Shotgun metagenomics from Monte Cristo cave (Brazil) reveals microbial metabolic potential related to iron and manganese biogeochemical cycles

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Abstract

Caves are among the most singular and understudied environments on Earth. Due to the harsh conditions observed in many caves, including scarcity of nutrients and low levels of light, these ecosystems are considered extreme environments (Gabriel and Northup 2012). Therefore, it may be worth paying special attention to the microbial communities existing in these unique systems. Previously, it has been suggested that the high levels of Manganese (Mn) and Iron (Fe) at Lechuguilla and Spider Caves in the United States may influence their microbial community structure in different ways (Northup et al. 2003, Carmichael and Bräuer 2015). In this context, caves are promising environments for investigating microbial functional capabilities in relation to these elements and the ecological interactions that allow these microbes to thrive. Monte Cristo cave (MCC) - in Diamantina, Brazil - was chosen for this study. The cave is located in a region known for historic mining activity and occurrences of Mn and Fe-rich rocks (Costa et al. 2003). With that in mind, our main goal is to investigate if within the microbial community of MCC there is evidence of taxa and genes associated with Fe and Mn metabolism. The samples were collected in 2018 from walls and saprolite deposits within MCC. Community DNA from three samples, P1b, P3 and P7, were independently sequenced using Illumina shotgun sequencing, and the data were analysed using conventional metagenomic pipelines and in-house python scripts. Taxonomic classification was assessed using Kraken2; Fe related genes with FeGenie; and Mn related genes were predicted using BlastP against a collection of manually curated Mn-oxidizing proteins. Environmental Mn and Fe concentrations were measured using ICP-OES. Our results suggest the presence of a microbial community potentially able to change Fe and Mn redox states. In sites P1b and P7, genes associated to Fe and Mn oxidation were identified, Fig. 1. Taxonomic

evidence for these metabolisms includes the presence of the taxa Comamonadaceae and Hyphomicrobiaceae, both families that were previously reported to harbour species able to oxidize Mn and Fe (Spring and Kämpfer 2015, Carmichael and Bräuer 2015). Our analysis also assigned contigs to the archaeal phyla Crenarchaeota, Euryarchaeota and Thaumarchaeota, whose presence has been associated with oligotrophic caves where archaea play a role in primary production (Ortiz et al. 2013) (Fig. 2). Moreover, the phylum Euryarchaeota harbours members that use Fe or Mn as electron acceptors during methane oxidation (Ettwig et al. 2016). Our results therefore contribute to understanding how microbial communities of MCC may be playing a role in the biogeochemical cycles of Fe and Mn under the conditions imposed by the subterranean environment, which might reflect similar processes in other caves yet to be explored by a metagenomics approach.

Keywords

molecular ecology, bioinformatics, mn-oxidizing, biogeochemisty

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Conflicts of interest

The authors have declared that no competing interests exist.

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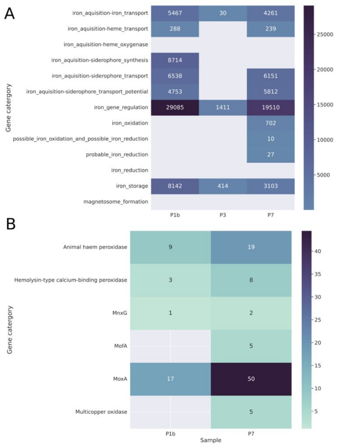

Figure 1.

Gene prediction for (A) iron metabolism and (B) manganese oxidation for all assembled contigs.

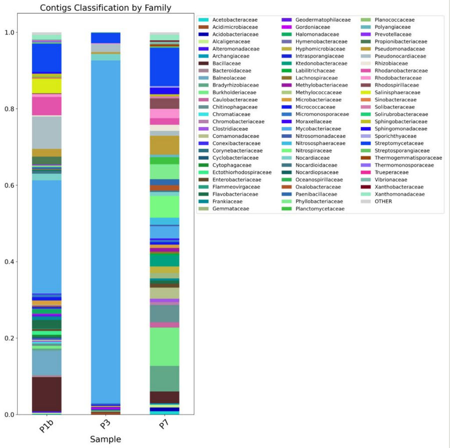

Figure 2.

The taxonomic classification was done using KRAKEN2 in all assembled contigs at the family level. We observed 67 classified families, 28 were exclusive to sample P7, and 7 to sample P1b.