O g^{-1} dry weight and that most molecules were in a glassy state at 0.03 g H_2O g^{-1} dry weight. We also note that different organisms use different molecules and mechanisms to maintain cell integrity during desiccation stress (Boothby and Pielak, 2017; Oliver *et al.*, 2020), not all of which involve vitrification of the cytoplasm.

5.1. Cellular metabolism in the anhydrobiotic state

Various studies have reported gene expression patterns from microbial desiccation/hydration. These studies have used a variety of experimental approaches, under different conditions and using different model organisms (Table 1). However, because the point of entry into the anhydrobiotic state is organism-specific, and probably specific to the spatiotemporal environmental conditions, it is often impossible to determine whether a particular study reports gene expression during desiccation/rehydration transitions or anhydrobiosis *per se* (Table 1). For clarification, we define the anhydrobiotic state as the 'steady state' between the processes of cellular desiccation and cellular rehydration, where cellular functions are restricted to basal metabolism (García, 2011). We note that while the two transitional phases will normally be relatively rapid, the anhydrobiotic steady state may exist for long periods.

Table 1. Studies exploring gene expression patterns in anhydrobiotic bacteria after desiccation/hydration experiments[a].

Organism	Desiccation conditions	Gene expression comparison	Gene expression technique	Study
Bradyrhizobium japonicum	Cell cultures were filtered. Filters were vacuum dried, placed in perforated polystyrene Petri dishes, and transferred in plastic 150 mm desiccators with distilled water (100% relative humidity) or saturated potassium acetate solution (27% relative humidity)	Comparison between cells desiccated for 4, 24 and 72 h	Microarray; RT-qPCR	Cytryn *et al.* (2007)
Salmonella enterica	Cell incubation in peanut oil (0.30 water activity)	Comparison between cells grown in peanut oil (at 72, 216 and 528 h after desiccation) or Luria–Bertani broth (12 and 312 h)	RNA-seq; RT-qPCR	Deng *et al.* (2012)
Salmonella enterica serovar Typhimurium	Cells were air-dried on steel for 4 h at 24°C in a laminar flow cabinet, at 45% relative humidity	Comparison between cells desiccated onto stainless steel for 4 h and cells cultured in static broth; cells were then rehydrated for 30 min	Microarray; RT-qPCR	Finn *et al.* (2013)
*Salmonella enterica*serovar Typhimurium	Cells were air-dried in a biosafety cabinet for 22 h at 25°C, at 40% relative humidity	Comparison between desiccated and non-desiccated cells incubated in the same conditions	RNA-seq; RT-qPCR	Gruzdev *et al.* (2012)
Anabaena sp. PCC7120	Cultured cells were spotted on filters. The latter were then desiccated in a Petri dish under room-light conditions at 30°C and 30% relative humidity	Comparison between desiccated and non-desiccated cells after desiccation for 0.5, 3, 10 and 22 h	Microarray; RT-PCR	Katoh *et al.* (2004)
Salmonella enterica serovar Typhimurium	Cultured cells were spotted on filters. Filters were air-dried for 24 h at room temperature and were then placed in desiccators with distilled water (100% relative humidity) or saturated solution of LiCl 99% (11% relative humidity)	Comparison between cells equilibrated at 0.11 water activity and 1 water activity	RNA-seq	Maserati *et al.* (2017)
Rhodococcus jostii RHA1	Cell cultures were filtered. Filters were placed in an air-tight cabinet maintained at 30°C and at either 20% or 100% relative humidity for the desiccated and control samples respectively	Comparison between control and desiccated cells after 0.5, 1, 3, 6, 12, 24, 48, 168, and 336 h	Microarray; RT-qPCR	LeBlanc *et al.* (2008)
Salmonella Tennessee and *Salmonella* Typhimurium LT2	Both strains were stored for 2 h in a sterile desiccator with LiCl (11% equilibrium relative humidity)	Comparison of *S.* Tennesse (highly desiccation resistant) and *S.* Typhimurium LT2 (weakly desiccation resistant) after 2 h drying	Microarray; RT-qPCR	Li *et al.* (2012)
Pseudomonas putida KT2440	Cells were dehydrated for 18 days in a desiccation chamber at 30°C and 50% relative humidity	Comparison of desiccated and non-desiccated cells after 20 min and 24 h rehydration	Microarray; RT-qPCR	López-Lara *et al.* (2020)
Pseudomonas putida KT2440	Massive Stamping Drop Plate method carried out at 30°C and 50% relative humidity	Comparison of cells before desiccation and after 20 min and 24 h rehydration; desiccation was performed for 6, 12 and 18 days	RT-qPCR	Pazos-Rojas *et al.* (2019)
Microcoleus vaginatus	In controlled mesocosms, the soil crusts were first hydrated simulating a rainfall event, and then were desiccated again	Comparison of gene expression in biological soil crusts subjected to rehydration, desiccation and two diel cycles	Microarray	Rajeev *et al.* (2013)

	after 72 h by decreasing room humidity using air conditioners		
Cronobacter sakazakii SP291	A 96-well microtiter plate was placed in a laminar-flow cabinet for 4 h	Comparison between cells in nutrient-rich medium early stationary phase and cells desiccated for 4 h	RNA-seq; RT-qPCR	Srikumar *et al.* (2019)

ᵃPlease note that most of these studies also performed additional analyses which are not reported here.

Cellular metabolic functions are limited in the anhydrobiotic state (Katoh *et al.*, 2004; LeBlanc *et al.*, 2008; Rapoport *et al.*, 2019). Deng *et al.* (2012) showed that less than the 5% of the *Salmonella enterica* genome was transcribed when the organism was cultured in peanut oil (0.30 water activity). For the nitrogen-fixing bacterium *Bradyrhizobium japonicum*, when desiccated at 27% relative humidity, expression of only a limited number of genes was detectable: pilin subunit (*ctpA*), heat-shock protein (*hsp20*), sigma factors, and transcriptional regulators (Cytryn *et al.*, 2007). Similar expression patterns were observed in *Salmonella enterica* serovar Typhimurium at 40% relative humidity (0.40 water activity) (Gruzdev *et al.*, 2012) and 11% relative humidity (0.11 water activity) (Maserati *et al.*, 2017). A decrease in stored trehalose after long-term desiccation (180 days) of *Saccharomyces cerevisiae* cells has been ascribed to ongoing basal metabolism, and is consistent with the observed activity of trehalases in low water-content cells (0.05 g H_2O g^{-1} dry weight) (Tapia and Koshland, 2014).

However, we note that the intracellular water activity has not been ascertained with certainty or accuracy in many such studies. Furthermore, gene expression patterns in anhydrobiotic cells should be interpreted with caution, as there is some disagreement in the literature on the stability of mRNA in a vitrified medium (Deng *et al.*, 2012; Minogue *et al.*, 2014; Sadler and Khodavirdi, 2015). We suggest that RNA-based studies in desiccated cells should incorporate comparative experiments and appropriate controls (e.g., fully hydrated cells) (Table 1), where the identification of significant differentially expressed genes (e.g., $p < 0.05$) provides some confidence in the assignment of water activity-related metabolic changes (Costa-Silva *et al.*, 2017). Further insights into the nature of gene expression during anhydrobiosis may be obtained by quantitating the turnover of RNA species using a time sensitive technique such as SLAM-seq (Herzog *et al.*, 2017) and others (Wissink *et al.*, 2019).

5.2. Enzymatic activity in the anhydrobiotic state

The retention of some metabolic functions during steady state anhydrobiosis implies that some enzymes continue to function in the very low water activity cytosol. Indeed, some enzymes, biochemical reactions and metabolic pathways retain functionality with an apparently negligible amount of water (Candotto Carniel *et al.*, 2021). For example, a ^3H_2O metabolic-labelling study of anhydrobiotic metabolism in lichens suggested that some basal metabolic processes, most notably amino acid biosynthesis (probably via transamination reactions from TCA cycle intermediates), occur at water activities as low as 0.30 (Cowan *et al.*, 1979), below the "complete desiccation" threshold (Alpert 2005). The precise threshold of water content required for protein function may depend on the amino acid composition and structure of the protein itself. It is also possible that residual cellular water is concentrated in subcellular compartments, rather than being homogeneously distributed throughout the cell. It has been reported that different components of proteins require at least 0.1-0.05 g H_2O g of protein^{-1} to maintain their conformation (Potts, 1994). Similarly, some lipases remain catalytically active in organic solvents at water contents as low as 1% (v/v); performing esterification, aminolysis, acyl exchange, thiotransesterification and oximolysis (Zaks and Klibanov, 1987), and subtilisin-catalysed transesterification reactions in >99% (v/v) tetrahydrofuran (Affleck *et al.*, 1992). The observation that solvent-free "melts" (e.g., molten sorbitol) support enzyme activity at sub-solvation levels and at very high temperatures (Brogan *et al.*, 2014) suggests a requirement for a liquid milieu, rather than an aqueous milieu *per se*, although the desiccated enzymes almost certainly retain bound (structural) water (Ball, 2017).

5.3. Energy generation in the anhydrobiotic state

For non-growing microorganisms, Morita (1997) proposed two modes of energy generation/metabolism:

- maintenance energy, which is required for osmotic regulation, maintenance of intracellular pH, futile cycles, turnover of macromolecules, motility, and energy dissipation by proton leak and ATP hydrolysis; and

- survival energy, which is required for the repair of macromolecular damage.

Assuming some metabolic functionality during the anhydrobiotic state is phylogenetically universal, a source of the maintenance energy is critical (Pirt, 1987; Morita, 1997; Leung *et al.*, 2020). Intracellular reserves of metabolic substrates are the most likely source, although the physiological utilisation of these substrates is not an equilibrium process (e.g., substrates will become depleted over time). The Gram-positive bacterium *Arthrobacter crystallopoietes* is metabolically active during desiccation, but at very low rates and approximately < 50% of stored carbon was catabolised over a 12-year period (Boylen, 1973).

The microbial oxidation of trace atmospheric gases (particularly H_2 and CO) is a key process for energy generation by hyper-oligotrophic soil microbiomes such as those of remote regions of terrestrial Antarctica (Ji *et al.*, 2017). This finding suggests a viable source of maintenance energy during anhydrobiosis, at least for some microorganisms. Thermodynamic calculations indicate that this autotrophic process (Greening *et al.*, 2016), which may actually be widespread in soil microbiomes (Bay *et al.*, 2021), can provide sufficient energy to meet the maintenance needs of these organisms (Bay *et al.*, 2021).

It has been noted that aerobic H_2 metabolism is hydrogenic (*sensu* water-generating: $2H_2 + O_2 = 2H_2O$) and that this process may be quantitatively significant as a source of cytoplasmic hydration in water-constrained habitats (Ortiz *et al.*, 2020). Although the underlying biochemistry is different, microbial growth appears to increase the water activity of the surrounding medium (Mossel, 1975; Reiß, 1978; Richard-Molard *et al.*, 1985). These studies further suggest that metabolically generated water may be an important source of water for microbial communities in hyper-arid habitats (Nagy, 2004). Given the importance of water for life, we speculate that the primary selective pressures driving the evolution of trace gas metabolism in desert microbiomes are oligotrophy and water insufficiency.

6. Metabolic responses of microbial communities to desiccation

Aridity affects microbial distribution in soils and reduces both microbial abundance and diversity in water-constrained ecosystems (Maestre *et al.*, 2015), including those with high concentrations

13

of salts (Lee *et al.*, 2018) or other solutes (Lievens *et al.*, 2015). However, the capacity of microorganisms to enter and persist for long periods in the anhydrobiotic state could play a pivotal role in maintaining biodiversity of dry environments; by increasing the longevity of individual cells and the sustainability of microbial populations (Pechter *et al.*, 2017).

The behaviour of microbial communities in desiccated environments may not be accurately predicted by the findings of laboratory studies, many of which have focused on pure cultures (Table 1). For example, biological soil crusts dominated by *Microcoleus vaginatus* continued to fix carbon at water potentials far below those where this activity had ceased in laboratory experiments (Rajeev *et al.*, 2013). The interpretation of the effects of desiccation in complex biological communities is likely to be affected by inter-species interactions. For example, microbial communities are known to improve and enhance plant growth under drought conditions (Khan *et al*., 2016; Gehring *et al*., 2017; Vilchez *et al.,* 2018). Root exudates have also been shown to accelerate the exit of microbes from the anhydrobiotic phase (Pazos-Rojas *et al*., 2019). Furthermore, mutualistic interactions are likely to exist between different microbial species (Manzanera, 2020).

Transcriptomic studies of a desiccated soil microbiome in the hyper-arid Namib Desert showed the presence of gene transcripts for enzymes driving inorganic nitrogen (nitrate) and organic carbon (3-P-glycerate) assimilation (soil relative humidity values ranged from 13 to 28%) (León-Sobrino *et al.*, 2019). We believe that these data constitute evidence of metabolic processes performed by microorganisms in an anhydrobiotic state in their natural habitat.

One likely reason for the respective responses of axenic laboratory cultures versus environmental microbial communities is the possible difference in the immediate cellular microenvironment. Microbes in spatially and temporally dynamic/complex natural habitats can be conditioned by changes in solutes and nutrients (Srikumar *et al.*, 2019), and by other environmental changes such as the formation of liquid films and microdroplets (Hallsworth, 2020; Orevi and Kashtan, 2021). In many soils (and other) biofilm communities, microorganisms respond to desiccation by secreting extracellular polymeric substances (EPS), which form complex three-dimensional

14

matrices in which the microbial cells are embedded (Rossi *et al.*, 2012; Costa *et al.*, 2018). Such EPS matrices retain water and reduce drying rates of cells (Roberson and Firestone, 1992; Manzoni and Katul, 2014; Schimel, 2018). EPS matrices are also hygroscopic, and have the capacity to absorb water from the gas phase (Cray *et al.*, 2013; Colica *et al.*, 2014; Flemming *et al.*, 2016). The water retention capacity of EPS matrices is thought to contribute to microbial community survival and function in desert soils (Adessi *et al.*, 2018; Costa *et al.*, 2018). Further, soil relative humidity values are typically substantially higher than those of the surface atmosphere, due to water vapour generated from sub-surface groundwater (in hot deserts) and melted permafrost (in cold deserts) (Stomeo *et al.*, 2012).

7. Discussion and Conclusions

In the last few decades, there has been substantial interest in the mechanisms involved in the desiccation-rehydration responses of microorganisms (García, 2011; Manzanera, 2020). Whereas the transition to and from the anhydrobiotic state has been well characterized for some species in culture-based systems, there is a general lack of information on the microbial anhydrobiotic state, especially for complex communities within their natural habitats.

Here, we suggest that microorganisms entering the anhydrobiotic state maintain some degree of basal metabolism to sustain cell repair systems and, possibly, other functions. However, the lower limits of desiccation-related metabolic activities have not been accurately defined, nor is it clear whether such limits might be organism-specific or generic. Furthermore, if rates of basal metabolism are extremely low then it may be impossible to measure these within the time frame of a typical research project, or even a human lifespan.

Despite these knowledge gaps, we argue that the available data, even if do not directly indicate an anhydrobiotic metabolism, could support the concept of "anhydrobiotic basal metabolism": upregulation of genes during microbial anhydrobiotic state (Cytryn *et al.*, 2007; Deng *et al.*, 2012), enzymatic activity at low cellular water content (Candotto Carniel *et al.*, 2021), the microbial oxidation of trace atmospheric gases (Greening *et al.*, 2016; Ortiz *et al.*, 2020). We also argue that

15

there is a need for more in-depth and rigorous studies on microbial functionality in the anhydrobiotic state. For example, high-resolution analyses of molecular turnover in desiccated cells (e.g., using SLAM-seq), coupled with analyses of temporal changes in the metaproteome and metabolome, would be highly informative, indicating which proteins and metabolic adaptations are necessary to maintain cellular viability during anhydrobiotic phase.

Understanding the molecular and functional basis of anhydrobiosis is important for a variety of reasons: increasing human population and climate change are exacerbating desertification on a global scale; ecology and survival in the absence of large-scale bodies of liquid water inform life-detection studies and other aspects of astrobiology; desiccated microbial structures can be used for long-term storage of microbial species or communities; and providing insights into ageing and longevity of cellular structures. Against this backdrop, there are other questions which remain unresolved: for example, how long can microorganisms persist in a desiccated state (presumably over geological timescales) before ionising radiation causes irreversible DNA damage; where is the dividing line between maintenance metabolism, activity of individual (xerotolerant) enzymes, and chemical processes (e.g., oxidation during anhydrobiosis); and what are the implications of long-term anhydrobiosis for evolutionary biology?

Box 1: Quantitation of the desiccated state

Various metrics are employed in the description and quantitation of water in physical and biological systems. Soil water content is traditionally designated by gravimetric H_2O content (θ_g), determined by heating and reweighing protocols (Bilskie and Campbell Scientific, 2001). An alternative, more modern and physiologically more relevant, metric is matrix water potential (Ψ_{soil}), a function of the attractive forces between hydrophilic polymers and/or charged surfaces (e.g., extracellular polymeric substances or clay particles) and water molecules (Chapin *et al.*, 2002; Seneviratne *et al.*, 2010), where non-wetted desert soils show typical values of between -40 and -95 MPa (Huang *et al.*, 2016). Other metrics include thermodynamic water activity (i.e., ρ/ρ^* where ρ is the partial vapour pressure of water in the solution, and ρ^* is the partial vapour pressure of pure water at the same temperature; from this it follows that water activity is unitless and that water activity of pure water is 1), and relative humidity (%) which is usually used to

describe water in the vapour phase and has values 100-fold greater than the concomitant water activity.

In soils, relative humidity values of < 70.0% (< 0.700 water activity) are generally considered to be dry, although most (non-xerophytic) plants wilt at around 0.950 water activity. By way of example, a 12-month 6-hourly sampling record of near-surface (0.5 cm) soil in the central hyper-arid Namib Desert gave a mean value of 26.1% ± 21.5% relative humidity (Cowan, unpublished data).

An important metric to understand water flow in and out biological cells is the osmotic or solute potential (Ψ_π), which indicates the potential of water to move from a hypotonic to a hypertonic solution, or vice versa. In desiccating soil, following an increase in solute concentration, water tends to flow out of the cells by osmosis. Microorganisms react to this trend with different mechanisms: for example, by accumulating solutes (stress protectants known as compatible solutes) to maintain the osmotic potential across the membrane (Wood *et al.*, 2001; Lebre *et al.*, 2017).

The physical state of water in biological systems differs substantially, depending on the degree of desiccation. Thirty years ago, based on thermodynamic estimates and moisture sorption isotherms (Vertucci, 1989, 1990), three different physical states of water in biological systems were proposed: Type I ("bound", meaning not readily removed), usually at a water activity between 0 and 0.250; Type II ("glass-forming", part of a hydration shell), usually occurring somewhere in the water activity range from 0.250 to 0.850 ; and Type III ("bulk water") usually in the water activity range from 0.850 to 1. Studies of water structure within fungal spores and dry plant-tissues indicate that residual water exists in a gel-like state: probably Type II (Wyatt *et al.*, 2015; Kuroki *et al.*, 2019). The anhydrobiotic condition in cells is thought to be primarily associated with the Type II water state, where the diffusion of metabolites and the conformational changes of macromolecules such as enzymes are limited (Greffe and Michiels, 2020).

Acknowledgements

References

Adessi, A., Cruz de Carvalho, R., De Philippis, R., Branquinho, C., and Marques da Silva, J. (2018) Microbial extracellular polymeric substances improve water retention in dryland biological soil crusts. *Soil Biol Biochem* **116**: 67–69.

Affleck, R., Xu, Z.F., Suzawa, V., Focht, K., Clark, D.S., and Dordick, J.S. (1992) Enzymatic catalysis and dynamics in low-water environments. *Proc Natl Acad Sci U S A* **89**: 1100–1104.

Allakhverdiev, S.I., Sakamoto, A., Nishiyama, Y., Inaba, M., and Murata, N. (2000) Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiol* **123**: 1047–1056.

Alpert, P. (2005) The limits on frontiers of desiccation-tolerant life. *Integr Comp Biol* **45**: 685–695.

Ball, P. (2017) Water is an active matrix of life for cell and molecular biology. *Proc Natl Acad Sci U S A* **114**: 13327–13335.

Bay, S.K., Dong, X., Bradley, J.A., Leung, P.M., Grinter, R., Jirapanjawat, T., et al. (2021) Trace gas oxidizers are widespread and active members of soil microbial communities. *Nat Microbiol* **6**: 246–256.

Benison, K.C., O'Neill, W.K., Blain, D., and Hallsworth, J.E. (2021) Water activities of acid brine lakes approach the limit for life. *Astrobiology* **21**: 1–12.

Berdugo, M., Vidiella Rocamora, B., Solé, R. V, and Maestre, F.T. (2021) Ecological mechanisms underlying aridity thresholds in global drylands. https://doi.org/10.32942/osf.io/w6n23

Billi, D., and Potts, M. (2002) Life and death of dried prokaryotes. *Res Microbiol* **153**: 7–12.

Bilskie, J., and Campbell Scientific (2001) Soil water status: content and potential. https://s.campbellsci.com/documents/us/technical-papers/soilh20c.pdf.

Boothby, T.C., and Pielak, G.J. (2017) Intrinsically disordered proteins and desiccation tolerance: elucidating functional and mechanistic underpinnings of anhydrobiosis. *BioEssays* **39**: 1–4.

Boylen, C.W. (1973) Survival of *Arthrobacter crystallopoietes* during prolonged periods of extreme desiccation. *J Bacteriol* **113**: 33–37.

Bremer, E., and Krämer, R. (2019) Responses of microorganisms to osmotic stress. *Annu Rev Microbiol* **73**: 313–334.

Brogan, A.P.S., Sharma, K.P., Perriman, A.W., and Mann, S. (2014) Enzyme activity in liquid lipase melts as a step towards solvent-free biology at 150°C. *Nat Commun* **5**: 5058.

Câmara, A.A. and Sant'Ana, A.S. (2021) Advances in yeast preservation: physiological aspects for cell perpetuation. *Curr Opin Food Sci* **38**: 62–70.

Candotto Carniel, F., Fernandez-Marín, B., Arc, E., Craighero, T., Laza, J.M., Incerti, G., et al. (2021) How dry is dry? Molecular mobility in relation to thallus water content in a lichen. *J Exp Bot* **72**: 1576–1588.

Cary, S.C., McDonald, I.R., Barrett, J.E., and Cowan, D.A. (2010) On the rocks: The microbiology of Antarctic Dry Valley soils. *Nat Rev Microbiol* **8**: 129–138.

Cesàro, A., De Giacomo, O., and Sussich, F. (2008) Water interplay in trehalose polymorphism.

Food Chem **106**: 1318–1328.

Chapin, F.S., Matson, P.A., and Mooney, H.A. (2002) Terrestrial Water and Energy Balance. In Principles of Terrestrial Ecosystem Ecology. New York: Springer, pp. 71–96.

Cherlet, M., Hutchinson, C., Reynolds, J., Hill, J., Sommer, S., and von Maltitz, G. (2018) World atlas of desertification, 3rd ed. Luxembourg: Publication Office of the European Union.

Chong, C.W., Pearce, D.A., and Convey, P. (2015) Emerging spatial patterns in Antarctic prokaryotes. *Front Microbiol* **6**: 1–14.

Clegg, J.S. (2005) Desiccation tolerance in encysted embryos of the animal extremophile, *Artemia*. *Integr Comp Biol* **45**: 715–724.

Clegg, J.S. (1967) Metabolic studies of crytobiosis in encysted embryos of *Artemia salina*. *Comp Biochem Physiol* **20**: 801–809.

Colica, G., Li, H., Rossi, F., Li, D., Liu, Y., and De Philippis, R. (2014) Microbial secreted exopolysaccharides affect the hydrological behavior of induced biological soil crusts in desert sandy soils. *Soil Biol Biochem* **68**: 62–70.

Costa-Silva, J., Domingues, D., and Lopes, F.M. (2017) RNA-Seq differential expression analysis: An extended review and a software tool. *PLoS One* **12**: 1–18.

Costa, O.Y.A., Raaijmakers, J.M., and Kuramae, E.E. (2018) Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. *Front Microbiol* **9**: 1–14.

Cowan, D.A., Green, T.G.A., and Wilson, A.T. (1979) Lichen metabolism. 1. The use of tritium labelled water in studies of anhydrobiotic metabolism in *Ramalina celastri* and *Peltigera polydactyla*. *New Phytol* **82**: 489–503.

Cowan, D.A., Hopkins, D.W., Jones, B.E., Maggs-Kölling, G., Majewska, R., and Ramond, J.B. (2020) Microbiomics of Namib Desert habitats. *Extremophiles* **24**: 17–29.

Cowan, D.A., Russell, N.J., Mamais, A., and Sheppard, D.M. (2002) Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. *Extremophiles* **6**: 431–436.

Cray, J.A., Russell, J.T., Timson, D.J., Singhal, R.S., and Hallsworth, J.E. (2013) A universal measure of chaotropicity and kosmotropicity. *Environ Microbiol* **15**: 287–296.

Crowe, J.H., Carpenter, J.F., and Crowe, L.M. (1998) The role of vitrification in anhydrobiosis. *Annu Rev Physiol* **60**: 73–103.

Crowe, J.H., and Cooper, A.F. (1971) Cryoptobiosis. *Sci Am* **225**: 30–37.

Crowe, J.H., Oliver, A.E., and Tablin, F. (2002) Is there a single biochemical adaptation to anhydrobiosis? *Integr Comp Biol* **42**: 497–503.

Crowe, J.H. (2014) Anhydrobiosis: an unsolved problem. Plant Cell Environ **37**: 1491–1493.

Cytryn, E.J., Sangurdekar, D.P., Streeter, J.G., Franck, W.L., Chang, W.S., Stacey, G., et al. (2007) Transcriptional and physiological responses of *Bradyrhizobium japonicum* to desiccation-induced stress. *J Bacteriol* **189**: 6751–6762.

Dechesne, A., Wang, G., Gülez, G., Or, D., and Smets, B.F. (2010) Hydration-controlled bacterial motility and dispersal on surfaces. *Proc Natl Acad Sci U S A* **107**: 14369–14372.

Deng, X., Li, Z., and Zhang, W. (2012) Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. *Food Microbiol* **30**: 311–315.

Dragone, N.B., Diaz, M.A., Hogg, I.D., Lyons, W.B., Jackson, W.A., Wall, D.H., et al. (2021) Exploring the boundaries of microbial habitability in soil. *J Geophys Res Biogeosci* **126**: e2020JG006052.

Dupont, S., Rapoport, A., Gervais, P., and Beney, L. (2014) Survival kit of *Saccharomyces cerevisiae* for anhydrobiosis. *Appl Microbiol Biotechnol* **98**: 8821–8834.

Evangelista, C.C.S., Guidelli, G.V., Borges, G., Araujo, T.F., De Souza, T.A.J., Neves, U.P. da C., et al. (2017) Multiple genes contribute to anhydrobiosis (tolerance to extreme desiccation) in the nematode *Panagrolaimus superbus*. *Genet Mol Biol* **40**: 790–802.

Farrant, J.M. (2000) A comparison of mechanisms of desiccation tolerance among three angiosperm resurrection plant species. *Plant Ecol* 151: 29–39.

Farrant, J.M., Cooper, K., Hilgart, A., Abdalla, K.O., Bentley, J., Thomson, J.A., et al. (2015) A molecular physiological review of vegetative desiccation tolerance in the resurrection plant *Xerophyta viscosa* (Baker). *Planta* **242**: 407–426.

Finn, S., Händler, K., Condell, O., Colgan, A., Cooney, S., McClure, P., et al. (2013) ProP is required for the survival of desiccated *Salmonella enterica* serovar Typhimurium cells on a stainless steel surface. *Appl Environ Microbiol* **79**: 4376–4384.

Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., and Kjelleberg, S. (2016) Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol* **14**: 563–575.

Fontana, A.J., and Carter, B.P. (2020) Measurement of water activity, moisture sorption isotherm, and moisture content of foods. In *Water activity in foods: fundamentals and applications, 2nd edition*. Barbosa-Cánovas, G.V., Fontana, A.J., Schmidt, S.J., and Labuza, T.P. (eds). John Wiley & Sons, pp. 207–226.

França, M.B., Panek, A.D., and Eleutherio, E.C.A. (2007) Oxidative stress and its effects during dehydration. *Comp Biochem Physiol - A Mol Integr Physiol* **146**: 621–631.

Gade, V.R., Traikov, S., Oertel, J., Fahmy, K., and Kurzchalia, T.V. (2020) *C. elegans* possess a general program to enter cryptobiosis that allows dauer larvae to survive different kinds of abiotic stress. *Sci Rep* **10**: 13466.

Gaff, D.F. (1997) Response of desiccation tolerant resurrection plants to water stress. In *Mechanisms of environmental stress resistance in plants*. Basra, A.S., and Basra R.K. (eds.). London: Harwood Academic Publishers, pp. 43–58.

García, A.H. (2011) Anhydrobiosis in bacteria: from physiology to applications. *J Biosci* **36**: 939–950.

Garcia-Fontana, C., Narvaez-Reinaldo, J.J., Castillo, F., Gonzalez-Lopez, J., Luque, I., and Manzanera, M. (2016) A new physiological role for the DNA molecule as a protector against drying stress in desiccation-tolerant microorganisms. *Front Microbiol* **7**: 1–12.

Gervais, P., Bensoussan, M., and Grajek, W. (1988) Water activity and water content: comparative effects on the growth of *Penicillium roqueforti* on solid substrate. *Appl Microbiol Biotechnol* **27**: 389–392.

Greening, C., Biswas, A., Carere, C.R., Jackson, C.J., Taylor, M.C., Stott, M.B., *et al.* (2016) Genomic and metagenomic surveys of hydrogenase distribution indicate H_2 is a widely utilised energy source for microbial growth and survival. *ISME J* **10**: 761–777.

Greffe, V.R.G. and Michiels, J. (2020) Desiccation-induced cell damage in bacteria and the relevance for inoculant production. *Appl Microbiol Biotechnol* **104**: 3757–3770.

Gruzdev, N., Mcclelland, M., Porwollik, S., Ofaim, S., Pinto, R., and Saldinger-Sela, S. (2012) Global transcriptional analysis of dehydrated *Salmonella enterica* serovar Typhimurium. *Appl Environ Microbiol* **78**: 7866–7875.

Guidetti, R. and Jönsson, K.I. (2002) Long-term anhydrobiotic survival in semi-terrestrial

micrometazoans. *J Zool* **257**: 181–187.

Gunde-Cimerman, N., Plemenitaš, A., and Oren, A. (2018) Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations. *FEMS Microbiol Rev* **42**: 353–375.

Hallsworth, J.E. (2020) Salt deliquescence can support extraterrestrial life. *Nat Astron* **4**: 739–740.

Hallsworth, J.E. (2018) Stress-free microbes lack vitality. *Fungal Biol* **122**: 379–385.

Hallsworth, J.E., Yakimov, M.M., Golyshin, P.N., Gillion, J.L.M., D'Auria, G., De Lima Alves, F., et al. (2007) Limits of life in $MgCl_2$-containing environments: Chaotropicity defines the window. *Environ Microbiol* **9**: 801–813.

Haranczyk, H., Gazdzinski, S., and Olech. M. (1998) Initial stages of lichen hydration observed by proton magnetic relaxation. *New Phytol* **138**: 191–202.

Herzog, V.A., Reichholf, B., Neumann, T., Rescheneder, P., Bhat, P., Burkard, T.R., et al. (2017) Thiol-linked alkylation of RNA to assess expression dynamics. *Nat Methods* **14**: 1198–1204.

Hibshman, J.D., Clegg, J.S., and Goldstein, B. (2020) Mechanisms of desiccation tolerance: themes and variations in brine shrimp, roundworms, and tardigrades. *Front Physiol* **11**: 1–19.

Huang, J., Yu, H., Guan, X., Wang, G., and Guo, R. (2016) Accelerated dryland expansion under climate change. *Nat Clim Chang* **6**: 166–171.

Hufnagel, L., Mics, F., Pálinkás, M., and Homoródi, R. (2018) Introductory chapter: global aspects and scientific importance of desert ecological research. In Community and Global Ecology of Deserts. Hufnagel, L. (ed). IntechOpen, pp. 137–144.

Ji, M., Greening, C., Vanwonterghem, I., Carere, C.R., Bay, S.K., Steen, J.A., et al. (2017) Atmospheric trace gases support primary production in Antarctic desert surface soil. *Nature* **552**: 400–403.

Kamilari, M., Jørgensen, A., Schiøtt, M., and Møbjerg, N. (2019) Comparative transcriptomics suggest unique molecular adaptations within tardigrade lineages. *BMC Genomics* **20**: 607.

Katoh, H., Asthana, R.K., and Ohmori, M. (2004) Gene expression in the cyanobacterium Anabaena sp. PCC7120 under desiccation. *Microb Ecol* **47**: 164–174.

Keilin, D. (1959) The problem of anabiosis or latent life: history and current concept. *Proc R Soc London Ser B, Biol Sci* **150**: 149–191.

Kieft, T. (2003) Hot desert soil microbial communities. In Encyclopedia of environmental microbiology. Hoboken: John Wiley & Sons, pp. 1576–1586.

Kosar, F., Akram, N.A., Sadiq, M., Al-Qurainy, F., and Ashraf, M. (2019) Trehalose: a key organic osmolyte effectively involved in plant abiotic stress tolerance. *J Plant Growth Regul* **38**: 606–618.

Koshland, D. and Tapia, H. (2019) Desiccation tolerance: an unusual window into stress biology. *Mol Biol Cell* **30**: 737–741.

Kranner, I., and Birtic, S. (2005) A modulating role for antioxidants in desiccation tolerance. *Integr Comp Biol* **45**: 734–740.

Kuliešienė, N., Žūkienė, R., Khroustalyova, G., Chang, C.R., Rapoport, A., and Daugelavičius, R. (2021) Changes in energy status of *Saccharomyces cerevisiae* cells during dehydration and rehydration. *Microorganisms* **9**: 1–13.

Kuroki, S., Tsenkova, R., Moyankova, D., Muncan, J., Morita, H., Atanassova, S., and Djilianov, D. (2019) Water molecular structure underpins extreme desiccation tolerance of the resurrection plant *Haberlea rhodopensis*. *Sci Rep* **9**: 1–12.

Larose, C., Dommergue, A., and Vogel, T.M. (2013) The dynamic Arctic snow pack: An unexplored environment for microbial diversity and activity. *Biology (Basel)* **2**: 317–330.

LeBlanc, J.C., Gonçalves, E.R., and Mohn, W.W. (2008) Global response to desiccation stress in the soil actinomycete *Rhodococcus jostii* RHA1. *Appl Environ Microbiol* **74**: 2627–2636.

Lebre, P.H., De Maayer, P., and Cowan, D.A. (2017) Xerotolerant bacteria: surviving through a dry spell. *Nat Rev Microbiol* **15**: 285–296.

Lee, C.J.D., McMullan, P.E., O'Kane, C.J., Stevenson, A., Santos, I.C., Roy, C., et al. (2018) NaCl-saturated brines are thermodynamically moderate, rather than extreme, microbial habitats. *FEMS Microbiol Rev* **42**: 672–693.

León-Sobrino, C., Ramond, J., Coclet, C., Kapitango, R., Maggs-Kölling, G., Cowan, D.A. (2021) Temporal dynamics of microbial transcription in wetted hyperarid desert soils. *bioRxiv* 1–46.

León-Sobrino, C., Ramond, J.B., Maggs-Kölling, G., and Cowan, D.A. (2019) Nutrient acquisition, rather than stress response over diel cycles, drives microbial transcription in a hyper-arid Namib desert soil. *Front Microbiol* **10**: 1–11.

Leprince, O., and Buitink, J. (2015) Introduction to desiccation biology: from old borders to new frontiers. *Planta* **242**: 369–378.

Leung, P.M., Bay, S.K., Meier, D. V., Chiri, E., Cowan, D.A., Gillor, O., et al. (2020) Energetic basis of microbial growth and persistence in desert ecosystems. *mSystems* **5**: 1–14.

Li, H., Bhaskara, A., Megalis, C., and Tortorello, M.L. (2012) Transcriptomic analysis of salmonella desiccation resistance. *Foodborne Pathog Dis* **9**: 1143–1151.

Lievens, B., Hallsworth, J.E., Pozo, M.I., Belgacem, Z.B., Stevenson, A., Willems, K.A., and Jacquemyn, H. (2015) Microbiology of sugar-rich environments: diversity, ecology and system constraints. *Environ Microbiol* **17**: 278–298.

de Lima Alves, F., Stevenson, A., Baxter, E., Gillion, J.L.M., Hejazi, F., Hayes, S., et al. (2015) Concomitant osmotic and chaotropicity-induced stresses in *Aspergillus wentii*: compatible solutes determine the biotic window. *Curr Genet* **61**: 457–477.

López-Lara, L.I., Pazos-Rojas, L.A., López-Cruz, L.E., Morales-García, Y.E., Quintero-Hernández, V., De La Torre, J., et al. (2020) Influence of rehydration on transcriptome during resuscitation of desiccated *Pseudomonas putida* KT2440. *Ann Microbiol* **70**: 54.

Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., Eldridge, D.J., Ochoa, V., Gozalo, B., et al. (2015) Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proc Natl Acad Sci U S A* **112**: 15684–15689.

Manzanera, M. (2020) Dealing with water stress and microbial preservation. *Environ Microbiol* **00**: 00–00.

Manzoni, S., and Katul, G. (2014) Invariant soil water potential at zero microbial respiration explained by hydrological discontinuity in dry soils. *Geophys Res Lett* **41**: 7151–7158.

Manzoni, S., Schimel, J.P., and Porporato, A. (2012) Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology* **93**: 930–938.

Maserati, A., Fink, R.C., Lourenco, A., Julius, M.L., and Diez-Gonzalez, F. (2017) General response of *Salmonella enterica* serovar Typhimurium to desiccation: a new role for the virulence factors *sopD* and *sseD* in survival. *PLoS One* **12**: 1–23.

Minogue, T.D., Kalina, W.V., and Coyne, S.R. (2014) Stabilization of biothreat diagnostic samples through vitrification matrices. *J Microbiol Methods* **101**: 81–85.

Mirzabaev, A., Wu, J., Evans, J., García-Oliva, F., Hussein, I.A.G., Iqbal, M.H., et al. (2019) Desertification. In *Climate change and land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems*. Shukla, P.R., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H.-O., Roberts, D.C., Zhai, P., Slade, R., Connors, S., van Diemen, R., Ferrat, M., Haughey, E., Luz, S., Neogi, S., Pathak, M., Petzold, J., Portugal Pereira, J., Vyas, P., Huntley, E., Kissick, K., Belkacemi, and Malley, M. (eds)., pp. 249–343. In press.

Morita, R.Y. (1997) Bacteria in oligotrophic environments, New York: Chapman & Hall.

Mosca, C., Rothschild, L.J., Napoli, A., Ferré, F., Pietrosanto, M., Fagliarone, C., et al. (2019) Over-expression of UV-damage DNA repair genes and ribonucleic acid persistence contribute to the resilience of dried biofilms of the desert Cyanobacetrium *Chroococcidiopsis* exposed to Mars-like UV flux and long-term desiccation. *Front Microbiol* **10**: 1–11.

Mossel, D.A.A. (1975) Water and Micro-organisms in Foods—A Synthesis. In Water relations of foods. London: Academic Press Inc., pp. 347–364.

Nagy, K.A. (2004) Water economy of free-living desert animals. *Int Congr Ser* **1275**: 291–297.

Naidoo, Y., Valverde, A., Pierneef, R.E., and Cowan, Y. (2021) Differences in precipitation regime shape microbial community composition and functional potential in Namib desert soils. *Microb Ecol*.

Narváez-Reinaldo, J.J., Barba, I., González-López, J., Tunnacliffe, A., and Manzanera, M. (2010) Rapid method for isolation of desiccation-tolerant strains and xeroprotectants. *Appl Environ Microbiol* **76**: 5254–5262.

Oliver, M.J., Farrant, J.M., Hilhorst, H.W.M., Mundree, S., Williams, B., and Bewley, J.D. (2020) Desiccation tolerance: avoiding cellular damage during drying and rehydration. *Annu Rev Plant Biol* **71**: 435–460.

Orevi, T., and Kashtan, N. (2021) Life in a droplet: microbial ecology in microscopic surface wetness. *Front Microbiol* **12**: 1–11.

Ortiz, M., Leung, P.M., Shelley, G., van Goethem, M.W., Bay, S.K., Jordaan, K., et al. (2020) A genome compendium reveals diverse metabolic adaptations of Antarctic soil microorganisms. *bioRxiv*.

Palud, A., Salem, K., Cavin, J., Beney, L., and Licandro, H. (2020) Identification and transcriptional profile of *Lactobacillus paracasei* genes involved in the response to desiccation and rehydration. *Food Microbiol* **85**: 103301.

Pazos-Rojas, L.A., Muñoz-Arenas, L.C., Rodríguez-Andrade, O., López-Cruz, L.E., López-Ortega, O., Lopes-Olivares, F., et al. (2019) Desiccation-induced viable but nonculturable state in *Pseudomonas putida* KT2440, a survival strategy. *PLoS One* **14**: e0219554.

Pechter, K.B., Yin, L., Oda, Y., Gallagher, L., Yang, J., Manoil, C., and Harwood, C.S. (2017) Molecular basis of bacterial longevity. *Am Soc Microbiol* **8**: 1–12.

Pedersen, B.H., Malte, H., Finster, K., and Ramløv, H. (2021) Respiration measurements of individual tardigrades of the species *Richtersius* cf *coronifer* as a function of temperature and salinity and termination of anhydrobiosis. *Astrobiology*.

Pirt, S.J. (1987) The energetics of microbes at slow growth rates: Maintenance energies and dormant organisms. *J Ferment Technol* **65**: 173–177.

Pointing, S.B., and Belnap, J. (2012) Microbial colonization and controls in dryland systems. *Nat*

Rev Microbiol **10**: 551–562.

Potts, M. (1994) Desiccation tolerance of prokaryotes. *Microbiol Rev* **58**: 755–805.

Potts M., Slaughter, S.M., Hunneke, F., Garst, J.F., and Helm R.F. (2005) Desiccation tolerance of prokaryotes: application of principles to human cells. *Integr Comp Biol* **45**: 800–809.

Proctor, M.C.F. (2003) Comparative ecophysiological measurements on the light responses, water relations and desiccation tolerance of the filmy ferns *Hymenophyllum wilsonii* Hook and *H. tunbrigense* (L.) Smith. *Ann Bot* **91**: 717–727.

Quinton, W.L., Hayashi, M., and Chasmer, L.E. (2011) Permafrost-thaw-induced land-cover change in the Canadian subarctic: implications for water resources. *Hydrol Process* **25**: 152–158.

Rajeev, L., Da Rocha, U.N., Klitgord, N., Luning, E.G., Fortney, J., Axen, S.D., et al. (2013) Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. *ISME J* **7**: 2178–2191.

Rapoport, A., Golovina, E.A., Gervais, P., Dupont, S., and Beney, L. (2019) Anhydrobiosis: inside yeast cells. *Biotechnol Adv* **37**: 51–67.

Ratnakumar, S. and Tunnacliffe, A. (2006) Intracellular trehalose is neither necessary nor sufficient for desiccation tolerance in yeast. *FEMS Yeast Res* **6**: 902–913.

Reiß, J. (1978) Mycotoxins in foodstuffs - XII. The influence of the water activity (a_w) of cakes on the growth of moulds and the formation of mycotoxins. *Z Lebensm Unters Forsch* **167**: 419–422.

Reuner, A., Hengherr, S., Brümmer, F., and Schill, R.O. (2010) Comparative studies on storage cells in tardigrades during starvation and anhydrobiosis. *Curr Zool* **56**: 1–13.

Richard-Molard, D., Lesage, L., and Cahagnier, B. (1985) Effect of water activity on mold growth and mycotoxin production. In *Properties in water foods*. Simatos, D., and Multon, J.L. (eds). Dordrecht: Springer, pp. 273–292.

Roberson, E.B., and Firestone, M.K. (1992) Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Appl Environ Microbiol* **58**: 1284–1291.

Rossi, F., Potrafka, R.M., Pichel, F.G., and De Philippis, R. (2012) The role of the exopolysaccharides in enhancing hydraulic conductivity of biological soil crusts. *Soil Biol Biochem* **46**: 33–40.

Ryabova, A., Cornette, R., Cherkasov, A., Watanabe, M., Okuda, T., Shagimardanova, E., et al. (2020) Combined metabolome and transcriptome analysis reveals key components of complete desiccation tolerance in an anhydrobiotic insect. *Proc Natl Acad Sci U S A* **117**: 19209–19220.

Sadler, T.R., and Khodavirdi, A.C. (2015) High-quality RNA extracted from biopsied samples dehydrated and stored dried at room temperature without chemical preservation for up to 3 months as evidenced by RT-PCR results. *Appl Immunohistochem Mol Morphol* **23**: 456–461.

Schimel, J.P. (2018) Life in dry soils: effects of drought on soil microbial communities and processes. *Annu Rev Ecol Evol Syst* **49**: 409–432.

Seneviratne, S.I., Corti, T., Davin, E.L., Hirschi, M., Jaeger, E.B., Lehner, I., et al. (2010) Investigating soil moisture-climate interactions in a changing climate: a review. *Earth-Science Rev* **99**: 125–161.

Singh, P., Jain, K., Desai, C., Tiwari, O., and Madamwar, D. (2019) Microbial community dynamics of extremophiles/extreme environment. In *Microbial diversity in the genomic era.* Das, S., and Dash, H.R. (eds), pp. 323–332.

Srikumar, S., Cao, Y., Yan, Q., Van Hoorde, K., Nguyen, S., Cooney, S., et al. (2019) RNA sequencing-based transcriptional overview of xerotolerance in *Cronobacter sakazakii* SP291. *Appl Environ Microbiol* **85**: 1–16.

Stevenson, A., Cray, J.A., Williams, J.P., Santos, R., Sahay, R., Neuenkirchen, N., et al. (2015) Is there a common water-activity limit for the three domains of life? *ISME J* **9**: 1333–1351.

Stevenson, A., Hamill, P.G., Medina, Á., Kminek, G., Rummel, J.D., Dijksterhuis, J., et al. (2017) Glycerol enhances fungal germination at the water-activity limit for life. *Environ Microbiol* **19**: 947–967.

Stevenson, A., Hamill, P.G., O'Kane, C.J., Kminek, G., Rummel, J.D., Voytek, M.A., et al. (2017) *Aspergillus penicillioides* differentiation and cell division at 0.585 water activity. *Environ Microbiol* **19**: 687–697.

Stomeo, F., Makhalanyane, T.P., Valverde, A., Pointing, S.B., Stevens, M.I., Cary, C.S., et al. (2012) Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley. *FEMS Microbiol Ecol* **82**: 326–340.

Sun, W.Q., and Leopold, A.C. (1997) Cytoplasmic vitrification and survival of anhydrobiotic organisms. *Comp Biochem Physiol - A Physiol* **117**: 327–333.

Tapia, H., and Koshland, D.E. (2014) Trehalose is a versatile and long-lived chaperone for desiccation tolerance. *Curr Biol* **24**: 2758–2766.

Trevors, J.T. (2011) Viable but non-culturable (VBNC) bacteria: Gene expression in planktonic and biofilm cells. *J Microbiol Methods* **86**: 266–273.

Torsvik V., and Øvreås L. (2008) Microbial diversity, life strategies, and adaptation to life in extreme soils. In *Microbiology of extreme soils*. Dion P., and Nautiyal C.S. (eds). Berlin: Springer, **13**.

Uritskiy, G., Getsin, S., Munn, A., Gomez-Silva, B., Davila, A., Glass, B., et al. (2019) Halophilic microbial community compositional shift after a rare rainfall in the Atacama desert. *ISME J* **13**: 2737–2749.

Vertucci, C.W. (1990) Calorimetric studies of the state of water in seed tissues. *Biophys J* **58**: 1463–1471.

Vertucci, C.W. (1989) The effects of low water contents on physiological activities of seeds. *Physiol Plant* **77**: 172–176.

Vilchez, J.I. Niehaus, K., Dowling, D.N., Gonzalez-Lopez, J., and Manzanera, M. (2018) Protection of pepper plants from drought by *Microbacterium* sp. 3J1 by modulation of the plant's glutamine and α-ketoglutarate content: a comparative metabolomics approach. *Front Microbiol* **9**: 1–19.

Voronina, T.A., Nesmelov, A.A., Kondratyeva, S.A., Deviatiiarov, R.M., Miyata, Y., Tokumoto, S., et al. (2020) New group of transmembrane proteins associated with desiccation tolerance in the anhydrobiotic midge *Polypedilum vanderplanki*. *Sci Rep* **10**: 1–13.

Vriezen, J.A.C., De Bruijn, F.J., and Nüsslein, K. (2007) Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. *Appl Environ Microbiol* **73**: 3451–3459.

Walters, C., Hill, L.M., and Wheeler, L.J. (2005) Dying while dry: kinetics and mechanisms of deterioration in desiccated organisms. *Integr Comp Biol* **45**: 751–758.

Wang, C., Grohme, M.A., Mali, B., Schil, R.O., and Frohme, M. (2014) Towards decrypting cryptobiosis - analyzing anhydrobiosis in the tardigrade *Milnesium tardigradum* using transcriptome sequencing. *PLoS One* **9**: 1–15.

Wharton, D.A. (2015) Anhydrobiosis. *Curr Biol* **25**: R1114–R1116.

Wissink, E.M., Vihervaara, A., Tippens, N.D., and Lis, J.T. (2019) Nascent RNA analyses: tracking transcription and its regulation. *Nat Rev Genet* **20**: 705–723.

Wood, J.M., Bremer, E., Csonka, L.N., Kraemer, R., Poolman, B., Van der Heide, T., and Smith, L.T. (2001) Osmosensing and osmoregulatory compatible solute accumulation by bacteria. *Comp Biochem Physiol - A Mol Integr Physiol* **130**: 437–460.

Wyatt, T.T., Golovina, E.A., van Leeuwen, R., Hallsworth, J.E., Wösten, H.A.B., and Dijksterhuis, J. (2015) A decrease in bulk water and mannitol and accumulation of trehalose and trehalose-based oligosaccharides define a two-stage maturation process towards extreme stress resistance in ascospores of *Neosartorya fischeri* (*Aspergillus fischeri*). *Environ Microbiol* **17**: 383–394.

Zaks, A. and Klibanov, A.M. (1987) Enzyme-catalyzed processes in organic solvents. *Ann N Y Acad Sci* **82**: 3192–3196.