# Exploiting a perchlorate-tolerant desert cyanobacterium to support bacterial growth for *in situ* resource utilization on Mars

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## Abstract

The presence of perchlorate in the Martian soil may limit *in-situ* resource utilization (ISRU) technologies to support human outposts. In order to exploit the desiccation, radiation-tolerant cyanobacterium *Chroococcidopsis* in Biological Life Support Systems based on ISRU, we investigated the perchlorate tolerance of *Chroococcidopsis* sp. CCMEE 029 and its derivative CCMEE 029 P-MRS. This strain was obtained from dried cells mixed with Martian regolith simulant and exposed to Mars-like conditions during the BIOMEX space experiment. After a 55-day exposure of up to 200 mM perchlorate ions, a tolerance threshold value of 100 mM perchlorate ions was identified for both *Chroococcidopsis* strains. After 40-day incubation, a Mars-relevant perchlorate concentration of 2.4 mM perchlorate ions, provided as a 60 and 40% mixture of Mg- and Ca-perchlorate, had no negative effect on the growth rate of the two strains. A proof-of-concept experiment was conducted using *Chroococcidopsis* lysate in ISRU technologies to feed a heterotrophic bacterium, i.e. an *Escherichia coli* strain capable of metabolizing sucrose. The sucrose content was fivefold increased in *Chroococcidopsis* cells through air-drying and the yielded lysate successfully supported the bacterial growth. This suggested that *Chroococcidopsis* is a suitable candidate for ISRU technologies to support heterotrophic BLSS components in a Mars-relevant perchlorate environment that would prove challenging to many other cyanobacteria, allowing a 'live off the land' approach on Mars.

## Introduction

Sustaining human outposts on Mars by providing life-support consumables from Earth is unrealistic mainly due to launch costs, travel times and failure risks. The development of Life Support Systems (LSS) is mandatory. On Earth, oxygenic photosynthesizers convert $CO_2$ to organic matter which serves as food, and $H_2O$ to $O_2$, processes that should play a key role in Biological Life Support Systems (BLSS) off the planet (Binot *et al.*, 1994; Wheeler *et al.*, 2008). An important aspect of LSS is the utilization of local materials, the so-called *in-situ* resource utilization (ISRU), although, to date, these technologies focused mainly on inorganic chemistry and building materials (Sridhar *et al.*, 2000).

A link between local resources and BLSS components, otherwise unable to live 'off the land' might be provided by oxygenic phototrophs, such as cyanobacteria, thanks to the development of Bio-ISRU technologies (Verseux *et al.*, 2016; Brown, 2018). Lithotrophic cyanobacteria have been proposed as ideal candidates for Bio-ISRU due to their capability of utilising rocks as a growth substrate (Olsson-Francis and Cockell, 2010).

The concept of using cyanobacteria to feed bacteria has been approached by genetically modifying the cyanobacterium *Anabaena* sp. 7120 in order to secrete sucrose to grow *Bacillus subtilis* (Moss and Ho, 2011; McCutcheon *et al.*, 2015). This approach was further developed into the NASA PowerCell space experiment, on-board the DLR Eu:CROPIS satellite (Hauslage *et al.*, 2018), aimed to germinate *Bacillus subtilis* spores by using *Anabaena* lysate under simulated Moon and Martian gravity (McCutcheon *et al.*, 2015).

It has been suggested that cyanobacteria could be used in BLSS using Martian local resources with relatively low hardware requirements and attenuation of surface conditions, such as low pressure and high flux of ionizing and UV radiation (Lehto *et al.*, 2006; Murukesan *et al.*, 2016). However, the presence in the Martian soil of a highly oxidizing agent such as perchlorate in the range of 0.4–0.6 wt % (Hecht *et al.*, 2009) provides an additional limiting factor for Bio-ISRU.

The exploitation of desert strains of *Chroococcidopsis* to support human space exploration has been proposed taking advantage of their remarkable desiccation- and radiation-tolerance (Billi, 2019). In particular, strain CCMEE 029 isolated from the Negev Desert has been

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extensively investigated and reported to survive years of desiccation (Billi, 2009), as well as high doses of ionizing (Billi et al., 2000; Verseux et al., 2017) and UV radiation (Baqué et al., 2013). In addition, strain CCMEE 029 was reported to cope with Mars-like conditions simulated in low Earth orbit, when exposed as dried cells mixed Martian regolith simulants (Billi et al., 2019a) or as dried biofilms (Billi et al., 2019b).

Although the strategies to withstand the above mentioned conditions have not been fully deciphered, for Chroococcidiopsis sp. CCMEE 029 the following mechanisms have been identified: (i) avoidance of protein oxidation during air-drying and ionizing radiation (Fagliarone et al., 2017); (ii) accumulation in response to desiccation of sucrose and trehalose that stabilize dried subcellular components, including ribosomal RNA and mRNAs codifying DNA repair proteins (Mosca et al., 2019; Fagliarone et al., 2020); and (iii) over-expression of DNA repair genes upon rehydration of dried cells exposed to a Mars-like UV flux (Mosca et al., 2019).

In the present work, we sought to address the hypothesis that as a consequence of its efficient antioxidant system, Chroococcidiopsis sp. CCMEE 029 might be perchlorate resistant and therefore a good candidate for Bio-ISRU on Mars. To test this hypothesis, we selected strain CCMEE 029 and its derivate CCMEE 029 P-MRS obtained from dried cells mixed with Phyllosilicatic Martian Regolith Simulant (P-MRS) and exposed to Mars simulations during the BIOMEX (BIOlogy and Mars EXperiment) space experiment performed during the EXPOSE-R2 space mission (Billi et al., 2019a). The perchlorate tolerance threshold of the two strains was identified by monitoring the growth rate during a 55-day exposure to 5, 50 and 100 mM Na-, Mg- and Ca-perchlorate. Once their perchlorate tolerance threshold was identified, the two strains were grown for 40 days in the presence of Mars-relevant perchlorate concentration, for example, 2.4 mM perchlorate ions supplied as a 60 and 40% mixture of Mg- and Ca-perchlorate, respectively, as reported by the NASA's Phoenix Mars Lander (Hassler et al., 2014). Then the biomass yielded by strain CCMEE 029 grown in 2.4 mM perchlorate ions was used to produce the lysate used to feed an E. coli strain capable of metabolizing sucrose.

## Material and methods

### Organisms and culture conditions

Chroococcidiopsis sp. CCMEE 029 was isolated by Roseli Ocampo-Friedmann from cryptoendolithic growth in the Negev Desert (Israel). The strain is part of the Culture Collection of Microorganisms from Extreme Environments (CCMEE), established by E. Imre and Roseli Ocampo-Friedmann, that is currently maintained at the Department of Biology, University of Rome Tor Vergata. Chroococcidiopsis sp. CCMEE 029 P-MRS was derived from dried cells of CCMEE 029 mixed with P-MRS and exposed to Mars simulations in low Earth orbit inside the EXPOSE-R2 facility: $CO_2$ atmosphere (780 Pa), $2.19 \times 10^2$ kJ m$^{-2}$ of UV$_{200-400 nm}$ radiation and 0.5 Gy of ionizing radiation (Billi et al., 2019a). Cyanobacterial strains were grown in BG11 medium (Rippka et al., 1979) by using 50 ml vented flasks, inside an incubator at 25°C, without shaking, under a photon flux density of 40 $\mu$mol m$^{-2}$ s$^{-1}$ provided by cool-white fluorescent lamp (4100 K) under continuous illumination.

Escherichia coli strain W (ATCC 9637) was purchased from the American Type Culture Collection (Manassas, VA, USA) and grown in Luria-Bertani broth (Sambrook et al., 1989) at 37°C with orbital shaking.

### Monitoring of growth rates

Cyanobacterial growth was monitored by determining the optical density at 730 nm (OD$_{730}$) of triplicates, each one consisting of 100 $\mu$l aliquot. Bacterial growth was determined by measuring the optical density at 600 nm (OD$_{600}$) of triplicates, each one consisting of 100 $\mu$l aliquot. Two calibration curves, one for OD$_{730 nm}$ versus cyanobacterial concentration and one for OD$_{600 nm}$ versus bacterial concentration, were obtained by determining cell concentrations (cells ml$^{-1}$) with a Burker's chamber.

### Determination of cyanobacterial perchlorate tolerance

One-ml aliquots of the two cyanobacterial cultures in the early stationary phase (about $1 \times 10^7$ cells) were centrifuged and pellets inoculated into 50 ml vented flasks containing 10 ml of liquid BG-11 with 5, 50 and 100 mM Na-, Mg- and Ca-perchlorate. Controls were obtained by inoculating 1 ml aliquots in liquid BG-11. During the 55 days of growth, the culture volume was kept constant by adding BG-11 or BG-11 with 5, 50 and 100 mM Na-, Mg- and Ca-perchlorate. The experiment was performed in triplicate.

### Cyanobacterial growth in Mars-relevant perchlorate

One-ml aliquots of the two cyanobacterial cultures in the early stationary phase (about $1 \times 10^7$ cells) were centrifuged, and the pellets inoculated into 50 ml vented flasks containing 10 ml of BG-11 with 2.4 mM ClO$_4^-$ provided as a 40% Mg-perchlorate and 60% Ca-perchlorate mixture. Each experiment was performed in triplicate. During the 40 days of growth, the culture volume was maintained constant by adding BG-11 or BG-11 with 2.4 mM ClO$_4$. The experiment was performed in triplicate.

### Cyanobacterial lysis

Six-ml aliquots (about $6 \times 10^8$ cells ml$^{-1}$) obtained from 40-day-old cultures in BG-11 and BG-11 containing 2.4 mM ClO$_4^-$ (as reported above) were centrifuged at 7000 $g$, at 20°C for 15 min. Pellets were washed twice in dd-H$_2$O, air-dried overnight under a laminar-flow hood, and weighted prior to and after drying. Air-dried cells (30 mg) were resuspended in 3 ml dd-H$_2$O and split into three aliquots. Pellets from each 1 ml aliquot were lysed as follows: (i) mortaring for 2 min, (ii) resuspension in 500 $\mu$l dd-H$_2$O and immersion in liquid N$_2$ for 1 min, (iii) thawing at 37°C for 10 min; (iv) centrifugation at 6000 $g$, for 10 min at 20°C to collect the soluble supernatant used as lysate medium.

### Escherichia coli growth with cyanobacterial lysate-based medium

One-ml aliquots of overnight E. coli were diluted to about $1 \times 10^6$ cells ml$^{-1}$, washed with 1X PBS, and resuspended in 1 ml lysate-based medium. As a positive control, $1 \times 10^6$ cells ml$^{-1}$ were washed with PBS and inoculated in 1 ml of M9 minimal medium (Sambrook et al., 1989) supplemented with 0.5% glucose. As negative controls, about $1 \times 10^6$ cells ml$^{-1}$ were washed with PBS and inoculated into 1 ml of sterile dd-H$_2$O and 1X PBS. Each sample was incubated in 1.5 ml Eppendorf tubes at 38°C
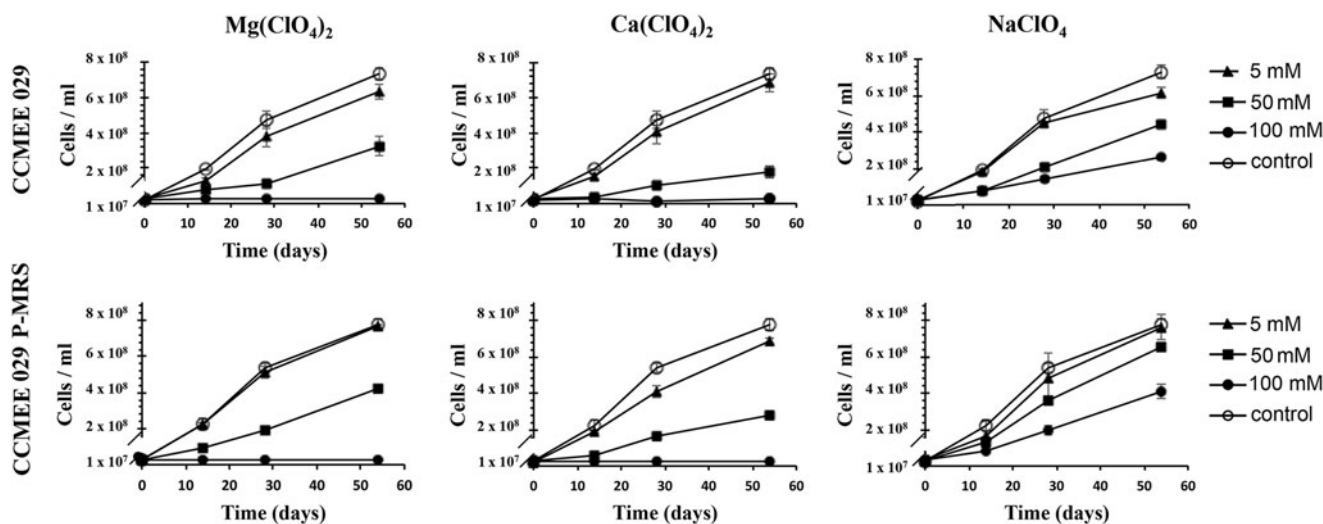
**Fig. 1.** Cell densities of cultures of *Chroococcidiopsis* sp. CCMEE 029 and CCMEE 029 P-MRS grown in BG-11 containing 5, 50 and 100 mM Mg-, Ca- and Na-perchlorate. Control cultures were grown in BG-11. Data are shown as mean ± standard deviation in three independent trials.

overnight, under continuous shaking of 180 rpm. The experiment was performed in triplicate.

### Perchlorate measurements

Perchlorate concentrations were determined by EPA Method 331.0 Rev. 1.0 – Liquid Chromatography/Electrospray Ionization/Mass Spectrometry (2012). Briefly, samples were diluted 1:20 with ultrapure $H_2O$ and 5 µl injected for analysis on a Waters ACQUITY I-Class UPLC/Xevo G2-XS QTof system. The quantification of perchlorate anion was based on Tof MRM negative acquisition mode of the following masses, under −25 V collision energy and by using a calibration curve that covered 0.01–10 mM concentration interval.

### Results

#### Cyanobacterial tolerance towards increasing perchlorate concentrations

The perchlorate tolerance of *Chroococcidiopsis* strains CCMEE 029 and CCMEE 029 P-MRS was investigated by exposure for 55 days in liquid BG-11 medium containing increasing concentration of Na-, Mg- and Ca-perchlorate up to 100 mM (Fig. 1). The presence of 5 mM of Na-, Mg- and Ca-perchlorate (5, 10 and 10 mM perchlorate ions, respectively) did not inhibit the growth of the two strains. While the presence of 50 mM perchlorate resulted in a reduced growth rate of both strains, that was more evident in Mg- and Ca-perchlorate (100 mM perchlorate ions), than in Na-perchlorate (50 mM perchlorate ions). The growth of both strains was reduced by the 100 mM concentration of Na-perchlorate, while no growth occurred in 100 mM Mg- and Ca-perchlorate (200 mM perchlorate ions) (Fig. 1).

#### Effects of increasing perchlorate concentrations on cyanobacterial morphology

The exposure to increasing concentration of Na-, Mg- and Ca-perchlorate did not show any severe morphological changes. For example, after 55 days of exposure in 100 mM Na-perchlorate,

*Chroococcidiopsis* sp. CCMEE 029 (Fig. 2a) and CCMEE 029 P-MRS (Fig. 2b) occurred as single cells and as four-celled aggregates as typical of strain CCMEE 029 when grown in BG-11 medium (Fig. 2c).

#### Effects of Mars-relevant perchlorate concentration on cyanobacterial growth

The ability of *Chroococcidiopsis* strains CCMEE 029 and CCMEE 029 P-MRS to grow in the presence of Mars-relevant perchlorate concentrations was investigated by incubation in liquid BG-11 containing 2.4 mM $ClO_4^-$ provided as a 40% Mg-perchlorate and 60% Ca-perchlorate mixture (Fig. 3). The combination of the two salts did not affect the growth rate. After 40-day incubation, strains CCMEE 029 and CMEE 029 P-MRS showed a slight increase in cell densities compared to control cells grown in BG-11 medium (Fig. 3).

#### Perchlorate content during cyanobacterial growth

The concentration of perchlorate ions in *Chroococcidiopsis* strains CCMEE 029 and CCMEE 029 P-MRS was measured at the beginning and after 40-day incubation in BG-11 medium with 2.4 mM perchlorate ions. In the overall, no significant variation occurred between the perchlorate concentrations in the BG-11 medium recovered after the cyanobacterial growth and that in control BG-11 medium with 2.4 mM perchlorate ions.

#### Escherichia coli *growth with cyanobacterial lysate-based medium*

A cyanobacterial lysate-based medium was derived from the soluble fraction of lysed cells of *Chroococcidiopsis* sp. CCMEE 029 grown for 40 days in BG-11 with 2.4 mM perchlorate ions (Fig. 4). When about $1 \times 10^6$ *E. coli* cells were inoculated in 1 ml of the lysate-based medium obtained from the lysis of about 30 mg of air-dried cyanobacteria, the cell density resulted increased to about $2 \times 10^8$ cells ml$^{-1}$ after overnight incubation. No significant difference occurred in *E. coli* cell density when using the lysate obtained from strain CCMEE 029 grown in
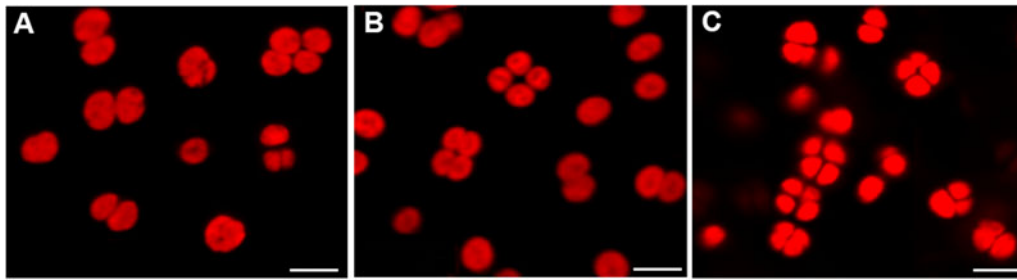
**Fig. 2.** CLSM images showing the morphology of Chroococcidiopsis sp. CCMEE 029 (A) and CCMEE 029 P-MRS (B) grown for 55 days in BG-11 medium containing 100 mM NaClO$_4$ and in control of strain CCMEE 029 (C) grown in BG-11 medium. In red autofluorescence of photosynthetic pigments. Bar scale = 10 μm.
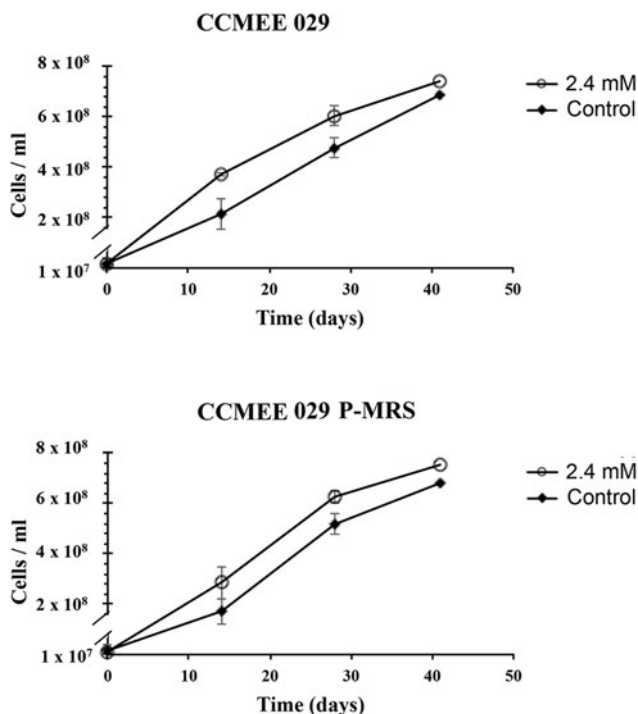


**Fig. 3.** Growth of Chroococcidiopsis sp. CCMEE 029 and CMEE 029 P-MRS in BG-11 and in BG-11 containing 2.4 mM perchlorate ions, provided as 40% Mg-perchlorate and 60% Ca-perchlorate mixture. Data are shown as mean ± standard deviation in three independent trials.



**Fig. 4.** Growth of E. coli supported by a cyanobacterium-based medium. Lysate 1: lysate of Chroococcidiopsis sp. CCMEE 29 grown in BG-11 containing 2.4 mM perchlorate ions; Lysate 2: lysate of Chroococcidiopsis sp. CCMEE 29 grown in BG-11. Positive controls: M9 minimal medium supplemented with 0.5% glucose and LB medium. Negative controls: dd-dH$_2$O and PBS.

BG-11 in the absence of 2.4 mM perchlorate ions. *Escherichia coli* reached an average density of $4 \times 10^8$ cells ml$^{-1}$ after incubation in M9 minimal medium (containing salts, nitrogen and 0.5% glucose) and about $1 \times 10^9$ cells ml$^{-1}$ in LB medium. No growth occurred when *E. coli* was inoculated in dd-H$_2$O or PBS (Fig. 4).

## Discussion

The aim of this work was to investigate whether the desert cyanobacterium *Chroococcidiopsis* could be used in Bio-ISRU technologies to support the growth of BLSS components, namely bacteria, that need fixed carbon and that might be perchlorate sensitive (Rothschild, 2016; Verseux *et al.*, 2016).

The incubation of *Chroococcidiopsis* sp. CCMEE 029 and CCMEE 029 P-MRS in increasing perchlorate concentrations identified a perchlorate threshold value at 100 mM perchlorate ions. No remarkable significant difference in the perchlorate tolerance occurred between CCMEE 029 and its space-derivate
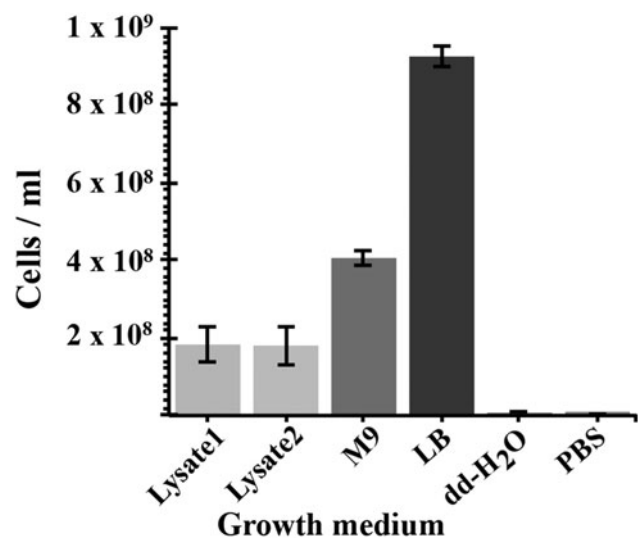
CCMEE 029 P-MRS, suggesting that the exposure to Mars simulations in low Earth orbit followed by rehydration on the ground after retrieval did not select any enhanced oxidative-stress resistance. Indeed on-going comparative genomic analysis between the genome sequence of strain CCMEE 029 and its space-derivate CCMEE 029 P-MRS will verify the eventual presence of differences between the two strains.

The identified perchlorate tolerance provided a first prerequisite for the use of *Chroococcidiopsis* in Bio-ISRU technologies. In contrast, at the Phoenix landing site, a concentration of about 2.4 mM perchlorate ions (0.5 wt %) was measured, whereas the Curiosity rover reported perchlorate concentrations up to 1% at the Gale Crater (Glavin *et al.*, 2013).

Long-term (55 days) exposure of the two *Chroococcidiopsis* strains to increasing perchlorate concentrations revealed that growth rate was: (i) not affected in 5 mM Na-, Mg- and Ca-perchlorate (5, 10 and 100 mM perchlorate ions); (ii) slightly reduced in 50 mM Na-perchlorate; (iii) further impaired in 100 mM Na-perchlorate; (iv) reduced in 50 mM Mg- and Ca-perchlorate (100 mM perchlorate ions); and (v) completely inhibited in 100 mM Mg- and Ca-perchlorate (200 mM perchlorate ions). No growth occurred in 300 mM Na-perchlorate (not shown).

The identified 100 mM perchlorate ion threshold was comparable to that of a halophilic bacterium isolated from Big Soda Lake, growing in 2% Na-perchlorate, that is, 160 mM perchlorate ions (Matsubara *et al.*, 2017). However, *Chroococcidiopsis* perchlorate tolerance was lower than that of several halophiles. For example, halophilic Archaea of the family Halobacteriaceae and *Halomonas elongata* showed a weak growth in 600 mM Na-perchlorate (Oren *et al.*, 2014), the growth of the archaeon *Halorubrum lacusprofundi* was still measurable in 800 mM perchlorate ions (Laye and DasSarma, 2018), while *Hydrogenothermus marinus* did not replicate in 400 mM Na-perchlorate (Beblo-Vranesevic *et al.*, 2017). So far the highest perchlorate tolerance was reported for the yeast *Debaryomyces hansenii* growing in 2.4 M Na-perchlorate (Heinz *et al.*, 2020), a value twice that of the bacterium *Planococcus halocryophilus* (Heinz *et al.*, 2019).

Short-term (15 min) exposure in Na-perchlorate highlighted $D_{10}$ values of 2.7, 1.3, 5 and >5 M, for *Deinococcus radiodurans*, *E. coli*, *B. subtilis* spores and *H. marinus*, respectively (Beblo-Vranesevic *et al.*, 2017). In the present work, *Chroococcidiopsis* strains were not exposed to short-term incubation in higher perchlorate concentrations because the main goal was to identify the tolerance threshold in long-term cultivation.

No significant morphological changes were observed in *Chroococcidiopsis* exposed up to 200 mM perchlorate ions. On the contrary, halophilic archaea occurred as swollen cells in perchlorate ion concentrations >200 mM (Oren *et al.*, 2014), *H. marinus* turned into long cell chains in Na-perchlorate concentrations >100 mM (Beblo-Vranesevic *et al.*, 2017), while *P. halocryophilus* formed large cell clusters in 1.1 M Na-perchlorate (Heinz *et al.*, 2019).

The two *Chroococcidiopsis* strains better tolerated Na- and Mg-perchlorate than Ca-perchlorate. Indeed Ca-perchlorate is a chaotropic agent, causing macromolecules destabilization and growth inhibition stronger than Mg-perchlorate and Na-perchlorate, the latter being a weak chaotropic agent (Cray *et al.*, 2013; Nagler and Moeller, 2015). Moreover, since *Chroococcidiopsis* sp. CCMEE 029 has been reported to bleach in 680 mM NaCl (Hershkovitz *et al.*, 1991), the observed slightly reduced growth in 100 mM Na-perchlorate might be ascribed to the oxidizing action of perchlorate.

Since 2.4 mM perchlorate ions were reported to occur on Mars as a 60 and 40% mixture of Mg- and Ca-perchlorate, respectively (Hassler *et al.*, 2014), the two *Chroococcidiopsis* strains were grown in Mars-relevant perchlorate ions as reported for the Phoenix landing site. The growth of the two *Chroococcidiopsis* strains was not impaired by 40-day incubations in 2.4 mM perchlorate ions, rather it increased slightly compared to control. This might be due to a lack of a synergistic negative effect of Mg- and Ca-perchlorate as reported for bacteria isolated from Big Soda Lake (Matsubara *et al.*, 2017), but also to a positive effect of the increased cation concentration. Indeed *Anabaena* sp. PCC 7120 was reported to grow in 1 mM $CaCl_2$ (Singh *et al.*, 2016). Here *Chroococcidiopsis* cells were grown in concentrations of 0.24 mM $CaCl_2$ and 0.30 mM $MgSO_4$ supplied by the BG-11 medium (Rippka *et al.*, 1979) while 0.5 mM $Mg^{2+}$ and 0.75 mM $Ca^{2+}$ were present in 2.4 mM perchlorate ions supplied as a 60 and 40% mixture of Mg- and Ca-perchlorate, respectively.

After 40-day growth in 2.4 mM perchlorate ions, no variation in the perchlorate content was observed; therefore, the perchlorate concentration in the lysate was not determined. Indeed cyanobacteria are non-perchlorate-reducing prokaryotes, although the presence of chlorite dismutase-like proteins has been reported in a few nitrogen-fixing species (Nerenberg, 2013; Schaffner *et al.*, 2015). Hence, the perchlorate tolerance of the two *Chroococcidiopsis* strains was likely a consequence of their efficient antioxidant system (Fagliarone *et al.*, 2017).

The observed *Chroococcidiopsis* capability to tolerate Mars-relevant perchlorate ions was further exploited to obtain a lysate-based medium to feed an *E. coli* strain, capable of utilizing sucrose as a carbon source (Lee and Chang, 1993). Therefore, after 40-day growth in 2.4 mM perchlorate, *Chroococcidiopsis* cells were air-dried to induce a fivefold increase in their sucrose content from, up to 5 mg/g dry weight (not shown), as previously reported (Fagliarone *et al.*, 2020). The lysate-based medium obtained from 10 mg of dried cyanobacteria supported an increase of the *E. coli* cell density from $1 \times 10^6$ to $2 \times 10^8$ cells after overnight incubation. A comparable increase of *E. coli* cell density was obtained using the lysate obtained from *Chroococcidiopsis* grown in standard BG-11 medium. This suggested that washing the cyanobacterial cells before lysis removed perchlorate and avoided a negative effect on *E. coli* growth. However, since a doubled cell density (about $4 \times 10^8$ cells) was supported by overnight growth in M9 minimal medium, an increased *E. coli* cell density is expected to be supported by lysing an increased amount of dried cyanobacteria.

However, results provided a first proof-of-concept of feeding a bacterium with a lysate-based medium obtained from *Chroococcidiopsis* grown in Mars-relevant perchlorate concentration. The use of this cyanobacterium as a pioneer for Bio-ISRU on Mars is further supported by its capability of lithotrophic growth on Mars regolith analogues, namely anorthosite and basalt (supplemented with $NaNO_2$), resulting in elemental release and biomass production (Olsson-Francis and Cockell, 2010).

Moreover, the feeding of *E. coli* with a cyanobacterial lysate is relevant since this bacterium is a typical chassis for genetic manipulation and metabolic engineering of bacteria was proposed to provide consumables to human outposts on Mars (Rothschild, 2016). Recently, the use of cyanobacterial lysate to support *B. subtilis* engineered to synthetize aromatic polymers for space application has been reported (Averesch and Rothschild, 2019) and tested in NASA's PowerCell payload aboard the DLR EuCROPIS Satellite (McCutcheon *et al.*, 2015).

In conclusion, *Chroococcidiopsis* might enable Bio-ISRU on Mars by combining its capability of converting $CO_2$ to organic compounds with its perchlorate resistance, and its use in Bio-ISRU might be further reinforced by synthetic biology (Montague *et al.*, 2012; Snyder *et al.*, 2019). Indeed, the development of *Chroococcidiopsis* CCMEE 029 as a chassis for synthetic biology will endow it with the ability to synthetize compounds not naturally produced and this approach will take advantage of the availability of a genetic system for its genetic manipulation (Billi *et al.*, 2001) and maintenance of plasmid DNA after prolonged air-dried storage (Billi, 2012).

# References

**Averesch NJH and Rothschild LJ** (2019) Metabolic engineering of *Bacillus subtilis* for production of para-aminobenzoic acid – unexpected importance of carbon source is an advantage for space application. *Microbial Biotechnology* **12**, 703–714.

**Baqué M, Viaggiu E, Scalzi G and Billi D** (2013) Endurance of the endolithic desert cyanobacterium *Chroococcidiopsis* under UVC radiation. *Extremophiles* **17**, 161–169.

**Beblo-Vranesevic K, Huber H and Rettberg P** (2017) High tolerance of *Hydrogenothermus marinus* to sodium perchlorate. *Frontiers in Microbiology* **8**, 1369.

**Billi D** (2009) Subcellular integrities in *Chroococcidiopsis* sp. CCMEE 029 survivors after prolonged desiccation revealed by molecular probes and genome stability assays. *Extremophiles* **13**, 49–57.

**Billi D** (2012) Plasmid stability in dried cells of the desert cyanobacterium *Chroococcidiopsis* and its potential for GFP imaging of survivors on Earth and in space. *Origins of Life Evolution of Biospheres* **42**, 235–245.

**Billi D** (2019) Desert cyanobacteria under space and planetary simulations: a tool for searching for life beyond Earth and supporting human space exploration. *International Journal of Astrobiology* **18**, 483–489.

**Billi D, Friedmann EI, Hofer KG, Grilli-Caiola M and Ocampo-Friedmann R** (2000) Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Applied and Environmental Microbiology* **66**, 1489–1492.

**Billi D, Friedmann EI, Helm RF and Potts M** (2001) Gene transfer to the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Journal of Bacteriology* **183**, 2298–2305.

**Billi D, Verseux C, Fagliarone C, Napoli A, Baqué M and de Vera J-P** (2019a) A desert cyanobacterium under simulated Mars-like conditions in low Earth orbit: implications for the habitability of Mars. *Astrobiology* **19**, 158–169.

**Billi D, Staibano C, Verseux C, Fagliarone C, Mosca C, Baqué M, Rabbow E and Rettberg P** (2019b) Dried biofilms of desert strains of *Chroococcidiopsis* survived prolonged exposure to space and Mars-like conditions in low Earth orbit. *Astrobiology* **19**, 1008–1017.

**Binot RA, Tamponnet C and Lasseur C** (1994) Biological life support for manned missions by ESA. *Advances in Space Research* **14**, 71–74.

**Brown I** (2008) Cyanobacteria to link closed ecological systems and in-situ resources utilization processes. In *37th COSPAR Scientific Assembly*, Montréal, Canada.

**Cray JA, Russell JT, Timson DJ, Singhal RS and Hallsworth JE** (2013) A universal measure of chaotropicity and kosmotropicity. *Environmental Microbiology* **15**, 287–296.

**Fagliarone C, Mosca C, Ubaldi I, Verseux C, Baqué M, Wilmotte A and Billi D** (2017) Avoidance of protein oxidation correlates with the desiccation and radiation resistance of hot and cold desert strains of the cyanobacterium *Chroococcidiopsis*. *Extremophiles* **21**, 981–991.

**Fagliarone C, Napoli A, Chiavarini S, Baqué M, de Vera J-P and Billi D** (2020) Biomarker preservation and survivability under extreme dryness and Mars-like UV flux of a desert cyanobacterium capable of trehalose and sucrose accumulation. *Frontiers in Astronomy and Space Sciences* **7**, 31.

**Glavin DP, Archer D, Brunner AE, Buch A, Cabane M, Coll P, Conrad P, Coscia D, Dworkin JP, Eigenbrode JL, Freissinet C, Mahaffy P, Martin M, McKay CP, Miller K, Ming DW, Navarro-González R, Steele A, Summons RE, Sutter B, Szopa C, Teinturier S and MSL Science Team** (2013) Investigating the origin of chlorohydrocarbons detected by the Sample Analysis at Mars (SAM) instrument at Rocknest. In 44th LPSC Lunar and Planetary Science Conference, The Woodlands, Texas, USA, p. 1080.

**Hassler DM, Zeitlin C, Wimmer-Schweingruber RF, Ehresmann B, Rafkin S, Eigenbrode JL, Brinza DE, Weigle G, Böttcher S, Böhm E, Burmeister S, Guo J, Köhler J, Martin C, Reitz G, Cucinotta FA, Kim M-H, Grinspoon D, Bullock MA, Posner A, Gómez-Elvira J, Vasavada A and Grotzinger JP and MSL Science Team** (2014) Mars' surface radiation environment measured with the Mars Science Laboratory's Curiosity rover. *Science (New York, N.Y.)*, **343**, 1244797.

**Hauslage J, Strauch SM, Eßmann O, Haag FW, Richter P, Krüger J, Stoltze J, Becker I, Nasir A, Bornemann G, Müller H, Delovski T, Berger T, Rutczynska A, Marsalek K and Lebert M** (2018) Eu:CROPIS – 'Euglena gracilis: combined regenerative organic-food production in space' – a space experiment testing biological life support systems under Lunar and Martian gravity. *Microgravity Science and Technology* **30**, 933–942.

**Hecht MH, Kounaves SP, Quinn RC, West SJ, Young SMM, Ming DW, Catling DC, Clark BC, Boynton WV, Hoffman J, DeFlores LP, Gospodinova K, Kapit J and Smith PH** (2009) Detection of perchlorate and the soluble chemistry of Martian soil at the Phoenix Lander Site. *Science (New York, N.Y.)* **325**, 64–67.

**Heinz J, Waajen AC, Airo A, Alibrandi A, Schirmack J and Schulze-Makuch D** (2019) Bacterial growth in chloride and perchlorate brines: halo-tolerances and salt stress responses of *Planococcus halocryophilus*. *Astrobiology* **19**, 1377–1387.

**Heinz J, Krahn T and Schulze-Makuch D** (2020) A new record for microbial perchlorate tolerance: fungal growth in $NaClO_4$ brines and its implications for putative life on Mars. *Life (Chicago, Ill)* **10**, 53.

**Hershkovitz N, Oren A and Cohen Y** (1991) Accumulation of trehalose and sucrose in cyanobacteria exposed to matric water stress. *Applied Environmental Microbiology* **57**, 645–648.

**Laye VJ and DasSarma S** (2018) An Antarctic extreme halophile and its polyextremophilic enzyme: effects of perchlorate salts. *Astrobiology* **18**, 412–418.

**Lee SY and Chang HN** (1993) High cell density cultivation of *Escherichia coli* W using sucrose as a carbon source. *Biotechnology Letters* **15**, 971–974.

**Lehto KM, Lehto HJ and Kanervo EA** (2006) Suitability of different photo-synthetic organisms for an extraterrestrial biological life support system. *Research in Microbiology* **157**, 69–76.

**Matsubara T, Fujishima K, Saltikov CW, Nakamura S and Rothschild LJ** (2017) Earth analogues for past and future life on Mars: isolation of per-chlorate resistant halophiles from Big Soda Lake. *International Journal of Astrobiology* **16**, 218–228.

**McCutcheon G, Kent R, Paulino-Lima I, Pless E, Ricco A, Mazmanian E, White B, Tapio E and Rothschild LJ** (2015) Powercells payload on EuCROPIS – measuring synthetic biology in space. 29th Annual AIAA/ USU Conference on Small Satellites. Logan, UT, USA.

**Montague M, McArthur GH, Cockell CS, Held J, Marshall W, Sherman LA, Wang N, Nicholson WL, Tarjan DR and Cumbers J** (2012) The role of synthetic biology for *in situ* resource utilization (ISRU). *Astrobiology* **12**, 1135–1142.

**Mosca C, Rothschild LJ, Napoli A, Ferré F, Pietrosanto M, Fagliarone C, Baqué M, Rabbow E, Rettberg P and Billi D** (2019) Over-expression of UV-damage DNA repair genes and ribonucleic acid persistence contribute to the resilience of dried biofilms of the desert cyanobacterium *Chroococcidiopsis* exposed to Mars-like UV flux and long-term desiccation. *Frontiers in Microbiology* **10**, 2312.

**Moss E and Ho J** (2011) PowerCell. http://2011.igem.org/Team:Brown-Stanford/PowerCell/Introduction.

**Murukesan G, Leino H, Mäenpää P, Ståhle K, Raksajit W, Lehto HJ, Allahverdiyeva-Rinne Y and Lehto K** (2016) Pressurized Martian-like pure $CO_2$ atmosphere supports strong growth of cyanobacteria, and causes significant changes in their metabolism. *Origins of Life and Evolution of Biospheres* **46**, 119–131.

**Nagler K and Moeller R** (2015) Systematic investigation of germination responses of *Bacillus subtilis* spores in different high-salinity environments. *FEMS Microbiology Ecology* **91**, fiv023.

**Nerenberg R** (2013) Microbiology. Breathing perchlorate. *Science (New York, N.Y.)* **340**, 38–39.

**Olsson-Francis K and Cockell CS** (2010) Use of cyanobacteria for *in-situ* resource use in space applications. *Planetary and Space Science* **58**, 1279–1285.

**Oren A, Elevi Bardavid R and Mana L** (2014) Perchlorate and halophilic pro-karyotes: implications for possible halophilic life on Mars. *Extremophiles* **18**, 75–80.

**Rippka R, Deruelles J, Waterbury JB, Herdman MS and Stanier RY** (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111**, 1–61.

**Rothschild LJ** (2016) Synthetic biology meets bioprinting: enabling technologies for humans on Mars (and Earth). *Biochemical Society Transaction* **44**, 1158–64.

**Sambrook J, Fritsch EF and Maniatis T** (1989) *Molecular Cloning: A Laboratory Manual*. 2nd Edn. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory.

**Schaffner I, Hofbauer S, Krutzler M, Pirker KF, Furtmüller PG and Obinger C** (2015) Mechanism of chlorite degradation to chloride and dioxygen by the enzyme chlorite dismutase. *Archives of Biochemistry and Biophysics* **574**, 18–26.

**Singh S, Verma E, Niveshika Tiwari B and Mishra AK** (2016) Exopolysaccharide production in *Anabaena sp*. PCC 7120 under different CaCl$_2$ regimes. *Physiology and Molecular Biology of Plants* **22**, 557–566.

**Snyder JE, Walsh D, Carr PA and Rothschild LJ** (2019) A makerspace for life support systems in space. *Trends in Biotechnology* **37**, 1164–1174.

**Sridhar KR, Finn JE and Kliss MH** (2000) In-situ resource utilization technologies for Mars life support systems. *Advances in Space Research* **25**, 249–255.

**Verseux C, Baqué M, Lehto K, de Vera J-P, Rothschild LJ and Billi D** (2016) Sustainable life support on Mars – the potential roles of cyanobacteria. *International Journal of Astrobiology* **15**, 65–92.

**Verseux C, Baqué M, Cifariello R, Fagliarone C, Raguse M, Moeller R and Billi D** (2017) Evaluation of the resistance of *Chroococcidiopsis* Spp. to sparsely and densely ionizing irradiation. *Astrobiology* **17**, 118–125.

**Wheeler RM, Mackowiak CL, Stutte GW, Yorio NC, Ruffe LM, Sager JC, Prince RP and Knott WM** (2008) Crop productivities and radiation use efficiencies for bioregenerative life support. *Advances in Space Research* **41**, 706–713.