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Multiplication of microbes below 0.690 water activity: implications for terrestrial and extraterrestrial life

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Summary

Since a key requirement of known life-forms is available water (water-activity; a_w), searches for signatures of past life in terrestrial and extraterrestrial environments have recently targeted places known to have contained significant quantities of biologically available water. The lower limit of water activity that enables cell division is ~0.605 which, until now, was only known to be exhibited by a single eukaryote; the sugar-tolerant, fungal xerophile Xeromyces bisporus. The first forms of life on Earth were, however, prokaryotic. Furthermore, early life on Earth inhabited high-salt environments, suggesting an ability to withstand low water-activity. Recent evidence indicates that some halophilic Archaea and Bacteria have water-activity limits more or less equal to those of X. bisporus. Regardless of species, cellular systems are sensitive to minute differences in water activity (of <0.005 a_w-units) so there is a need to determine water-activity values to three decimal places. We discuss water activity in relation to the limits of Earth's present-day biosphere; the possibility of microbial multiplication by utilizing water from thin, aqueous films or non-liquid sources; whether prokaryotes were the first organisms able to multiply at the 0.605-a_w limit; and whether extraterrestrial aqueous milieu of ≥0.605 a_w can resemble fertile microbial habitats found on Earth.

Introduction

Given the fact that water is one of the principal ingredients of cellular life (Daniel et al., 2004), insights into the minimum water requirements of cells are imperative to understanding the functionality of living-systems at every level (from biomacromolecule to biosphere), as well as the origins of life, in an environmental context. The generally held opinion is that life appeared independently on Earth and, possibly, elsewhere in the Solar System (Clancy et al., 2005); though one other explanation for the presence of life on Earth is that it appeared on another planet and was transported here in the form of prokaryotes or their ancestors (an idea known as panspermia; Thomson, 1871). Until recently, eukaryotic microbes have held the record for life under water-constrained conditions, as some species are capable of cell division down to a water activity (a_w)[1] of 0.605 at high sugar concentrations (Pitt and Christian, 1968; Williams and Hallsworth, 2009). Whereas such data have formed the basis of international policy for planetary protection in relation to space-exploration missions (see

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[1] Water activity, the mole fraction of water, is defined by an equation (water activity = vapour pressure of the solution/vapour pressure of the water) which is derived from Raoult's Law; this parameter and its derivation are discussed in detail by Brown (1990) and Grant (2004).

below), sugar-rich substrates have very limited applicability to those extraterrestrial habitats with which we are familiar. Historically, the accepted limit for cell division of prokaryotic microbes has been 0.755 a_w; this applied to a small fraction of halophilic species at high salt concentrations (for references, see Grant, 2004). However, both culture-based and culture-independent studies provide evidence for multiplication and metabolic activity of halophilic Archaea and Bacteria in the range 0.680 to 0.605 a_w, both in their natural habitats in situ, and in vitro (Javor, 1984; Yakimov et al., 2014; A. Stevenson et al., submitted). Other studies have shown that, whereas the vast majority of yeasts and fungi are active somewhere within the range 1 to 0.720 a_w (Pitt, 1975; Brown, 1976), only ~12 species have been observed to grow and/or germinate at < 0.700 a_w (Williams and Hallsworth, 2009; A. Stevenson et al., submitted). Here, we discuss the evidence for microbial activity below at or below 0.690, which represents the very edge of the functional biosphere on Earth. Low water activity is also discussed in relation to early life on Earth, the plausibility of cell division in habitable extraterrestrial environments in which biologically available water is present, and a series of unanswered scientific questions.

Water-activity at the outer edges of the microbial biosphere

The primary physical determinants of the habitable space on Earth are temperature and water activity; these parameters are also used to designate the ‘Special Regions’ of Mars in which microbial cell-division might feasibly take place (Beaty et al., 2006; Kminek et al., 2010; J. D. Rummel et al., unpublished)[2]. The temperature window over which microbes are, collectively, capable of cell division (i.e. from -18 to +122℃; Takai et al., 2008; Chin et al., 2010) spans ≤ 40% of the entire range of temperatures to which life-systems on Earth can be exposed; i.e. from approximately -90℃ to ≥ 250℃ (for some hydrothermal vents; Fig. 1a). By contrast, environmental water-activity values range from 1 to 0 and most cellular systems of known life-forms on Earth are only active in the range, or a segment of the range, 1 to 0.900 a_w (Fig. 1b; Brown, 1976; Grant, 2004). For example, there is a drop-off in measurable metabolic activity in many soils at ≤ 0.890 a_w (Moyano et al., 2012; 2013; Stevenson and Hallsworth, 2014). However, metabolic activity and cell-division has been

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[2] Planetary protection in relation to space missions aims to protect those planets where spacecraft are landed, as well as Earth, from accidental contamination with non-native life-forms (Kminek et al., 2010; 2014). Mars Special Regions have been defined according to the activities of the NASA Mars Exploration Program Analysis Group (MEPAG), Special Regions-Scientific Analysis Group 1 (SR-SAG1) and the Committee on Space Research (COSPAR), which is part of the International Council for Science. Both these committees conservatively recommended 0.500 a_w as the limit beyond which no known terrestrial microorganism is capable of multiplication; implying that any environment of Mars with a water activity of > 0.500 may potentially enable proliferation of xerophilic microbes if they happened to arrive as accidental passengers on spacecraft sent from Earth (Fig. 1; Beaty et al., 2006; Kminek et al., 2010). A revised analysis of Mars Special Regions is currently underway by the MEPAG SR-SAG2 (J. D. Rummel et al., unpublished).

reported below 0.900 a_w for a great number of xerotolerant/philic and halotolerant/philic microbes (Brown, 1976; Grant, 2004), and even below 0.755 a_w for both eukayotic and prokaryotic species (Javor, 1984; Williams and Hallsworth, 2009; Yakimov et al., 2014; A. Stevenson et al., submitted). Of the microbes known to multiply below 0.720, the majority (unlike X. bisporus) are not obligate osmophiles that must inhabit sugar-rich substrates; these include halophilic prokaryotes and xerophilic fungi such as Aspergillus penicilliodes and Eurotium herbarorium (Samson and Lustgraaf, 1978; Williams and Hallsworth, 2009; Yakimov et al., 2014; A. Stevenson et al., submitted)[3]. Even for the most xerophilic microbes thus far characterized (see Pitt, 1975; Javor, 1984; Williams and Hallsworth, 2009; A. Stevenson et al., submitted), rates of cell division typically decrease by an order of magnitude between 0.870 and 0.770 a_w, and by a further order of magnitude between 0.770 and 0.670 a_w (Stevenson and Hallsworth, 2014; A. Stevenson et al., submitted). There are only reports of cell division for between 20 and 30 microbial species or communities at $\leq$ 0.690 a_w (see Javor, 1984; Yakimov et al., 2014; A. Stevenson et al., submitted). Whereas all of these species are extreme, obligately xerophilic eukaryotes or extreme, obligately halophilic prokaryotes which have low rates of cell division - or are incapable of growth - close to 1 a_w, the ultimate limit for multiplication of even the most resilient strains appears to be ~0.61 a_w (Pitt and Christian, 1968; A. Stevenson et al., submitted). For microbes on Earth, therefore, biotic activity spans approximately 40% of the available water-activity range, thus emphasizing the potency of water as a determinant of the functional biosphere. The overwhelming majority of microbial systems are metabolically active somewhere within the ranges 5 to 40°C, and 1 to 0.900 a_w, which represent even smaller portions of the environmentally pertinent temperature and water-activity ranges; i.e. only 10% in each case (Fig. 1). Of the microbial systems characterized thus far, the 20 to 30 known to be active at $\leq$ 0.690 a_w (Fig. 1; Javor, 1984; Yakimov et al., 2014; A. Stevenson et al., submitted) represent the most extreme forms of life to have penetrated these kinds of hostile environment[4].

Some reports have alluded to the possibility of microbial growth and metabolism at the otherwise unprecedented water-activity values of 0.382 (for deep-sea halophiles in $MgCl_2$-saturated brine; van der Wielen et al., 2005), < 0.450 (for halophiles in the $CaCl_2$-rich, Antarctic Don Juan Pond; Siegel, 1979), 0.500 (Actinobacteria isolated from algal mats and cultured in soil-based substrates; Doroshenko et al., 2005; 2006; Zvyagintsev et al., 2009;

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[3] This has implications for preventing contamination of other planetary bodies (see above) which, as far as we know, lack sugar-ruch environments, during space exploration missions.

[4] Habitats which have sufficiently low water-activity to exclude almost all forms of life on Earth and, therefore, have a characteristically low biodiversity (especially those of < 0.690 a_w) are fertile habitats for those extremophiles which thrive there due to minimal competition and, frequently, a lack of grazers and predators (for references, see Cray et al., 2013b). Such low-water activity habitats are, however, typically too biologically hostile and insufficiently biodiverse to act as open habitats for microorganisms (Cray et al., 2013b; Lievens et al., 2014; Oren and Hallsworth, 2014).

2012), 0.570 (for halophiles in acidic saline lakes; Mormile et al., 2009), 0.600 (for germination of Wallemia sebi [a xerophilic basidiomycete] on high-sugar substrates; Frank and Hess, 1941) and 0.600 (reported value for optimum growth of halophiles (Jaenicke and Bohm, 1998), and biotic activity in salt lakes; Cobucci-Ponzano et al., 2006). Some of these values were hypothetical (see below), and the other claims have not been accepted or have been refuted by authors of a number of subsequent studies (Pitt and Christian, 1968; Wynn-Williams, 1996; Beaty et al., 2006; Hallsworth et al., 2007; Kminek et al., 2010; Oren, 2011; Stevenson and Hallsworth, 2014; A. Stevenson et al., submitted; J. D. Rummel et al., unpublished). The Don Juan Pond (located within the McMurdo Dry Valleys, Antarctica) is a $CaCl_2$-saturated brine-pool situated in a closed basin and fed by seasonal melt-water streams and deliquescent seepages, both of which are thought to deliver $CaCl_2$ to the lake (Dickson, 2013). Its volume fluctuates but is typically ~3000 m^3 (slightly larger than an Olympic swimming pool), and it is amongst the most saline large-scale bodies of water known on Earth. This pond rarely, if ever, freezes despite winter temperatures of $\leq$ -51ºC (Siegel 1979; Marion 1997; Grant, 2004). While annual temperatures of the pond's water and the surrounding sediments are occasionally above 0℃, they remain below -20°C for the majority of the year (Samarkin et al., 2010) so it is highly unlikely that microbial life could multiply there (for references, see Chin et al., 2010; Kminek et al., 2010; J. D. Rummel et al., unpublished). Saturated solutions of $CaCl_2$, as found in the Don Juan Pond, are highly chaotropic and are therefore likely to prevent microbial growth (and may even be sterile environments; Duda et al., 2004; 2005; Hallsworth et al., 2007; Cray et al., 2013a; 2013b; Oren, 2013; Yakimov et al., 2014). Nitrous oxide emissions recorded from the surrounding sediments, frequently attributed to the biological transformation of nitrogenous compounds, are apparently the result of abiotic reactions between brine nitrates and Fe^{II}-bearing minerals (Samarkin et al., 2010). The water activity of the $MgCl_2$-dominated, deep-sea hypersaline brine studied by van der Wielen et al. (2005) is ~0.382 at the in situ temperature of 14.5°C (Winston and Bates, 1960; Hallsworth et al., 2007). Culture-dependent and culture-independent studies of this brine, and investigations into the biophysics of macromolecular interactions, indicate that both its potent chaotropicity (even at water-activity values which would otherwise be permissive for cell division) and exceptionally low water-activity prohibit life processes (Hallsworth et al., 2007; Yakimov et al., 2014), as these parameters do for solutions of comparable salts (Winston and Bates, 1960; Duda et al., 2004; Hallsworth et al., 2003a; Kminek et al., 2010; Oren, 2011; Cray et al., 2013a; 2013b). Speculations that microbial metabolism and cell division occur at ~5 M $MgCl_2$ are inconsistent with the virtual sterility of the Dead Sea when $MgCl_2$ concentrations become elevated (Oren, 1999; 2010; Oren, 2013) or the $CaCl_2$-dominated Don Juan Pond (Siegel et al., 1983; Samarkin et al., 2010; Oren, 2013) where concentrations of divalent chloride salts reach critical concentrations which are prohibitive for all life processes (Hallsworth et al., 2007; Cray et al., 2013a; Oren, 2013; Yakimov et al., 2014). Although there is a theoretical possibility that some microbes have evolved specialised structures which isolate cells from such hostile habitats whilst permitting biotic activity, to our knowledge no such structures have yet been reported for any microbial species in any type of extremely chaotropic (e.g. Hallsworth et al., 2007; Yakimov et al., 2014) or low water-activity (≤ 0.600) environment.

Reports of germination and subsequent cell division during germ-tube formation of several Actinobacteria (i.e. Streptomyces albidoflavus [syn. Streptomyces odorifer], Streptomyces rectiviolaceus, and a Micromonospora strain) at 0.500 a_w (which were carried out by one group: Doroshenko et al., 2005; 2006; Zvyagintsev et al., 2009; 2012) are apparently erroneous (see Stevenson and Hallsworth, 2014). Independent studies have demonstrated that none of these species was capable of growth below 0.895 a_w, and the theoretical water-activity minimum for the most xerotolerant (a strain of Streptomyces albidoflavus) was ~0.870 (Stevenson and Hallsworth, 2014). Proposed limits of 0.570 or 0.600 a_w for biotic activity of halophiles were speculative (i.e. not derived from determinations of water-activity; Jaenicke and Bohm, 1998; Mormile et al., 2009; Cobucci-Ponzano et al., 2006), and sources of experimental error in studies of W. sebi germination have been discussed previously (Pitt and Christian, 1968). Furthermore, apparent microbial growth within terrestrial brine lakes which can reach values of ≤ 0.600 a_w may have actually occurred at higher water-activity values given the seasonal and other temporal fluctuations of the in situ salt concentrations (Oren, 1988; 1993; Cobucci-Ponzano et al., 2006; Mormile et al., 2009).

Although the established temperature minima for multiplication of the most psychrophilic microbes are in the region of -15 to -18℃ (for references, see Chin et al., 2010; Kminek et al., 2010), there are numerous sources of evidence for metabolic activity considerably below this range (Kminek et al., 2010; J. D. Rummel et al., unpublished). By contrast, there is a paucity of data to demonstrate metabolic activity below the accepted water-activity minimum for microbial cell division (i.e. 0.605; Kminek et al., 2010; Yakimov et al., 2014; A. Stevenson et al., unpublished; J. D. Rummel et al., unpublished). In relation to the water-activity limit for life, it is noteworthy that trehalose, a hygroscopic substance which accumulates in desiccated microbial cells and may facilitate the acquisition and retention of water, cannot efficiently absorb water from the vapour phase at equilibrium relative humidities of less than ~50%, equivalent to 0.500 a_w (Fakes et al., 2000). Whereas some enzymes can remain catalytic at water activities of < 0.500 (Kurkal et al., 2005; Lopez et al., 2010), there is evidence that DNA becomes disordered, and is therefore no longer transcribable, below a water activity of 0.550 (Falk et al., 1963). Furthermore, strand breaks have been recorded at 0.530 a_w in bacterial cells (Asada et al., 1979). It has, therefore, long-been considered unlikely that cellular systems could function at water activities substantially lower than 0.600 (e.g. Pitt, 1975; Brown, 1976; 1990; Sutton and Hildebrand, 1985; J. D. Rummel et al., unpublished). However, interactions between the various factors which determine the biophysical limits for cellular integrity and biotic activity at low water-activity are complex and have yet to be fully elucidated. Macromolecular integrity and functionality can depend on the net effect of prevailing conditions such as temperature, chao-/kosmotropicity, pressure and water activity (Hallsworth, 1998; Hallsworth et al., 2007; Williams and Hallsworth, 2009; Bhaganna et al., 2010; Chin et al., 2010; Yakimov et al., 2014) and it may be possible that, in some as-yet-undiscovered environments, cells are capable of metabolism at < 0.600 a_w.

Microbial cell division via utilization of water which is not in the bulk liquid-phase

Water is more or less ubiquitous on Earth and in other parts of the Solar System (Bradley et al., 2014; Küppers et al., 2014); it may be present within the atmospheres, subsurface, rocks and regolith, polar ice-sheets, glaciers, and/or subsurface oceans of planetary bodies, in vapour plumes extruded into space, and – indeed – within space itself[5]. Whereas here on Earth, we tend to be most familiar with water in its bulk-liquid phase, in both terrestrial and extraterrestrial environments, it can also be present in a variety of forms. In addition to ice and vapour, these include thin aqueous films on/at various types of surfaces and interfaces, or as molecules hydrating mineral, organic, and other substances (Kminek et al., 2010; Toner et al., 2014; J. D. Rummel et al., unpublished). Liquidity of water is determined by temperature, pressure, the presence of solutes and/or gases, and molecular interactions between other materials or substances and water molecules - as well as processes such as salt deliquescence, sublimation of ice, frost formation, condensation or dew-formation on surfaces or within the gaseous phase, aerosol formation, and precipitation (Watanabe and Mizoguchi, 2002; Jepsen et al., 2007; Möhlmann; 2008; 2009; 2012; Argyris et al., 2008; Chin et al., 2010; Pavlov et al., 2010; Bing and Ma, 2011).

Thin aqueous films can exist on various surfaces including those of ice and biological and mineral structures, and the water within these films can remain in the liquid phase under a wide range of conditions (Pearson and Derbyshire, 1974; Raviv et al., 2001; Wolfe et al., 2002; Jepsen et al., 2007; Möhlmann; 2004; 2008; 2009; 2011; 2012; J. D. Rummel et al.,

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[5] See Waite et al. (2006); Nimmo et al. (2007); Tosca et al. (2008); Campins et al. (2010); Sohl et al. (2010); Carter et al. (2013); Martínez and Renno (2013); and Bradley et al. (2014).

unpublished). The depth of thin films can range from > 1 mm to a monolayer of water molecules (~0.3 nm; Möhlmann, 2004; 2005), and they can be stable (Möhlmann, 2012) or highly ephemeral (Burkhardt and Hunsche, 2013). At the temperatures and pressures which typically prevail in Earth's biosphere, aqueous films of ~1 mm are primarily made up of water which is biologically available (e.g. Qvit-Raz et al., 2008, Burch et al., 2013). Whereas we speculate that single-monolayer films do not provide water that can be accessed by cellular systems. It has, however, been suggested that microbes can utilize fluid films with a mean thickness equivalent to that of three water molecules (Harris, 1981; Beaty et al., 2006); a hypothesis that may be inconsistent with the lack of solute diffusion in very thin films (Derjaguin and Churaev, 1986; Hu and Wang, 2003) which indicate that the water in films as thin as this is not in the liquid phase[6]. Despite the circumstantial evidence (see also Rivkina et al., 2000), there is a paucity of data thus far available which convincingly demonstrate that water in thin films that are equivalent to between one and three water molecules in depth is biologically available.

There are three possible sources of liquid water in otherwise desiccated and cold areas such as those which are characteristic of Mars: (1) interfacial water as a thin film (several water-molecular in depth) forming on mineral surfaces by adsorption or, on ice, as pre-melted ice (Dash et al. 2006, Möhlmann, 2011); (2) brines forming on salt crystals via deliquescence; and (3) subsurface melt-water below an ice covering due to a solid-state 'greenhouse' effect (Möhlmann, 2011). Process (2) is a particularly effective mechanism by which liquid water can be generated on Earth and, almost certainly, in extraterrestrial locations (Möhlmann, 2011). The condensing water vapour can potentially reach the dry weight of the deliquescent salt, and will exceed it if the humidity exceeds the deliquescence relative humidity. Deliquescence of NaCl, as equilibrium relative humidity increases from 65 to 80%, can be observed in Movie S1. Most salts (and, indeed, many organic substances) are hygroscopic and will attract water to their surface at equilibrium relative humidities of ≤ 100%. Each salt becomes deliquescent at a specific relative humidity, thereby dissolving as the water vapour condenses. The deliquescence relative humidity for a given salt and its (usually slight) temperature-dependence quantitatively correspond to both the water activity values of, and equilibrium relative humidity values for, saturated solutions of a given salt (Winston and Bates, 1960). If the equilibrium relative humidity is higher than a salt's deliquescence relative humidity, the water activity of the salt solution will equilibrate with the relative humidity of the atmosphere, so the salt solution will become more dilute. Mixtures of substances (e.g. mixtures of different salts or salts plus sugars) will have a deliquescence

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[6] This inconsistency also raises the possibility that the high water-activity values associated with very thin films (Harris, 1981; Papendick and Campbell, 1981) could be a consequence of methodological error.

relative humidity below that of each individual component (Mauer and Taylor, 2010). In addition to the reduced water activity, salts also reduce the freezing point, and cryobrines may be stable far below the melting point of water, e.g. under Martian conditions (Möhlmann, 2011, Martínez and Renno, 2013).

Within the Earth's biosphere, brine formation may play a role for diverse microbial species – especially those that are halotolerant or halophilic – which are located within bioaerosols, or on mineral or biological surfaces (e.g. leaf surfaces) and are exposed to humid air (Potts, 1994). For example, adapted species can reproduce within the phyllosphere of salt-exuding desert plants (Qvit-Raz et al., 2008, Burch et al., 2013) and, at subzero temperatures, in supercooled water in the atmosphere (Sattler et al., 2001). Pseudomonas syringae, which is not haliophilic, is a species widely transported within bioaerosols and its cells are highly effective as ice nuclei because they have protein coatings that cause water to freeze at relatively warm temperatures (Christner et al., 2008; Morris et al., 2014). Being surrounded by ice, they may benefit from the solid-state greenhouse effect which involves the internal formation of thin films due to the penetration and retention of shortwave radiation within the ice.

Microbes can obtain water from the vapour-phase, a process which has been observed in lichens (Lange et al., 2006; Pintado and Sancho, 2002) as well as the propagules of various species (Waldham and Halvorson, 1954; Pasanen et al., 1991; Reponen et al., 1996). Other studies have demonstrated that microbial cells also generate considerable quantities of water via their metabolic activity (Oriol et al., 1988; Nagel et al., 2001; Marcano et al., 2002; Kreuzer-Martin et al., 2005; 2006; de Goffau et al., 2011), up to 70% of the cell's water according to radio-labelled gas uptake experiments (Kreuzer-Martin et al. 2005; 2006). Spore germination of powdery mildews, such as by the Erysiphe and Uncinula species, has been observed at low equilibrium relative humidities (0 to 10%) without a visible extracellular source of liquid water (Brodie and Neufield, 1942; Manners and Hossain, 1963; Carroll and Wilcox, 2003); although it is not clear whether condensation processes and/or thin films might act to shuttle water to the cell. Desiccated lichens are able to absorb water at an equilibrium relative humidity of ≥ 82% and thereby commence photosynthesis (Pintado and Sancho, 2002; Lange et al. 2006). Various lines of evidence suggest that microorganisms may be capable of cell division without an extracellular supply of liquid water (see also Miller and Chibnall, 1932; Yarwood, 1950; Peterson and Cowling, 1972; Lange et al., 1986; 1994). However, there is a paucity of convincing data to irrefutably affirm this hypothesis. Furthermore, systematic studies of water-activity limits for cell division of phylogenetically diverse extremotolerant and extremophilic microbes suggest that cell division would be implausible at values much below 0.600 a_w (i.e. 60% equilibrium relative humidity) (Pitt and Christian, 1968; Brown, 1976; Williams and Hallsworth, 2009; A. Stevenson et al., unpublished). This question is equally pertinent to life on Earth, and the aqueous milieu found elsewhere in the Solar System (not least in relation to planetary protection; see above).

Implications for the evolution of microbial life on Earth

The most solute-tolerant Bacteria and Archaea (i.e. extreme, obligate halophiles) are only able to grow at their water-activity minima under hypersaline conditions. Some of these organisms thrive under conditions which resemble those that would have been available on the early Earth; indeed, many of the extreme halophiles thus far studied exhibit their water-activity minimum for cell division at elevated temperatures (Robinson et al., 2005). There is some debate regarding the temperature of the early seas; earlier estimates of 70-80℃ (Knauth and Lowe, 2003) are now considered to be too high (the $\delta^{18}O$ values on which the calculations were based were skewed due to increased seawater temperatures which resulted from inputs of hydrothermal fluids from the crust). More recent estimates based on analysis of oxygen and hydrogen isotopes (i.e. $\delta^{18}O$ and δD, respectively) are about 40℃ (Blake et al., 2010). However, the high mantle heat flow on the early Earth drove a highly active hydrothermal circulatory system that contributed hot, salty (de Ronde et al., 1997), silica-rich fluids to the local environment (Westall, 2012). It has been proposed that primordial life may have first occurred within saline environments on early Earth (Dundas, 1998), and recent evidence suggests that the abiotic formation of primitive proteins can indeed occur under saline conditions (Longo et al., 2013; Longo and Blaber, 2014). Understanding the way in which water-condensing chemical reactions could have led to the emergence of key biomolecules (eg: peptides and nucleic acids) is essential to understanding the origins of life (da Silva and Holm 2014 and references therein). Prokaryote life (anaerobic) was relatively abundant in these early environments and left behind numerous signatures of its presence (Westall, 2012). There are stratified salt deposits of various ages across large regions of the Earth, indicating that concentrated salt-waters/brines have existed across the planet’s geologic history (Warren, 2010). Direct association of an early photosynthetic microbial community with evaporitic conditions is documented in 3.33 billion-year-old volcanic sands from the Barberton greenstone belt, South Africa (Figure 2; Westall et al., 2006, 2011). The uppermost layers of a desiccated biofilm, formed on sediments deposited in shallow waters that were partially exposed to air, are interlayered with tiny evaporate crystals (microns in size and including aragonite, gypsum, halite and magnesium calcite; Figure 2). Evaporitic precipitates have been described from other formations on the early Earth, including the 3.42 billion-year-old Buck Reef Chert in Barberton (Lowe and Fisher-Worrell, 1999) and the 3.43 billion-year-old Strelley Pool Chert of the Pilbara in Australia (Allwood et al., 2007). The early terrestrial phototrophs were quite advanced on the evolutionary scale compared to chemotrophs. Although, to date, no direct association of chemotrophic biosignatures with the early evaporitic deposits has been identified, these more primitive organisms were nevertheless also common (Westall, 2012; Westall et al., 2013). If primitive cells did reach the early Earth through panspermia, experiments simulating the entry of meteorites containing microorganisms into the Earth's atmosphere have shown (1) that phototrophs could not have been transported to Earth by these means (Cockell et al., 2007) and (2) that, if resilient forms of life were hidden in meteorites, they would need to be buried at depths of at least 5 cm in cracks within the meteorite in order to withstand the heat of entry (Foucher et al., 2010).

Regardless of how (and where) life originated, it seems most likely that it was prokaryotes (known to have preceded eukaryotes by ~2 billions years), in saline environments, which first reached the 0.605-a_w limit. Some of the oldest known fossils are those of prokaryotic cells (dating from ~3.5 billion years ago [Frances, is this the same environment as the 3.33 billion above? And if so, do we need to use the same number?....also is there any repetition between these sentences concerning the Barberton work here and those in the paragraph above? If not then that's fine.]) which apparently lived in salt-rich environments, as evaporite minerals such as magnesium calcite and halite were found embedded in the biofilm of an extant [Frances, was this mat fossilized or alive?] microbial mat, discovered in the Barberton greenstone belt, South Africa [Frances, given that the Barberton greenstone belt and it's location were mentioned above does this need repeating here?] (Westall et al., 2001; 2006), and similarly within ancient stromatolite columns from the Pilbara Craton, Western Australia (Allwood et al., 2007). Intriguingly, molecular analysis of modern stromatolite communities revealed that 74% of archaeal clones were closely related to the Halobacteria (Burns et al., 2004), which frequently dominate hypersaline environments (Oren, 2002). These prokaryotic halophiles were exposed to, and presumably inhabited [Frances should there be a comma here…] evaporitic environments containing […or a comma here?] elevated concentrations of magnesium and characterized by water activities of considerably less than 0.755 (and can, indeed, be considerably below 0.600 a_w, depending on salt concentrations; Winston and Bates, 1960; Hallsworth et al., 2007; Yakimov et al., 2014; A. Stevenson et al., submitted). Indeed, the signatures of past life forms, including stromatolites, can be common in evaporitic deposits (Rothschild and Mancinelli, 2001).

Much later, and presumably in terrestrial locations, the Eukarya must have developed a similar resilience during growth at high concentrations of solutes which are produced via biogenic activity; namely sugars and polyols. Indeed, extremophilic Eukarya are considerably less salt-tolerant than their bacterial and archaeal counterparts, and it may be that the prokaryotes are yet to evolve an ability to grow at low water-activity in non-saline substrates (their current record is in the range 0.850 to 0.800; Lievens et al., 2014; R. Santos et al., submitted; A. Stevenson et al., submitted). Microbial, and indeed all biological, cells are not pure-water reactors with water activity of 1 (Trevors and Pollack, 2005), but consist of gels within which modulation of water activity along with speciation as a result of the solute-exclusion principle are central to effective cellular function. Indeed, a metabolic ability to maintain the cellular system at his level is one of the fundamental, defining characteritstics of life itself.

Extraterrestrial, aqueous milieu which resemble fertile habitats on Earth

Liquid water was, and may still be, present in numerous locations in the Solar System. On Mars, for example, there is abundant geomorphological evidence for the presence of liquid water on the planet in the past (Carr, 2006) and possibly even, ephemerally, in the present (Möhlmann, 2011; McEwan et al., 2014; J. D. Rummel et al., unpublished). Such evidence includes the formation of secondary minerals through the aqueous alteration of the basaltic rocks that cover the surface of the planet (e.g. Carter et al., 2013; Martínez and Renno, 2013). It has been calculated that the water activities of evaporite deposits and bodies of saline water on early Mars were as high as 0.780 to 0.860 (Tosca et al., 2008), which is well within the ranges for microbial species from each Domain-of-life (Javor, 1984; Grant, 2004; Williams and Hallsworth, 2009; Stevenson et al., submitted).

The various brines on Jupiter's moon Europa are composed primarily of water and salts such as $MgSO_4$, Na_2SO_4, and/or Na_2CO_3 (and, in some cases also contain sulfuric acid; Muñoz-Iglesias et al., 2013). Saturated solutions of these salts have water-activity values of 0.900, 0.930 and 0.920 respectively (at 20℃, 1 atm; Winston and Bates, 1960), although it is currently unclear what the values would be under the prevailing conditions on Europa. At the lower temperatures, and the in situ pressures, on Europa the solubility of ions and, conversely, the precipitation of salts can also vary leading to increases in water activity (Marion et al., 2003; 2005), the water activity of a saturated Na_2CO_3 solution at 10℃, for example, is 0.990 (Winston and Bates, 1960). Whereas water-activity values for individual brines will vary according to their ionic composition (and pH, which also influences solubilities of some salts), it seems likely that the in-situ water activities are sufficiently high to span the entire range for known life (Javor, 1984; Williams and Hallsworth, 2009; Stevenson et al., submitted).

Water has also been identified in asteroidal materials, for example the Monahans (1998) H5 chondrite which contained hypersaline fluid inclusions composed predominantly of saturated NaCl (Zolensky et al., 1999) having a water activity of 0.760 at 20℃ and 0.750 at 2℃ at 1 atm (Winston and Bates, 1960), although these values will vary with pressure. Fluid inclusions have been identified in an increasing number of asteroidal specimens including the Zag (1998) meteorite (Rubin et al., 2002). Furthermore, organic molecules have been detected in the fluid inclusions of some of these asteroidal bodies (e.g. Fries et al., 2012), so the composition of these asteroidal fluids can be close to those of the media and substrates in which halophiles occur. For instance, halophiles in hypersaline fluid inclusions of salt crystals from evaporite deposits contain Archaea, Bacteria, and algae (Dunaliella species)[7]. Many NaCl-saturated habitats contain a remarkably high microbial biomass and are characterised by intense competition (Antón et al., 2002; Daffonchio et al., 2006; Baati et al., 2008; Elevi Bardavid et al., 2008; Khemakhem et al., 2010) during which some species - which are known as “microbial weeds” (Cray et al., 2013b; Oren and Hallsworth, 2014) - achieve dominance of the communities including Archaea, Bacteria and Eukarya (e.g. Haloquadratum walsbyi, Salinibacter ruber, and Dunaliella salina; for references see Cray et al., 2013b; Oren and Hallsworth, 2014). The microbes that dominate and/or are most frequently isolated from the fluid inclusions of salt crystals found in evaporite deposits include a number of species known to be capable of cell division in the range 0.710 to 0.605 (or their close relations, such as Dunaliella, Halocarcula, Halobacterium, Halococcus, Halorubrum and Natrinema spp.: Stan-Lotter et al., 2000; Schubert et al., 2009b; Lowenstein et al., 2011; Gramain et al., 2011; A. Stevenson et al., submitted). In relation to water activity, the biotic activity of microorganisms - including halophiles – is plausible for some of the aqueous milieu found in extraterrestrial environments. Indeed, some of these locations resemble highly fertile habitats for known halophiles (see also A. Stevenson et al., submitted).

Planets which are neither too close to nor too far from a star and could, theoretically at least, accommodate active biological systems are said to be in the Circumstellar Habitable Zone or Goldilocks Zone of their respective solar system (Strughold, 1953). This designation is based on criteria, such as size of the planet and its absolute distance from the star it orbits, whether illuminosity could permit photosynthesis, having surface temperatures which

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[7] See McGenity et al. (2000); D'Hondt et al. (2002); Schubert et al. (2009a); Gramain et al. (2011); Lowenstein et al. (2011); Lomstein et al. (2012); Valentine (2013). Cyanobacteria are know to be metabollically active in evaporite deposits (the in situ water-activity limit for this activity has yet to be determined; Rothschild et al., 1994).

are biologically permissive for at least some of the time (variously defined as 0 to 100℃, or -25 to +122℃; Franck et al. 2007; Takai et al., 2008; Kminek et al., 2010; Harrison et al., 2013), and/or whether they have liquid water (Rampino and Caldeira, 1994; von Bloh et al., 2011). However, these criteria (and indeed the habitable-zone concept) have limited applicability or validity for a variety of reasons. Ecosystems exist on Earth which do not depend on photosynthetic activity (Chivian et al., 2008; Teixeira et al., 2013) and, indeed, the earliest forms of life were not photosynthetic (Westall, 2012); furthermore, there is circumstantial evidence that an extracellular source of liquid water is not obligatory for microbial life (see above). What is more, biologically permissive conditions may prevail in specific environments or substrates on otherwise hostile planetary bodies (for examples in relation to moons of Saturn, see Raulin, 2006; Nimmo et al., 2007; Parkinson et al., 2008). And finally, various activities of solutes can both prevent freezing of water and expand biotic windows of microbes and may do so to a degree yet to be determined (see below; Chin et al., 2010; J. D. Rummel et al., unpublished).

Water can remain liquid at temperatures far lower than those known to permit microbial cell-division (i.e. approximately -18℃; see references in Chin et al., 2010). Liquid water (in various forms, from thin films to underground oceans) may be found in many environments on Mars as well as planetary moons (Europa, Ganymede, Enceladus, etc). Diverse lines of evidence suggest that both photosynthetic and non-photosynthetic microbes may be capable of metabolism and cell division by hygroscopic absorption of water vapour and/or acquiring water from their substratum (as a sole extracellular source of water) both in vitro and in their natural habitats on Earth[8], and utilize a variety of mechanisms for the acquisition and retention of water (e.g. production and accumulation of trehalose and other hygroscopic substances which optimize the acquisition and retention of water, morphological changes which minimize water loss, hydrotactic responses, inhabiting high-humidity niches, and construction of soil features to enhance water capture and retention; Garcia-Pichel and Pringault, 2001; Garvie et al., 2008; de Goffau et al. 2011; Williams et al., 2012; Rajeev et al., 2013; Zakharova et al., 2013). Furthermore, as noted above, some microbial cells can generate vast quantities of water via their metabolic activities (Miller, 1932; Peterson and Cowling, 1973; Oriol et al., 1988; Nagel et al., 2001; Marcano et al., 2002; Hocking, 2003; Kreuzer-Martin et al., 2005; 2006). Indeed, studies of bacterial cells demonstrate that up to 70% of intracellular water can be derived in this way (Kreuzer-Martin et al., 2005; 2006) and other studies demonstrate that cells can maintain higher intracellular water-activity than that of the environment; de Goffau et al. (2011).

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[8] E.g. fungi, lichens and cyanobacteria (Snow, 1949; Armolick and Dickson, 1956; Pitt and Christian, 1968; Ayerst, 1969; Bootsma et al., 1973; Drewello and Weissmann, 1997; Shomari and Kennedy, 1999; Lange et al., 2006; Wierzchos et al., 2011; Zakharova et al., 2013).

The atmosphere of Saturn's moon Enceladus can contain ≥ 90% water vapour (Waite et al., 2006) and, whereas its terrestrial surface is approximately -200℃ (Brown et al., 2006), plumes of water vapour and ice which are released into space are thought to originate in subsurface oceans that have temperatures in the range -23 to -3℃ (Nimmo et al., 2007; Parkinson et al., 2008); i.e. temperatures which are permissive for the metabolic activity of psychrotolerant and psychrophilic microbes (Collins and Buick, 1989; Chin et al., 2010, Kminek et al., 2010, Mykytczuk et al., 2013). Various salts, nitrogenous compounds, and organic substances have been identified in the atmosphere of Enceladus and E-ring ice grains of Saturn (which may originate from Enceladus) including NaCl, $NaHCO_3$, $NaCO_3$, N_2, ammonia, hydrogen cyanide, CO and CO_2, methane, acetylene, and propane (Matson et al., 2007; Postberg et al., 2009; 2011). Under conditions prevalent on Earth, bioaerosols can be fertile habitats characterized by high levels of microbial diversity, biomass, and metabolic activity (Fahlgren et al., 2010; Womack et al., 2010; 2012). In relation to the atmosphere of Enceladus and/or the watery plumes which it emits into space, it is intriguing to speculate what the water activity of liquid droplets in, or the humidity of, the gaseous phase might be (presumably close to 100%) and whether the temperatures within these plumes can ever be considerably higher than -200℃. It should be noted that, whereas definitive evidence from culture-based studies of microbial systems on Earth indicate limits for cell division of approximately +122℃ or -18℃ (Collins and Buick, 1989; Takai et al., 2008; Chin et al., 2010; Harrison et al., 2013), circumstantial evidence from other biochemical or geochemical data suggest biotic activity under more extreme conditions (down to about -40℃, and up to approximately +140℃; Parkes et al., 2000; Kminek et al., 2010; J. D. Rummel et al., unpublished).

Although the Earth is located within the region allocated as the Goldilocks Zone of our own Solar System, it hosts many environments which do not permit life process and are therefore essentially sterile due to, for example, low water activity, high chaotropicity, excessively high or low temperatures, pH of > 12, plus combinations of conditions such as high salt and low pH or high temperature and high pH (e.g. Brown, 1990; Hallsworth, 1998; Parkes et al., 2000; Grant, 2004; Hallsworth et al., 2007; Harrison et al., 2013; Yakimov et al., 2014). Under all these conditions cells also need adequate energy sources and nutrients for maintenance and growth which may require electron donors and acceptors for respiration etc. Some combinations of conditions can slightly extend extremes for growth, such as high pressure and temperatures; furthermore survival can occur under conditions where growth cannot[9]. Conversely, planetary bodies which are basically hostile to life may nevertheless harbour small-scale, biologically permissive domains (Kminek et al., 2010; J. D. Rummel et al., unpublished). Solute activities represent one of the determinants for potential habitability on Earth; for example, chaotropicity can enable cellular function at low temperatures and kosmotropicity may enable cellular function in high-temperature environments or those dominated by chaotropic substances[10]. The ways in which water activity and other solute activities can interact to determine the physicochemical limits for life (e.g. Williams and Hallsworth, 2009; Chin et al., 2010) have yet to be fully characterized. Furthermore, there is little information on the way in which availability of nutrients and other resources can determine tolerance limits to physicochemical stress parameters (e.g. Daffonchio et al., 2006; J. P. Harrison et al., submitted). Once the interactions between such factors are better understood, the currently accepted criteria for habitability will require revision (Beaty et al., 2006; Marion et al., 2003; Marion and Kargel, 2008; Tosca et al., 2008; Kminek et al., 2010; Harrison et al., 2013; J. D. Rummel et al., unpublished).

How sensitive are cells to minute changes in water activity? And other unanswered questions

In their environmental context, microbes are exposed to complexity at multiple levels; in relation to (i) the dynamics of physical and chemical parameters, (ii) the antimicrobials and other substances produced by other cells in the vicinity, (iii) varying availability of resources, and countless other factors. Water activity, in particular, can oscillate (Cray et al., 2013b; Lievens et al., 2014), and may do so across a range of timescales from a fraction of a second, for example to days or longer. The majority of stress-biology studies which quantify water activity do so to either one or two decimal places. We propose here that water activity ought to be determined to an accuracy of three decimal places (Winston and Bates, 1960; Williams and Hallsworth, 2009; A. Stevenson et al., submitted) as this is more closely aligned with the sensitivity of cellular systems. All technologies used to quantify the water activity of undefined substrates are associated with some degree of error (see Winston and Bates, 1960, Greenspan, 1977, Hallsworth and Nomura, 1999, Yu et al., 2009). Commercially available apparatus for water-activity determination are associated with a net

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[9] The propagules/cells of many microbes are highly resilient to exposure to extremes of temperature, uv, pH, chaotropicity, desiccation and other stresses (e.g. Wyatt et al., 2014; R. Santos et al., submitted), even over long timescales, and so are capable of surviving conditions found in extraterrestrial locations (see above).

[10] See Hallsworth (1998a); Hallsworth et al. (1998b; 2003a; 2003b; 2007); Williams and Hallsworth (2009) Bhaganna et al. (2010); Chin et al. (2010); McCammick et al. (2010); Bell et al. (2013); Cray et al. (2013a; 2013b); Lievens et al. (2014); Yakimov et al. (2014). Whereas chaotropic substances are typically less polar than water and disorder biomacromoelcules, kosmotropic substances are usually more polar than water and thereby structure or rigidify macromolecular systems (see Cray et al., 2013a, and references therein).

variation (accounting for both accuracy and repeatability) of ±0.010 to 0.020 water-activity units (A. Stevenson et al., submitted). At 0.600 water activity, this is equivalent to variations of water potential between ± -2.3 and -4.5 MPa respectively). For the purposes of biological and food-related research it has been suggested, that levels of accuracy of ±0.010 (Labuza et al., 1976; Roa and Tapia, 1998), ±0.020 (Troller and Christian, 1978; Sereno et al., 2001), ±0.005 (Ferro Fontán and Chirife, 1981; Hallsworth and Nomura, 1999), or ±0.001 a_w are appropriate (Winston and Bates, 1960). Our earlier studies (Williams and Hallsworth, 2009; A. Stevenson et al., submitted) suggest that microbial cells can be sensitive to differences/changes of < 0.010 water activity. For example, water-activity differences of < 0.005 units have impacted growth rates for diverse strains of xerophilic fungi by between 40 and 80% (A. Stevenson et al., submitted) which, in turn, implies fundamental differences at every level of the cellular system, from gene expression to physiological and developmental processes. On glycerol-supplemented media at water activities of 0.799 and 0.795 growth-rates for A. penicillioides varied between 1.13 and 0.642 mm d^{-1} for strain JH06THH and between 1.20 and 0.732 mm d^{-1} for strain JH06THJ; and on $MgCl_2$-supplemented media at water activities of 0.915 and 0.907 rates for X. bisporus varied between 3.96 and 1.43 mm d^{-1} for strain FRR 0025, 2.55 and 0.533 mm d^{-1} for strain FRR 2347, and 2.13 and 0.800 mm d^{-1} for strain FRR 3443 (A. Stevenson et al., submitted). These data raise the tantalizing question of whether microbial cells are sensitive to water-activity differences down to the fourth, or even fifth, decimal place[11]. It is noteworthy that, for a hypothetical microbial species which has a temperature window for cell division spanning from 5 to 40℃ (i.e. a 35℃ range), a temperature change of 10, 1 or 0.1℃ would represent a 1/3.5, 1/35 and 1/350 fraction of this window, respectively. If the water-activity window for this microbe spanned from 1 to 0.900 a_w (i.e. 0.100 a_w-units in total), 1/3.5, 1/35- and 1/350-portions of this window would correspond to 0.02857, 0.00286 and 0.00029 a_w units, respectively. This underlines the fact that water-activity determinations to one decimal place (equivalent, in this example, to ~29℃) can lack biological meaning, and those made to two decimal places (equivalent to an accuracy level of up to 2.9℃) are far less accurate than we would accept for biological studies of temperature or other environmental parameters. In relation to microbial multiplication on Earth, the water-activity and temperature windows for life span 0.400 a_w-units and 140℃, respectively (Fig. 1). In the context of stress biology, and at the scale of the

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[11] Based on the use of Novasina technnology (Axair Ltd., Pfäffikon, Switzerland) and a protocol incorporating a range of precautionary measures we achieve an accuracy of ±0.001 water-activity units (A. Stevenson et al., submitted). Whereas calculations can be carried out to enable the expression of water-activity values to the fourth decimal place, these have been based on a number of assumptions which, collectively, result in unacceptable levels of uncertainty (Greenspan, 1977; Yu et al., 2009). Such a level of accuracy would be highly desirable in many spheres of biological research but empirical determinations of water activity to the fourth decimal place are currently unattainable.

biosphere, the expression of water activity to decimal place leads to an unacceptable level of accuracy, as 0.100 a_w units equates to a temperature of 35℃. Even water-activity determinations to three decimal places (equivalent to an accuracy level of ~0.3℃) are imposed by technological limitations rather than being dictated by the sensitivity level of the cell.

It remains unclear whether microorganisms are capable of subsistence without an extracellular supply of liquid water, and the biological availability of water in various types of aqueous film has also yet to be quantified. Cells may be able to acquire and retain water (de Goffau et al., 2011) which can be utilized when water activity falls below biologically permissive levels (for instance, see the studies of powdery mildew cited above) but there is no definitive evidence that this does indeed occur (and, if so, what mechanisms are involved) at present (J. D. Rummel et al., unpublished). Culture-independent studies are needed for high-solute, and other low-water activity, habitats to establish whether metabolic activity below the threshold for cell division (0.605 a_w) is commonplace at different locations within the microbial biosphere. In contrast with the increasing understanding of molecular-level adaptations in many other forms of extremophile, there is a paucity of information in relation to physiological, biochemical, and genetic mechanisms which facilitate halophile/xerophile function at < 0.690 a_w[12]. Further work is also needed to elucidate the roles that low water-activity substrates have played, and continue to play, in the evolution of both prokaryotic and eukaryotic systems. In the context of habitability, work is also needed to elucidate the interactions between type and concentration of ions, chao-/kosmotropicity, and water activity in relation to complex brines such as current those found in various locations on Earth (Siegel et al., 1983; Oren, 1988; Hallsworth et al., 2007; Yakimov et al., 2014) and those likely to have existed on early Earth or ancient Mars (Tosca et al., 2008). For ecosystems located in extremely hostile habitats, some reports hint that microbial life can be discontinuous and fragmented (Hopkins et al., 2005). In some low water-activity habitats, it may be that active cells can be located in otherwise biologically non-permissive zones, and pockets of sterility exist within otherwise inhabited zones. Furthermore, in some locations microbes may be inactive for most of the time and yet functional for short periods. It has yet to be determined, for example, whether slow cell divisions (over 100s or 1000s years) can occur in microbial communities which may subsist in nature at water activities below the known 0.605 a_w limit. In relation the water-activity limits for microbial life, we know much about the outer edges of Earth's biosphere yet, in the relation to the in situ conditions of microbial habitats, we still know relatively little.

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[12] This also acts as a barrier to the biotechnological exploitation of these extremophiles and the macromolecular systems derived from them.

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Figure legends

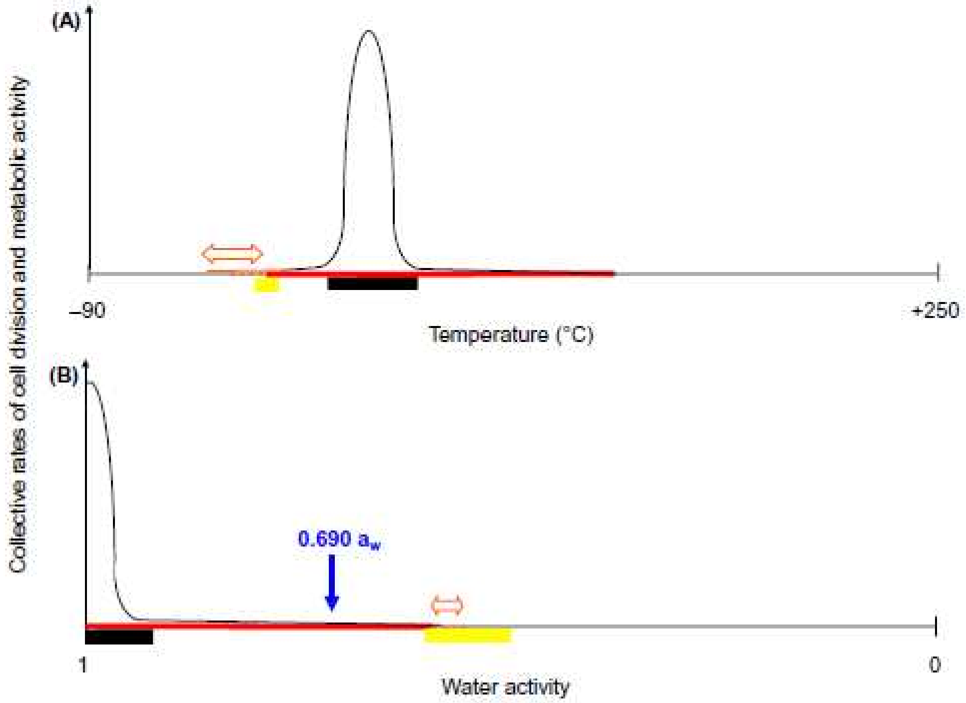

Figure 1. Diagrammatic representation of collective biological activity (compound rates cell division and metabolic activity) for microbes on Earth in relation to prevailing environmental (**a**) temperatures and (**b**) water activities. Red bars indicate the known range for cell division of microbes (-18 to +122℃, and 1 to 0.605 a_w), and orange dotted lines indicate for (**a**) the established limit for cellular metabolism (33℃), and (**b**) the known limit for physiological function of DNA (down to 0.530 a_w). Black bars indicate the range in which the overwhelming majority of microbial activity takes place, and curves represent collective biotic activity of microbes on Earth. Yellow bars indicate safety margins used for the designation of 'Special Regions' on Mars (down to -25℃ and 0.500 a_w; Kminek et al., 2010) in relation to international policy on planetary protection. Horizontal orange arrows indicate zones in which cell division may take place over extended timescales (10s to 1000s years) though there is a paucity of data on this topic; this zone for temperature extends considerably below -33℃ because of the possibility that chaotropic substances may enhance flexibility of macromolecular systems and thereby reduce the temperature minima for microbial activity by a further 10 to 20℃ (Chin et al., 2010).

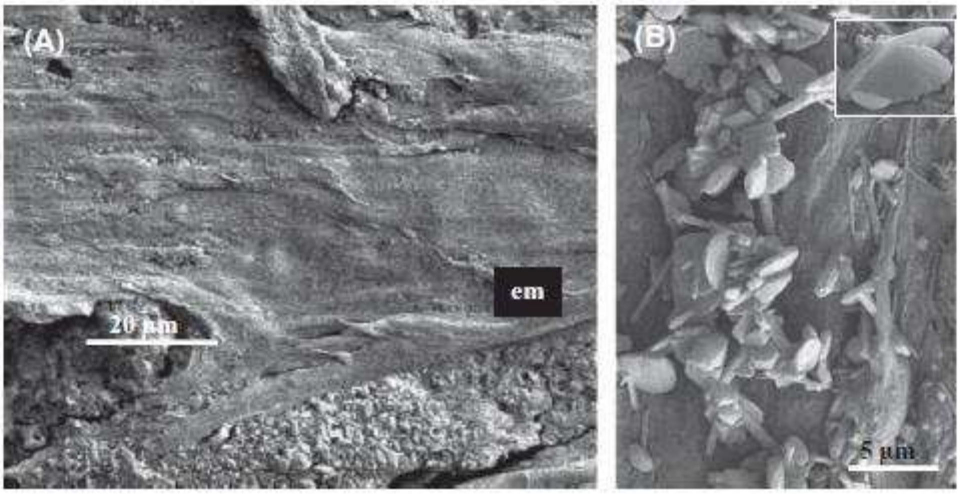

Figure 2. Early Archaean microbes and evaporites; example from the 3.33 billion-year-old Josefsdal Chert, Barberton Greenstone Belt: (**a**) layer of evaporite minerals interbedded with layers of a photosynthetic microbial biofilm, (em) evaporite minerals, and (**b**) details of the diversity of minerals encrusted on the surface of the biofilm. They include here pseudomorphs (silica replaced) of acicular aragonite and losenge-shaped gypsum. Reproduced from Westall et al. (2006) with permission from The Royal Society Press.

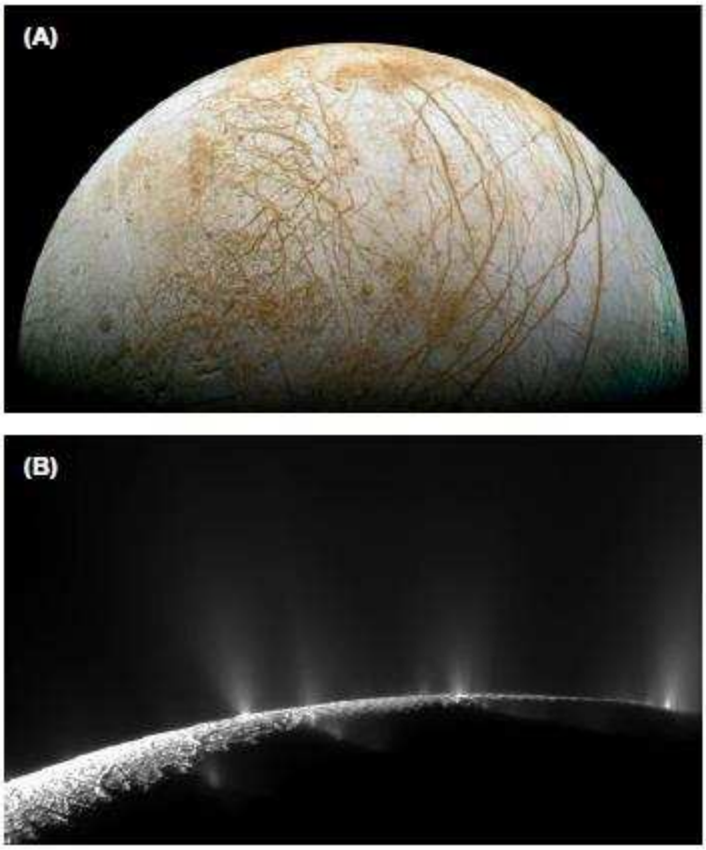

Figure 3. Views of two planetary moons which are known to have an abundance of water, some of which may be present as subsurface oceans: (**a**) the icy surface of Europa, and (**b**)

jets composed of water vapour, ice particles and organic compounds released from beneath the surface of Enceladus. Courtesy NASA/JPL-Caltech.

Supplemental information

Movie S1. Deliquescence of NaCl crystals on the surface of a pine needle (Pinus sylvestris) as humidity rises from approximately 65 to 80% equilibrium relative humidity. The deliquescence point of NaCl is approximately 75.0% equilibrium relative humidity at 2℃. An epistomatal chamber is visible but the guard cells are located below this section and cannot, therefore, be seen. The recording was made using an environmental scanning electron microscope and equilibrium relative humidity was controlled experimentally within a chamber (see Burkhardt and Hunsche, 2013).