MICROBIAL DIVERSITY IN SURFACE IRON-RICH AQUEOUS ENVIRONMENTS: IMPLICATIONS FOR SEEKING SIGNS OF LIFE ON MARS. I.I. Brown[1], C.C. Allen[2], S. G. Tringe[3], C. G. Klatt[4], D.A. Bryant[5], S.A. Sarkisova[1], D.H. Garrison[1] and D.S. Mckay[2]. [1]SARD/JSC, Mail Code: JE 23, ESCG, P. O. Box 58447, Houston, TX. [2]NASA JSC, Mail code KA, 2101 NASA Road One, Houston, TX, 77058. [3]DOE Joint Genome Institute. [4]Montana State University. [5]The Pennsylvania State University.

Introduction: The success of selecting future landing sites on Mars to discover extinct and/or extant extraterrestrial life is dependent on the correct approximation of available knowledge about terrestrial paleogeochemistry and life evolution to Martian (paleo) geology and geochemistry.

It is well known that both Earth and Mars are Fe rich. This widespread occurrence suggests that Fe may have played a key role in early life forms, where it probably served as a key constituent in early prosthetic moieties in many proteins of ancient microbes on Earth and likely Mars. The second critical idea is the premise that Life on Mars could most likely have developed when Mars experienced tectonic activity [1] which dramatically decreased around 1 bln years after Martian creation. After that Martian life could have gone extinct or hibernated in the deep subsurface, which would be expensive to reach in contrast to the successful work of Martian surface rovers.

Here we analyze the diversity of microbes in several terrestrial Fe rich surface environments in conjunction with the phylogeny and molecular timing of emergence of those microbes on Earth. Anticipated results should help evaluate future landing sites on Mars in searches for biosignatures.

Material and methods: Bacterial diversity in Chocolate Pots iron depositing hot spring (CP IDHS) was evaluated by clone library construction as well as by metagenomic analysis. Microbial diversity in the Rio Tinto River is discussed on the base of relative publications.

Results: Among contemporary environments, iron-depositing hot springs may represent the most appropriate natural models [2] for insights into microbial diversity, since life may have originated on Earth and possibly Mars in association with high concentration of Fe^{2+} [3-7] and hydrothermal activity [8]. This hypothesis received additional support from recent discovery of remnants of hydrothermal springs on Mars [9]. The detection of hydrothermal activity on Mars is extremely significant since these environments could represent ideal habitats for microorganisms that obtain their carbon and energy from inorganic sources and light. They might host extant life as well as the fossilized traces of its ancestors.

The ability to oxidize Fe is broadly distributed among prokaryotes, but only phototrophic prokaryotes reduce CO_2 using Fe^{2+} as a reductant which leads to the generation of biomolecules and ferrihydrite [10]. This process can be accompanied by Fe isotope fractionation [11] which provides a biosignature. Ferrihydrates produced by phototrophs are represented by goethite and/or hematite [12]. Consequently, amorphous and crystalline ferrihydrates, including hematite, can mineralize and preserve microfossils and physical biomarkers [13]. It was also found that cyanobacteria and different thermophilic phototrophs oxidize Fe^{2+} in IDHS producing different forms of FeOx [14, Brown et al, this meeting]. This finding is very important for future missions to Mars because of significant progress in the identification of preserved remnants of cyanobacteria [15].

Thus, our first conclusion is that remnants of thermal springs associated with FeOx deposits might be an appropriate environment for identifying signatures of life.

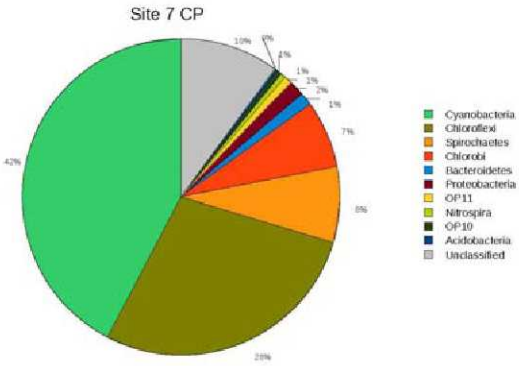

Fig. 1. Microbial diversity in a cyanobacterial mat determined by 16S clone libraries in IDHS Chocolate Pots (Yellowstone National Park, WY, USA). T- 52°C, $[Fe^{2+}]$ – 77 μM, pH – 5.7.

The highly acidified Rio Tinto River (Iberian Belt, Spain) has also been proposed as an analog to possible habitable environments on early Mars [16].

What environment (Fe rich near-neutral vs. Fe rich with very low pH) is more promising for the search of extinct or extant life on Mars?

Chocolate Pots (CP) IDHS is mainly populated with prokaryotic phototrophs, including membersof the Cyanobacteria, *Chloroflexi* and *Chlorobi*. The latter two phyla include are well-known oxidizers of Fe^{2+} [17, 18]. However, CP is nearly completely devoid of

α- and γ-proteobacteria (Fig. 1). *Candidatus* Chloroacidobacterium thermophilum (*Acidobacteria*) [19], which is likely involved in Fe^{2+} oxidation, also occurs within this community.

In contrast microbial communities in the Rio Tinto River are mainly represented by eukaryotic algae such as *Bacillariophyta, Chlorophyta* and *Euglenophyta* and chemolithoautotrophic α-, γ-proteobacteria, *Nitrospirae* and *Bacilli* [20; 21, 22] (Fig.2). None of those species have been shown to carry out Fe mineralization. Moreover, microbial fossils in Rio Tinto river should be hydrolyzed very quickly because of the very low pH.

Paleobiological data, combined with recent "tree of life" interpretations, suggest that phototrophic eukaryotes evolved no earlier than 2.5 – 2.8 Gy after Earth's accretion (4.6 Ga), while cyanobacteria and /or their iron-tolerant predecessors evolved between 1 - 1.5 Gy after accretion [23].

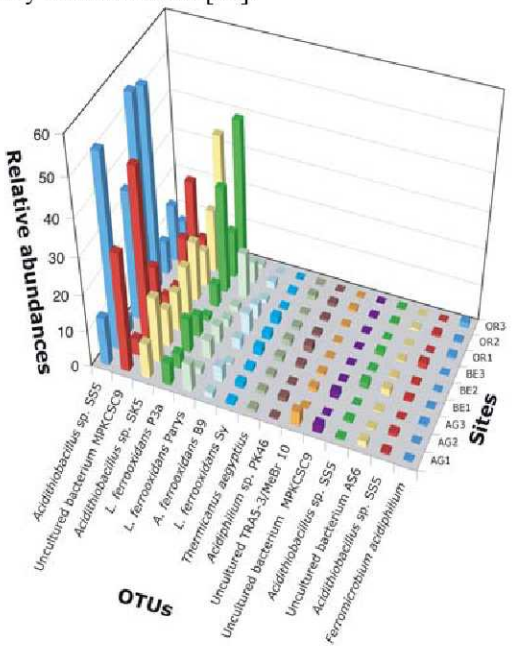

Fig. 2. Relative abundances of dominant OTUs at Rio Tinto River study sites. (Reprinted from [22]).

Conclusion: The facts and/or hypothesis which should be considered during the selection of future landing sites to search for signatures of Martian life are: 1) terrestrial life emerged in warm, near-neutral, Fe^{2+}-rich ocean or terrestrial springs; 2) microbial life on Mars' surface could have been common for more than 1-1.5 Gy after Mars' accretion (also 4.6 Ga), after which life may have retreated under ground as surface water dried up; 3) near-neutral wet environments prevailed at approximately this time, as suggested by recent multispectral mapping of Mars [24]; 4) thermal springs on Mars have been found; 5) microbial lineages, especially phototrophs, found in near neutral IDHS emerged on Earth significantly earlier that the lineages found in Rio Tinto River.

Therefore, we believe that near neutral IDHS such as Chocolate Pots should be considered appropriate analogs to ancient living environments for life in the Martian surface. Expected mineralogy might include iron oxides precipitated or assisted by microbial activity, including magnetite and ferrihydrite, both of which are known to be associated with siderophilic bacteria on earth. By analogy with terrestrial hot springs, silica-rich opal-like deposits, carbonates, and evaporates such as gypsum might also be expected [9] Such sites may be identified in the future, based on high-resolution orbital images and geologic mapping [9], combined with the identification of characteristic hot spring minerals based on orbital spectroscopy. Identifying remnants of IDHS in the Martian surface holds great promise for the success of future missions to search for signatures of life on Mars.

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