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Desiccation of the Extreme Thermoacidophile *Metallosphaera sedula* Grown on Terrestrial and Extraterrestrial Materials

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Prokaryotes are among the most versatile organisms on Earth and their ability to adsorb metals for nutrient, energy, or protection purposes can be noted in many different environments on our planet. The extreme thermoacidophilic archaeon *Metallosphaera sedula* is a metal-mobilizing archaeon capable of redox transformations during chemolithoautotrophic growth on diverse metal-bearing compounds. Examining the interfaces of this extreme metallophilic archaeon with various metal-bearing substrates of terrestrial and extraterrestrial origin, we have detected its selective preservation after desiccation. Cultivated on specific metal-bearing materials, e.g., tungsten-bearing scheelite, tungsten-bearing polyoxometalate, multimetallic waste products, and the NWA 1172 meteorite, cells of *M. sedula* can be preserved after dehydration, and therefore can potentially serve as a microbial fingerprint of the presence and/or activity of metal-transforming microorganisms. Preservation of desiccated *M. sedula* cells reported in this study has a discriminatory character, depending on the content and nature of the metal-containing compound used for cultivation of this metallophilic microorganism. The achieved preservation of dehydrated *M. sedula* cells facilitates our survivability studies with this desiccated microorganism during future space exposure experiments and under simulated space environmental conditions.

Keywords: desiccation, meteorite, *Metallosphaera sedula*, metals, SEM

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INTRODUCTION

Liquid water is a vital requirement for life on our planet and is the main research focus for scientists looking for life beyond Earth. Nonetheless, various organisms, including unicellular and multicellular life forms, can be preserved in dehydrated settings for long periods. However, dehydration remains a severe stress that leads to cellular perturbation interfering with survival. It has been widely accepted that intracellular compatible solutes impact microbial tolerance to desiccation. A potential positive influence of compatible solutes, e.g., trehalose or sucrose, on cell membranes during dehydration has been shown by Hincha and Hagemann (2004). The efficient ability of trehalose to preserve the function of dried biological macromolecules is widely used for protecting cell membranes and proteins from extreme temperatures, desiccation, and osmotic shock in a wide range of microorganisms. Trehalose biosynthesis genes were described in the genomes of thermophilic members of the *Sulfolobaceae* family, including *Metallosphaera* spp. (Seo et al., 2008; Okazaki et al., 2012; Moon et al., 2016). In addition to metabolic

defense with non-reducing sugars, key molecular elements for the repair of dehydration effects (e.g., oxidative stress and DNA damage) might also be engaged by microorganisms. Apart from intracellular molecular protectants, production of stress-induced biofilms can have a substantial influence on the dehydration tolerance of thermophilic archaea and bacteria, physically preventing water loss in the cells (La Paglia and Hartzell, 1997; Mortel and Halverson, 2004; Chang et al., 2007; Beblo et al., 2011). Another strategy, an inorganic protection by sulfidic ore particles, has been described for the extreme thermophile *Hydrogenothermus marinus* exposed to a combination of desiccation and irradiation (Beblo et al., 2011), asserting a "shielding by ore" effect.

A multitude of multifunctional biogenic structures emerged repeatedly and independently over the course of Earth's history. Armor as a protective mechanism against harsh environmental conditions has been particularly successful. Multicellular organisms, e.g., chitons, have taken advantage of metal-encrusted armor for more than 200 million years (Li et al., 2015). Metal-bearing cell walls of single-celled microorganisms can serve as a protective armor against phytoplankton predators. Microbial survival in extreme environments with high metal concentrations is usually connected with cell surface absorption and/or extracellular polymeric substance (EPS) complexation of various surrounding metals (Schultze-Lam et al., 1996; Loaëc et al., 1998; Gupta and Diwan, 2017; Hickman-Lewis et al., 2019). Moreover, for endolithic and metal-respiring microorganisms, metal accumulation on the cell surface can serve as a mechanism for nutrient and energy source. Certain cell wall structures serve as a nucleation site and might enhance the metal accumulation process by providing a surface that promotes adsorption or precipitation of metals. Prokaryotes (e.g., cyanobacteria, proteobacteria, archaea) are able to adsorb metals, and even radionuclides on their surfaces (proteinaceous S-layer, sheaths, capsules, cell walls), which can serve as an extracellular nucleation site (van Gemerden, 1986; Beveridge, 1989; Ehrlich, 1999). The potential to withstand and promote metal accumulation followed by subsequent cell preservation has been examined using different microbial strains (archaea and bacteria); however, only limited reports exist revealing archaeal–metal interactions. To gain deeper insight into what drives microbial metal accumulation, precipitation, and subsequent cellular preservation, microbes from the *Bacteria* and *Archaea* kingdoms have been artificially encrusted (impregnated) with metals since the 1970s. Cyanobacteria have been successfully silicified, which leads to microbial structures that are well-preserved and thoroughly embedded in a crystalline silica matrix (Oehler and Schopf, 1971). The first experimental silicification of archaeal strains was performed with two strictly anaerobic and hyperthermophilic microorganisms, *Methanocaldococcus jannaschii* and *Pyrococcus abyssi* that could have inhabited hydrothermal environments on the early Earth and probably the early Mars as well. The obtained observations demonstrated that not all (micro)organisms are susceptible to metal precipitation and preservation; they behave differently even though they are closely related phylogenetically. Although both strains possess similar cell wall structures, most *M. janaschii* cells could not withstand silicification and lysed quickly, whereas silica precipitated on the cell wall of *P. abyssi* and its cells were well-preserved (Orange et al., 2009). Experimental silicification of the Gram-positive bacterial species *Geobacillus* SP7A over a period of 5 years led the authors to the conclusion that this species impregnates with silica faster than other thermophilic or mesophilic Gram-negative bacteria and archaea, due to a Gram-positive characteristic thick peptidoglycan layer, containing abundant anionic functional groups as primary silica binding sites (carbonyl, hydroxyl, phosphoryl groups). Furthermore, fast and efficient metal accumulation processes seem to be crucial to prevent degradation of organic material and to preserve the structural integrity of cellular material throughout a long period of time (Li et al., 2014; Orange et al., 2014). Rapid and high-precision preservation of microorganisms in the geological record is of utmost importance for the successful fossilization process (Orange et al., 2014). Experimental fossilization as a tool to study the preservation potential of different Bacterial and Archaeal communities could improve our understanding of the nature of Earth's earliest fossils. Importantly, the experimental fossilization approach enables thorough investigation of the influence of geological/mineralogical settings on microbial preservation potential. To extend the search of preserved biomarkers from Earth to Mars, the polyextremotolerant bacterium *Yersinia intermedia* was artificially encrusted by silica and gypsum under cold and anoxic settings, similar to current Martian conditions. *Yersinia* cells interacted immediately with the metal-bearing materials, which favors the preservation of cells during aging due to early entombment in a metal-bearing matrix (Gaboyer et al., 2017). The capacity of biosorption depending on a specific cell wall structure has been examined in two halophilic archaeal strains of *Halobacterium noricense*, which sequesters uranium in cell agglomerates and structures (Bader et al., 2017).

The extreme thermoacidophilic archaeon *Metallosphaera sedula* is a metal-mobilizing organism capable of redox transformations of a variety of metal-bearing substrates (Huber et al., 1989; Peeples and Kelly, 1995; Auernik and Kelly, 2008, 2010a; Maezato et al., 2012; Mukherjee et al., 2012; Wheaton et al., 2016; Kölbl et al., 2017; Blazevic et al., 2019; Milojevic et al., 2019a,b). Our recent studies indicate that this metallophilic archaeon can form a metal-bearing crust encasing its S-layer (Blazevic et al., 2019; Milojevic et al., 2019a,b). Examining the physiology of this extreme metallophilic archaeon cultivated with diverse metal-bearing substrates of terrestrial (Blazevic et al., 2019; Milojevic et al., 2019a) and extraterrestrial origin (Kölbl et al., 2017; Milojevic et al., 2019b), we have observed its selective preservation under the conditions of desiccation. Here, we report on several cases of the metal-grown polyextremophilic archaeon *M. sedula*, which were particularly beneficial to preserve its viability and cellular integrity after long-term desiccation.

MATERIALS AND METHODS

Strain and Media Composition

Metallosphaera sedula (DSMZ 5348) cultures were grown aerobically as described before (Kölbl et al., 2017) in DSMZ88

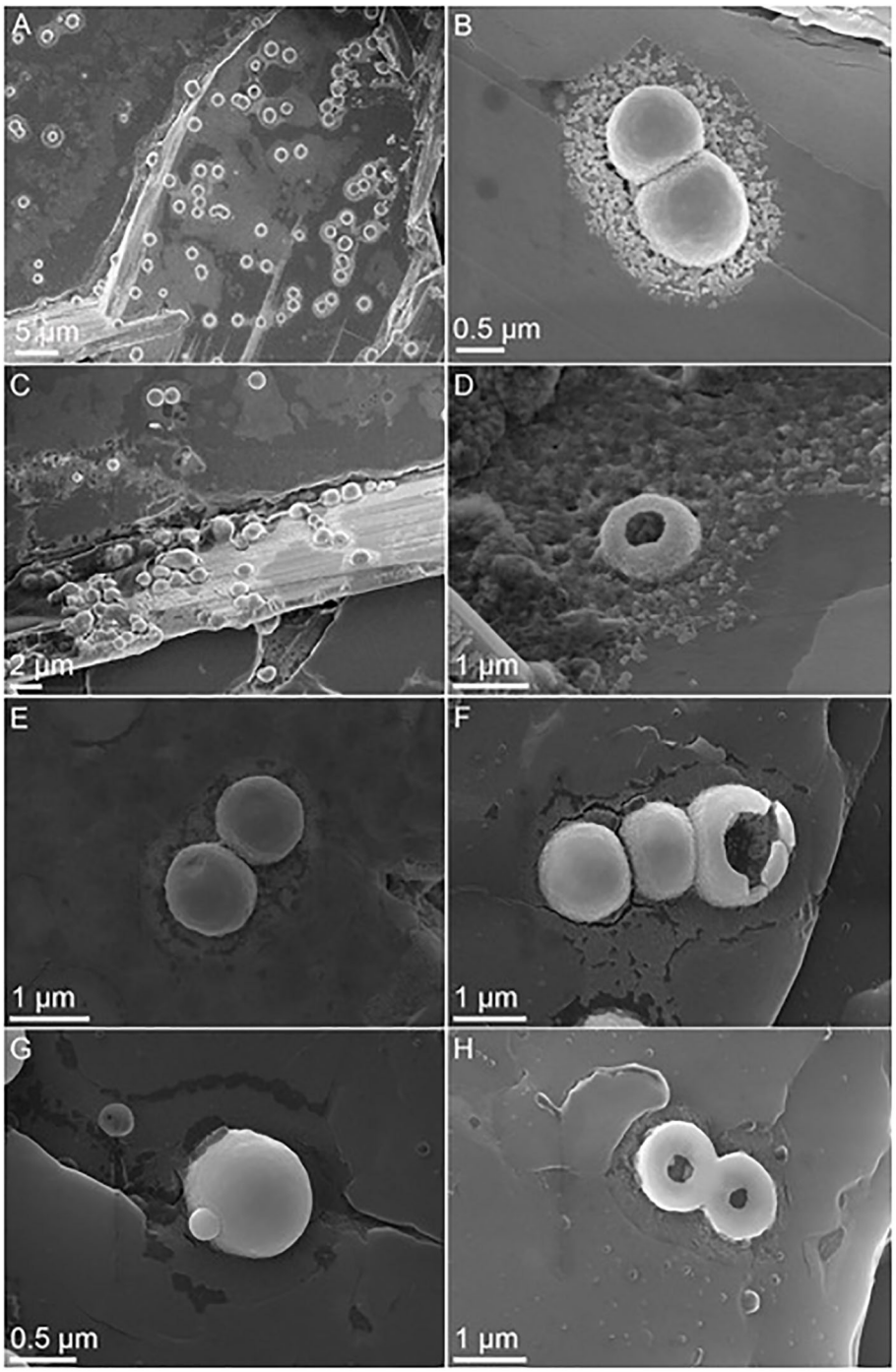

FIGURE 1 | Scanning electron microscopy (SEM) images of desiccated *M. sedula* cells grown on tungsten (W) ore scheelite and preserved after 2 months of dehydration. **(A,C)** SEM images of dehydrated colonies of *M. sedula* cells grown on scheelite. **(B)** Magnified SEM image showing single scheelite-grown cells of *M. sedula* preserved after dehydration. **(D–H)** SEM images of intact cells of *M. sedula* maintaining the integrity after dehydration and broken dried cells of *M. sedula* revealing their cellular interior.

Sulfolobus medium containing 0.28 g KH_2PO_4, 1.3 g $(NH_4)_2SO_4$, 0.07 g $CaCl_2{\cdot}2\ H_2O$, 0.25 g $MgSO_4{\cdot}7\ H_2O$, and 0.02 g $FeCl_3{\cdot}6\ H_2O$ dissolved in 1 L of water. After autoclaving, Allen's trace elements solution was added to 1 L of media resulting in 4.50 mg $Na_2B_4O_7{\cdot}10\ H_2O$, 1.80 mg $MnCl_2{\cdot}4\ H_2O$, 0.05 mg $CuCl_2{\cdot}2\ H_2O$, 0.22 mg $ZnSO_4{\cdot}7\ H_2O$, 0.03 mg $VSO_4{\cdot}2\ H_2O$, 0.03 mg $Na_2MoO_4{\cdot}2\ H_2O$, and 0.01 mg $CoSO_4$ (final concentration). The pH was adjusted with 10 N H_2SO_4 to 2.0.

Cultivation Setup

Chemolithoautotrophic cultivation of *M. sedula* was performed in DSMZ88 *Sulfolobus* medium defined above as described earlier (Milojevic et al., 2019a,b). Chemolithoautotrophic cultures were supplemented with 10 g/L of (i) a tungsten-bearing scheelite ore containing impurities of Mn and Fe oxides (Blazevic et al., 2019; see **Supplementary Table 1** for chemical composition); (ii) the Dawson-type tungsten-bearing polyoxometalate W-POM ($K_6[\alpha\text{-}P_2W_{18}O_{62}]\cdot nH_2O$) (Contant et al., 2007; Milojevic et al., 2019a); (iii) the stony meteorite NWA 1172 (H5 ordinary chondrite Northwest Africa 1172) (Russell et al., 2002; Milojevic et al., 2019b; see **Supplementary Tables 1, 2** for chemical and mineralogical composition); (iv) multimetallic waste products (voestalpine BÖHLER Edelstahl GmbH & Co KG); and (v) multimetallic Martian regolith simulants (MRSs; Kölbl et al., 2017; see **Supplementary Tables 1, 2** for chemical and mineralogical composition). The metal-containing compounds were temperature sterilized in a heating chamber (180°C) for a minimum of 24 h prior to autoclaving (121°C for 20 min). Abiotic controls containing uninoculated culture media with all aforementioned metal-bearing sources were included through all the experiments. Growth of cells was examined by phase contrast/epifluorescence microscopy and metal release (Kölbl et al., 2017; Milojevic et al., 2019a,b).

Dehydration Experiments

Dehydration of *M. sedula* cultures grown on various metal-bearing materials was performed under oxic laboratory conditions and under atmospheric pressure. For the dehydration experiments, cultures of *M. sedula* autotrophically cultivated on metal-bearing materials were harvested at middle stationary phase (**Supplementary Table 3**) omitting centrifugation and concentration, deposited by spreading evenly on glass plates (VWR International, Ø7 cm), and desiccated at room temperature within 60 days. Abiotic controls containing uninoculated culture media with the corresponding metal-bearing sources were included throughout the experiments. The morphology of the desiccated cells of *M. sedula* and crystalline and amorphous precipitates were examined by means of scanning electron microscopy (SEM). The contents of glass plates with desiccated cells were transferred into the culture media supplemented with 1% tryptone extract and incubated heterotrophically in a shaking orbital bath at 73°C. Growth of the cells was monitored during 1-week post-inoculation by phase-contrast/epifluorescence microscopy with 60× and 100× magnification (**Supplementary Figure 1**).

Scanning Electron Microscopy

The precipitates obtained after dehydration experiments were examined with a Zeiss Supra 55 VP scanning electron microscope, operated with a field emission gun (Schottky-FE, DENKA). Prior to SEM, the dehydrated samples were coated with a 3 nm Au/Pd layer (spincoater Laurell WS-650-23).

Statistical Analysis

The Excel 2016 (version 7.0) and Sigma plot (version 13.0) software packages were used to perform statistical analysis and graphical representation of the obtained data.

RESULTS

Dehydration of *M. sedula* Cells

The cultures of *M. sedula* chemolithoautotrophically grown on various metal-bearing materials (**Supplementary Table 2**, Kölbl et al., 2017; Blazevic et al., 2019; Milojevic et al., 2019a,b) were dehydrated for 60 days at room temperature under atmospheric laboratory conditions. The surface of the precipitates obtained after dehydration was examined using SEM. The cell cultures were deposited and dehydrated in their respective glassware as monolayers composed of single cells (**Figures 1–3**), thus avoiding protective "shielding" effect of cellular multilayers during dehydration. In the case of *M. sedula* grown on MRSs, no preserved cells were detected after the 60-day period of dehydration (**Figures 3G–J**).

Preservation of Desiccated *M. sedula* Cells Grown on Tungsten (W) Ore Scheelite

SEM analysis revealed that after a long-term treatment under dehydration conditions (up to 2 months), most *M. sedula* cells grown on scheelite tungsten ore as the sole energy source (Blazevic et al., 2019) remained intact, did not show significant alteration in the overall morphology, and preserved their cellular integrity, maintaining the structural stability and exposing a fine-scale irregular surface (**Figures 1A–C**). The cells of *M. sedula* with budding vesicles attached to the cell surface occurred in this case as well (**Figure 1G**). To a minor extent, lysed and broken cells were clearly recognizable too, revealing their empty interior content (evidence of cell lysis, **Figures 1D,F,H**).

Preservation of Desiccated *M. sedula* Cells Grown on Tungsten Polyoxometalate (W-POM)

Dehydration by evaporation (up to 2 months) has been applied toward microbial cultures harvested after cultivation of *M. sedula* with W-POM (Milojevic et al., 2019a). The crystalline material obtained after dehydration was examined by SEM. Colonies of *M. sedula* cells were found attached to the surface of the obtained material (**Figures 2A–D**), revealing that after long-term treatment under dehydrating conditions, cells of *M. sedula* visually remained undamaged, did not show SEM-detectable alterations in the overall morphology, and preserved their cellular integrity in frames of SEM detection, exposing a fine-scale irregular surface (**Figures 2C,D**). Particularly in regard with W-POM grown cells, no broken cells or cells with a damaged cell surface were detectable after a long-term dehydration. Physical reorganization of cellular structures after dehydration did not occur, nor was structural evolution of the cell surface after the dehydration process observed in this case (**Figure 2**). In addition, the desiccated cells forming budding vesicles were recognizable too (**Figure 2**).

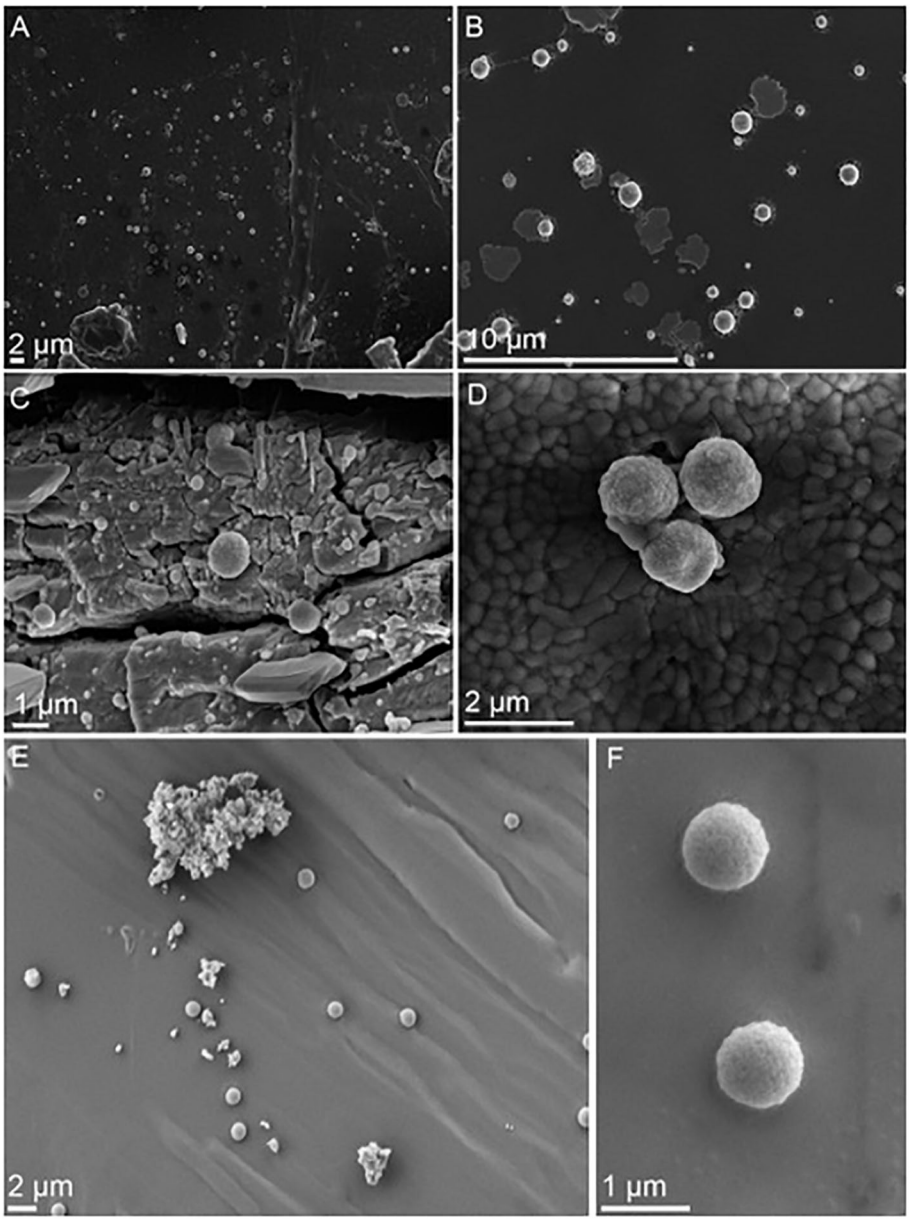

FIGURE 2 | SEM images showing desiccated cells of *M. sedula* cultivated with W-POM and multimetallic waste material after 2 months of dehydration. **(A–D)** SEM images of dehydrated *M. sedula* cells cultivated with W-POM. **(E,F)** SEM images of dehydrated *M. sedula* cells cultivated with multimetallic waste material.

Preservation of Desiccated *M. sedula* Cells Grown on Multimetallic Waste Material

Cells were harvested after cultivation with multimetallic waste material as the sole energy source and subjected to dehydration by slow evaporation for up to 2 months. Extensive post-dehydration monitoring of these cultures was performed. SEM-assisted evaluation of the post-dehydration structural integrity revealed intact colonies of dried *M. sedula* cells attached to the surface of the obtained dehydrated material (**Figures 2E,F**). Single undamaged dried cells were inspected after a long-term treatment with dehydrating conditions. The cells did not show alterations in their overall morphology and preserved their cellular integrity, exposing a fine-scale irregular surface (**Figures 2E,F**).

Preservation of Desiccated *M. sedula* Cells Grown on the NWA 1172 Meteorite

Cultures of *M. sedula* grown on the stony meteorite Northwest Africa 1172 (NWA 1172; an H5 ordinary chondrite; Milojevic et al., 2019b) were subjected to a dehydration procedure for 60 days. Dehydration of *M. sedula* cultures by evaporation resulted in the formation of amorphous and crystalline materials.

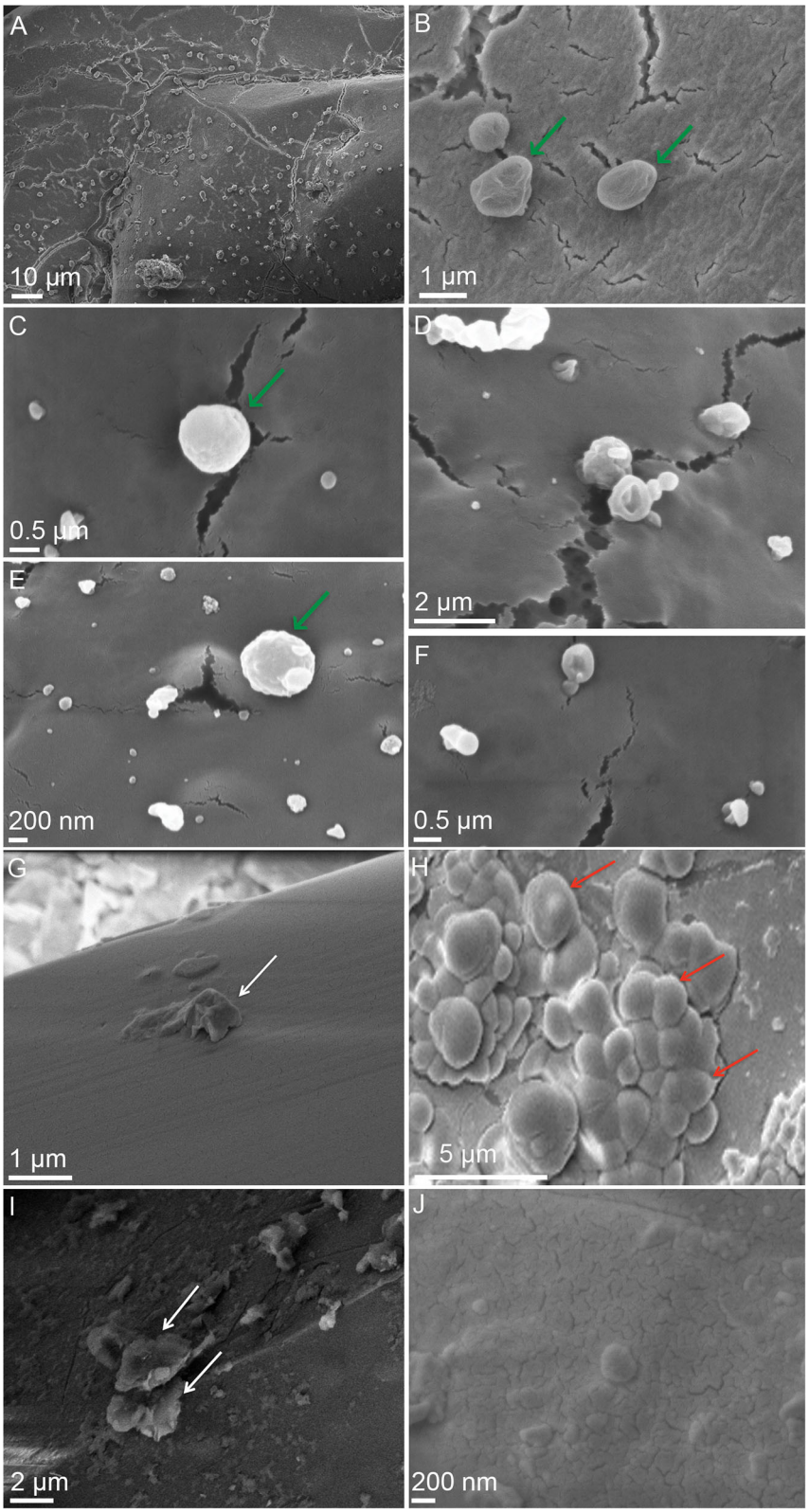

FIGURE 3 | SEM images showing desiccated cells of *M. sedula* cultivated with the stony meteorite NWA 1172 and surfaces of Martian regolith simulants (MRSs) dehydrated for 2 months after cultivation with *M. sedula*. **(A–F)** SEM images of dehydrated *M. sedula* cells cultivated with NWA 1172. **(B,C,E)** Magnified SEM images *(Continued)*

FIGURE 3 | showing single cells of *M. sedula* preserved after dehydration. **(D,F)** Magnified SEM images of broken dried cells of *M. sedula* revealing their cellular interior. **(G–J)** SEM images of MRSs dehydrated for 2 months after cultivation with *M. sedula*. **(G)** Scanning electron image showing a surface of precipitate obtained after the cultivation of *M. sedula* on JSC 1A. **(H)** Scanning electron image showing a surface of precipitate obtained after the cultivation of *M. sedula* on P-MRS. **(I)** Scanning electron image showing a surface of precipitate obtained after the cultivation of *M. sedula* on S-MRS. **(J)** Scanning electron image showing a surface of precipitate obtained after the cultivation of *M. sedula* on MRS07/52. *M. sedula* cells are shown with green arrows. White arrows indicate cell debris (e.g., cells and EPS remnants). Al-rich microspheroids are depicted with red arrows. Representative SEM-EDS analysis of carbon-rich cells, cell debris, and Al-rich microspheroids is provided in **Supplementary Figure 2**.

Our SEM analysis, performed after the dehydration period, displayed *M. sedula* cells deposited on the surface of the obtained amorphous and crystalline material (**Figures 3A–F**; see **Supplementary Figure 2C** for SEM-EDS analysis). Most of these desiccated cells remained intact on the level of SEM detection and visually preserved their cellular and structural integrity, exposing a fine-scale irregular surface (**Figures 3B,C,E**). Flourishing and dividing cells occurred as well; however, lysed and broken cells were clearly recognized too, revealing their empty interior content (evidence of cell lysis) (**Figures 3D,F**). Precipitated nanoglobules were detected attached to the surface of *M. sedula* cells in this case as well (**Figures 3D,E**).

Dehydration of *M. sedula* Grown on MRSs

Cultures of *M. sedula* grown on multimetallic MRSs as the sole energy sources (Kölbl et al., 2017) were subjected to dehydration by slow evaporation for 2 months at room temperature under atmospheric laboratory conditions. Neither preserved cells, nor cellular-like morphologies with broken, damaged, or lysed exterior were detected on the surface of dehydrated MRSs precipitates (**Figures 3G–J**). In the case of MRSs, we solely observed the formation of the aluminum/chlorine containing microspheroids published previously (Kölbl et al., 2017; **Figure 3H** and **Supplementary Figure 2A**). These hemispheroid formations were mostly composed of oxygen, chlorine, and aluminum and were characterized as nearly carbon-free metal inclusions, thus excluding the cellular nature of these morphologies (Kölbl et al., 2017; **Figure 3H** and **Supplementary Figure 2A**). Apart from aluminum/chlorine containing microspheroids, the deposition of cell debris materials was frequently detected in dehydrated cultures of *M. sedula* grown on MRSs (**Figures 3G,I** and **Supplementary Figure 2B**).

Survivability of *M. sedula* After Desiccation

The cells of *M. sedula* grown on various metal-bearing substrates after desiccation treatment for 2 months were transferred into the culture medium and were allowed to recover heterotrophically for 120 h. The cells of *M. sedula* grown and desiccated on NWA 1172, multimetallic waste, and tungsten-bearing materials (scheelite and W-POM) possess the ability to survive desiccation (**Figure 4**). The cells of *M. sedula* grown on four MRSs (JSC-1A, P-MRS, S-MRS, and MRS 07/52) showed impeded recovery after desiccation treatment when compared to NWA 1172, multimetallic waste, and tungsten-bearing materials scheelite and W-POM (**Figure 4**). Overall, heterotrophic re-cultivation of desiccated *M. sedula* cells on NWA 1172 yielded almost 2-fold higher cell number after 120 h compared to multimetallic waste, scheelite, and W-POM, showing a comparable re-cultivation capacity of cells grown on these substrates. The reduction of cell number after the desiccation treatment ("0" time point) and the subsequent impeded recovery of cells (120 h) was observed with all four types of tested MRSs (**Figure 4**). The cells grown and desiccated on MRS-07/52 exhibited the highest recovery among all four types of tested MRSs, which was however about two orders of magnitude lower than the recovery of the cells grown and desiccated on NWA 1172 and again, a 70- to 100-fold lower when compared to multimetallic waste and both tested tungsten-bearing materials (**Figure 4**). Among all examined metal-bearing substrates, the highest number of recovered cells after 120 h of post-desiccation incubation was observed in the case of NWA 1172 (**Figure 4**).

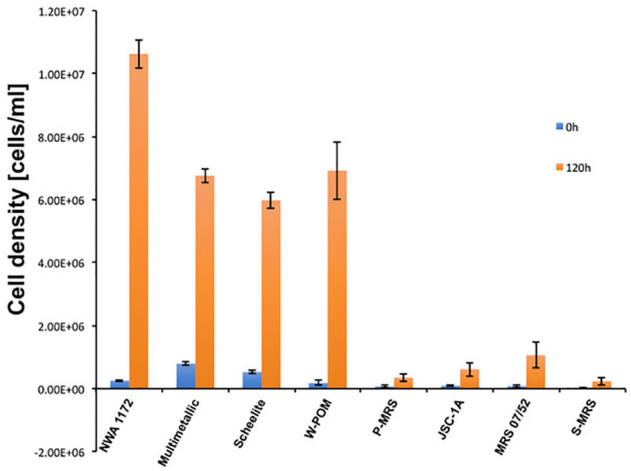

FIGURE 4 | Cell densities of *M. sedula* cells recovered after desiccation for 2 months. Blue bars represent cell counts after immediate re-cultivation in heterotrophic medium ("0"), orange bars represent cell counts after 120 h of growth after re-culturing. Columns and error bars show the mean and error-represented standard deviation, respectively, of $n = 3$ biological replicates. If not visible, error bars are smaller than symbols.

DISCUSSION

Our results showed that cells of *M. sedula* cultivated with certain metal-bearing materials (tungsten-bearing substrates, the stony chondrite meteorite NWA 1172, and multimetallic waste product) can be well-preserved (**Figures 1–3**) and recovered after dehydration up to 60 days under atmospheric conditions (**Figure 4**). Dehydrated cells of *M. sedula* cultivated on the solid metal-bearing materials were dried in a single cell layer

to avoid microbial cell-aggregates, such that a "cell-by-cell shielding effect" expected in cellular multilayers is minimized. We have previously shown that metal incorporation by the cell surface of *M. sedula* leads to the formation of a metal crust around a cell (Blazevic et al., 2019; Milojevic et al., 2019a,b). Such metal-bearing crust might protect cell integrity, providing an additional barrier against complete water loss by the cell and thus retains a minimal level of the water phase, with implication for microbial preservation in severe dehydrated conditions. Various metal-containing precipitates and accumulations were reported previously on the S-layer of other extremophilic bacteria and archaea (Orange et al., 2011, 2014; Oggerin et al., 2013; Sanchez-Roman et al., 2015; Kish et al., 2016). Interestingly, cell surface deposition of metals and subsequent cellular encrustation does not necessarily lead to the cell entombment and death. Microorganisms capable of metal sorption and encrustation have developed strategies to deal with such micro-barriers. Recent work conducted with the archaeon *S. acidocaldarius* (closely related to *M. sedula*) demonstrated that this microorganism forms encrusted outer membrane vesicles when heavily encrusted with Fe-containing accumulations (Kish et al., 2016). This strategy is likely attributed to the removal of impaired S-layer proteins to enable substitution with new, precipitate-free proteins. Elimination of damaged encrusted S-layer portions may serve as an important strategy for *Sulfolobales* members to regenerate their cell wall structure and survive in extreme environments.

Moreover, differences in preservation of dried *M. sedula* cells were observed depending on the nature of metal-bearing materials used for cultivation of this chemolithotroph. In contrast to cultures grown on tungsten-bearing substrates, the NWA 1172 meteorite, and multimetallic waste products, no cells after desiccation could be recovered or detected when *M. sedula* was cultivated on sulfide ores, elemental sulfur, molecular hydrogen (Beblo et al., 2009, 2011), volcanic glass (**Supplementary Figure 3**), and synthetic extraterrestrial materials such as MRSs (**Figures 3G–J**). The chemolithotrophic growth of *M. sedula* on all these metal-bearing matrixes has been well documented (Huber et al., 1989; Auernik and Kelly, 2008, 2010a,b; Kölbl et al., 2017; Blazevic et al., 2019; Milojevic et al., 2019a,b). Similar to our observations, Beblo et al. (2009) also reported no survival after long-term desiccation of *M. sedula* grown on elemental sulfur, sulfide ores, or on solfatara sand. In these cases, it is possible that even when cells of *M. sedula* were subjected to metal sorption, the composition of these metal-containing substrates did not provide a sufficient protection layer to resist dehydration. The observed selective preservation of *M. sedula* cells after desiccation in these cases can be explained by a discriminating "shielding by metal nano-precipitates" effect. Apparently, only certain cell wall–metal interactions can strengthen prokaryotic cell envelopes and augment their structural stability implicating in cell preservation during harsh long-term dehydration. In this regard, the tungsten incorporation by the cell envelope of *M. sedula* (Blazevic et al., 2019; Milojevic et al., 2019a) may certainly serve as an efficient strengthening strategy, as this is a hard element with the highest melting point and extraordinary properties among all metals. Being suitable for high-temperature applications in energy and lighting technology, and in the space industry, it is also used as alloys, superalloys, and radiation-shielding. We have previously reported that *M. sedula* (cultivated on scheelite and W-POM) mineralizes its S-layer via encrusting with crystalline nanoparticles containing tungsten carbide-like structures (Blazevic et al., 2019; Milojevic et al., 2019a). Tungsten exhibits a hardness of ∼9–9.5 on the Mohs hardness scale (Tabor, 1954) and can potentially provide an efficient barrier against water loss, warranting preservation of cell integrity after desiccation. *M. sedula* cells grown on the NWA 1172 meteorite are also heavily mineralized with the amorphous crust containing a $Cu_xFe_yO_z$(SPNiAl)–SiO_2 product (Milojevic et al., 2019b). This multimetallic crust of mixed Ni/Al/Si content might have implications for the preservation of desiccated cells. Growth on elemental sulfur and related sulfur-bearing substrates (Beblo et al., 2009, 2011) as energy sources would imply sulfur incorporation by S-layer of *M. sedula*. Sulfur is a soft and very brittle material [2 on the Mohs scale of mineral hardness Tabor, 1954]. Therefore, sulfur-bearing crust might not provide sufficient mechanic protection against desiccation and sulfur-supplemented growth represents a contrasting case in which the substrate does not provide the required raw materials for defense against desiccation stress.

In environments of low organic carbon content, high temperature, acidic pH, and iron-rich surroundings, microorganisms face multiple challenges maintaining their cell population and growth. The four MRSs used in this study were modeled to represent global Martian regolith chemistry and are therefore limiting possible microbial–mineral interactions compared to natural minerals (e.g., NWA 1172, scheelite ore). In addition, these synthetic mixtures do not take Mars' actual Fe^{2+}/Fe^{3+} ratios into account (Ramkissoon et al., 2019), generating regolith simulants high in Fe^{3+} rather than abundantly detected and metabolically feeding Fe^{2+} on the planet's surface (Boynton et al., 2008; Nixon et al., 2013). Elevated cultivation temperatures for *M. sedula*, the high ferric iron content, and the synthetic and therefore trace metal-depleted nature of the used MRSs (**Supplementary Table 1**) could have played a major role in the degradation of organic matter, since organic carbon is thermodynamically unstable in the presence of abundant Fe^{3+} (Sumner, 2004; Hays et al., 2017), even though there is evidence for organic matter-Fe coprecipitation and conservation over extended timescales (Lalonde et al., 2012). Our SEM observations (**Figures 3G–J**) and subsequent re-culturing under heterotrophic conditions show that thermoacidophilic *M. sedula* cells were not entombed on the surface of the MRSs mineral mixtures under the given experimental circumstances.

Apart from abundant Fe^{3+} content, additional reasons may contribute to the observed low efficiency of MRSs in the preservation of dehydrated cells. Elemental and mineralogical comparison of MRSs with genuine extraterrestrial and terrestrial materials shows that natural minerals (e.g., NWA 1172 and scheelite) exhibit a rich and complex mixture of elements in bulk as well as in trace concentrations (**Supplementary Table 1**). After cultivation of *M. sedula* on the ordinary chondrite

NWA 1172, HAADF-STEM analysis detected complex mixed mineral phases (e.g., Cu, Fe, and Al) encasing individual cells (Milojevic et al., 2019b). Such complexity of encapsulated cell wall may contribute to cellular preservation under dehydrating conditions in the case of NWA 1172. Furthermore, NWA 1172 is characterized by much pronounced Mg and Ni elemental content (**Supplementary Table 1**) and its microbially mediated biotransformation leads to the formation of magnesium and nickel sulfates $MgSO_4x7H_2O$ and $NiSO_4x6H_2O$ (Milojevic et al., 2019b). These sulfate salts may assert their kosmotropic effect, bringing order to the surrounding solution by accumulating several water layers around them and acting as potential protein (organic matter) stabilizers under desiccation stress conditions (Okur et al., 2017; Kang et al., 2020). On the contrary, each regolith simulant lacks an authentic composition and subsequently diminishes the possibilities of more complex mineral–microbe interactions. Since growth of *M. sedula* on simulated Martian regolith was successful and the hydrogeological and iron-rich makeup of Mars could have supported iron and/or sulfur-transforming, chemolithoautotrophic microorganisms at one point, the identification of their putative (iron-) relevant fingerprints is of general interest (Amils et al., 2011; Nixon et al., 2012; Kölbl et al., 2017). Despite not being able to withstand dehydrating conditions in this study, we suggest that further investigations with alternative MRSs/analogs varying in overall and iron chemistry [reviewed by Ramkissoon et al. (2019)] should be performed. Since *M. sedula* cells were preserved after growth and desiccation on the meteorite NWA 1172, which exhibits a similar chemical composition to synthetic MRSs, most valuable would be the investigation of chemolithotrophs grown on real Martian meteorite materials.

Metal-organic associations in the fossil record are key indicators of past life and can be crucial in assessing whether microstructures found in rocks are of biological origin. Metabolic activity of certain microorganisms leads to mineral precipitation extracellularly; however, in metal-laden environments, e.g., associated with contamination or natural enrichments of S, Fe, Mn, and other metals, cells are subjected to metal sorption and mineral nucleation followed by metal encrustation of the cell surface (Schultze-Lam et al., 1996). Cell surface biomineralization can serve as a powerful microbial fingerprint and potential biosignature for the presence and/or activity of metal-transforming microorganisms. Assigning biogenicity to a certain structure or signature in ancient rocks remains very challenging due to probable degradation of microbial remains during diagenesis or microbial-like morphologies being produced abiotically (Gaboyer et al., 2017). The heavily encrusted desiccated cells depicted in our study may serve as relevant biosignatures to be looked for in the geological record, if they are not destroyed during diagenetic or metamorphic processes and are intact during different stages of fossilization. With regard to the subject of the preservation potential of desiccated microbes, many fascinating questions remain to be answered, including the influence of complex environmental parameters (e.g., the environmental chemistry, the rapidity of mineral encapsulation, and various post-diagenetic factors) on biomineralized cell wall. Such investigations may serve in establishing mineralogical and morphological criteria for the identification of metal-containing microfossils. The results of our study also suggest that desiccation-resistant and heavily encrusted cell walls might be identified in other representatives of archaeal order *Sulfolobales*, including fossil species where the S-layer could be preserved due to biomineralization, and remain intact.

CONCLUSION

Communities of archaea grown on tungsten-bearing materials, the NWA 1172 meteorite, and a multimetallic waste product provide well-preserved and recoverable cells of *M. sedula* under dehydrating conditions with NWA 1172 grown cells exposing the highest number of recovered cells after the desiccation treatment. Preservation of desiccated *M. sedula* cells described in the present study appears to be a discriminatory process, which depends on the nature and content of metal-bearing source used for growth of this metal-oxidizing archaeon. Our research report emphasizes the importance of considering microorganisms in their geological/mineralogical setting during investigations of geobiological environmental constrains. More importantly, the preservation of dehydrated *M. sedula* cells on metal-bearing substrates described herein suggests that this polyextremophilic archaeon is an ideal candidate for further survivability studies during future space exposure experiments and under the simulated space environmental conditions, including the testing of *M. sedula* persistence in a vacuum and after a combination of multiple stressors, for example, vacuum conditions combined with UV and gamma irradiation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

DK, AB, MA, and TM performed experiments. DK and TM performed, planned, and interpreted experiments described in this article. All authors provided editorial contribution to the manuscript, critically revised the report, and accepted the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fspas.2020.00041/full#supplementary-material

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Conflict of Interest: CF was employed by company Voestalpine BÖHLER Edelstahl GmbH & Co KG, Austria.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.