Algal Research 71 (2023) 103044

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Feasibility as feedstock of the cyanobacterium *Chroococcidiopsis* sp. 029 cultivated with urine-supplemented moon and mars regolith simulants

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ARTICLE INFO

Keywords:
Life support
Feedstock
Cyanobacteria
Regoliths
ISRU
Urea

ABSTRACT

Biology-driven in situ resource utilization (bio-ISRU) should provide a breakthrough in supporting long-term human permanence on the Moon and Mars that will rely on the ability to produce consumables on site. Lithotrophic cyanobacteria are gaining interest for linking ISRU to life-support systems: In the “PowerCell” concept, cyanobacteria are grown with raw materials available on the Moon and Mars and used to feed bacteria producing consumables. Here we showed that lunar and martian regolith simulants as well as water-released minerals (supplemented with a nitrogen source) can support planktonic and biofilm growth of the desert cyanobacterium *Chroococcidiopsis* sp. 029. Such a growth versality is relevant to meet different technological requirements of future cultivation hardware to be used on the Moon (and eventually on Mars). We showed that this cyanobacterium can use urea as a nitrogen source and be cultivated with lunar and martian regolith simulants supplemented with synthetic human urine. Notably, the biomass yielded from each experimental set up could be used as feedstock for bacteria. Results provided a proof-of-concept of the feasibility of *Chroococcidiopsis* grown with raw materials available on site on the Moon and Mars and human waste, to “power” bio-production by feeding bacteria useful for bioprocesses to support human settlements beyond Earth.

1. Introduction

Biological Life support systems (BLSS) are key enabling technologies for human space exploration since future human outposts on the Moon and Mars cannot rely on re-supplies from Earth to be affordable [1]. Biology-driven use of local resources based on microorganisms, a concept known as bio-ISRU, will offer a breakthrough in supporting long-term human presence on the Moon and Mars [2,3]. A steppingstone for human mission to Mars will be provided by the NASA's Artemis program that is planning to maintain a long-term human presence on the Moon by 2028 [4]. Indeed bio-ISRU under lunar gravity and radiation environment will expand our knowledge on how these factors will affect BLSS on Mars.

When developing bio-ISRU strategies no single organism/metabolism will serve all purposes, therefore several complementary systems will be required to advance toward technological readiness [5]. Almost 10 years ago the relevance of cyanobacteria for bio-ISRU was highlighted by showing their growth with Mars and Moon regolith simulants [6]. Recently, the concept of “living off the land” has gained a momentum: The general strategy being to link BLSS to ISRU by using cyanobacteria grown on extraterrestrial regoliths, as feedstock for bacteria, or as biofertilizer for plants, both employed in BLSS [7,8]. In such an endeavor, a screening of five *Anabaena* isolates was performed in order to identify the best candidate for growth on Mars regolith simulant and lysate suitability to cultivate the heterotrophic bacterium *Escherichia coli* and the macrophyte *Lemna minor* [9]. While the testing of *Anabaena cylindrica, Arthrospira platensis* and *Nostoc muscorum*, identified the former cyanobacterium as the one with enhanced growth in aqueous extracts of a martian regolith simulant and lysate suitability as biofertilizer of *Lemna minor* [10].

Importantly, cyanobacteria-based technologies for BLSS in space, where constraints on resources are pushed to an extreme, could pave the way toward more sustainable solutions on Earth. Cyanobacteria-based technologies might find applications in resource-poor areas, like

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https://doi.org/10.1016/j.algal.2023.103044
Received 20 December 2022; Received in revised form 15 February 2023; Accepted 3 March 2023
Available online 8 March 2023
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deserts, where mineral resources and solar energy could be transformed into oxygen and food. In addition, cyanobacteria-based technologies might contribute to industrial processes with a reduced use of natural resources and pollutant emission [8]. Similarly, the use of cyanobacteria as feedstock for BLSS in space, could be adapted to Earth applications. For example, microalgae capable of growing under anoxic conditions and releasing O_2, were proposed for mitigating the greenhouse effect on Earth and atmosphere recycling in closed human settlements on other planets [11].

Cyanobacteria which thrive in extreme environments such as deserts, hold promises for space and Earth applications [12]. Nevertheless, their potential to "power" bio-production by feeding bacteria used to produce consumables for human settlements on the Moon and Mars, a bio-ISRU concept known as "PowerCell", remains to be unlocked [7,13].

The desert cyanobacterium *Chroococcidiopsis* sp. 029 possesses key features for bio-ISRU: i) it can grow on lunar and martian regolith simulants if supplemented with nitrate [6]; ii) its biomass can be used as feedstock for bacteria [14]; and iii) it tolerates up to 100 mM sodium perchlorate [14]. Indeed perchlorate is a powerful oxidant agent present in the martian soil at a concentration ranging from 0.6 to 0.12 wt% [15,16]. Hence, when using 0.2 g/ml of martian soil to culture cyanobacteria, according to the sampling site, a perchlorate ion concentration ranging from 12 and 2.4 mM, will be present.

In addition, *Chroococcidiopsis* sp. 029 possesses features critical to move microbial-based technologies from Earth to space [17]: i) it is extremely desiccation and radiation tolerant [18]; ii) it efficiently repairs space-induced DNA damage [19], and iii) dried biofilms are more tolerant against Mars-like conditions than planktonic counterpart [20].

The aim of the present work was to further reduce the knowledge gap in using *Chroococcidiopsis* sp. 029 as "PowerCell" in bio-ISRU technologies on the Moon and Mars. First, since only its planktonic growth with lunar and martian regolith simulants was tested to date [6], its growth capability as biofilm on regolith simulants was evaluated. In addition, the effect of perchlorate enrichment of the martian simulant was investigated as well. Then, the possibility of using water-released minerals for its cultivation in both planktonic and biofilm lifestyle was evaluated. The suitability as feedstock of the lysate obtained from each experimental set up was tested. Furthermore, in order to overcome the limitation imposed by the inability of this cyanobacterium to fix nitrogen, we verified its capability to use urea in the form of synthetic human urine – a nitrogen-containing compound that will be readily available on human missions. Finally, the suitability of the cyanobacterial lysate obtained from each experimental set up was tested as feedstock for *E. coli* W, a bacterium used in industrial biotechnology [21]. Results provided a proof-of-concept of a bio-ISUR production system going from raw materials available on the Moon and Mars and human waste through to the growth of a heterotrophic "production cell".

2. Materials and methods

2.1. Microorganisms and culture conditions

Chroococcidiopsis sp. CCMEE 029 (hereafter *Chroococcidiopsis*) 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, and currently maintained at the Department of Biology, University of Rome Tor Vergata. Routinely, *Chroococcidiopsis* sp. CCMEE 029 was grown in BG11 medium [22] at 25 °C, without shaking, under a photon flux density of 40 μmol/m^2/s provided by a cool-white fluorescent lamp under continuous illumination. The culturing this strain using Mars and Moon regoliths was investigated by cultivation in both the planktonic and biofilm lifestyle, using double-distilled water (ddH_2O) containing 0.2 g/ml of regolith analogs or using water-released minerals, both supplemented with $NaNO_3$ (see Section 2.2). The capability of using synthetic urine as a nitrogen source was tested by determining the optimal urea concentration to be added to $BG11_0$ medium (BG11 lacking $NaNO_3$) and then using a diluted synthetic urine as growth medium (see Section 2.3).

Escherichia coli W (ATCC 9637). A fast-growing strain that utilizes sucrose as a carbon source [21], was purchased from the American Type Culture Collection (Manassas, VA, USA) and grown in Luria-Bertani (LB) broth or in M9+ minimal medium supplemented with 0.5 % glucose [23] at 37 °C with orbital shaking.

2.2. Media based on lunar and martian regolith simulants and water-released minerals

The Lunar Regolith Simulant (LRA) was provided by the Museum für Naturkunde, MfN, Berlin, Germany and the Martian regolith simulant (JSC Mars-1A) was provided by Orbital Technologies Corporation (Madison, WI, USA). Their composition is reported in Table 1. Before use the regolith simulants were autoclaved and two different media were prepared with ddH_2O as follows: i) media based on regolith simulants were prepared by adding 0.2 g/ml of LRA or JSC Mars-1A to sterile ddH_2O for planktonic cultures, or to sterile ddH_2O containing 1.5 % agar for biofilm growth; ii) media based on water-released minerals were prepared by adding 0.2 g/ml of LRA or JSC Mars-1A to sterile ddH_2O, shaking at 6 r.p.m. overnight at room temperature, and collecting the supernatant after centrifugation at 100 *g* for 10 min. The collected supernatant was used for planktonic and biofilm cultures. Each medium was supplemented with 17 mM $NaNO_3$ or with synthetic human urine (see Section 2.3). When needed JSC Mars-1A was supplemented with 2.4 mM perchlorate ions (JSC Mars-1A + ClO_4^-), provided as a 40 % Mg-perchlorate and 60 % Ca-perchlorate salt mixture as previously reported [14].

2.3. Cyanobacterial growth media with urea and synthetic human urine

Urea-based medium was prepared by adding 1 or 10 mM urea, providing 28.0 and 280.75 mg N/l, respectively, to sterile $BG11_0$ medium. Synthetic human urine (SHU) medium was prepared as reported [24], modified by adding K_2HPO_4 at a final 0.04 g/l concentration as present in BG11 medium. A SHU-derived medium containing 10 mM urea was obtained after a 25-fold dilution. Negative and positive controls were obtained using $BG11_0$ (lacking $NaNO_3$) and BG11 medium (containing 248 mg N/l nitrate).

2.4. Cyanobacterial planktonic and biofilm culture conditions

For planktonic growth, aliquots of about 1×10^8 *Chroococcidiopsis* cells were collected from exponential cultures, washed twice with

Table 1
Lunar and martian analogue mineral mixtures.

Lunar regolith simulant components (de Vera et al., 2019)	(% wt)	JSC-Mars-1A components (Allen et al., 1998)	(wt%)
Dunite—Olivine Fo96 (A° heim, Norway)	5.7	Silicon dioxide	34.5–44
CPx—Diopside (Kragerö, Norway)	8.9	Titanium dioxide	3–3.8
OPx—Hyperstehn (Egersund, Norway)	5.7	Aluminum oxide	18.5–23.4
Anorthosite—Plagioclase (Larvik, Norway)	66.8	Ferric oxide	9–12
Apatite (Minas Gerais, Brasil)	1.1	Iron oxide	2.5–3.5
Illmenite (Flekkefiord, Norway)	1.1	Magnesium oxide	2.5–3.5
Iron (Fe)	1.3	Calcium oxide	5–6
Volcanic slag (Aeolian islands, Italy)	9.4	Sodium oxide	2–2.5
		Potassium oxide	0.5–0.6
		Manganese oxide	0.2–0.3
		Diphosphorus pentoxide	0.7–0.9

ddH_2O, and used to inoculate 25-ml culture flasks containing 10 ml of medium based on lunar and martian regolith simulants, or 10 ml of water-released minerals, supplemented with 17 mM $NaNO_3$ as in BG11 medium. For biofilm growth, aliquots of about 1×10^7 *Chroococcidiopsis* cells were washed twice with ddH_2O and plated on the top of 3-cm diameter Petri disks containing 1.5 % agarized medium based on lunar and martian regolith simulants, or water-released minerals, supplemented with 17 mM $NaNO_3$. Negative and positive controls were ddH_20 containing 17 mM $NaNO_3$ and BG11 medium, respectively.

2.5. Measurement of cyanobacterial biomass

In order to avoid the interference of regolith with dry weight and optical density measurements, the growth rate was evaluated by determining chlorophyll *a* (Chl *a*) - to-dry biomass ratio. A standard curve was prepared by measuring Chl *a* content in samples ranging from 0.1 to 10 mg dry weight (Supplementary Fig. S1) as previously reported [25]. The growth rate of cells used to inoculate urea-based media, was evaluated by optical density readings at 730 nm (OD_{730}) with a spectrophotometer.

2.6. Cyanobacterial lysate

Lysates were obtained from biomass produced by 21 day-old planktonic cultures and 35-day-old biofilms grown in media based on lunar and martian regolith simulants, or water-released minerals. After washing twice with ddH_2O, pellets were air-dried overnight under a laminar flow chamber and lysed as described [14] with minor modifications. Briefly, each pellet was resuspended in 500 μl ddH_2O with half volume glass beads and subjected to 3 cycles of freeze-thawing consisting of 1 min freezing in liquid nitrogen, thawing at 37 °C, followed by 1 min of vigorous vortexing. After centrifugation at 6000 *g* for 5 min, the supernatant was collected and the lysis procedure was repeated by adding 500 μl ddH_2O to the remaining pellet and finally, supernatants were pooled. Lysates at a concentration of about 1 mg/ml were used as substrate for growing *E. coli* W.

The quantitative analysis of sucrose content in lysates was performed by reverse phase ion pair (RPIP)-HPLC48 on a Luna 5lC18 reversed phase column (150 × 4.6 mm) (Phenomenex, Torrance, CA, USA) as previously described [26].

2.7. Bacterial growth with cyanobacterial lysate

One-ml aliquots of overnight cultures of *E. coli* cells were diluted to about 1×10^7 cells/ml, washed with PBS, and resuspended in 1 ml of cyanobacterial lysate. Positive controls were cells washed with PBS and used to inoculate 1 ml of Luria Bertani (LB) broth or into minimal medium supplemented with 0.5 % glucose (M9+) [23]. For negative control, *E. coli* cells were washed with PBS and used to inoculate 1 ml of sterile ddH_2O. Each sample was incubated overnight under continuous orbital shaking at 38 °C. Cell densities were determined by optical density readings at 600 nm (OD_{600}) with a spectrophotometer using a calibration curve determined by cell counting with a Burker's chamber.

2.8. Data analysis

Each experiment included 3 independent biological replicates and three technical replicates. Statistical tests were performed using GraphPad PRism version 8.0.2 for Windows, by GraphPad Software (San Diego, CA, USA). Shapiro-Wilk test (p-value > 0.05) was used to assess the normality of data. One-way ANOVA (ANalysis Of VAriance) with Tukey multiple-comparison test (p-value < 0.05) was used to assess the significance of differences between pairs of group means.

3. Results

3.1. Biomass from cyanobacterial planktonic cultures grown with regoliths

After 21 days cultivation of *Chroococcidiopsis* in ddH_2O containing lunar regolith simulant (LRA, supplemented with 17 mM $NaNO_3$), the yielded biomass was about 50 % (2.6 g/l dry weight) of control biomass grown in BG11 (Fig. 1). After the same cultivation period with martian regolith simulant (JSC Mars1A, supplemented with 17 mM $NaNO_3$), the yielded biomass was about 24 % (1.3 g/l dry weight) of the control biomass (Fig. 1). Media based on water-released minerals from LRA and JSC Mars-1A (supplemented with 17 mM $NaNO_3$) supported a biomass corresponding to about 40 % (2.1 g/l dry weight) and 23 % (1.2 g/l dry weight) of the control biomass, respectively (Fig. 1). When adding 2.4 mM perchlorate ions to each JSC Mars1A-based medium no significant differences in biomass yield occurred (Fig. 1). The biomass production during 21 days of cultivation in the different media is reported in Supplementary Fig. S2.

3.2. Lysate from cyanobacterial planktonic cultures as bacterial feedstock

When about 1×10^7 *E. coli* cells were cultured with 1 ml of lysate from *Chroococcidiopsis* planktonic cultures grown in lunar (LRA), martian regolith simulants (JSC Mars-1A with perchlorate) and control BG11, cell concentrations of about 9.5×10^7, 8.0×10^7 and 9.0×10^7 cells/ml, respectively, were obtained (Fig. 2). Slightly reduced bacterial cell concentrations were obtained when using the lysate from planktonic cells grown in water-released minerals from LRA, reaching a final concentration of about 7.5×10^7 (Fig. 2). The lysate from cyanobacterial cells grown in water-released minerals from JSC Mars-1A (with perchlorate) yielded about 9.0×10^7 cells/ml (Fig. 2). Higher *E. coli* cell concentrations of about 1.1×10^9 and 8.0×10^8 cells/ml were obtained when using LB and M9+ medium, respectively (Fig. 2).

3.3. Biomass from cyanobacterial biofilms grown on regolith

After 5 weeks culturing *Chroococcidiopsis* biofilms on 1.5 % agar with lunar regolith simulant (LRA, supplemented with 17 mM $NaNO_3$), a biomass of about 39 % (2.1 g/l dry weight) of control biofilm on BG11, was obtained (Fig. 3). After 5 weeks culturing on martian regolith simulants (JSC Mars-1A, supplemented with 17 mM $NaNO_3$), biofilms produced a biomass of about 47 % (2.6 g/l dry weight) of control biofilm on BG11 (Fig. 3).

The biomass yielded from biofilms grown on water-released minerals from LRA and JSC-Mars1A was about 80 % (4.38 g/l dry weight), 45 % (2.4 g/l dry weight) of control biofilms (Fig. 3). No significant differences in biomass yield occurred when adding 2.4 mM perchlorate ions to each JSC Mars1A-based medium (Fig. 3). The biomass production after 35 days of cultivation in the different agarized media is shown in Supplementary Fig. S3.

3.4. Lysate from cyanobacterial biofilms as bacterial feedstock

When about 1×10^7 *E. coli* cells were incubated in 1 ml of lysate from *Chroococcidiopsis* biofilms grown on lunar (LRA), martian regolith simulants (JSC Mars-1A with perchlorate) and BG11 medium, cell concentrations of about 1.1×10^8 cells/ml were reached after overnight incubation (Fig. 4). Comparable cell concentrations were obtained with the lysate of *Chroococcidiopsis* biofilms grown with minerals released from LRA and JSC Mars1A, supplemented with perchlorate (Fig. 4).

3.5. Cyanobacterial growth with regoliths and synthetic human urine

The growth rate of *Chroococcidiopsis* planktonic cultures in $BG11_0$ medium (BG11 lacking $NaNO_3$) supplemented with 10 mM urea was

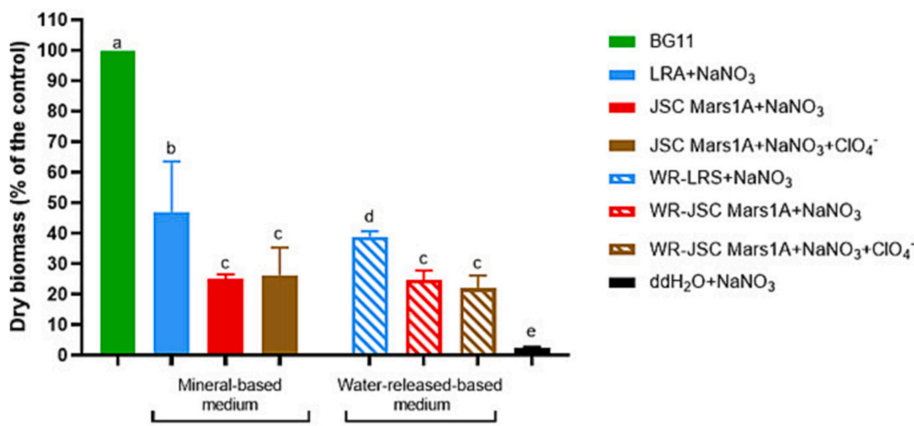

Fig. 1. Biomass produced by 21 day-old planktonic cultures of *Chroococcidiopsis* sp. 029 grown in media based on lunar (LRA) or martian regolith simulants (JSC Mars-1A) and water-released (WR) minerals. The JSC Mars-1A medium was enriched with 2.4 mM perchlorate ions (JSC Mars-1A + $ClO4^-$). Each medium was supplemented with 17 mM $NaNO_3$. Positive control: BG11 medium. Negative control: ddH_2O containing 17 mM $NaNO_3$. Data are means ± standard deviation ($n = 3$ biological replicates). Different lowercase letters indicate statistically significant differences between growth media ($p < 0.05$).

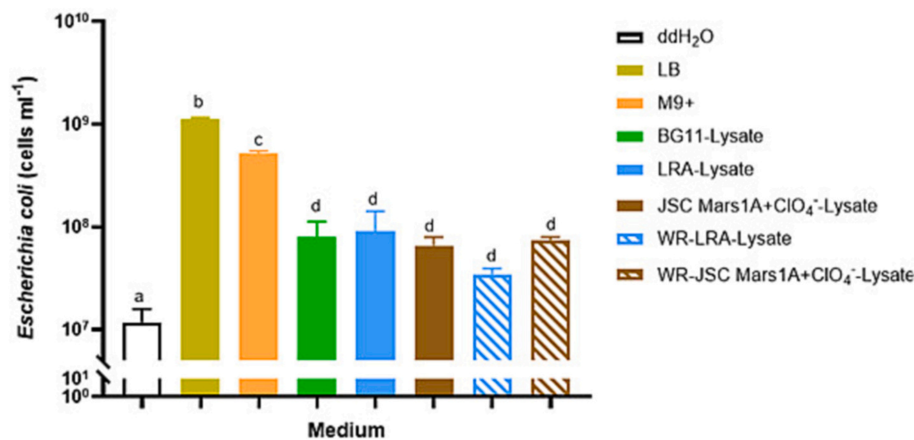

Fig. 2. Growth of *E. coli* W with lysate obtained from 21 day-old planktonic cultures of *Chroococcidiopsis* sp. 029 grown in media based on lunar (LRA) or martian regolith simulants (JSC Mars-1A) and water-released (WR) minerals. JSC Mars-1A medium was enriched with 2.4 mM perchlorate ions (JSC Mars-1A + $ClO4^-$). Each medium was supplemented with 17 mM $NaNO_3$. Positive controls: M9+ and LB medium. Negative control: ddH_20. Data are means ± standard deviation ($n = 3$ biological replicates). Different lowercase letters indicate statistically significant differences between growth media ($p < 0.05$).

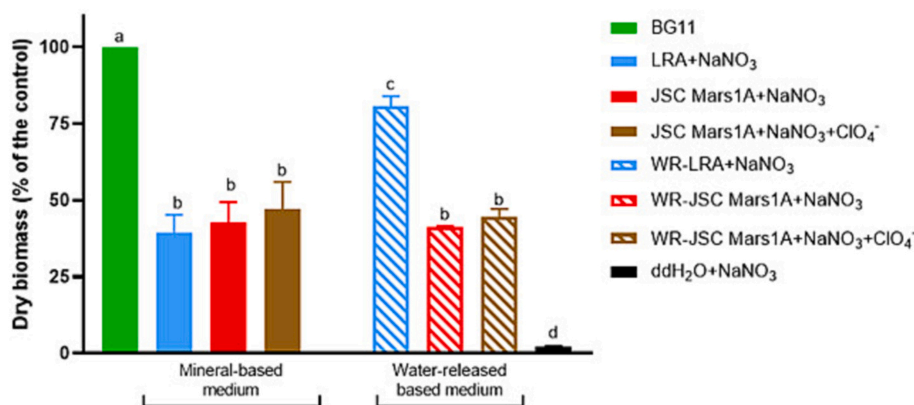

Fig. 3. Biomass produced by 35-day-old biofilms of *Chroococcidiopsis* sp. 029 grown on media based on lunar (LRA) or martian regolith simulants (JSC Mars-1A) and water-released (WR) minerals from LRA and JSC Mars-1A. The JSC Mars-1A medium was enriched with 2.4 mM perchlorate ions (JSC Mars-1A + $ClO4^-$). Each medium was supplemented with 17 mM $NaNO_3$. Positive control: BG11 medium. Negative control: ddH_20 with 17 mM $NaNO_3$. Data are means ± standard deviation ($n = 3$ biological replicates). Different lowercase letters indicate statistically significant differences between growth media ($p < 0.05$).

comparable to that in BG11 medium (not shown). Hence planktonic cultures were cultivated with a 25-fold diluted synthetic human urine (25-SHU) containing 10 mM urea. After 21 days the yielded biomass was about 36 % of the control in BG11 medium (Fig. 5). No significant differences occurred when adding 2.4 mM perchlorate ions to 25-SHU (Fig. 5). When 25-SHU was used to supplement ddH_2O containing lunar regolith simulant, after 3 weeks, the produced biomass was about 26 % (1.4 g/l dry weight) of the BG11 control. While a biomass of about 41 % (2.2 g/l dry weight) of the BG11 control, was produced using the martian regolith simulant (with perchlorate) supplemented with 25-SHU (Fig. 5). The biomass produced during 21 days of cultivation with 25-SHU based media is reported in Supplementary Fig. S4.

3.6. Lysate from cyanobacteria grown with regoliths and synthetic human urine as bacterial feedstock

When about 1×10^7 *E. coli* cells were incubated in 1 ml of lysate of *Chroococcidiopsis* biofilms grown in lunar (LRA) or martian (JSC Mars-1A with perchlorate) regolith simulants supplemented with 25-fold diluted SHU, and in control BG11, cell concentrations of about 8.5×10^7, 1.2×10^8, and 8.6×10^7 cells/ml, respectively, were obtained (Fig. 6).

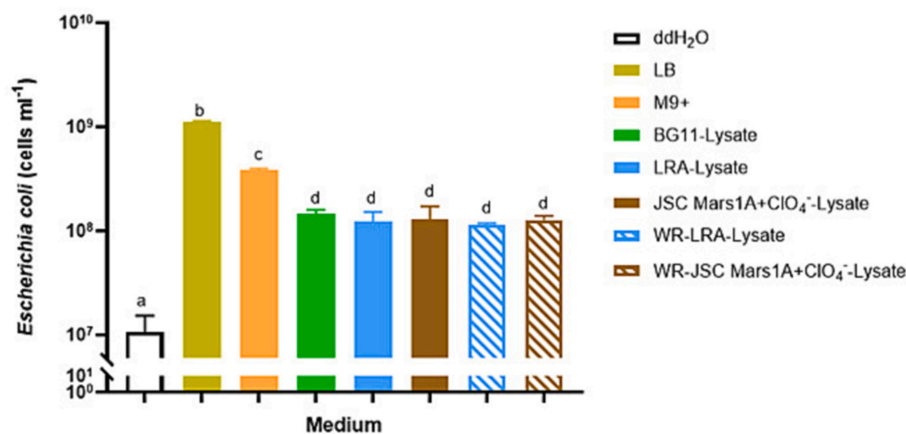

Fig. 4. Growth of *E. coli* W using the lysate obtained from 35 day-old biofilms of *Chroococcidiopsis* sp. 029 grown on media based on lunar (LRA) or martian regolith simulants (JSC Mars-1A) and water-released (WR) minerals. The JSC Mars-1A medium was enriched with 2.4 mM perchlorate (JSC Mars-1A + ClO_4^-). Each medium was supplemented with 17 mM $NaNO_3$. Positive controls: M9+ and LB media. Negative control: ddH_20. Data are means ± standard deviation (n = 3 biological replicates). Different lowercase letters indicate statistically significant differences between growth media ($p < 0.05$).

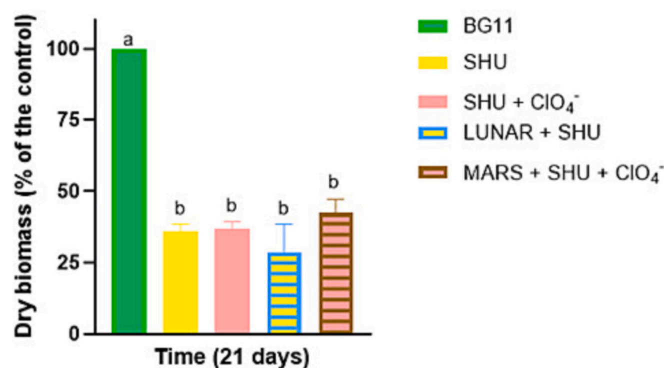

Fig. 5. Biomass yielded from 21-day-old *Chroococcidiopsis* planktonic cultures grown using 25-fold diluted human synthetic urine (SHU) as a nitrogen source. Cells were grown in SHU, SHU enriched with perchlorate (SHU + $ClO4^-$), lunar (LRA) and martian regolith simulants (JSC Mars-1A with perchlorate) supplemented with SHU. Positive control: BG11 medium. Data are means ± standard deviation (n = 3 biological replicates). Different lowercase letters indicate statistically significant differences between growth media ($p < 0.05$).

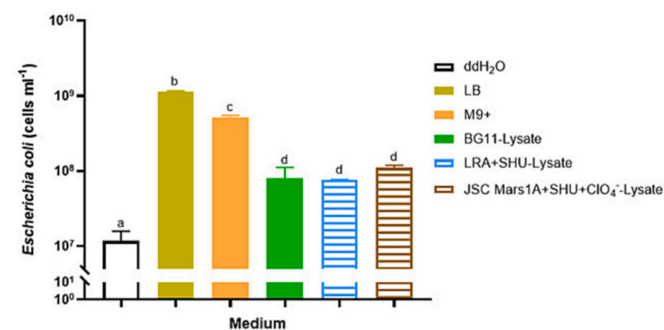

Fig. 6. Growth of *E. coli* W using the lysate obtained from planktonic cultures of *Chroococcidiopsis* sp. 029 grown in media derived from lunar or martian (JSC Mars-1A with 2.4 mM perchlorates) regolith simulants supplemented with 25-fold diluted human synthetic urine (SHU). Positive controls: M9+ and LB medium. Negative control: ddH_20. Data are means ± standard deviation (n = 3 biological replicates). Different lowercase letters indicate statistically significant differences between growth media (p < 0.05).

4. Discussion

The sustainability of human outposts on the Moon and Mars will rely on the ability to produce consumables on site. A game changing is offered by developing bio-ISRU system based on rock-leaching cyanobacteria, a concept known as "PowerCell". This approach is based on cyanobacteria grown with resources available on site to "power" bioproduction carried out by bacteria [7,8].

Here we further reduced the knowledge gap to use the desert cyanobacterium *Chroococcidiopsis* sp. 029 as a PowerCell in bio-ISRU by showing: i) its growth capability in planktonic and biofilm lifestyle, in water containing lunar and martian regolith simulants as well as in water-released minerals (supplemented with nitrate); iii) its capability to use human synthetic urine as a nitrogen source; and iv) its lysate suitability as feedstock for bacteria.

In the present work *Chroococcidiopsis* planktonic cultures grew better with lunar regolith simulant rather than martian regolith simulant, yielding a biomass of about 50 % and 30 %, respectively, of the control biomass in BG11. Biofilms developed better on martian regolith simulant than on lunar, producing a biomass of about 47 % and 39 % respectively, of the control. Water-released minerals supported both planktonic and biofilm growth. The yielded biomass with martian-released minerals was comparable to the counterpart with regolith, while the biomass produced with lunar-released minerals was twice the counterpart with regolith. Indeed, it was reported that planktonic cultures of cyanobacteria grew better with lunar regolith simulant rather than martian regolith simulant [6]. It was also reported that cultivating cyanobacteria with extraterrestrial regoliths is impaired by various factors, like the regolith particle size that limits light incidence and gas exchange, the regolith mineral composition and the cyanobacterial leaching capabilities [27–29].

The cultivation of *Chroococcidiopsis* as planktonic and biofilm forms in water-released minerals is relevant to meet different technological requirements of future cultivation hardware to be used on the Moon (and eventually on Mars): i) the integration of water-released minerals into the bioreactor might be preferred to that of regolith; ii) planktonic cultures in water-released minerals might facilitate the downstream biomass-regolith separation; and iii) biofilm lifestyle might be advantageous under reduced gravity conditions, since the leaching activity might be enhanced by a tight cell-mineral interaction [3,30].

Notably, the lysate of *Chroococcidiopsis* cells cultivated in the different experimental set up, equally supported *E. coli* W growth. This highlighted the potential of this cyanobacterium as interface between resources available on the Moon or Mars and microbial bioprocesses used in life support systems.

The planktonic and biofilm growth of *Chroococcidiopsis* was not affected by the enrichment of the martian regolith simulant with 2.4 mM perchlorate ions. This is relevant since such a concentration would be present in 200 g/l martian regolith containing 0.12 wt% perchlorate, as occurring at the John Klein outcrop [15,16]. Attempt to grow *Anabaena* sp. PCC 7938 with increasing amount of martian regolith simulant

identified as optimal a 3 mM perchlorate ion concentration that corresponded to 50 g/l martian regolith containing 0.6 wt% perchlorate concentration, as measured at the Phenix landing site [28]. Hence, considering that *Chroococcidiopsis* sp. 029 can tolerate up to 100 mM sodium perchlorate it is anticipated that increased perchlorate concentration will not impair its use in bio-ISRU on Mars [14].

In the present work, the limitation imposed by *Chroococcidiopsis* inability to fix atmospheric nitrogen, was overcome by demonstrating its ability to use urea as a nitrogen source. It is well known that urease-producing cyanobacteria can grow on urea by catalyzing the hydrolysis of urea to ammonia and carbamate [31,32]. After 21 days of cultivation in $BG11_0$ supplemented with 10 mM urea (containing 280.75 mg N/l), *Chroococcidiopsis* sp. 029 showed a growth rate comparable to that in BG11 (containing 248 mg N/l sodium nitrate). This is remarkable since a similar urea concentration (247 mg N/l) yielded a minimal growth of *Anabaena* PCC 7120 and *Synechocystis* PCC 6803 [33,34]. While *Spirulina platensis* grown with 373 mg N/l urea produced a higher biomass than with the same nitrogen amount provided as nitrate [35].

Since urine will be readily available on human space settlements, the possibility of using organic nitrogen in bio-ISRU based on *Chroococcidiopsis* is relevant. Indeed, when planktonic cultures were cultivated with lunar and martian regolith simulants supplemented with diluted synthetic human urine (280 mg N/l urea), a biomass of about 26 % and 41 %, respectively, of the BG11control, was produced. Notably, the lysate of cells grown with lunar and martian regolith simulants supplemented with synthetic human urine yielded a bacterial growth comparable to that supported by the lysate of cells grown with control BG-11.

This result provided the first proof-of-concept in using cyanobacteria to link bacterial bioprocesses to resources available on the Moon or Mars and human urine. Indeed *E. coli* W is a strain that can grow with only sucrose and magnesium sulphate [36]. The sucrose content of the lysate obtained from air-dried biomass of *Chroococcidiopsis* cells cultivated with lunar and martian regolith simulants, was about 6 mg/L (not shown). Feeding *E. coli* W with a cyanobacterial lysate is relevant since it can be used as chassis for synthetic biology/biotechnology applications to produce on-demand consumables to support human outposts on the Moon and Mars [7,8].

5. Conclusions

Here we further reduced the gap to employ *Chroococcidiopsis* sp. 029 as a “PowerCell” in bio-ISRU. The lysate of cells grown in the planktonic and biofilm forms with lunar and martian regoliths was used to “power” bio-production by feeding bacteria. Its growth versability on lunar and martian regoliths, as well as in water released minerals, is relevant to meet different technological requirements of future cultivation hardware on the Moon (and eventually on Mars). The suitability as feedstock of cells cultivated with regolith simulants supplemented with synthetic human urine, provided a proof-of-concept for linking resources available on the Moon and Mars and human urine to bacterial bioprocesses used in life support systems.

CRediT authorship contribution statement

Daniela Billi and Lynn J. Rothschild = Conception.

Beatriz Gallego Fernandez, Claudia Fagliarone and Salvatore Chiavarini = Methodology and formal Analysis.

Beatriz Gallego Fernandez = Writing original draft.

Daniela Billi and Lynn J. Rothschild = Writing - review & editing.

Funding

This research was supported by the Italian Space Agency (ReBUS – In-situ Resource Bio-Utilization to Support Life in Space, grant n° 2019-4-U.0 WP: Cyanobacteria for ISRU, to D.B.)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2023.103044.

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