IRON-TOLERANT CYANOBACTERIA AS A TOOL TO STUDY TERRESTRIAL AND EXTRATERRESTRIAL IRON DEPOSITION. I. I. Brown[1], D. Mummey[2], K.E. Cooksey[3], D.S. McKay[1], [1]NASA JSC; [1]Mail Code KA13, NASA JSC, 2101 NASA Parkway, Houston, TX 77058, USA (igor.i.brown1@jsc.nasa.gov); [2]Microbial Ecology Program, Division of Biological Sciences, University of Montana, 32 Campus Dr. #4824, Missoula, MT 59812, USA; [3]The Department of Microbiology, Montana State University, 109 Lewis Hall, Bozeman, MT, 59717, USA.

Introduction: We are investigating biological mechanisms of terrestrial iron deposition as analogs for Martian hematite recently confirmed by [1]. Possible terrestrial analogs include iron oxide hydrothermal deposits, rock varnish, iron-rich laterites, ferricrete soils, moki balls, and banded iron formations (BIFs) [2, 3]. With the discovery of recent volcanic activity in the summit craters of five Martian volcanoes [4], renewed interest in the iron dynamics of terrestrial hydrothermal environments and associated microorganisms is warranted. *In this study we describe a new genus and species of **CB** exhibiting elevated dissolved iron tolerance and the ability to precipitate hematite on the surface of their exopolymeric sheathes.*

Although the role of photosynthetic microorganisms in the generation of BIFs was acknowledged about 30 years ago [5], there is still no consensus about how ancestral photosynthetic organisms which are participated in Fe^{2+} oxidation during the Precambrian period The generally accepted assumption is that cyanobacteria (**CB**) were the source of oxygen for BIFs generation however, this hypothesis has recently been called into question by [6]. Based on phylogenetic analyses, [6] proposed that **CB** with oxygenic photosynthesis capabilities evolved just 2.2 Ga ago, much later than the time at which BIFs were formed. However, the potential role of **CB**-mediated anoxygenic photosynthesis [7] was not accounted for in these analyses.

While both phototrophic proteobacteria [8] and sulfide-resistant **CB** [9] are capable of the oxidation of Fe^{2+} through anoxygenic photosynthesis, the ability of **CB** to perform anoxygenic photosynthesis under conditions relevant to the Precambrian has not been thoroughly explored. One of the few studies to address this issue [10] found that Fe^{2+} stimulated photosynthesis in iron-tolerant, thermophilic cyanobacterial mat communities inhabiting iron-rich hot springs. They [10] hypothesized direct oxidation of Fe^{2+} in photosystem II (PS II), although the biochemistry of this process has not been studied in detail. However, it has been postulated that iron-dependent photosynthesis might have been an important step in the evolution of oxygenic photosynthesis in ancestral **CB** [11]. Thus, atmospheric oxygen would not be required in this direct microbial oxidation process and significant BIFs could be formed in anoxic conditions. *Hence, examination of iron-tolerant and thermophilic **CB** may shed light on ancient terrestrial iron deposition processes and may be applicable to the understanding the presence of certain mineral signatures on Mars.*

There is also a strong need for inclusion of iron-resistant thermophilic strains in cyanobacterial phylogenies, many areas of which are poorly defined [12]. Since there is a paucity of sequence data pertaining to thermophilic iron-resistant strains, **CB** phylogenetic analyses, including those suggesting that oxygenic photosynthesis evolved 2.2 Ga ago, are potentially omitting key species. This is especially important for determination of relationships between ancient and contemporary **CB**, since there is general agreement that temperatures [13], iron concentrations of Precambrian waters [14] and pH [15] were generally much greater than what is typical today.

Results and discussion. Ten unialgal cultures of iron-tolerant **CB** were isolated from two iron-depositing hot springs in Great Yellowstone area, i.e. LaDuke and Chocolate Spots Hot Springs (chemical descriptions of both springs can be found in [16, 17]). They are represented by both unicellular and filamentous species, all of which appear to exhibit elevated resistance to ferric (Fe^{3+}) iron (up to 400 µM) and to be potential reservoirs for precipitated hematite (Fig. 1). Representative of these strains is the isolate 5.2 s.c.1, which is the focus our analyses.

Phylogenetic analysis of isolate 5.2 s.c.1 supported placement within Cyanobacteria but failed to support placement within any established cyanobacterial group. These results indicate that 5.2 s.c.1 isolate diverges independently from any other genus-level cyanobacterial group and, therefore, merits genus status. Interestingly, isolate 5.2 s.c.1 exhibited the greatest sequence homology to the *Microcoleus*, a genus with expressed anoxygenic photosynthesis capabilities [18]. Future research will address the issue as to whether isolate 5.2 also has this capability.

The isolate 5.2 s.c.1 is a unicellular cyanobacterium with cells typically packed as duplexes, or in clumps or small chains, covered with extracellular polymeric substance (EPS). Cells exhibit a bladder-like shape.

Although the morphology of 5.2 s.c.1 cells is reminiscent of the *Synechocystis* genus, a salient difference is that isolate 5.2 s.c.1 has an EPS cover what may reach ~ 1μm.

No growth without added combined nitrogen occurred, suggesting that 5.2 s.c.1 isolate is unable to fix atmospheric nitrogen, thus providing a physiological distinction between isolate 5.2 s.c.1 and the genus *Nostocales* [19].

Two weeks after inoculation with 5.2 s.c.1 culture, DH media supplemented with 400 μM $FeCl_3.6H_2O$ exhibited a nearly 3 fold decrease in total soluble iron concentration compared to uninoculated media, indicating that 5.2 s.c.1 facilitates the removal of soluble iron from growth medium. While the EPS layer of cells incubated in the presence of 400 μM of iron exhibited orange-brown coloration (Fig. 1), this coloration was absent in cells grown in the presence of only 40 μM of iron. Additionally, crystals observed in light and SEM micrographs were confirmed to contain iron using Energy Dispersive X-ray Spectroscopy, further suggesting that the EPS layer of 5.2 s.c.1 is a repository for iron. Extracellular accumulation of iron by iron-resistant **CB** may potentially serve three functions: to decrease the chemical potential of active (accessible for cells) iron [20], to produce a pool of reserve iron for times of low iron availability [14], and as protective sunscreen in hostile solar environments [21]. Iron/EPS casts are preserved after cell death (Fig. 2), potentially resulting in the preservation of microfossils, applicable to ancient terrestrial and possibly, Martian rocks and soils.

Presented results indicate that isolate 5.2 s.c.1 is morphologically, physiologically, and phylogenetically distinct from any other cyanobacterial genus and belongs to a novel genus and represents a novel species of this genus. The name "Chroogloeocystis siderophila" (i.e., "C. siderophila") is proposed for this novel cyanobacterial isolate. This organism was isolated from, and is physiologically well adapted to, an environment likely similar to that expected for Precambrian environments.

Other isolates are currently the subject of phylogenetic, morphological and biochemical examination. We plan to verify conditions appropriate for ferrous iron oxidation by either PSII or/and PS I in iron-tolerant cyanobacteria. Studies to elucidate the role of iron-tolerant cyanobacteria in EPS-mediated iron deposition, as well as for the microfossil generation, are currently being conducted.

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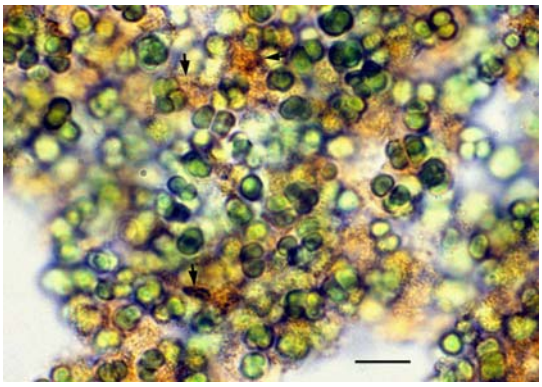

Fig. 1. Light micrograph of the isolate 5.2 s.c.1 grown in liquid medium DH supplemented with 400 μM Fe^{3+}, indicating iron-containing precipitates on cell surfaces (black arrows). Bar = 5 μm.

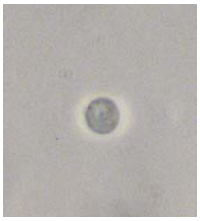

Fig.2. Iron/EPS cast (no Chl "a" fluorescence was observed) preserved after "C. siderophila" cells death. Same conditions as Fig. 1.