INFERRING PROPERTIES OF ANCIENT CYANOBACTERIA FROM BIOGEOCHEMICAL ACTIVITY AND GENOMES OF SIDEROPHILIC CYANOBACTERIA.
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Introduction: Interrelationships between life and the planetary system could have simultaneously left landmarks in genomes of microbes and physicochemical signatures in the lithosphere. Verifying the links between genomic features in living organisms and the mineralized signatures generated by these organisms 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 probably Mars in association with hydrothermal activity [2,3]. IDHS also seem to be appropriate models for studying certain biogeochemical processes that could have taken place in the late Archean and/or early Paleoproterozoic eras [4, 5].

It has been suggested that inorganic polyphosphate (PPi), in chains of tens to hundreds of phosphate residues linked by high-energy bonds, is environmentally ubiquitous and abundant [6]. Cyanobacteria (CB) react to increased heavy metal concentrations and UV by enhanced generation of PPi bodies (PPB) [7], which are believed to be signatures of life [8]. However, the role of PPi in oxygenic prokaryotes for the suppression of oxidative stress induced by high Fe is poorly studied.

Here we present preliminary results of a new mechanism of Fe mineralization in oxygenic prokaryotes, the effect of Fe on the generation of PPi bodies in CB, as well as preliminary analysis of the diversity and phylogeny 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. The Fe mineralization process by CB cultures was studied *in vitro* using field-emission STEM. Genomic and metagenomic results were obtained by standard methods.

Results: The cultivation of three siderophilic (Fe loving) CB with 0.6 mM Fe^{3+} led to the generation of extracellular FeOx precipitate 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 Hot Spring, Yellowstone National Park (YNP), the external Fe precipitate was mostly composed of FeOx, while intracellular particles were mainly composed of amorphous ferric phosphate with inclusions of Ca and Al. TEM/EDS study of Fe precipitate generated in sterile medium of similar composition revealed that this Fe precipitate was richer in P than the extracellular precipitate on CB cells (not shown). This indicates that siderophilic CB may extract P from precipitated ferric phosphates.

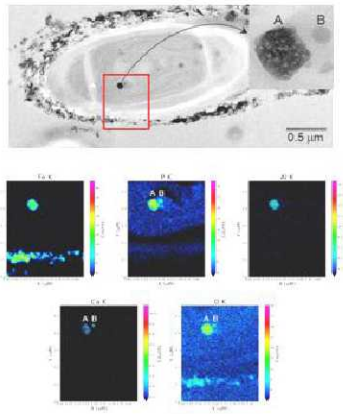

Fig. 1. Extra- and intracellular Fe-rich particles found in culture of siderophilic CB JSC-1. TEM sample was not stained with Os. Mineralogical information was obtained using selected area electron diffraction (SAED). Quantitative 2-D maps were collected for elements $8 \leq Z \leq 26$, at a spatial resolution of < 100 nm. Upper panel: A – intracellular iron rich phase; colored panels reflect relative quantity Fe, P, Al, Ca and O outside and inside of JSC-1 cells.

Thus, the results obtained suggested that both extracellular and intracellular Fe rich particles found in siderophilic CB have a biogenic origin but are distinctive in mineral composition. Further studies on the diagenesis of FeOxP generated by siderophilic CB will show whether these minerals can be used as the signatures of siderophilic CB.

We also carried out a comparative analysis of the abundance of the proteins dealing with the maintenance of intracellular Fe homeostasis in organisms from an IDHS and two non-Fe depositing hot springs.

It was found that CB are the most abundant organisms in the bacterial community in Chocolate Pots (CP), an IDHS where the [Fe] is about 77 µM. On the other hand, the fraction of oxygenic phototrophs in Octopus (OS) and Mushroom (MS) springs, where the Fe concentrations are 300 to 700 times lower, was less than the fraction of anoxygenic phototrophs. This result suggests that the genomes of siderophilic CB posses specific molecular features which make them a dominant group in IDHS and probably in the late Archean ocean. This observation should be taken into account in discussing the role of both oxygenic and anoxygenic phototrophs in the generation of global biosignatures such as Banded Iron Formation.

It was also found that *Synechococcus* sp. JA-2-3B'a (2-13), which has been sequenced recently [9], was dominant in a CB mat isolated from CP. This species is considered as the most deeply rooted organism in CB phylogeny [9]. Metagenomic analysis also revealed the presence of another relatively deeply rooted CB clone SM1F09 in this mat. Thus, one may speculate that molecular features of deeply rooted species of siderophilic CB help them withstand very high concentrations of Fe^{2+} and oxidative stress it stimulates [10].

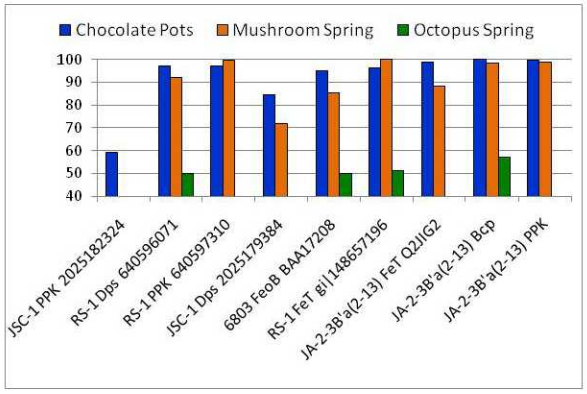

Fig. 2. Percent of identity of proteins involved in the maintenance of Fe homeostasis in organisms found in IDHS to metagenomes of CB mats in Chocolate Pots, Mushroom and Octopus hot springs. Proteins with identity above 50% were used for the analysis. Abbreviations are explained in the text.

Comparing the identity of proteins found in the genomes of some phototrophic inhabitants of IDHS to the metagenomes for CP, MS and OS generated a mosaic picture (**Fig. 2**). The sequences of proteins such as DNA-binding ferritin-like protein (**Dps**), Polyphosphate kinase (**PPK**) and Ferric ABC transporter permease (**FeT**) from *Roseiflexus* sp. RS-1 have similar identity to proteins in metagenomes from CP and MS but not for OS. **FeT** and **PPK** found in the genome of *Synechococcus* sp. JA-2-3B'a (2-13) gave very high identities to CP and MS metagenomes but were not found in OS metagenome. However, the identity of bacterioferritin comigratory protein (**Bcp**) in *Synechococcus* sp. JA-2-3B'a(2-13) genome was close to 100% in CP and MS metagenomes but below 60% in OS metagenome. Proteins with similar role for Fe homeostasis from 5 non-siderophilic CB displayed less than 50% identity to all metagenomes studied. Surprisingly, ferrous iron transport protein B (**FeoB**) from *Synechococcus* sp PCC 6803 displays substantial identity to CP and MS metagenomes (**Fig.2**). These observations may suggest that physico-chemical parameters in each spring studied render specific sets of proteins to maintain Fe homeostasis in organisms inhabiting those springs.

Since PPi bodies in CB are supposed to contribute to Fe homeostasis we studied the effect of Fe on the number and stability of PPi bodies in siderophilic CB JSC-1 and non-siderophilic *Synechocystis* sp. PCC 6803. It was found that Fe stimulates the generation of PPi bodies in JSC-1 when the medium had low [P] (0.04 mM) (**Fig.3**) and preserved PPBs degradation in JSC-1 cells transferred to a medium without added P (not shown). Iron did not seem to affect the number of PPBs in cells of *Synecocystis* sp. PCC 6803. The different reactions of siderophilic and non-siderophilic CB could be explained by the fact that *S.* sp. 6803 has only one type of PPK while JSC-1 has 4 variants of this enzyme (not shown). These observations suggest that fossils of ancient siderophilic CB, which existed before Great Oxidation Event [11] might be richer with ferric phosphates than their descendants inhabiting Fe^{2+} poor water bodies.

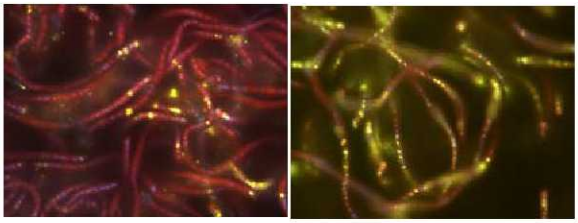

Fig. 3. Fe (600 µM) effect on the generation of polyphosphate bodies (PPB) in siderophilic CB JSC-1. PPB were stained with DAPI and displayed by fluorescent microscopy.

Conclusion: Our studies revealed significant differences between sequences of proteins involved in the maintenance of Fe homeostasis and oxidative stress protection in siderophilic and non-siderophilic CB. The results suggest that siderophilic CB use phosphates for Fe sequestration in the cells of CB and that these FeOxP can be defined as potential biosignatures. Comparative analyses of metagenomes and the genomes of siderophilic CB with non-siderophilic ones may link physical signatures to their molecular determinants.

References: [1] Pierson B.K. and Parenteau M.N. (2000) FEMS Microb. Ecol., 32, 181-196 . [2] Hausrath E.M.et al. (2008) Astrobiology, 8, 1079-1092. [3] Allen C.C. and Oehler D. (2008) Astrobiology, 8, 1093-1112. [4] Nisbet E.G., Sleep N.H. (2001) Nature, 409, 1083-1091. [5] Shi T., Falkowski P.G. (2008) PNAS, 105, 2510-25. [6] Brown M.R.V. and Kornberg A. (2004) PNAS, 101, 16085-16087. 15. [7] Seufferheld M.J. et al. (2008) AEM, 74, 5867-5874. [8] Douglas S. et al. (2008) Icarus, 90, 2620-636. [9] Bhaya D. et al. (2007) ISME J. 8, 703-713. [10] Shcolnick S. et al. (2009) 150, 2045-56. [11] Anbar A.D. et al. (2007) Science, 317, 1903-1906.