

Studying prokaryotic communities in iron depositing hot springs (IDHS): implication for early Mars habitability.

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Introduction: The success of selecting future Martian landing sites to discover extinct and/or extant extraterrestrial life is strongly dependent on the extent to which ancient and/or present habitable zones may have existed or presently exist on Mars. It has been suggested that early Mars could have been supportive for life since it was wet and warm [1, 2] similar to conditions on early Earth. Moreover, Mars is also rich with iron, a key element for the majority of biochemical pathways in terrestrial organisms [3]. The recent discovery of thermal springs remnants on Mars [4] gave additional support to the hypothesis that life may have originated on Earth, and possibly on Mars, in association with hydrothermal activity and high [Fe²⁺] [5, 6]. However, it is still not clear how an iron-rich thermal hydrosphere is hospitable to microbes because reduced iron appears to stimulate oxidative stress in all domains of life [7] and first of all in oxygenic phototrophs [8]. Therefore, the study of microbial diversity in iron-depositing hot springs (IDHS), the biogeochemical activity of these microbes and the mechanism of oxidative stress suppression may help to define purported habitable zones on Mars.

Here we describe (1) physico-chemical parameters in Chocolate Pots IDHS, Yellowstone National Park (YNP); (2) microbial diversity in that spring; (3) preliminary analysis of the diversity of the proteins involved in the suppression of oxidative stress in thermophilic microbes, and (4) iron mineralization by iron-tolerant CB. These preliminary results have important implications in defining potential habitable zones on early Mars.

Main results. The analysis of CP microbiome revealed that oxygenic prokaryotes, CB, were the main group among different inhabitants of Chocolate Pots (CP) IDHS despite the concentration in the spring source being [Fe²⁺] = 70 uM. Two deeply rooted cyanobacterial species *Synechococcus* sp. JA-2-3B'a (2-13) and clone SM1F09 (GenBank access # AF445691) were dominant in CP. Thus, we suggest molecular features of deeply rooted species of iron-tolerant CB help them withstand very high [Fe²⁺] and the resulting oxidative stress. Comparative analysis of microbiomes in both Chocolate Pots IDHS and low iron White Creek hot spring, also YNP, showed that the proteins predicted to maintain intracellular Fe homeostasis in freshwater and marine CB demonstrated a lower rate of identity and clustering with homologous proteins found in genomes of freshwater CB. In contrast, the proteins predicted to maintain Fe homeostasis genes found in White Creek spring where the Fe concentrations are 300 to 700 times lower, exhibited a higher level of identity and clustering with homologous proteins in fresh-water and marine CB. Thus, a well known system of Fe homeostasis in fresh water or marine CB seems to be different from homologous system in CB inhabiting IDHS and probably on early Earth and Mars.

The cultivation of three siderophilic CB with 0.6 mM Fe³⁺ led to the formation of extracellular Fe-oxide nanoparticles (typically < 50 nm in size) and intracellular FeOxP particles. The non-siderophilic CB *Synechocystis* sp. PCC 6803 neither accumulated bulk Fe precipitate on the cellular sheath nor generated intracellular Fe-rich particles. These results suggest that siderophilic CB use phosphates for internal Fe sequestration.

Conclusion: We speculate that both external and intracellular iron precipitate in iron-tolerant CB might be involved in oxidative stress suppression shown by [9]. Significant differences are apparent between a set of proteins involved in the maintenance of Fe homeostasis and oxidative stress protection in iron-tolerant and fresh-water and marine CB. Correspondingly, these properties may help to make iron-tolerant CB as dominant organisms in IDHS and probably on early Earth and Mars. Further comparative analyses of hot springs metagenomes and the genomes of iron-tolerant microbes versus fresh-water/marine ones may point out to different habitable zones on early Mars.

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