The Cyanobacterium *Chroococcidiopsis* and Its Potential for Life on Mars

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Abstract

The unicellular *Chroococcidiopsis* is extremely resistant to desiccation, UV-irradiation, salt toxicity and high temperature. It requires only low light intensities for growth, and is protected against destruction from high energy UV-light by pigments such as carotenoids and scytonemin. Cyanobacteria, such as *Chroococcidiopsis*, have minimal nutrient requirements and can meet their nitrogen demand by means of the conversion of the atmospheric dinitrogen molecule to ammonium ions (nitrogen fixation). Water is the electron donor for photosynthesis, but can possibly be replaced by molecular hydrogen gas. A member of the genus *Chroococcidiopsis* is likely the best organism for exploring the potential for life on Mars. For this goal to be achieved, however, many strains exist on Earth that still must be explored to find the best suited one.

Keywords: Exploring life on Mars, cyanobacteria, extremophiles, *Chroococcidiopsis*, stress resistance, life demand for water, salt tolerance, nitrogen fixation, drought tolerance, protection against UV-light

1. Introduction

The evidence for past and current existence of life on Mars has recently been extensively summarized and with much expertise in this journal (Joseph et al., 2019).The ability of various organisms to survive in Mars-like environments has been researched (de Vera et al., 2019). Cyanobacteria may have begun constructing stromatolites on Mars (Rizzo and Cantasano, 2009, 2016; Ruff and Farmer, 2016) at around the same time as on Earth (Kremer, 2006; Sharma and Shukla, 2019). Although conclusive evidence of cyanobacteria has not yet been detected on Mars, several teams of investigators have identified sedimentary structures which closely resemble stromatolites which may have been constructed 3.2 - 3.7 billion years ago (Noffke 2015, Rizzo and Cantasano, 2016). The formation of Fe_2O_3 structures, and other sediments on Mars, including hematite (Joseph et al., 2019) may have been fashioned by prokaryotes and fungi adapted to life in hot springs and under higher pressures. However, all positive evidence for any existence of life on Mars (Joseph, 2016; Joseph et al., 2019) remains circumstantial, unproven and debated (Armstrong 2019; Kidron, 2019) with some scientists arguing life is unlikely under the present conditions, but may have existed in endolithic crusts in the past (Warren-Rhodes et al., 2006).

Life on Mars may have arisen through transfer by meteorites from Earth and other planets (Horneck et al., 2008). The transferred organisms could have been shielded from the harsh conditions of space by being embedded a few centimetres under the surface thus enhancing survivability (Cockell et al., 2007; Foucher et al., 2010, Smith et al., 2014, Gomez-Silvo, 2018). Organics might have also been delivered through asteroid and comets impact (Flynn, 1996; Ten Kate, 2010; Frantseva et al., 2018). The

high potential for survival on Mars has been repeatedly assessed in the laboratory, where extreme harsh Mars-like conditions, such as temperature, UV radiation, gas pressure, wind exposure and others, were mimicked (Cockell et al., 2005; Baque et al., 2013; 2016; Onofri et al., 2019). Also, field sites with extremely harsh conditions for life have been explored for the occurrence and survival of particular adapted organisms (Wynn-Williams and Edwards, 2000; Wierzchos et al., 2005, Warren-Rhodes et al., 2006; Liu et al., 2008; Davila et al., 2008, Stivalettta et al., 2012; Azua-Bustos et al., 2012; Gomez-Silvo, 2018). Both prokaryotes (Baque et al., 2016) and eukaryotes (Onofri et al., 2019; Pacelli et al., 2019) have been examined. An Antartic lichen rapidly adapts to Martian niche conditions (De Vera et al., 2014). Organics might have delivered to planet through asteroid and comets impact (Flynn, 1996; Ten Kate, 2010; Frantseva et al., 2018). Therefore, heterotrophic organisms such as the spore forming *Bacillus subtilis* (Averesch and Rothschild, 2019; Cortesao et al., 2019) may have a chance to survive on Mars, although organics for growth may currently not be available on Mars. My personal view is shared by several authors (Olsson-Francis and Cockell, 2010, Verseux et al., 2017; Baque et al., 2013; 2016) that the best prospects for exploring growth on Mars are offered by the cyanobacteria.

Many species of cyanobacteria, formerly called blue-green algae, of the order Nostocales, such as *Anabaena cylindrica*, form akinetes. These resting cells are resistant to drought, high temperature and UV exposure and can withstand harsh conditions over longer periods (Olsson-Francis et al., 2009; Sharma and Shukla, 2019). However, after germination of akinetes, such species generally form long unbranched filaments of conjoined single cells. These filaments can undergo a complex cell differentiation into vegetative cells that perform photosynthetic CO_2-fixation and O_2-evolution, and heterocysts. The latter harbour the nitrogenase complex that catalyses the conversion of atmospheric nitrogen gas to ammonium ions. These filaments are unable to withstand harsh stress conditions, if not embedded in a thick slime layer. However, there is no evidence of slime layers on Mars.

Non-akinetes forming species of the order Pleurocapsales, particularly the genus *Chroococcidiopsis*, have a much greater likelihood of survival on Mars. The literature on the use of this genus for extraterrestrial survival is remarkable (e.g., Cockell et al., 2005; Olsson-Francis and Cockell, 2010; Baque et al., 2013; 2016; Verseux et al., 2017). In the present review, major facets of this topic, supplemented with novel and, as yet, largely unappreciated considerations will be summarized.

Instead of cyanobacteria, lichens particularly with a tripartite symbiosis consisting of a fungus, a green alga and a N_2-fixing cyanobacterium may be considered as probe for life on Mars. They grow in or on extreme habitats (Armstrong and Smith, 1993; Armstrong 2019; Kidron, 2019) but their slow growth might hinder their successful use on Mars in contrast to *Chroococcidiopsis*.

2. Biology and world-wide occurrence of *Chroococcidiopsis*

By multiple fissions, most *Chroococcodiopsis* species exist either as aggregates of 16 cells or multiples thereof; but single cells are also observed (Fig. 1). Large parental cells within the consortium form very small cells, called baeocytes, by rapid multiple fissions (Herdman and Rippka 1988). Baeocytes do not grow to a larger size and are not covered by a thick cell-wall layer. Non-motile baeocytes are characteristic of the genus *Chroococcidiopsis* (Büdel and Henssen, 1983). Their primary function is dispersal such as via wind, and they are released from sessile parental cells in large quantities (Herdman and Rippka, 1988). The genus *Chroococcidiopsis* includes both salt-tolerant and salt-sensitive species, some of which even require salt for growth (Cumbers and Rothschild, 2014). *Chroococcidiopsis* is species polyphyletic (Cumbers and Rothschild, 2014). Separation into species by molecular methods awaits assignment.

Figure 1. *Chroococcidiopsis* as isolated from underground of the shards of the gypsum rock of Bad Sachsa, Harz mountains, Germany.

A marked feature of *Chroococcidiopsis* is its resistance to desiccation. The organism occurs in driest places world-wide, in deserts, such as the Atacama of Chile (Azua-Bustos et al., 2012; Gomez-Silva, 2018), the Mojave in the south western part of the United States (Smith et al., 2014) and the Antarctic (Friedmann, 1982). Water availability by rainfall is extremely low at such sites and organisms survive mainly from the atmospheric moisture brought in with the fog. They protect themselves against the powerful irradiation by living within rocks, the so-called endolithic mode of life, mostly a few millimetres beneath the surface or at the underside of rocks. *Chroococcidiopsis* also survives near the surface of salt (halite) deposits (Stivaletta, Barbieri and Billi, 2012; Finstad et al., 2017), occupying spaces among salt crystals in the Atacama desert (Wierzchos, Ascaso and McKay, 2006). Water that condenses onto the halite crusts is apparently sufficient for life (Davila et al., 2008).

A particular binding of *Chroococcidiopsis* to gypsum soils has repeatedly been reported, e.g. in the Atacama and Mojave deserts, in the Al-Jafr Basin of Jordan (Dong et al., 2007) or in mound evaporite

deposits in Tunisia (Stivaletta and Barbieri, 2009). Gypsum, bassanite and anhydrite might occur on Mars (Vaniman et al., 2018). A peculiar occurrence of *Chroococcidiopsis* is at the gypsum rocks located at the southern rim of the German Harz mountains. The soil parameters of this site, which is famous for the presence of rare, endangered plants and mycorrhizal fungi, have been published (Landwehr et al., 2002). In the Earth's Devonian period, anhydrite ($CaSO_4$) was deposited there by ocean organisms.

Figure 2. Shard of the gypsum rock of Bad Sachsa, showing the distinct blue-greenish layer formed by*Chroococcidiopsis*.

Upon long duration exposure to water, anhydrite at the surface of the rocks is converted to gypsum ($CaSO_4.2H_2O$), a process that continues to this day. Due to the expansion by the uptake of water, the gypsum rock surface breaks into small pieces and the resulting flakes or shards can easily be peeled off by hand (Fig. 2). Beneath the shards, a distinct blue-greenish layer is discernible in a zone where both low, but still sufficient, light intensities for photosynthesis are available and where microaerobic (low O_2-tension) conditions prevail. The blue-greenish layer consists of cyanobacteria, more than 90% of which (as determined by sequencing data) belong to the genus *Chroococcidiopsis* (Boison et al., 2004). These cyanobacteria near the rock surface are exposed to large fluctuations in rainfall and, thus, can have a low water supply. In the summer of 2003, with its long drought period, the blue-greenish layer was almost dried out to a faint green band, but the band rapidly resumed its original colour and broadness after rainfall. Furthermore, there is scarcely any fine earth under the shards, which indicates that the cyanobacteria must live mainly on nutrients brought in by wind and rain.

3. Biochemical aspects of *Chroococcidiopsis* in the Martian environment

First, the ryolith (rocky material near the surface of other planetary bodies) contains silica, which could allow for growth of organisms on Mars (Olsson-Francis and Cockell, 2010). Both the ryolith and the regolith (surface with loosely packed material) might support life of extant organisms, such as cyanobacteria, on Mars (Liu et al., 2008; Olsson-Francis and Cockell, 2010).

Second, *Chroococcidiopsis* strains are not dependent on a supply of combined nitrogen (e.g., NO_3^-, NH_4^+ or organic nitrogen). It can meet its demand for biologically usable nitrogen by nitrogen fixation, which is the conversion the atmospheric dinitrogen molecule to the ammonium ion catalysed by the enzyme complex nitrogenase. Microorganisms can express combinations of several different nitrogenases; two different Mo- and Fe-containing nitrogenases, a V- and Fe-based enzyme and another enzyme that contains Fe as its only metal in the prosthetic group (Bothe et al., 2010a). *Chroococcidiopsis* possesses only one nitrogenase, the more rarely occurring of the two Mo-containing nitrogenases, which was first detected in the filamentous *Plectonema boryanum* (Stewart and Lex, 1970). It has since been shown to be expressed only under microaerobic conditions, in some unicellular forms and in vegetative cells of heterocystous cyanobacteria.
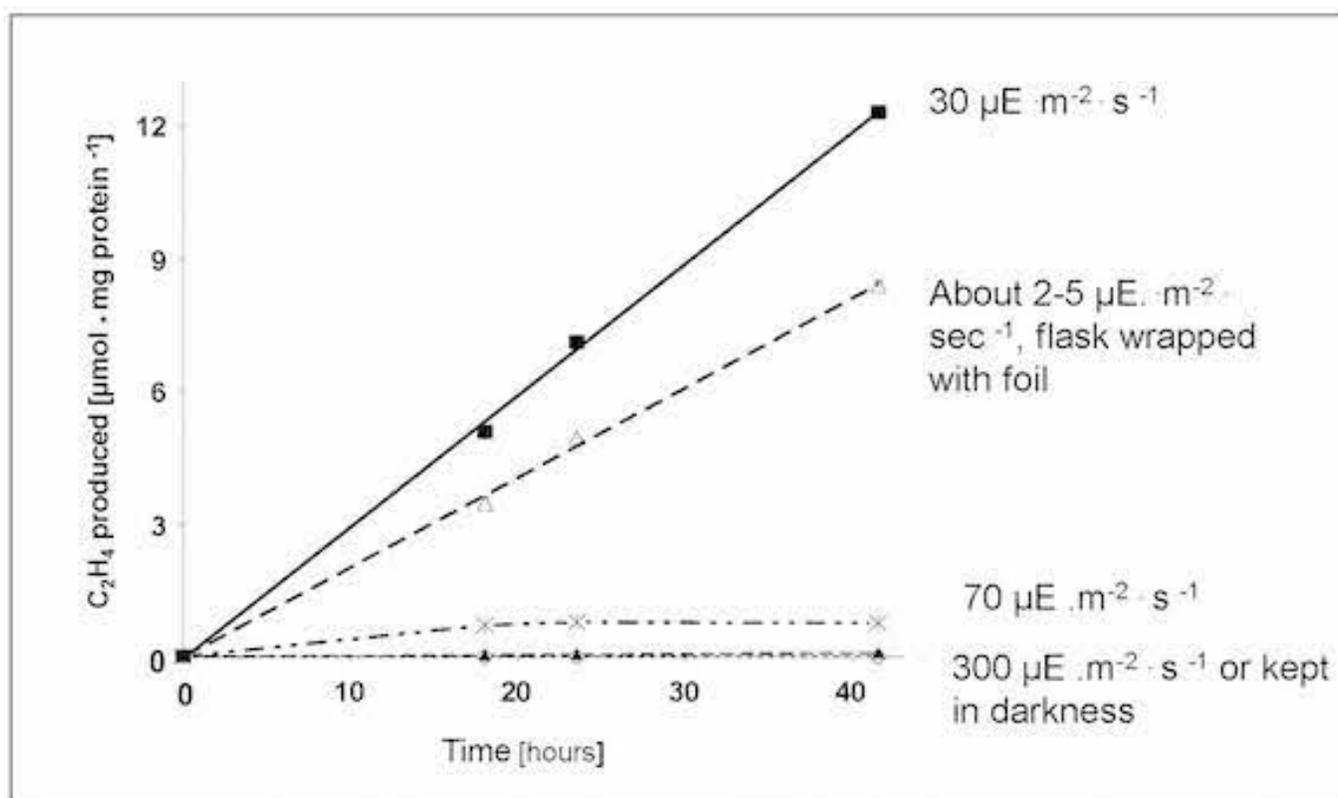

Figure 3. Light-dependence of C_2H_2-reduction (nitrogen fixation) by *Chroococcidiopsis* PCC 7203 (from Boison et al., 2004).

Using acetylene (C_2H_2), which is an alternative reducible substrate for all nitrogenases, nitrogen-fixation activity can easily be assessed by gas-chromatography. Suspension cultures

of *Chroococcidiopsis* PCC 7203 catalyse the formation of C_2H_4 from C_2H_2 in a process that is strictly dependent on optimal irradiation (Fig. 3). High light intensities are inhibitory (Fig. 3). Low light intensities are necessary for the formation of organic carbon by photosynthesis, and these low light intensities may be available in areas of Mars.

In contrast, *Chroococcidiopsis* cells isolated from a hot desert rock in Israel could not grow in an N-depleted medium (Billi and Caiola, 1996). Thus some *Chroococcidiopsis* strains may be unable to meet their N-demand by nitrogen fixation, but the extreme light sensitivity of cells growing under N_2-fixing conditions or with nitrate as N-source may be problematic for assigning N_2-fixation in such physiological experiments.

The apparent Michaelis (half saturation) constant for growing nitrogen-fixing bacteria corresponds to a low P_{N2} of 0.02 to 004 atm. N_2 (Postgate, 1972). N_2-fixation may have played a role in the primordial Martian atmosphere, but the possible lack of even such low levels of dinitrogen in the present Martian atmosphere may forbid nitrogen fixation to proceed nowadays (Klingler, Mancinelli and White, 1989). On the other hand, molecular nitrogen (N_2) may make up 1.9-2.7 % of the Mars atmosphere, but combined nitrogen (nitrate, ammonium ions, organic nitrogen) is apparently not available on the Mars surface crust (according to the Wikipedia article about Mars). Other N-compounds such as hydrazine, hydroxylamine, nitrous oxide, hydrogen cyanide, methyl isocyanide or azide may be reduced by nitrogenase to produce ammonium ions. Ammonia was likely abundant on the early anaerobic history of Earth. It has been speculated that the earliest function of nitrogenase on Earth was to detoxify our planet from toxic compounds such as hydrogen cyanide and also acetylene (Postgate, 1972), and this may apply also to Mars.

Third, *Chrooccocidiopsis* can protect itself against high UV doses by the formation and self-embedment into large amounts of slime (Almon and Böger, 1988). It is also able to grow or survive in biofilms (Baque et al., 2013; Billi et al., 2019). Strains can grow as endosymbiont in lichens (Büdel and Henssen, 1988) or endolithically (e.g. Boison et al., 2004). Even more important is the formation of pigments, such as carotenoids and scytonemin, which protect against UV radiation (Vitek et al., 2014). The latter compound, an aromatic indole alkaloid, is specifically synthesized by cyanobacteria and is highly effective as a sunscreen. Its synthesis is enhanced upon periodic desiccation in one *Chroococcidiopsis* strain but not in another (Fleming and Castenholz, 2007). Recently, Couradeau et al., (2016) found that cyanobacterial crusts warm the soil surface by as much as 10 °C through the production and accumulation of scytonemin. This effect is due to the dissipation of the absorbed photons into heat by scytonemin. Such a finding could have major implications when the potentials for life on Mars are explored.

Fourth, desiccation tolerance. Prolonged desiccation leads to the destruction of proteins, DNA and membrane components, mainly by oxidation and the formation of reactive oxygen species (Potts, 1999). Desiccation tolerance is rare in higher plants, but occurs in some pteridophytes, such as *Ceterach officinarum* (= *Asplenium ceterach*), and the higher plant *Ramonda myconi* dwelling in rock fissures in southern Europe.

Desiccation tolerance is wide-spread in lower organisms, such as algae, lichens and cyanobacteria (Kranner et al., 2008). Such microorganisms rapidly reduce photosynthesis and respiration to a minimum upon drought onset and resume these activities more or less immediately with rainfall. A fine example of this poikilohydrous behaviour is *Chroococcidiopsis*. After a long period of drought, shards from the Bad Sachsa gypsum rock with *Chroococcidiopsis* beneath were assayed for nitrogen fixation (C_2H_2-reduction) activity in the laboratory (Fig. 4). They showed no activity within the first week. Then water was added to the assay flask. Thereafter, C_2H_4-formation rapidly commenced within one day and

remained constant over the next 2 weeks. The activity was light-dependent and was best at lower O_2-levels in the flasks.
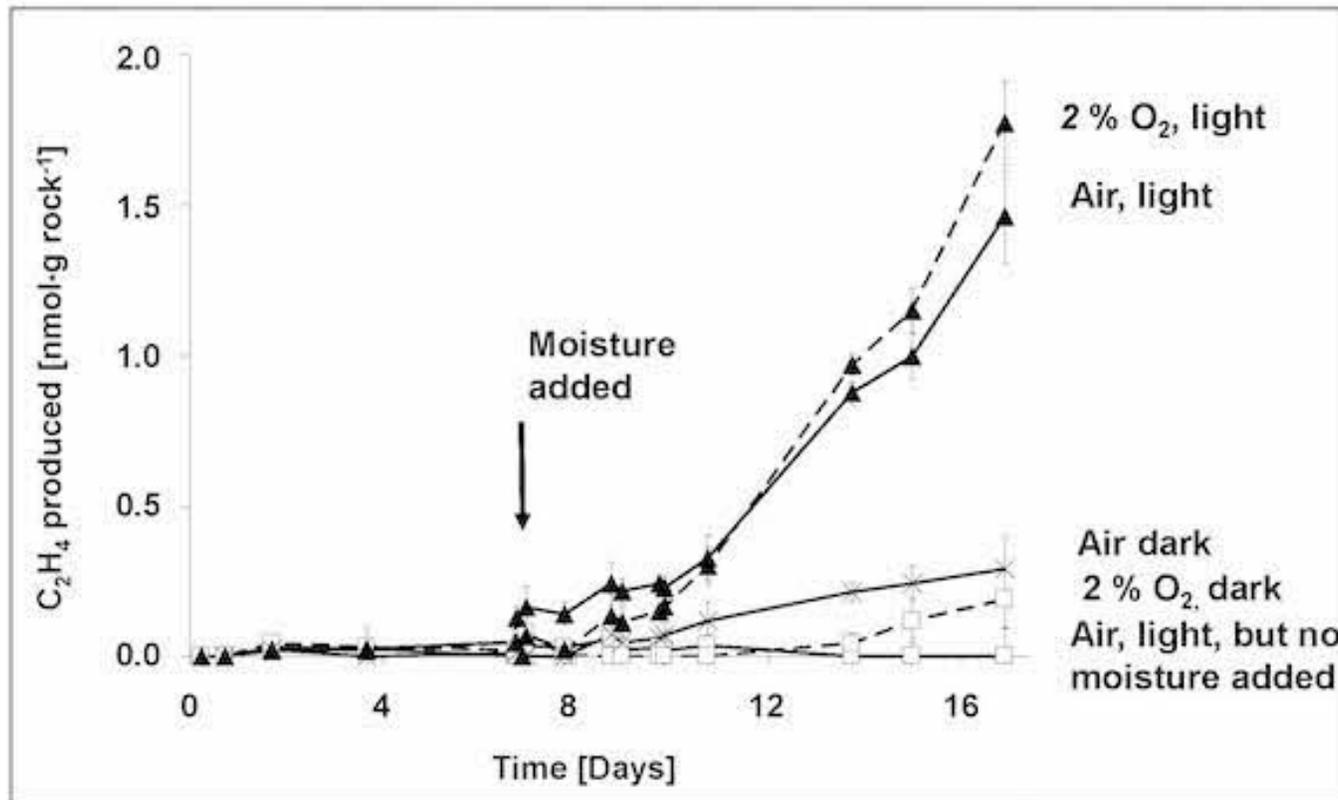

Figure 4. C_2H_2-reduction by the *Chroococcidiopsis*-harbouring shards from the Bad Sachsa gypsum rock after a long period of drought (from Boison et al., 2004).

Fifth, *Chroococcidiopsis* still performs photosynthesis at a temperature of 51 °C (Tracy et al., 2010) and can endure higher atmospheric pressures (Thomas et al., 2008). The survival rate at higher temperatures is greater when desiccated than when hydrated (Cockell et al., 2017). It is also acid tolerant over the large range between pH 3 and 7 as shown for at least one enzyme (Del Arco et al., 2018). Cyanobacteria can grow even under very high atmospheric CO_2-concentrations which might exist on Mars (Murusekan et al., 2016).

4. Reduced requirement of water for life of *Chroococcidiopsis*?

Some N_2--fixing unicellular cyanobacteria of the oceanic picoplankton have been found to lack a functional photosystem II and are, therefore, not dependent on water as electron donor for photosynthetic electron transport (Bothe et al., 2010b). They cannot be cultivated as yet, and their mode of life, particularly their acquisition of organic carbon, has not been elucidated. Thus, their potential use in Mars missions is currently uncertain. *Chroococcidiopsis* possesses both photosystems and splits water concomitantly with the formation of molecular oxygen in the generation of reductant for photosynthetic CO_2-fixation. However, the role of water could be substituted by molecular hydrogen, using the system outlined below.

Cyanobacteria possess two types of Ni-containing hydrogenase, both of which catalyse the

reaction: $H_2 \leftrightarrow 2\,H^+ + 2e^-$ (Bothe et al., 2010a). The so-called "uptake hydrogenase" is only involved in the consumption of dihydrogen gas under physiological conditions and is confined to N_2-fixing species. The "bidirectional hydrogenase" performs both the uptake and the evolution of H_2 and is widespread in cyanobacteria. It catalyses the reduction of NAD(P)+ by H_2 (Schmitz and Bothe, 1996). The expression of this protein is under control of the *LexA* transcriptional activator (Gutekunst et al., 2005). Its (main) physiological function is to dispose of excess reductant generated by fermentation during darkness (Bothe et al., 2010a). Also present in cyanobacteria is a NAD(P)H-dependent oxidoreductase, which feeds electrons into photosynthesis at the plastoquinone site. As this entry point occurs after any participation by photosystem II, reductant input via this oxidoreductase would negate the need for photosystem II and, therefore, for water (Fig. 5).
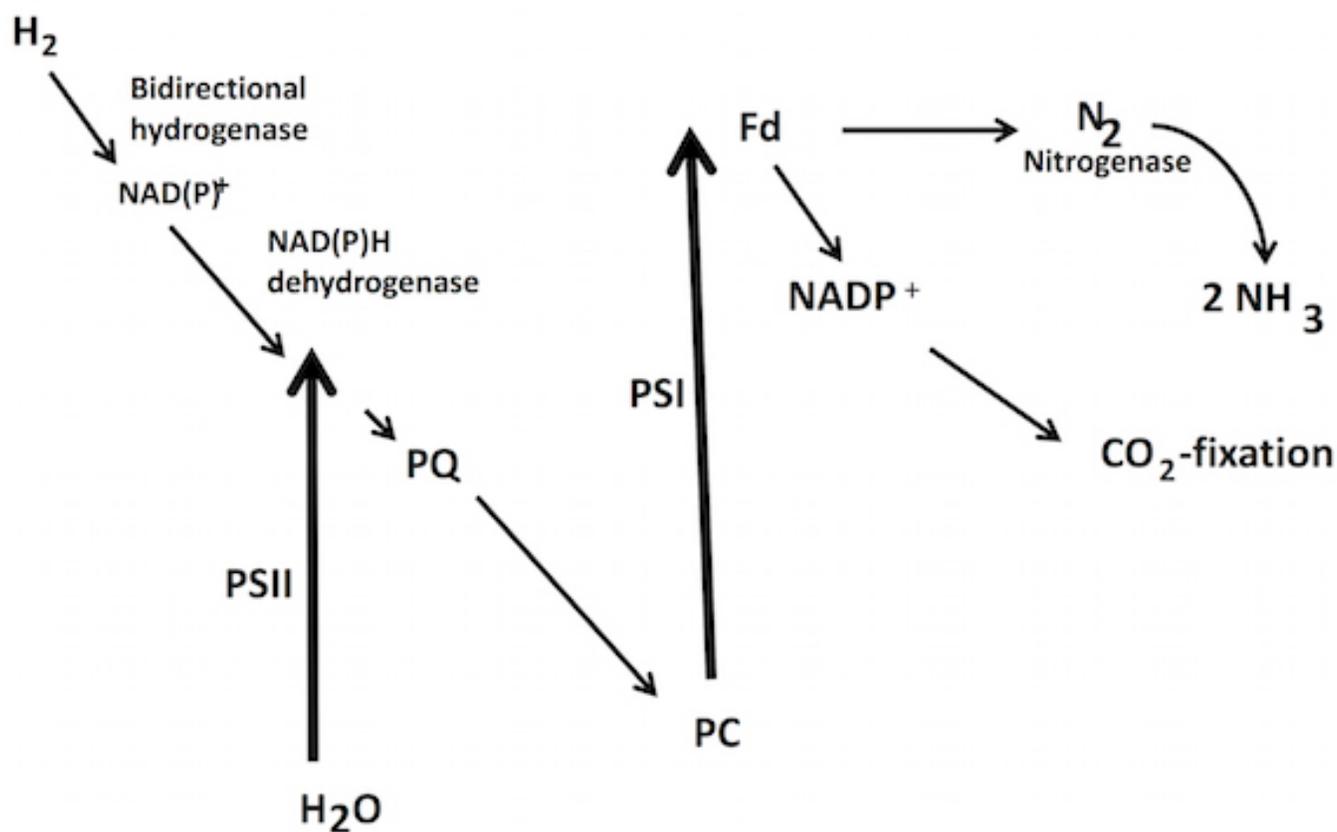

Figure 5. A simple scheme of cyanobacterial photosynthetic electron transport indicating that water as electron donor may be replaced by molecular hydrogen.

In non-photosynthetic bacteria, H_2- and O_2-dependent N_2-fixation has been demonstrated for *Alcaligenes latus* (Malik et al., 1981) and H_2-dependent growth and CO_2- fixation for *Bradyrhizobium (Rhizobium) japonicum* (Lepo et al., 1980). *Chroococcidiopsis* is able to utilize H_2 (Almon and Böger (1988), but the type of hydrogenase present (uptake or bidirectional enzyme) has not been investigated. However, the bidirectional enzyme is very likely to occur in *Chroococcidiopsis* as

it is found in almost all cyanobacteria investigated so far.

From the perspective of its potential of life on Mars, the discovery of CO_2-fixation that is dependent on only H_2 and photosystem I would be of enormous value and could provide the pathway for growth of *Chroococcidiopsis*. If H_2 were not available it could be produced from non-photosynthetic bacteria transported as by-pack of *Chroococcidiopsis*. These bacteria could digest and live on dying *Chroococcidiopsis* cells. This suggestion is supported by experiments with the shards from the Bad Sachsa rock. They showed that H_2 is produced at the *Chroococcidiopsis* layer, likely by contaminant fermentative bacteria (Boison et al., 2004).

Alternatively, electron donors other than H_2 such as H_2S can be envisaged. Dinatrium sulfide was shown to support anoxygenic photosynthesis of extant cyanobacteria (Garlick et al., 1977, Belkin and Padan, 1978, Shimizu et al., 2017).

Although photosynthesis would then be independent on a supply of water on Mars, some water must be available as solvent for the constituents in the cells. On Earth, cyanobacteria show a marked capacity to withstand lack of water (Potts, 1999). This could be enhanced on Mars if water, as the photosynthetic electron donor could, indeed, be replaced by H_2 on Mars.

5. Conclusions

All the features summarized in the present review speak for *Chroococcidiopsis* as the organism that has the potential to thrive on Mars. However, it is not yet clear which strain(s) combine(s) the best of all the properties needed to withstand the different and harsh conditions on Mars. As already mentioned,*Chroococcidiopsis* thrives world-wide in many deserts, each with differing harsh conditions. However, in almost all of the studies, the description of the occurrence is not complemented with an investigation of the physiological, biochemical and molecular properties that enable the cells to survive and grow in the specific habitat.

The ability to culture a variety of *Chroococcidiopsis* strains might be a difficult task. For example, the strain*Chroococcidiopsis thermalis* PCC 7203 could well be cultured in our laboratory. The generation time of this isolate was not exactly determined, but it is between 2 and 3 hrs and thus in the same range as that for the filamentous *Anabaena variabilis*. In contrast, the *Chroococcidiopsis* isolate from the gypsum shards of Bad Sachsa (Boison et al., 2004) grew very slowly, was clumpy and rapidly died out, making it a very poor candidate for physiological characterization in the laboratory. A detailed investigation to find a good growing strain, which can cope with all the adverse Martian conditions, such as resistance to desiccation followed by rapid resurrection, protection against high load of salt, acid, UV-radiation, high pressure, high CO_2-concentrations, availability of only low light intensity and a marginal water and gaseous dinitrogen supply, etc., is urgently needed to maximise the potential for success of organic growth on Mars.

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