BIOGEOCHEMICAL ACTIVITY OF SIDEROPHILIC CYANOBACTERIA AND INSIGHTS FROM THEIR GENOMES: IMPLICATION FOR THE DEVELOPMENT OF NEW BIOSIGNATURES. I.I. Brown[1], D.A. Bryant[2], K.L. Thomas-Keprta[1], S. G. Tringe[3], S.A. Sarkisova[1],C. Galindo Jr[1]., K. Malley[4], O. Sosa[4], D.H. Garrison[1], and D.S. Mckay[5]. [1]SARD/JSC, Mail Code: JE 23, ESCG, P. O. Box 58447, Houston, TX; [2]The Pennsylvania State University, University Park, PA 16802; [3]DOE Joint Genome Institute; [4] NASA/USRP; [6]NASA JSC.

Introduction: Verifying the links between genomic features in living organisms and their mineralization/demineralization activity will help to reveal traces of life on Earth and beyond.

Among contemporary environments, iron-depositing hot springs (IDHS) may represent one of the most appropriate natural models [1] for insights into ancient life since organisms may have originated on Earth and possibly Mars in association with hydrothermal activity and high [Fe^{2+}] [2-6]. Siderophilic or "iron-loving" cyanobacteria (CB) inhabiting IDHS may have genomic features and properties similar to those of ancient organisms because abundant Fe^{2+} in IDHS has a strong potential to increase the magnitude of oxidative stress [7]. That is why specific and/or additional proteins involved in Fe mineralization [8, 9] by siderophilic CB are expected.

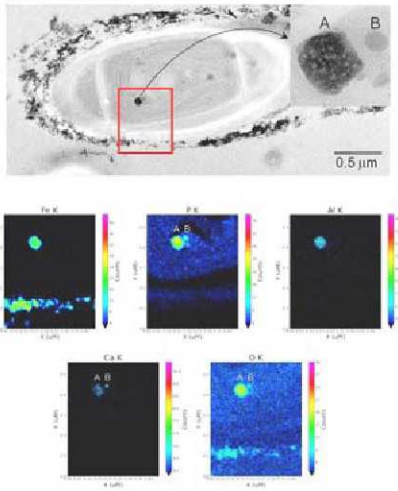
Inorganic polyphosphates (PPi) are known to increase the viability of prokaryotes under heavy metal concentrations and UV stress conditions [10, 11]. PPi have also been proposed as biosignatures [12]. Ancient CB could have also been stressed by occasional migrations from the Fe^{2+}-rich Ocean to the basaltic land which was almost devoid of dissolved Fe^{2+}. Thus, the study of the adaptation reactions of siderophilic CB to fluctuation of dissolved *Fe level* may shed light on the paleophysiology of ancient oxygenic prokaryotes. Moreover, bioweathered Fe, Al, P, Cu, Ti and rare earth elements can be thought of as candidate organomarkers that document the effects of organic molecules in weathered rocks. However, the molecular mechanisms of the maintenance of Fe homeostasis in siderophilic CB, the role of PPi for this process and bioweathering activities are poorly understood.

Here we present preliminary results describing a new mechanism of Fe mineralization in siderophilic CB, the effect of Fe on the generation of PPi bodies in siderophilic CB, their bioweathering activity and preliminary analysis of the diversity of proteins involved in the prevention of oxidative stress in phototrophs inhabiting IDHS.

Material and methods: CB and environmental DNA studied were isolated from IDHS located in the Greater Yellowstone Area (USA). The Fe mineralization process by CB cultures and their bioweathering activity were studied *in vitro* using TEM, SEM and EDS analysis. The genome of *Cyanobacterium* JSC-1 is currently being sequenced in collaboration with the DOE Joint Genome Institute.

Results: *Formation of Fe-Oxides*. The cultivation of three siderophilic CB with 0.6 mM Fe^{3+} led to the formation of extracellular Fe-Oxide nanoparticles (typically < 50 nm in size) and intracellular Fe-rich particles (Fig. 1). The non-siderophilic CB *Synechocystis* sp. PCC 6803 neither accumulated bulk Fe precipitate on the cellular sheath nor generated intracellular Fe-rich particles. In a medium with low [P], which is typical in IDHS such as Chocolate Pots (CP) Hot Spring in Yellowstone National Park, the external Fe-oxides have a texture (fine grain/amorphous to poorly crystalline) and composition (Fe, O) consistent with ferrihydrite (Fig.1). Intracellular Fe-rich particles are also amorphous but contain additional elements including P, Al and Ca (Fig.1).

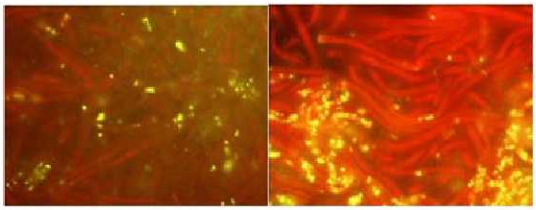
Fig. 1. Extra- and intracellular Fe-oxide particles associated with siderophilic CB JSC-1. *Top*: TEM view of a single cell encased in electron dense Fe-Oxides. Two intracellular components are highlighted in the ROI (red box): (A) intracellular Fe-oxide; and, (B) a spatially related internal body. *Lower*: Element maps for Fe, P, Al, Ca, O of the ROI showing the extracellular Fe-oxides are essentially free of P while the internal Fe-oxide (A) contains P, Al, and Ca and the associated body (B) is composed of P, Ca, and O.



We suggest the Fe-rich particles located within the cells are biogenic in nature and that those Fe-oxides located on the external sheaths have an inorganic ori-

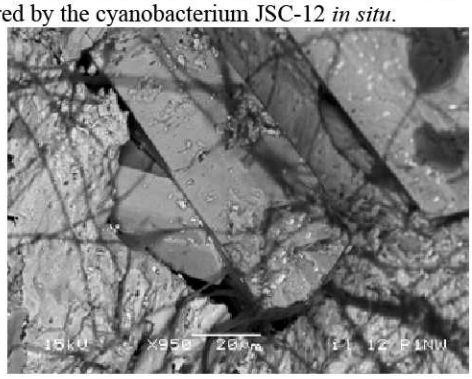
gin. However, they could be subjected to further modification by microbial cells, *e.g.* phosphorous leaching.

Fig. 2. The effect of Fe on the generation of polyphosphate bodies (PPB) in siderophilic CB JSC-1. *Left*: No added Fe; *Right*: 600 µM Fe.



PPi analysis. Fe stimulated the generation of PPi bodies in JSC-1 when the medium had low [P] (0.04 mM) (Fig.2). Iron did not seem to affect the number of PPi bodies in cells of *Synechocystis* sp. PCC 6803. The different reactions of siderophilic and non-siderophilic CB could be explained by the fact that *Synechocystis* sp. 6803 has only one type of PPK while the JSC-1 genome has 4 paralogous ORFs predicted to encode this enzyme (not shown). These observations suggest that fossils of ancient siderophilic CB [13], which existed before the Great Oxidation Event, [14] might be richer with ferric phosphates than their descendants inhabiting Fe^{2+} poor water bodies.

Fig. 3. SEM micrograph of natural sample of ilmenite (FeTiO3) with imbedded crystal of rutile (TiO2) weathered by the cyanobacterium JSC-12 *in situ*.



SEM-EDS studies of the interaction of siderophilic cyanobacteria with Fe-rich minerals and rocks revealed, for the first time, their ability to leach ilmenite, olivine, *FeS*, *ZnS* and ferrosilicates, perhaps because the cyanobacteria studied can secrete *2-oxo-glutarate* and *malate* which possess chelating properties (not shown).

Genome analysis. Comparative analysis of the genes encoding enzymes predicted to maintain intracellular Fe homeostasis in siderophilic CB and several non-siderophilic CB revealed that siderophilic *Cyanobacterium* JSC-1 possesses 2 proteins containing the Bacterioferritin (Bfr) domain, 4 proteins belonging to the DNA-binding ferritin-like protein (Dps) super family and 4 proteins belonging to the Polyphosphate kinase (PPK) family. CB *Synechococcus* sp. JA-2-3B'a (2-13), which inhabits hot springs with low and high [Fe], possesses 1 protein containing the Bfr domain and 1 PPK but 3 proteins belonging to the Dps superfamily. In contrast, such non-siderophilic CB as *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 possess only 1 copy of the genes encoding proteins belonging to Bfr domain, Dps superfamily and PPK family.

Conclusion: We suggest that Fe-rich particles located within the siderophilic CB cells are formed biologically. These results suggest that siderophilic CB use phosphates for internal Fe sequestration and that these FeOxP can be defined as useful biosignatures. In addition, significant differences are apparent between a set of proteins involved in the maintenance of Fe homeostasis and oxidative stress protection in siderophilic and non-siderophilic CB. Further comparative analyses of IDHS metagenomes and the genomes of siderophilic CB versus non-siderophilic ones may determine the link between physical and molecular signatures. Finally, the ability to leach *Fe*-rich minerals could have supported the expansion of ancient CB onto basaltic land.

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