

## Metagenomic evidence for microbial Fe(II) oxidation in iron mats at the Kallisti Limnes CO<sub>2</sub>-rich seafloor hydrothermal pools, Santorini

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Combined metagenomic, FeOx-microfossil and geochemical evidence for microbially-driven Fe(II) oxidation involving the zetaproteobacterium *Mariprofundus ferrooxydans*, in marine hydrothermal Fe-rich ecosystems, is rare. Here we present, combined shotgun metagenomics analysis of pool water and Fe-mat, indicating that FeOx-biominerals of Fe(-Si)-mats in the Kallisti Limnes (KLI) low-temperature, CO<sub>2</sub>-rich, hypoxic pools, of the Santorini caldera seafloor hydrothermal field<sup>1</sup>, are directly related to the metabolic activity of Fe-oxidizing *M. ferrooxydans*. This is confirmed by: (i) The abundance of the molybdopterine oxidoreductase encoding genes, which are important in the Fe(II) oxidation by *M. ferrooxydans*<sup>2</sup> and (ii) The sequences of the abundant cbb3-type and aa3-type cytochrome c oxidase encoding genes, that are involved in aerobic respiration and facilitate Fe(II) oxidation, which were related to the *Mariprofundus* genus. Moreover, the KLI metagenomes featured full sets of genes encoding the bc1 cytochrome and the Mtr protein complexes that several FeOB employ to transfer the electrons gained by Fe(II) oxidation, across the periplasm. These are supported with the presence of FeOx minerals, morphologically consistent with known Fe-(bio)oxyhydroxides<sup>1</sup>, and the dissolved KLI fluid micromolar O<sub>2</sub> concentration<sup>1</sup>, which agrees with O<sub>2</sub> growth level at which *M. ferrooxydans* PV-1 has been found in the lab to outpace abiotic Fe(II)-oxidation<sup>3</sup>. KLI, may constitute potential analog for geobiological formation mechanisms of Fe-rich chemical sediments in the rock record<sup>4</sup>.

[1] Camilli *et al* (2015) *Sci. Rep.* **5**, 12152 [2] Singer *et al* (2013) *Front Microbiol* **4**, 1–9 [3] McAllister *et al* (2019) *FEMS microbiology ecology* DOI: 10.1101/416842 [4] Chan *et al.* (2016) *Geobiology* **14**, 509-528.