Zweitveröffentlichung/
Secondary Publication

https://media.suub.uni-bremen.de

Ramalho Tiago P. ; Chopin Guillaume ; Pérez-Carrascal Olga M. ; Tromas Nicolas ; Verseux Cyprien

Selection of Anabaena sp. PCC 7938 as a Cyanobacterium Model for Biological ISRU on Mars

Journal Article as: peer-reviewed accepted version (Postprint)

DOI of this document*(secondary publication): https://doi.org/10.26092/elib/3172

Publication date of this document: 01/08/2024

* for better findability or for reliable citation

Recommended Citation (primary publication/Version of Record) incl. DOI:

Ramalho TP, Chopin G, Pérez-Carrascal OM, Tromas N, Verseux C.2022.Selection of Anabaena sp. PCC 7938 as a Cyanobacterium Model for Biological ISRU on Mars. Appl Environ Microbiol 88:e00594-22. https://doi.org/10.1128/aem.00594-22

Please note that the version of this document may differ from the final published version (Version of Record/primary publication) in terms of copy-editing, pagination, publication date and DOI. Please cite the version that you actually used. Before citing, you are also advised to check the publisher's website for any subsequent corrections or retractions (see also https://retractionwatch.com/).

This document is made available under a Creative Commons licence.

The license information is available online: https://creativecommons.org/licenses/by/4.0/

Take down policy

If you believe that this document or any material on this site infringes copyright, please contact publizieren@suub.uni-bremen.de with full details and we will remove access to the material.

Selection of *Anabaena* sp. PCC 7938 as a Cyanobacterium Model for Biological ISRU on Mars

Tiago P. Ramalho,[a] Guillaume Chopin,[a] Olga M. Pérez-Carrascal,[b] Nicolas Tromas,[c,d] Cyprien Verseux[a]

[a]Center of Applied Space Technology and Microgravity (ZARM), University of Bremen, Bremen, Germany
[b]Department of Integrative Biology, University of California, Berkeley, California, USA
[c]Département des Sciences Biologiques, Université de Montréal, Montréal, Quebec, Canada
[d]LS2N, Faculté des Sciences et Techniques (FST), Université de Nantes, Nantes, France

Tiago P. Ramalho and Guillaume Chopin contributed equally to this article. Author order was determined by tossing a coin.

ABSTRACT Crewed missions to Mars are expected to take place in the coming decades. After short-term stays, a permanent presence will be desirable to enable a wealth of scientific discoveries. This will require providing crews with life-support consumables in amounts that are too large to be imported from Earth. Part of these consumables could be produced on site with bioprocesses, but the feedstock should not have to be imported. A solution under consideration lies in using diazotrophic, rock-weathering cyanobacteria as primary producers: fed with materials naturally available on site, they would provide the nutrients required by other organisms. This concept has recently gained momentum but progress is slowed by a lack of consistency across contributing teams, and notably of a shared model organism. With the hope to address this issue, we present the work performed to select our current model. We started with preselected strains from the *Nostocaceae* family. After sequencing the genome of *Anabaena* sp. PCC 7938—the only one not yet available—we compared the strains' genomic data to determine their relatedness and provide insights into their physiology. We then assessed and compared relevant features: chiefly, their abilities to utilize nutrients from Martian regolith, their resistance to perchlorates (toxic compounds present in the regolith), and their suitability as feedstock for secondary producers (here a heterotrophic bacterium and a higher plant). This led to the selection of *Anabaena* sp. PCC 7938, which we propose as a model cyanobacterium for the development of bioprocesses based on Mars's natural resources.

IMPORTANCE The sustainability of crewed missions to Mars could be increased by biotechnologies which are connected to resources available on site via primary producers: diazotrophic, rock-leaching cyanobacteria. Indeed, this could greatly reduce the mass of payloads to be imported from Earth. The concept is gaining momentum but progress is hindered by a lack of consistency across research teams. We consequently describe the selection process that led to the choice of our model strain, demonstrate its relevance to the field, and propose it as a shared model organism. We expect this contribution to support the development of cyanobacterium-based biotechnologies on Mars.

KEYWORDS space exploration, biological life-support systems (BLSS), perchlorate resistance, geomicrobiology, *in situ* resource utilization (ISRU)

Crewed missions to Mars are foreseen within the coming decades (1). While astronauts of the first expeditions will likely spend less than 2 years at the surface (2), longer stays are expected to follow, up to permanently inhabited outposts—perhaps

akin to current research stations in Antarctica. This will require the supply of large amounts of consumables, from oxygen to food to drugs, and the costs per mass of a payload sent to Mars will limit the feasibility of importing those consumables from Earth. An alternative would consist in producing them on site, and biological systems could there play a key role—especially after being engineered (3–6). However, biological systems must be fed and the feedstock, as other consumables, would best be sourced from Mars. In addition to decreasing the mass of imported payloads, this would provide the flexibility required to address changing mission demands and help reduce the dependence of outposts on costly and uncertain resupply missions (e.g., 5).

A solution has been proposed which lies in using diazotrophic, rock-weathering cyanobacteria (7–9). Their physiology is such that they could, it seems, be fed with materials available on site: water mined from the ground or atmosphere; carbon and nitrogen sourced from the atmosphere; and the local regolith (the Martian soil), from which it has been argued that they could extract the other necessary nutrients (7, 8, 10, 11). The cultured cyanobacteria could then produce various consumables directly, such as O_2 and dietary supplements (12, 13), but also support the growth of secondary producers (plants or microorganisms [9, 11]). The latter could, in turn, generate a wide range of critical consumables, ranging from food to structural materials to pharmaceuticals to fuels (e.g., 3, 14–16).

The number of research teams contributing to this concept, or closely related concepts, has increased significantly over the past 2 years (11, 16–21). Swift progress can consequently be expected in the near future. We argue, however, that this progress would benefit from a higher consistency across research groups. In particular, a shared model organism would help focus resources, compare results from separate projects, and ensure that studies from different teams can build upon one another. The work presented here is aimed at selecting such a model.

It has been suggested that desert cyanobacterium species were particularly suited to the utilization of local resources (*in situ* resource utilization, ISRU) on Mars (17, 19), as extremophilic properties may be an asset in case of exposure to harsh environmental conditions. While agreeing that such traits might provide an advantage, we put a stronger emphasis on other features, with the assumption that cultivation hardware would provide conditions mild enough for less resistant species. We do not anticipate, for instance, exposure to Mars-ambient UV flux, temperatures, or atmospheric pressure. Accordingly, we focused on the *Nostocaceae* family: some species within are (i) diazotrophic; (ii) able to use regolith as a source of mineral nutrients (8, 10, 11, 22); (iii) capable of fast growth; (iv) edible; and/or (v) amenable to genetic engineering.

Five strains from this family, preselected based on growth rates and robustness, were considered. After comparing their genomic data to determine their relatedness and provide insights into their physiology, we performed a series of assays to select among them a model organism. The main criteria we applied can be divided into two categories: (i) ISRU capabilities (efficiency of growth on regolith and perchlorate resistance); and (ii) suitability of the biomass as feedstock for other organisms, here assessed using models of heterotrophic bacteria (*Escherichia coli* W) and aquatic higher plants (*Lemna* sp.). Tendencies to aggregate were also taken into account because biofilms could interfere with photobioreactor operations.

This work led to the selection of *Anabaena* sp. PCC 7938, for which we provide a genome sequence that will help facilitate future investigations. We are relying on it for ongoing studies and suggest that other teams include it in their efforts, even if only to provide a point of comparison.

RESULTS

Genome-based comparison. When the present study was initiated, genome sequences were available for four of the five preselected strains. After sequencing the remaining genome, that of *Anabaena* sp. PCC 7938 (hereafter, PCC 7938), genome data from the five

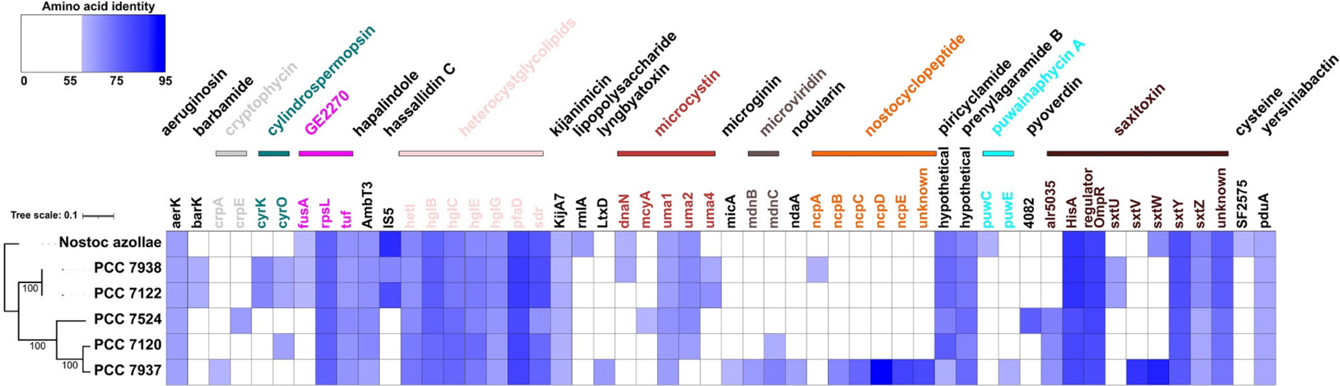

FIG 1 Distribution of biosynthetic gene clusters across PCC 7120, 7122, 7524, 7937, and 7938, and phylogenetic tree of the five strains. The array shows the presence (blue) or absence (white) of genes involved in the production of secondary metabolites, as well as a heatmap denoting amino acid identity with reference sequences (blue gradient). Only genes present in at least one strain are listed (see Data set S1 for a complete list of genes which were screened). The phylogenetic tree uses *Nostoc azollae* (NC_014248.1) as an outgroup. PCC 7122 and 7938 have a phylogenetic distance below 0.00005.

strains (Table S1) were compared to determine their relatedness and provide insights into their physiology.

PCC 7938 and *Anabaena* sp. PCC 7122 (hereafter, PCC 7122) were found to have a high genomic similarity (ANI $>$ 99.9% and phylogenetic distance $<$ 0.00005; Fig. 1; Fig. S1), although 220 flexible genes (i.e., genes found in only one of both) were identified. *Anabaena* sp. PCC 7120 (hereafter, PCC 7120) and *Anabaena* sp. PCC 7937 (hereafter, PCC 7937) had the second most similar pair of genomes, with an ANI value $>$ 93%. *Nostoc* sp. PCC 7524 (hereafter, PCC 7524) had ANI values with the other genomes ranging from 77% to 81%.

PCC 7938 and PCC 7122 appeared to have the same number of complete metabolic pathways and similar functional profiles (Fig. S2), although more pathways involved in nitrate reduction were found in the former (Fig. S3). Pathways related to galactose degradation, nucleotide sugar biosynthesis, and sulfate reduction were found to have a higher degree of completeness in PCC 7122 and PCC 7938 than in the other strains.

Genome-scale metabolic models were constructed to predict potential essential metabolites and complement the functional profiles (primarily to differentiate between PCC 7122 and PCC 7938). The relationships between those models were characterized with a correspondence analysis, based on metabolic potential. As shown in Fig. S4, biosynthetic pathways involved in nitrate reduction, production of L-lactate and salicylic acid, as well as inositol phosphate metabolism, were found in PCC 7122 and PCC 7938 but not in the other genomes.

Fifty-two genes involved in biosynthetic gene clusters (BGCs) were identified, belonging to 23 BGCs (Fig. 1). Only the heterocystglycolipid gene cluster (present in all genomes) is complete. The BGC for nostocyclopeptide (a putative anti-toxin [23]) is nearly complete in PCC 7937, as well as GE2270 (an inhibitor of bacterial protein synthesis [24]) in PCC 7122 and PCC 7938. However, none of the cyanotoxin BCGs is complete and, for most (including microcystin and saxitoxin), essential core genes were found to be missing (25).

Regolith-dependent growth. In order to assess the abilities of the five tested strains to utilize Martian regolith as a source of mineral nutrients, we quantified the biomass obtained after 28 days of cultivation in water containing simulants of that regolith: MGS-1 Mars Global Simulant (MGS-1); MGS-1C Clay ISRU (MGS-1C); and MGS-1S Sulfate ISRU (MGS-1S). Analogues of regolith from the lunar highlands and maria provided additional points of comparison: LHS-1 Lunar Highlands Simulant (LHS-1) and LMS-1 Lunar Mare Simulant (LMS-1), respectively.

Biomass concentrations varied across both regolith types and strains (Fig. 2). For all strains, growth was highest in LMS-1 and lowest in LHS-1. Intermediate values were obtained with MGS-1 and its derivatives, and which among those regolith types most supported growth was strain-dependent. The most productive strain on regolith was

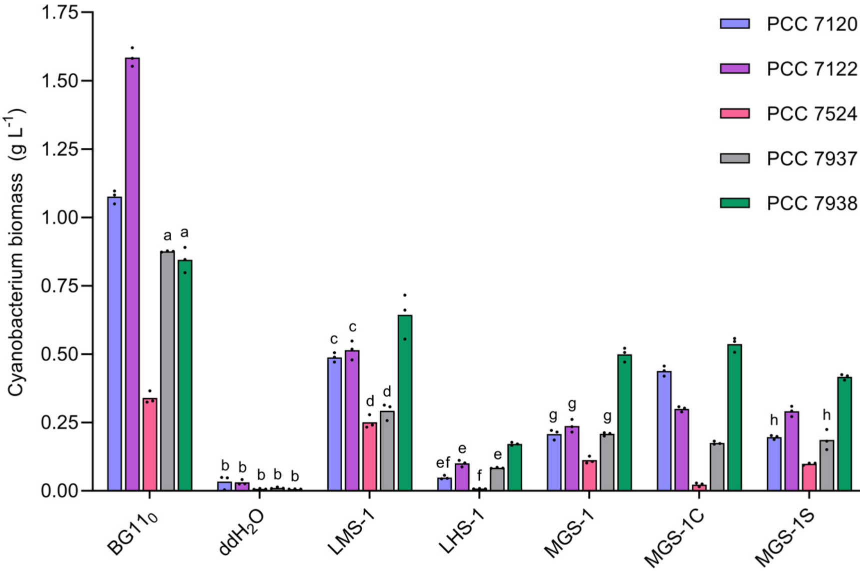

FIG 2 Biomass of PCC 7120, 7122, 7524, 7937, and 7938 after 28 days of cultivation in $BG11_0$, double-distilled and deionized water (ddH_2O), or ddH_2O supplemented with 200 kg m^{-3} of one among five regolith simulants: MGS-1 Mars Global Simulant (MGS-1); MGS-1C Clay ISRU (MGS-1C); MGS-1S Sulfate ISRU (MGS-1S); LHS-1 Lunar Highlands Simulant (LHS-1); or LMS-1 Lunar Mare Simulant (LMS-1). Columns represent mean values of three biological replicates (dots). Means obtained within a given medium and which do not share a letter are significantly different (Tukey test, adjusted $P < 0.05$).

PCC 7938: its final biomass was highest, regardless of the regolith type. In MGS-1, in particular, it yielded more than twice the biomass of any other strain (0.5 ± 0.02 g L^{-1}). Second overall came PCC 7120 and PCC 7122, which yielded the second- and third-highest biomass across all simulants (which among both came second was dependent on regolith type). Their biomass was either matched (LHS-1, MGS-1, and MGS-1S) or closely followed (LMS-1 and MGS-1C) by that of PCC 7937. PCC 7524 produced the least biomass under all conditions.

Perchlorate resistance. Perchlorate resistance was assessed by comparing biomass concentrations after 28 days of cultivation in the presence of perchlorate ions (3, 6, or 12 mM) with control values obtained without perchlorate (Fig. 3). The ranking of strains based on fitness varied with concentration. PCC 7122 reached the highest concentration in proportion to no-perchlorate controls in 3 and 6 mM perchlorate ions (85% ± 6% and 78% ± 11%, respectively), although the difference with PCC 7938 was not significant in 3 mM. However, PCC 7524 displayed a higher resistance in 12 mM, reaching 62% ± 9% of the control's final biomass. This strain showed the lowest variation within the tested range of perchlorate concentrations. PCC 7120 and PCC 7938 had intermediate (and similar) resistance levels. Finally, PCC 7937 exhibited the lowest perchlorate resistance: growth was barely detectable at any of the tested concentrations.

Culture homogeneity. Culture homogeneity—a lack of a tendency to form surface biofilms or aggregates, which may interfere with the foreseen bioprocesses—was assessed qualitatively. Under standard conditions, cultures of PCC 7122 tended to develop free-floating cell aggregates or biofilms attached to the walls of culture vessels; all other strains led to nearly homogeneous cultures, or formed loose aggregates which could be dismantled with gentle shaking. In the presence of regolith simulant, clusters of cells and regolith appeared in all cultures but those of PCC 7924. With PCC 7937, in particular, the degree of aggregation was such that cells and regolith sedimented immediately after inverting tubes, leaving a clear supernatant (as opposed to the turbid suspension that persisted in other samples). The other strains adhered less strongly to the regolith (Fig. S5).

Suitability as a nutrient source for other organisms. The suitability of cyanobacterium strains as a nutrient source for other organisms was evaluated by cultivating a heterotrophic bacterium (*Escherichia coli* W) and an aquatic higher plant (*Lemna* sp.) in a filtered lysate of cyanobacterium biomass (25 g_{DW} L^{-1} before filtration).

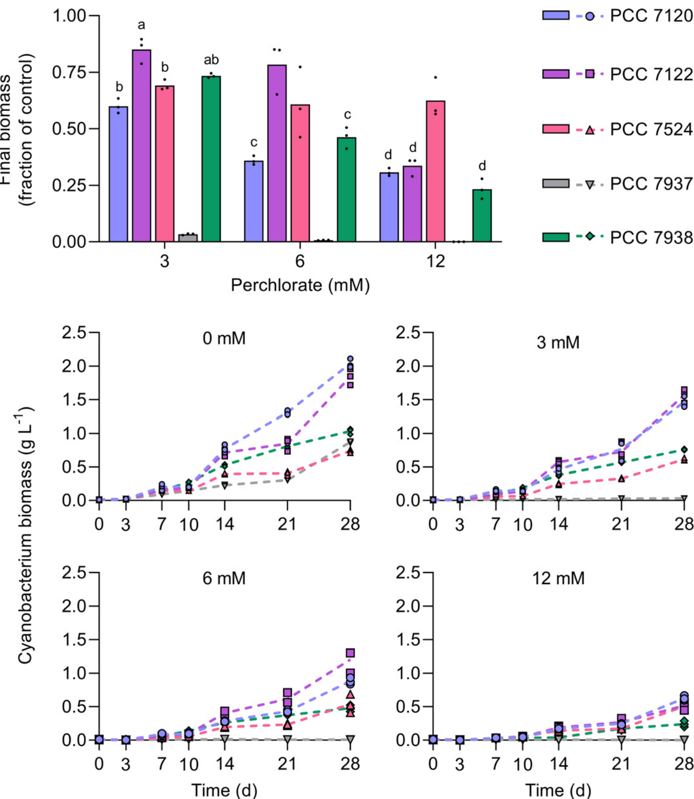

FIG 3 Growth of PCC 7120, 7122, 7524, 7937, and 7938 in the presence of perchlorates. Top: Biomass obtained after 28 days of cultivation in $BG11_0$ spiked with 3, 6, or 12 mM perchlorate ions (parent salt: calcium perchlorate), as a fraction of the biomass obtained in perchlorate-free $BG11_0$. Columns represent mean values of three biological replicates (dots). Means obtained within a given perchlorate concentration and which do not share a letter are significantly different (Tukey test, adjusted $P < 0.05$). Bottom: Biomass at culture onset and after 3, 7, 10, 14, 21, and 28 days of cultivation in $BG11_0$ spiked with 0, 3, 6, or 12 mM perchlorate ions. Symbols represent biological replicates.

Filtered lysates from all strains supported the growth of *E. coli* (Fig. 4). After overnight cultivation, *E. coli* cell concentrations reached levels equivalent to (PCC 7120, 7122, and 7937) or significantly higher than (PCC 7524 and 7938) those in LB medium, where they reached $9.2 \times 10^9 \pm 3.2 \times 10^8$ CFU mL^{-1}. Their range remained narrow across filtered lysates: from $8.1 \times 10^8 \pm 2.9 \times 10^7$ (PCC 7120) to $2.6 \times 10^9 \pm 4.2 \times 10^8$ (PCC 7524) CFU mL^{-1}.

The biomass produced by *Lemna* sp. within 2 weeks of cultivation varied significantly across filtered lysates (Fig. 5). The highest value (1.14 ± 0.15 mg) was obtained with PCC 7938, which was followed by PCC 7122 (0.66 ± 0.19 mg), PCC 7524 (0.44 ± 0.15 mg), and PCC 7937 (0.37 ± 0.02 mg). Fronds placed in the filtered lysate from PCC 7120 did not multiply but became chlorotic. In all cases, biomass yields were significantly lower than in Hoagland solution (1.70 ± 0.22 mg).

DISCUSSION

To be sustainable, settlements on Mars should rely on consumables produced on site rather than imported from Earth. It has been suggested that cyanobacteria could serve this purpose by feeding bioprocesses: after being cultivated using the planet's natural resources, they would produce nutrients for other organisms of biotechnological interest (9). Proofs-of-concept have been obtained and suggest that such a trophic chain could be implemented. However, systematic characterization and modeling are required to determine whether this solution would be cost-effective. Such efforts require a model organism which, ideally, would be used by a majority of the contributing

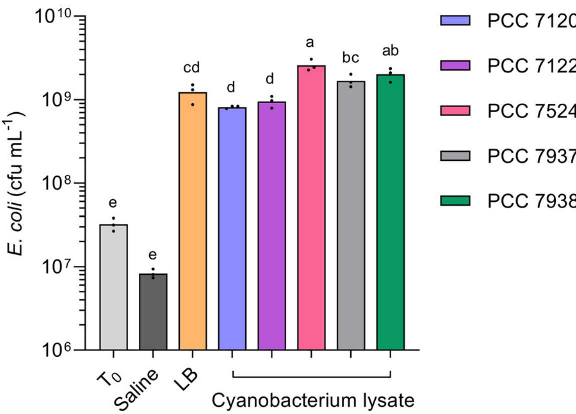

FIG 4 Cell concentrations of *E. coli* W at culture onset (T_0) and after overnight incubation in saline solution (saline), LB medium (LB), or a filtered lysate of PCC 7120, 7122, 7524, 7937, or 7938. Columns represent mean values of three biological replicates (dots). Means that do not share a letter are significantly different (Tukey test, adjusted $P < 0.05$).

research groups. After assessing candidate strains based on features relevant to the foreseen processes, we propose *Anabaena* sp. PCC 7938 as a model cyanobacterium for studies pertaining to the development of biological ISRU on Mars.

Our work started with five strains from the Pasteur Culture Collection of Cyanobacteria: *Anabaena* spp. PCC 7120, 7122, 7937, and 7938, and *Nostoc* sp. PCC 7524 (other designa-

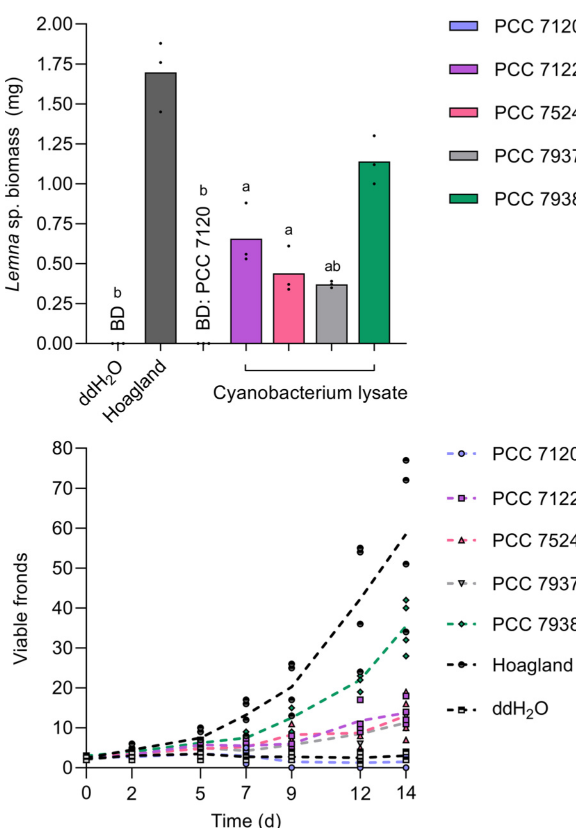

FIG 5 Growth of *Lemna* sp. in filtered lysates of PCC 7120, 7122, 7524, 7937, or 7938, in distilled and deionized water (ddH_2O), or in Hoagland solution (Hoagland). Top: Biomass of *Lemna* sp. after 14 days of cultivation. Columns represent mean values of three biological replicates (dots). Means that do not share a letter are significantly different (Tukey test, adjusted $P < 0.05$). BD, below detection. Bottom: Number of viable fronds at culture onset and after 2, 5, 7, 9, 12, and 14 days of cultivation. Symbols represent biological replicates.

tions can be found in literature: classification within *Nostocaceae* can be tentative and evolves rapidly). Prior to performing experiments, we sequenced the genome of PCC 7938—the others were already available at the time—and compared genomic data of the five strains. One motivation was to determine their relatedness; most striking was the genomic closeness of PCC 7122 and 7938, which is consistent with the overall similarity of the results obtained, for those strains, in the assays described below. Another motivation was a search for toxic secondary metabolites whose associated gene clusters are known. Indeed, toxins may affect the suitability of cyanobacteria as a nutrient source for secondary producers, let alone their edibility (if they are to be used as a dietary supplement). While genes belonging to toxin-associated clusters were identified, essential core genes were missing, and the production of no specific toxin was consequently predicted. This way of assessing toxicity, however, only gives limited insights: toxins may be produced that our approach failed to identify. Experimental validation would be required to conclude on a lack of toxicity.

Candidates were then compared with a series of experiments. The first pertained to the strains' abilities to feed on Martian regolith: these abilities will largely determine the efficiency of cyanobacterium growth on Mars, where the regolith would be the source of all nutrients not provided as atmospheric gases. A comparison was performed by growing each strain with three mineral mixtures analogous to Martian regolith, in high-purity water and under ambient air, with no other source of nutrients. Those mixtures were MGS-1—a simulant based on windblown soil at Rocknest (26)—and its sulfate-rich and smectite-rich derivatives. Simulants of regolith from lunar maria and highlands were included as well, for two main reasons. First, they provided additional points of comparison (helping assess capacities to grow on regolith of different compositions). Second, cyanobacteria are being considered for applications on the Moon: while our satellite lacks a substantial atmosphere, mineral nutrients (as well as water) could be sourced from the regolith and help reduce the dependency on Earth of bioprocesses. Regardless of the simulant, PCC 7938 was the most productive strain. In MGS-1, which is particularly relevant as it represents a type of regolith widespread on Mars, that strain reached biomass concentrations at least twice as high as any other strain.

The Martian regolith will not be a source of nutrients only: it contains toxic compounds, among which perchlorates are presumably the most critical. Those oxychlorine species have been detected and quantified at several locations on Mars (e.g., 27–29). They are likely ubiquitous at the surface, and how their concentration changes with depth is unknown (30). If no remediation is performed, using regolith as a source of nutrients means exposing cyanobacteria to perchlorates; resistance to those compounds is consequently expected to influence the efficiency of ISRU-based cyanobacterium cultivation. To compare the perchlorate resistance of the five preselected strains, the latter were grown in $BG11_0$ spiked with concentrations of calcium perchlorate (a likely parent salt at Rocknest; [31]) leading to 3, 6, and 12 mM perchlorate ions. These concentrations correspond to those that would result from using 50, 100, and 200 kg m^{-3} of regolith containing 0.6 wt% of perchlorate ions (a seemingly typical concentration on Mars). We used the decrease in growth induced by perchlorates as the metric for comparison. Perchlorate resistance varied among strains, although none largely outperformed the others. Under all conditions, four of the five strains reached biomass concentrations above 20% of the no-perchlorate controls—the only exception being PCC 7937.

One major application suggested for cyanobacteria grown using materials available on Mars is their use as a nutrient source for other organisms. These secondary producers could then perform a wide range of functions, including the production of drugs, fuels, biomaterials, and various industrially useful chemicals; metal leaching; and food processing for taste improvement (4, 5). We thus compared the suitability of lysed and filtered biomass from the five preselected cyanobacterium strains as feedstock for two distant organisms: a heterotrophic bacterium and a higher plant.

Owing in large part to their dependence on organics, whose availability on Mars remains unknown but is expected to be very low, heterotrophic organisms most likely

could not be fed directly with Mars's natural resources. The biomass of some cyanobacteria, however, can be used as a nutrient source: heterotrophic bacteria (*E. coli* W, *E. coli* K-12, *Bacillus subtilis* 168, and *B. subtilis* SCK6) were previously grown in a filtrate of ground PCC 7120 (32), and *E. coli* W in a filtrate of ground PCC 7938 (11) or of ground *Chroococcidopsis* sp. CCMEE 029 (17). Here, we use *E. coli* W as a model, owing both to its being a widespread model organism and to its versatility in biotechnologies. After overnight cultivation, *E. coli* W reached concentrations in all cyanobacterium lysates comparable to those reached in LB medium, with little difference among strains.

While plants are autotrophs and could rely on atmospheric (rather than organic) carbon, they are generally expected not to be able to grow in unprocessed Martian regolith, among others because of a low bioavailability of mineral nutrients, low amounts of fixed nitrogen, poor water-holding potential, and excess salts and toxins (33–35). It has been suggested that cyanobacteria could be used as a nutrient source, whether as a supplement in processed regolith or in a solution for soilless cultivation (9), although, to our knowledge, that higher plants can be cultivated using cyanobacterium-extracted compounds as the only nutrient source beside water and atmospheric gases remained to be demonstrated. Here, we use duckweed (*Lemna* sp.), a small floating macrophyte, as a model higher plant. This was primarily motivated by its ease of cultivation in a microbiology laboratory. However, duckweeds are being considered as a food source in space due to their high nutrient density, lack of inedible parts, and high productivity (36–38). Growth of *Lemna* sp. in cyanobacterium lysates varied largely across cyanobacterium strains. PCC 7938 led to the highest biomass and frond numbers (ca. twice that of the second most productive strain); it was followed by PCC 7122, 7524, and 7937. PCC 7120 did not support the growth, or even survival, of *Lemna* sp.; what in that strain may be the cause currently cannot be ascertained.

It should be pointed out that the quantitative results obtained here were only meant for comparing strains: they should not be relied on to assess the productivity of cyanobacterium-based ISRU processes. Cultivation conditions in regolith, for instance, were not optimized; nor were steps taken to increase the resistance of cyanobacteria to perchlorates, or to remove the latter. As another example, the cyanobacterium biomass was simply ground and filtered before being fed to *E. coli* or *Lemna* sp., leaving out the large fraction of the biomass retained by the filters, and no attempt was made to convert nonmetabolizable compounds into useable forms.

The cyanobacterium strains' tendency to form aggregates and surface-bound biofilms was accounted for in the present selection, although it was assessed only qualitatively. Biofilms may be desired for some applications; examples may be the control of Martian dust over enclosed areas (39) or the use of biofilm cultivation chambers which, for some applications, may be more resource-efficient than planktonic photobioreactors. Here, however, we considered aggregation a drawback: in a bioprocess based on the production of planktonic biomass, biofilms can affect fluid dynamics, lead to localized changes in culture parameters, hinder downstream processes, and foul or damage equipment (40). In addition, it may make the separation of biomass and regolith (to collect the former or renew the latter) highly challenging. This criterion decreased the perceived suitability of PCC 7122—which even under routine conditions developed large, free-floating aggregates and formed biofilms on cultivation equipment—and of PCC 7937, which created large lumps of cells and grains when grown on regolith.

After considering the results as a whole (Table 1), we elected to use PCC 7938 as a cyanobacterium model for future studies pertaining to biological ISRU on Mars. First, because—in addition to features common to all preselected strains, such as diazotrophy—it led to the highest biomass productivity when grown on Martian or lunar regolith simulants, and appeared to be most suitable as a substrate for other organisms. Second, because it did not reveal any major flaw pertaining to other criteria, such as a high sensitivity to perchlorates or a strong tendency to aggregate.

PCC 7938 may not be the cyanobacterium which would eventually be used for practical applications: testing and comparing a large number of candidates from various

TABLE 1 Overview of the results from the comparative tests which led to the selection of *Anabaena* sp. PCC 7938 as a model strain

Strain (PCC ID)	7120	7122	7524	7937	7938
Regolith-dependent growth	+	+	–	+/–	++
Perchlorate resistance	+/–	+	+	–	+/–
Suitability as a feedstock for heterotrophs	+	+	+	+	+
Suitability as feedstock for aquatic higher plants	–	+	+	+/–	++
Culture homogeneity (lack of biofilms/aggregates)	+/–	–	+	–	+

genera may lead to the identification of more suitable isolates, and improvements may be brought using bio-engineering. It is, however, the most suitable strain identified so far, and it provides a good basis for assessing and increasing the resource-efficiency of cyanobacterium ISRU on Mars.

MATERIALS AND METHODS

Strains and growth conditions. *Anabaena* spp. PCC 7120, 7122, 7937, and 7938, and *Nostoc* sp. PCC 7524, were obtained from the Pasteur Culture Collection of Cyanobacteria (Paris, France). They were maintained inside a poly klima PK 520-LED photo-incubator at 25°C, in $BG11_0$ medium, with a light intensity of 5 to 10 $\mu mol_{ph}\ m^{-2}\ s^{-1}$ (16 h/8 h day/night cycle). Prior to experiments, cultures to be used for inoculation were cultivated on a rotary shaker at 100 rpm under a light intensity of 15 to 20 $\mu mol_{ph}\ m^{-2}\ s^{-1}$.

Duckweed (*Lemna* sp.) was isolated from a stream in the Bürgerpark (Bremen, Germany). Axenic cultures were obtained by treatment with a sodium hypochlorite solution, as recommended by Sree and Appenroth (41). Routine cultivation was performed in Hoagland medium and otherwise under the same conditions as for the cyanobacteria.

Escherichia coli W (DSM 1116) was acquired from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Prior to experiments, samples from glycerol stocks were streaked on LB-agar plates and incubated overnight at 37°C.

Genome sequencing of *Anabaena* sp. PCC 7938. Genomic DNA of *Anabaena* sp. PCC 7938 was extracted using Macherey-Nagel's NucleoSpin Soil kit and sequenced using an Illumina NovaSeq 6000 S4, with 150-bp paired-end reads. A genomic library (with an average fragment size of 477 bp) was prepared using the NEB Ultra II kit (New England Biolabs). Low-quality reads and adapter sequences were trimmed using fastp (v0.20.1; [42]). Cleaned reads were then assembled with SPAdes (v3.11.1), using --careful parameter to reduce the potential number of mismatches and short indels (43). Reads were mapped back to SPAdes contigs using bowtie2 (v2.4.4; [44]) and contigs were filtered, clustered and binned using Anvi'o (v7.0; [45]). Each bin was taxonomically classified using Centrifuge (v1.04; [46]). Combining information such as coverage, tetranucleotide frequencies, GC content, and taxonomic affiliation, contaminants were screened for and the quality of the genome sequence was assessed. Completeness and contamination were estimated using Anvi'o (97.18% completeness, 8.45% redundancy) and checkM (v1.1.3; [47]; 99.4% completeness, 0% contamination).

Genome-based comparison of the cyanobacterium strains. Gene prediction in, and annotation of, the genomes of PCC 7120, 7122, 7524, 7937, and 7938 (Table S1) were done using Prokka (v1.14.6; [48]). A total of 298 core genes (shared by all strains, with blastp identity $>$ 90%) were identified with Roary (v3.13.3; [49]) and aligned with --mafft Roary parameters. A phylogenic tree was built in RAxML-NG (v. 1.1.0; 50) using the GTRGAMMA model, with 100 bootstraps. The average nucleotide-identity distances of the five genomes were estimated using FastANI (v1.32; [51]).

The five genomes were compared at the gene and functional levels using MicrobeAnnotator (v.2.0.5; [52]; --full mode), which relies on the databases KOfam (53), UniProt's Swissprot and trEMBL (54), and NCBI's RefSeq (55). Genome-scale metabolic models were reconstructed using *gapseq* (v1.2; [56]), with --doall parameters and an autotrophic medium (gapseq media resources) for the gapfill step. gapseq relies on the databases MetaCyc (57), ModelSEED (58) and the Transporter Classification Database (59). The metabolic components were then compared using Metage2Metabo (v1.5.0; [60]), with --iscope parameters and a growth medium (seed) predicted from PCC 7938. A correspondence analysis was performed using the FactoMineR and factoextra R packages to compare models based on their metabolic potential.

In order to determine whether BGCs known to enable the production of toxins were found in any of the five genomes, a database of BGCs found in cyanobacteria and associated bacteria was generated. It gathered 94 BGCs and 460 genes (Data set S1). BCGs were predicted using ANTISMASH (v5.1.2; [61]), and the corresponding reference sequences (of nucleotides and amino acids) were retrieved from the NCBI or ANTISMASH databases. An all-against-all diamond BLASTP analysis (v2.0.8; [62]) was applied to find the best reciprocal hits between elements in the database and the cyanobacterium genome sequences (identity $>$ 60%, and length of the sequences aligned $>$ 30%). A presence-absence matrix of BGC in the five cyanobacterium strains was generated and combined with the phylogenetic tree using iToL (v6; [63]).

Regolith-dependent growth. The five cyanobacterium strains were cultivated in 24-well plates containing 1.5 mL of double-distilled and deionized water (ddH_2O), $BG11_0$, or ddH_2O supplemented with 200 kg m^{-3} of one among the following regolith simulants: MGS-1 (26), MGS-1S, MGS-1C, LHS-1, and

LMS-1 (64). The simulants were purchased from the Center for Lunar and Asteroid Surface Science (Orlando, FL, USA); their bulk chemistry is given in Table S2. The inoculum, prepared by washing a preculture twice in ddH_2O, was delivered to an optical density at 750 nm of 0.02. All conditions were prepared in triplicate. The inoculated plates were sealed with parafilm to reduce evaporation and slits were cut to enhance gas exchange. Plates were incubated for 28 days at 25°C, with a light intensity of 15 to 20 μmol_{ph} m^{-2} s^{-1} (16 h/8 h day/night cycle) and, to avoid shading from suspended regolith grains, without shaking. Chlorophyll a was extracted with ethanol from the whole of each sample and quantified based on optical density at 665 nm (65). The final biomass in each sample was inferred from chlorophyll a concentrations, using a conversion factor determined experimentally (Table S3). The significance of the differences in biomass concentration between cyanobacterium strains was assessed using a two-way ANOVA followed by Tukey's multiple-comparison test (adjusted p, 0.05).

Perchlorate resistance. Strains were grown in $BG11_0$ without perchlorates or spiked with calcium perchlorate (Sigma-Aldrich, Merck) at concentrations leading to 3, 6, and 12 mM perchlorate ions. The biomass obtained under each condition was determined after 0, 3, 7, 10, 14, 21, and 28 days. Cultivation was prepared and performed, and biomass quantified and compared, as described above (section "Regolith-dependent growth").

Culture homogeneity. Tendencies to form surface biofilms and aggregates were assessed qualitatively. Under routine conditions, this was performed by mere visual inspection. When regolith was used as a source of nutrients, this was performed as follows. Cells were grown for 28 days in ddH_2O supplemented with 200 kg m^{-3} of MGS-1. Samples were transferred to 1.5 mL Eppendorf tubes and left to sediment. Tubes were then inverted 5 times and left standing for 30 s, after which the abundance of sedimentary materials was assessed visually. Samples were further examined for the presence of cyanobacteria-regolith aggregates under an inverted microscope (Bresser Science IVM 401); images were then acquired using a mounted camera (Bresser MikroCam 5.0) and the associated software (MicroCamLabII). Additionally, fluorescence images were obtained from samples (fixed with 2% glutaraldehyde) of 14-day-old cultures grown under the same conditions. This was performed using a Zeiss Axioscope 5/7 microscope (Zeiss, Germany; equipped with a Solid-State Light source Colibri 3, type RGB-UV, an Axiocam 702 mono camera, and a 90 HE LED filter set) with the 470 and 625 nm light channels. Z-stacks were captured with a motorized stage, deconvoluted, and collapsed into a Z projection, using the manufacturer's Zen software (version 3.0).

Suitability as a nutrient source for other organisms. Filtered lysates of each strain were prepared from 25 g of dry biomass per L, as previously reported (11). An estimate of carbohydrate and protein concentrations in the lysates is given in Table S4.

E. coli W was cultivated overnight in the cyanobacterium filtered lysates, and final cell densities were assessed, as previously described (11). Differences in cell concentrations across filtered lysates and across controls were assessed with a one-way ANOVA followed by Tukey's multiple-comparison test (adjusted *p*, 0.05).

Assays with *Lemna* sp. were conducted as follows. Fronds from a culture in exponential phase were washed by dipping them successively in three vessels containing sterile ddH_2O. They were then transferred to 24-well plates (one colony of two to three fronds per well) containing 1.5 mL of distilled water, Hoagland medium, or one among the cyanobacterium filtered lysates. Each condition was prepared in triplicate. Plates were then incubated in conditions similar to the routine ones but with a light intensity of 15 to 20 μmol_{ph} m^{-2} s^{-1}. The number of fronds was determined every 2 to 3 days (each new frond was counted from the moment when it was unambiguously visible with a 10x magnifying glass). After 14 days of cultivation, fronds were rinsed with ddH_2O, dried at 60°C, and weighed. The dry weight between conditions was compared with a one-way ANOVA followed by Tukey's multiple-comparison test (adjusted p, 0.05).

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 9.2.0 for Windows, by GraphPad Software (San Diego, California).

Data availability. PCC 7938 contigs are available in GenBank under Bioproject number PRJNA812045.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 2 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.

ACKNOWLEDGMENTS

We thank Muriel Gugger (Pasteur Institute, Paris) for her insights when preselecting cyanobacterium strains; Klaus Juergen Appenroth (Jena University, Germany) for instructions on the obtention of axenic duckweed cultures; Clémence Frioux (INRIA Bordeaux) for her advice on GEM reconstruction; Sven Kerzenmacher (UFT, Bremen University) for access to fluorescence microscope facilities; and Guillaume Pillot (UFT, Bremen University) for support in operating these facilities.

This research work was supported by a Humboldt Research Fellowship for postdoctoral researchers to C.V. The project it belongs to is part of the "Humans on Mars Initiative" funded by the Federal State of Bremen and the University of Bremen.

REFERENCES

1. ISECG. 2018. The global exploration roadmap, 3rd edition. NP-2018–01-2502-HQ. NASA Headquarters, Washington, DC.
2. Drake BG. 2009. Human exploration of Mars: design reference architecture 5.0. NASA/SP–2009–566. NASA Headquarters, Washington, DC.
3. Berliner AJ, Hilzinger JM, Abel AJ, McNulty MJ, Makrygiorgos G, Averesch NJHH, Sen Gupta S, Benvenuti A, Caddell DF, Cestellos-Blanco S, Doloman A, Friedline S, Ho D, Gu W, Hill A, Kusuma P, Lipsky I, Mirkovic M, Luis Meraz J, Pane V, Sander KB, Shi F, Skerker JM, Styer A, Valgardson K, Wetmore K, Woo S-GG, Xiong Y, Yates K, Zhang C, Zhen S, Bugbee B, Clark DS, Coleman-Derr D, Mesbah A, Nandi S, Waymouth RM, Yang P, Criddle CS, McDonald KA, Seefeldt LC, Menezes AA, Arkin AP. 2021. Towards a biomanufactory on Mars. Front Astron Sp Sci 8.
4. Hendrickx L, Mergeay M. 2007. From the deep sea to the stars: human life support through minimal communities. Curr Opin Microbiol 10:231–237. https://doi.org/10.1016/j.mib.2007.05.007.
5. Nangle SN, Wolfson MY, Hartsough L, Ma NJ, Mason CE, Merighi M, Nathan V, Silver PA, Simon M, Swett J, Thompson DB, Ziesack M. 2020. The case for biotech on Mars. Nat Biotechnol 38:401–407. https://doi.org/10.1038/s41587-020-0485-4.
6. Poulet L, Engeling K, Hatch T, Stahl-Rommel S, Velez-Justiniano Y-A, Castro-Wallace S, Bunchek J, Monje O, Hummerick M, Khodadad CLM. 2022. Large-scale crop production for the Moon and Mars: current gaps and future perspectives. Front Astron Sp Sci 8:733944. https://doi.org/10.3389/fspas.2021.733944.
7. Brown I, Sarkisova S, Garrison D, Thomas-Keprta K, Allen C, Jones J, Galindo C, McKay D. 2008. Bio-weathering of lunar and Martian rocks by cyanobacteria: a resource for Moon and Mars exploration. Lunar and Planetary Sciences.
8. Olsson-Francis K, Cockell CS. 2010. Use of cyanobacteria for in-situ resource use in space applications. Planet Space Sci 58:1279–1285. https://doi.org/10.1016/j.pss.2010.05.005.
9. Verseux C, Baqué M, Lehto K, De Vera JPP, Rothschild LJ, Billi D. 2016. Sustainable life support on Mars - the potential roles of cyanobacteria. Int J Astrobiol 15:65–92. https://doi.org/10.1017/S147355041500021X.
10. Arai M, Tomita-Yokotani K, Sato S, Hashimoto H, Ohmori M, Yamashita M. 2008. Growth of terrestrial cyanobacterium, *Nostoc sp.*, on Martian regolith simulant and its vacuum tolerance. Biol Sci Space 22:8–17. https://doi.org/10.2187/bss.22.8.
11. Verseux C, Heinicke C, Ramalho TP, Determann J, Duckhorn M, Smagin M, Avila M. 2021. A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars. Front Microbiol 8:733944.
12. Gòdia F, Albiol J, Montesinos JL, Pérez J, Creus N, Cabello F, Mengual X, Montras A, Lasseur C. 2002. MELISSA: a loop of interconnected bioreactors to develop life support in space. J Biotechnol 99:319–330. https://doi.org/10.1016/S0168-1656(02)00222-5.
13. Poughon L, Laroche C, Creuly C, Dussap C-G, Paille C, Lasseur C, Monsieurs P, Heylen W, Coninx I, Mastroleo F, Leys N. 2020. *Limnospira indica* PCC8005 growth in photobioreactor: model and simulation of the ISS and ground experiments. Life Sci Space Res (Amst) 25:53–65. https://doi.org/10.1016/j.lssr.2020.03.002.
14. McNulty MJ, Xiong Y, Yates K, Karuppanan K, Hilzinger JM, Berliner AJ, Delzio J, Arkin AP, Lane NE, Nandi S, McDonald KA. 2021. Molecular pharming to support human life on the moon, Mars, and beyond. Crit Rev Biotechnol 41:849–864. https://doi.org/10.1080/07388551.2021.1888070.
15. Rothschild LJ. 2016. Synthetic biology meets bioprinting: enabling technologies for humans on Mars (and Earth). Biochem Soc Trans 44:1158–1164. https://doi.org/10.1042/BST20160067.
16. Kruyer NS, Realff MJ, Sun W, Genzale CL, Peralta-Yahya P. 2021. Designing the bioproduction of Martian rocket propellant via a biotechnology-enabled in situ resource utilization strategy. Nat Commun 12:6166. https://doi.org/10.1038/s41467-021-26393-7.
17. Billi D, Fernandez BG, Fagliarone C, Chiavarini S, Rothschild LJ. 2021. Exploiting a perchlorate-tolerant desert cyanobacterium to support bacterial growth for in situ resource utilization on Mars. Int J Astrobiol 20:29–35. https://doi.org/10.1017/S1473550420000300.
18. Cycil LM, Hausrath EM, Ming DW, Adcock CT, Raymond J, Remias D, Ruemmele WP. 2021. Investigating the growth of algae under low atmospheric pressures for potential food and oxygen production on Mars. Front Microbiol 12:733244. https://doi.org/10.3389/fmicb.2021.733244.
19. Rzymski P, Poniedziałek B, Hippmann N, Kaczmarek Ł. 2022. Screening the survival of cyanobacteria under perchlorate stress. Potential implications for Mars in situ resource utilization. Astrobiology 22:672–684. https://doi.org/10.1089/ast.2021.0100.
20. Tomita-Yokotani K, Kimura S, Ong M, Ajioka R, Igarashi Y, Fujishiro H, Katoh H, Hashimoto H, Mita H, Yokobori S. 2020. Tolerance of dried cells of a terrestrial cyanobacterium, *Nostoc* sp. HK-01 to temperature cycles, helium-ion beams, ultraviolet radiation (172 and 254 nm), and gamma rays: primitive analysis for space experiments. Eco-Engineering 32:47–53.
21. Mapstone LJ, Leite MN, Purton S, Crawford IA, Dartnell L. 2022. Cyanobacteria and microalgae in supporting human habitation on Mars. Biotechnol Adv 59:107946. https://doi.org/10.1016/j.biotechadv.2022.107946.
22. Olsson-Francis K, Simpson AE, Wolff-Boenisch D, Cockell CS. 2012. The effect of rock composition on cyanobacterial weathering of crystalline basalt and rhyolite. Geobiology 10:434–444. https://doi.org/10.1111/j.1472-4669.2012.00333.x.
23. Jokela J, Herfindal L, Wahlsten M, Permi P, Selheim F, Vasconçelos V, Døskeland SO, Sivonen K. 2010. A novel cyanobacterial nostocyclopeptide is a potent antitoxin against microcystins. Chembiochem 11:1594–1599. https://doi.org/10.1002/cbic.201000179.
24. Selva E, Beretta G, Montanini N, Saddler GS, Gastaldo L, Ferrari P, Lorenzetti R, Landini P, Ripamonti F, Goldstein BP, Berti M, Montanaro L, Denaro M, Montanaro L. 1991. Antibiotic GE2270 a: a novel inhibitor of bacterial protein synthesis. I. Isolation and characterization. J Antibiot 44:693–701. https://doi.org/10.7164/antibiotics.44.693.
25. Dittmann E, Fewer DP, Neilan BA. 2013. Cyanobacterial toxins: biosynthetic routes and evolutionary roots. FEMS Microbiol Rev 37:23–43. https://doi.org/10.1111/j.1574-6976.2012.12000.x.
26. Cannon KM, Britt DT, Smith TM, Fritsche RF, Batcheldor D. 2019. Mars global simulant MGS-1: a Rocknest-based open standard for basaltic Martian regolith simulants. Icarus 317:470–478. https://doi.org/10.1016/j.icarus.2018.08.019.
27. 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, Smith PH. 2009. Detection of perchlorate and the soluble chemistry of Martian soil at the phoenix lander site. Science 325:64–67. https://doi.org/10.1126/science.1172466.
28. Kounaves SP, Chaniotakis NA, Chevrier VF, Carrier BL, Folds KE, Hansen VM, McElhoney KM, O'Neil GD, Weber AW. 2014. Identification of the perchlorate parent salts at the Phoenix Mars landing site and possible implications. Icarus 232:226–231. https://doi.org/10.1016/j.icarus.2014.01.016.
29. Sutter B, McAdam AC, Mahaffy PR, Ming DW, Edgett KS, Rampe EB, Eigenbrode JL, Franz HB, Freissinet C, Grotzinger JP, Steele A, House CH, Archer PD, Malespin CA, Navarro-González R, Stern JC, Bell JF, Calef FJ, Gellert R, Glavin DP, Thompson LM, Yen AS. 2017. Evolved gas analyses of sedimentary rocks and eolian sediment in Gale Crater, Mars: results of the Curiosity rover's sample analysis at Mars instrument from Yellowknife Bay to the Namib Dune. J Geophys Res Planets 122:2574–2609. https://doi.org/10.1002/2016JE005225.
30. Carrier BL. 2017. Next steps forward in understanding Martian surface and subsurface chemistry. J Geophys Res Planets 122:1951–1953. https://doi.org/10.1002/2017JE005409.
31. Millan M, Szopa C, Buch A, Summons R, Navarro-Gonzalez R, Mahaffy P, Johnson SS. 2020. Influence of calcium perchlorate on organics under SAM-like pyrolysis conditions: constraints on the nature of Martian organics. J Geophys Res Planets 125:e2019JE006359.
32. Verseux C. 2018. Resistance of cyanobacteria to space and Mars environments, in the frame of the EXPOSE-R2 space mission and beyond. PhD thesis, University of Rome "Tor Vergata".
33. Eichler A, Hadland N, Pickett D, Masaitis D, Handy D, Perez A, Batcheldor D, Wheeler B, Palmer A. 2021. Challenging the agricultural viability of Martian regolith simulants. Icarus 354:114022. https://doi.org/10.1016/j.icarus.2020.114022.
34. Maggi F, Pallud C. 2010. Space agriculture in micro- and hypo-gravity: a comparative study of soil hydraulics and biogeochemistry in a cropping unit on Earth, Mars, the moon and the space station. Planet Space Sci 58:1996–2007. https://doi.org/10.1016/j.pss.2010.09.025.
35. Oze C, Beisel J, Dabsys E, Dall J, North G, Scott A, Lopez AM, Holmes R, Fendorf S. 2021. Perchlorate and agriculture on Mars. Soil Syst 5:37. https://doi.org/10.3390/soilsystems5030037.
36. Escobar CM, Escobar AC, Power GJ, Nabity JA. 2020. μG-LilyPondTM: preliminary design of a floating plant pond for microgravity. Proceedings of the 50th International Conference on Environmental Systems. ICES-2020-246. ICES, Emmaus, PA.

37. Gale J, Smernoff DT, Macler BA, MacElroy RD. 1989. Carbon balance and productivity of *Lemna gibba*, a candidate plant for CELSS. Adv Sp Res 9: 43–52. https://doi.org/10.1016/0273-1177(89)90027-6.
38. Romano LE, Aronne G. 2021. The world smallest plants (*Wolffia* sp.) as potential species for bioregenerative life support systems in space. Plants 10:1896. https://doi.org/10.3390/plants10091896.
39. Liu Y, Cockell CS, Wang G, Hu C, Chen L, De Philippis R. 2008. Control of lunar and Martian dust — experimental insights from artificial and natural cyanobacterial and algal crusts in the desert of inner Mongolia, China. Astrobiology 8:75–86. https://doi.org/10.1089/ast.2007.0122.
40. Dzianach PA, Dykes GA, Strachan NJC, Forbes KJ, Pérez-Reche FJ. 2019. Challenges of biofilm control and utilization: lessons from mathematical modelling. J R Soc Interface 16. https://doi.org/10.1098/rsif.2019.0042.
41. Sree SK, Appenroth K-J. 2017. Cultivation, sterilization and stock cultivation of duckweeds; an update. In International Steering Committee on Duckweed Research and Applications (eds.), Duckweed Forum 5:32–54. http://www.ruduckweed.org/uploads/1/0/8/9/10896289/iscdra-duckweedforum_issue17-2017-04.pdf.
42. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560.
43. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
44. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.
45. Eren AM, Esen OC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO. 2015. Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ 3:e1319. https://doi.org/10.7717/peerj.1319.
46. Kim D, Song L, Breitwieser FP, Salzberg SL. 2016. Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res 26:1721–1729. https://doi.org/10.1101/gr.210641.116.
47. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114.
48. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
49. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, Fookes M, Falush D, Keane JA, Parkhill J. 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31:3691–3693. https://doi.org/10.1093/bioinformatics/btv421.
50. Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35:4453–4455. https://doi.org/10.1093/bioinformatics/btz305.
51. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9:1–8. https://doi.org/10.1038/s41467-018-07641-9.
52. Ruiz-Perez CA, Conrad RE, Konstantinidis KT. 2021. MicrobeAnnotator: a user-friendly, comprehensive functional annotation pipeline for microbial genomes. BMC Bioinformatics 22:11–16. https://doi.org/10.1186/s12859-020-03940-5.
53. Aramaki T, Blanc-Mathieu R, Endo H, Ohkubo K, Kanehisa M, Goto S, Ogata H. 2020. KofamKOALA: KEGG Ortholog assignment based on profile HMM and adaptive score threshold. Bioinformatics 36:2251–2252. https://doi.org/10.1093/bioinformatics/btz859.
54. Bateman A. 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47:D506–D515.
55. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao Y, Blinkova O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McGarvey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, et al. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745. https://doi.org/10.1093/nar/gkv1189.
56. Zimmermann J, Kaleta C, Waschina S. 2021. gapseq: informed prediction of bacterial metabolic pathways and reconstruction of accurate metabolic models. Genome Biol 22:1–35. https://doi.org/10.1186/s13059-021-02295-1.
57. Caspi R, Billington R, Fulcher CA, Keseler IM, Kothari A, Krummenacker M, Latendresse M, Midford PE, Ong Q, Ong WK, Paley S, Subhraveti P, Karp PD. 2018. The MetaCyc database of metabolic pathways and enzymes. Nucleic Acids Res 46:D633–D639. https://doi.org/10.1093/nar/gkx935.
58. Henry CS, Dejongh M, Best AA, Frybarger PM, Linsay B, Stevens RL. 2010. High-throughput generation, optimization and analysis of genome-scale metabolic models. Nat Biotechnol 28:977–982. https://doi.org/10.1038/nbt.1672.
59. Saier MH, Reddy VS, Tamang DG, Västermark Å. 2014. The transporter classification database. Nucleic Acids Res 42:D251–D258. https://doi.org/10.1093/nar/gkt1097.
60. Belcour A, Frioux C, Aite M, Bretaudeau A, Hildebrand F, Siegel A. 2020. Metage2metabo, microbiota-scale metabolic complementarity for the identification of key species. eLife 9:1–38. https://doi.org/10.7554/eLife.61968.
61. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87. https://doi.org/10.1093/nar/gkz310.
62. Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60. https://doi.org/10.1038/nmeth.3176.
63. Letunic I, Bork P. 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 49:W293–W296. https://doi.org/10.1093/nar/gkab301.
64. Isachenkov M, Chugunov S, Landsman Z, Akhatov I, Metke A, Tikhonov A, Shishkovsky I. 2022. Characterization of novel lunar highland and mare simulants for ISRU research applications. Icarus 376:114873. https://doi.org/10.1016/j.icarus.2021.114873.
65. Ritchie RJ. 2008. Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. Photosynt 46: 115–126. https://doi.org/10.1007/s11099-008-0019-7.