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Mars’ surface is not universally biocidal

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Summary
The Mars surface/near-surface is often considered to be biocidal. Here, diverse lines of evidence are presented indicating that some terrestrial microbes can survive the in-situ conditions albeit in an inactive state. For the purposes of planetary protection, it is important to consider what we mean by a planetary ‘surface’; this term has qualitatively distinct definitions for different scientific disciplines, and can also have different meanings from a human viewpoint versus that of a microbial cell. Most microbial cells spores or other cells deposited on Mars, even those that initially fall on the outward-facing part of the absolute surface, will fall within pores of the regolith or become covered by its dust. They are, therefore, protected from ultra-violet radiation. Desiccating conditions and low temperatures (−40 to −70 °C) can act to preserve rather than kill all microbes, potentially maintaining cellular viability – especially for certain extremophiles – over geological timescales. Whereas salts are ubiquitous on Mars, many terrestrial microbes are highly tolerant to NaCl and other salts, and these substances (including potentially inhibitory chaotropes such as MgCl₂ and perchlorates) cannot access cells in the absence of a liquid milieu. Whereas the Mars regolith is nutrient-deplete and conditions may be acidic in places, oligotrophic conditions per se are not biocidal and many terrestrial microbes can thrive in acidic conditions (some acidophiles can proliferate at or below pH 0). The low temperatures of Mars’ surface are not conducive to metabolic activity, but the biophysical sophistication and robust stress biology of many terrestrial microbes, and the protection afforded by Martian conditions, are likely to ensure the long-term viability of some extremophilic microbes if transported to Mars.

Studies of potential habitability of Mars by terrestrial microbes often focus on substances and parameters that are biocidal. Indeed, Mars itself is considered by some to be more-or-less biocidal (Berry et al., 2010; Schuerger et al., 2013, 2020; Spry et al., 2021). Berry et al. (2010) describe ‘the biocidal nature of the Martian terrain’ and Spry et al. (2021) state that ‘Numerous biocidal factors persist in the Martian [environment]...The top 7 most biocidal factors for inactivation of Earth microbes [are]:...UVC and UVB radiation,... desiccation (i.e., low aᵦ),... UV-induced volatile oxidants,..., high salt concentrations... (e.g., MgCl₂, NaCl, FeSO₄ and MgSO₄), acidic conditions in many regoliths, solar particle events and low-pressure...’. The article by Spry et al. is a 2021 Decadal Survey Planetary Protection White Paper relating to planetary-protection policy. Indeed, in relation to both space exploration and planetary protection, there may have been an assumption that the Mars’ surface/near-surface as more biocidal than it really is.

‘Biocide’ refers to substances able to kill living things; the suffix -cide is derived from the Latin cīda meaning ‘killer’. An argument is presented here that the Mars ‘surface’ can be life-preserving rather than biocidal; to be more precise, the near-surface or beneath dust where cells can be shadowed. This classification ‘biocide’ most-commonly describes substances designed to kill microbes indiscriminately, including liquids such as bleach and 70% (v/v) ethanol that are used to clean surfaces. Substances that are essential for some microorganisms, such as NaCl (even saturated, 5-M NaCl brines...
often host phylogenetically diverse and biomass-dense microbial communities; Lee et al., 2018), can be inhibitory (biostatic) or even lethal for other types of microbe if present at sufficiently high concentration. However, cellular life in general requires the presence of some NaCl so we rarely refer to this salt as a biocide. Indeed, the terms ‘biocide’ and ‘NaCl’ are historically used as mutually exclusive (Vasseur et al., 2001). Furthermore, halophile cells and their communities are thought to survive long periods – even over geological timescales – within the NaCl-saturated fluid inclusions of halite (McGenity et al., 2000; Kminek et al., 2003; Fendrihan et al., 2012; Jaakkola et al., 2016; Thompson et al., 2021).

Various parameters on Mars have been designated in the published literature as being biocidal, including low temperature, high NaCl, low nutrient concentration and other parameters that are listed above (Schuerger et al., 2012, 2013, 2020; Mickol and Kral, 2018; Spry et al., 2021). These studies are generally based on mesophilic, copiotrophic and/or biophysically fragile bacteria, such as Escherichia coli and Senatia liquefaciens, commonly closely associated with plant- or animal hosts (a problem discussed by Hallsworth (2018) and Cavicchioli (2019)) yet with an implied and misconstrued notion that all terrestrial microbes would not survive (see Craven et al., 2021).

Of these parameters, ultra-violet light is known to kill microbes (UVC more than UVB) if sufficiently intense and prolonged, and depending on the type of microorganism but especially vegetative cells. Indeed, ultra-violet radiation can be used to sterilize many types of surface. However, in the context of accidental contamination of Mars’ surface with terrestrial microorganisms, ultra-violet is unlikely to eradicate microorganisms that are shadowed. This is because there is no planar surface on which microbes may come to rest; for the most part there is a highly porous regolith (Wood, 2020). Therefore, the majority of microbes released would fall into these pores (Marshall and Mancinelli, 2011) or mix with dust so gain protection from ultra-violet radiation (Mancinelli and Klovstad, 2000, Hansen et al., 2009). Such microbes will come to land some distance below the actual surface (at depths likely to range from μm to cm) so would almost certainly be shielded from exposure to solar radiation (Moore et al., 2007; Osman et al., 2008).

They may further be protected from ultra-violet radiation if crystals of salt, water ice, or CO2 ice form above them, or if wind-blown particles or other microbial spores/cells are deposited onto them (Mancinelli and Klovstad, 2000; Rummel et al., 2014). In the context and at the scale of the microbial cell, the ‘near-surface’ of Mars can refer to only several microns beneath the absolute surface (or greater depths); from a depth of about 10 μm according to Mancinelli and Klovstad (2000). Any microbes on the underside of rocks and spacecraft or other anthropogenic structures would also be safe from ultra-violet radiation. Furthermore, some studies on microbial tolerance of ultra-violet radiation are carried out on hydrated, physiologically active cells at temperatures far higher than those found on or close to Mars’ surface. A number of reports indicate that some organic molecules and microbial cells that are desiccated and/or frozen can resist ultra-violet damage (Gerakines and Hudson, 2015; Onofri et al., 2018; Mosca et al., 2019; Feshangsz et al., 2020) but such studies were not designed to test a monolayer of cells (that are not mixed with anything). In relation to the Mars, the absolute (outward-facing) surface that is directly exposed to high levels of UVC radiation will theoretically be sterilised; at least for any microbial cells which remain exposed (Horneck et al., 2010). Sources of ionizing radiation (such as solar energetic particles and cosmic radiation) can also be lethal but may take a million years or more to eradicate the most-resistant cells (Hassler et al., 2014).

Parameters such as suboptimal temperature and low water-activity can induce stress in physiologically active cells (Hallsworth, 2018) and can even prevent metabolism if sufficiently extreme. However, there is little evidence that low temperatures, desiccation (as opposed to a rehydration event), or low water-activity per se act as biocidal factors; e.g., see Crowe et al. (1984) and Mancinelli (2015). Indeed, desiccation (lyophilization) and freezing to −70°C are the primary techniques used for the long-term preservation of microbial cells (Ayala-Zermeño et al., 2017; Bircher et al., 2018, 2020). Moreover, microbes can remain viable over geological timescales in frozen and desiccated terrestrial environments (Steven et al., 2006; Price, 2007; Margesin and Collins, 2019; Knief et al., 2020). The low surface-temperatures of Mars (generally in the range −40°C to −70°C) are likely to preserve rather than kill microbial cells, albeit that microbial metabolism under these conditions is not known (Rummel et al., 2014).

In liquid solutions, including brines, chemically diverse salts can act as cellular stressors – even for halophiles – so can limit terrestrial life and can even define the biophysical boundary of Earth’s biosphere (Hallsworth, 2019). Brines are life-limiting due to low water-activity (Stevenson et al., 2015), high ionic strength (Fox-Powell et al., 2016), extreme chaotropicity (Hallsworth et al., 2007), and/or multiple extremes (Benison et al., 2021). In relation to some types of brine, whereas they are sufficiently stressful to prevent metabolism or growth of halophiles (i.e., they are biostatic), there is no evidence that halophile cells are killed/eradicated. In brines containing sulphate salts such as FeSO4 and MgSO4, for example, sulphate ions stabilize cellular membranes due to their kosmotropic activity (Fox-Powell et al., 2016). By contrast, chaotropic salts such as MgCl2 increase the entropic disorder of macromolecular proteins.
systems to a degree that the cellular system is no longer viable due to either lysis or cellular mummification (Duda et al., 2004; Hallsworth et al., 2007; Santos et al., 2015). Chaotropicity, therefore, is a potentially biocidal parameter whether induced by salts or organics such as aliphatic alcohols (Cray et al., 2015). However, it should be noted that biocidal activity is concentration-dependent for all chaotropic substances (Hallsworth et al., 2003; 2007; Santos et al., 2015).

Acidity is not biocidal for many terrestrial microbes; for example, acid brines host biodiverse communities (Zaikova et al., 2018) and other types of acidic habitat contain species able to retain metabolic activity and proliferate at pH values down to −0.06 (Schleper et al., 1995), although recent analyses suggest that their acid-tolerance limit may actually be determined by water activity (Hallsworth et al., 2021). Similarly, low pressure does not kill all microbes. Most microbes on Earth are thought to occur in the subsurface where nutrient concentrations are low and microbial communities subsist, without cell-division events, over geological timescales. There is no evidence that oligotrophic conditions are directly biocidal and, whereas some papers use the term ‘spacecraft microbe’, any microbe that occurs in Earth’s biosphere could potentially occur as an accidental contaminant of spacecraft (Rummel et al., 2014). For some microorganisms, the combination of nutrient deprivation and multiple types of stress can impair vigour and reduce percentage viability (Harrison et al., 2015; Craven et al., 2021), most especially when cellular damage is coupled with an inability to generate energy for self-repair (Moger-Reischer and Lennon, 2019). This said, the ubiquity of microbes within Earth’s biosphere that are able to survive for indefinite periods under nutrient-deplete and biophysically hostile conditions is testament to the tenacity of microbial life.

Planetary protection is concerned with potential microbial contamination of the planetary environment and not only with microbes within spacecraft or habitations (Kminek et al., 2010; Rummel et al., 2014). In this context, microbial cells or biomass, including waste materials that will be generated from human habitations on Mars (Nangle et al., 2020), cannot be assumed to be eradicated by Martian conditions outside the habitation. There are some substances and parameters that can kill at least some microbes effectively, but the action of these may not be pertinent at the low temperatures of the Martian surface/near-surface where brines are for the most part absent. For example, cells that are desiccated, frozen and inactive typically do not take in potentially biocidal substances. Furthermore, chaotropic salts that can be biocidal when present at high concentrations and physiological temperatures (e.g., MgCl₂; Hallsworth et al., 2007) must be dissolved in liquid water to act in this way. In general, biocidal substances do not kill cells that are desiccated (i.e., water is absent); they cannot gain access to the cell because there is no continuum between extracellular liquid water, hydrated plasma membrane and cytosol (McCammick et al., 2010) and cellular macromolecules are not hydrated so are not in a vulnerable condition. Chaotropicity, for example, operates only in a liquid milieu where cells that are neither frozen nor desiccated (Bhaganna et al., 2010; Cray et al., 2013; Ball and Hallsworth, 2015). Furthermore, even at low temperatures and under conditions that otherwise permit cellular metabolism, MgCl₂ solutions (and those of other chaotropic salts such as perchlorates) become less chaotropic as temperature decreases (Chin et al., 2010). Therefore, the inhibitory activities of such substances that are observed in culture-based experiments at temperatures of about 20 to 37°C cannot be assumed to apply for temperatures below 0°C. Whereas perchlorates can decompose to produce reactive oxygen species, this only occurs in the region of 300°C so is not pertinent to the Mars surface/near-surface.

Human activity on Mars can potentially create conditions conducive for the revival of inactive microbes, for example due to heat generated within or close to a human habitation or due to a spacecraft collision (Rummel et al., 2014). However, different types of microbe (also depending on a cell’s physiological condition and life history) exhibit differences in longevity and ability to survive rehydration, thawing, or other aspects of the revival process. Recently, novel terrestrial microbes have been found in space (on the International Space Station; Bijiani et al., 2021) and we know nothing of the stress phenotypes of the vast majority microbes on Earth. For both these reasons, we cannot know how microbes inadvertently taken to Mars might fare once they arrive there. The Mars surface is not habitable in as much as no terrestrial microbe of any kind is thought capable of proliferating there (Rummel et al., 2014). This does not mean, however, that the surface/near-surface conditions actively kill all microbial life (Mancinelli, 1989). The biophysical limits-for-active life in the terrestrial biosphere, which is characterised by complexity, are in reality defined according to the capabilities of oligotrophic and extremophilic microbes that can survive indefinite periods of inactivity and of which we have incomplete knowledge.

The conundrum created by the term ‘Mars’ surface’ is consistent with the nebulous etymology of ‘surface’. The latter has distinct meanings in common parlance versus in scientific use, as well as across different disciplines: ‘SURFACE. n. [Fr. sur; upon, and face] The exterior part of anything that has length and breadth; one of the limits that terminates a solid; the superficies; outside…; an even or an uneven surface… A smooth or rough surface… In geom., [that which is] distinguished from a line which has length only, and from a solid, which has length, breadth…’
and thickness...we have surfaces of the first order, or plane surfaces, and surfaces of the second order, or curved surfaces. In physics, a surface is supposed to be composed of a number of material particles, places together side by side, without any opening or interstice between them. Such a surface, therefore, cannot be said to be absolutely destitute of thickness, but may be regarded as a film of matter whose thickness is indefinitely small. In common language, the word surface is often used to signify not merely the outside or exterior boundary of any substance, but also a certain thickness of the exterior material part. In this way we speak of the surface of the earth...&c.' (Ogilvie, 1868). Accordingly, the scientific literature pertaining to Mars does include lithospheric-scale usages of 'the Mars surface' (e.g., Górski et al., 2018).

The meaning of this term, and whether we has important implications for planetary protection. Planetary protection committees usually consist of experts from diverse disciplines including planetary science, geochemistry, engineering, space-exploration policy and microbiology (Kminek et al., 2010; Rummel et al., 2014; Spry et al., 2021) where each person may have a different interpretation of what the Mars surface is. In relation to microorganisms, the evidence suggests that conditions at the Mars surface/near-surface – especially where cells are shadowed from ultra-violet radiation – can preserve, rather than kill, cellular systems.

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