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Metagenomic signatures of a tropical mining-impacted stream reveal complex microbial and metabolic networks

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HIGHLIGHTS

- The microbiome of a metal-rich tropical stream sediment was studied by metagenomics.
- The metagenome showed complexity of microbial interaction and metabolic processes.
- Functional analysis highlighted nitrogen and methane metabolic pathways.
- Metal stress seems to contribute to distinct life strategies in this environment.

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ABSTRACT

Bacteria from aquatic ecosystems significantly contribute to biogeochemical cycles, but details of their community structure in tropical mining-impacted environments remain unexplored. In this study, we analyzed a bacterial community from circumneutral-pH tropical stream sediment by 16S rRNA and shotgun deep sequencing. Carrapatos stream sediment, which has been exposed to metal stress due to gold and iron mining (21 [g Fe]/kg), revealed a diverse community, with predominance of Proteobacteria (39.4%), Bacteroidetes (12.2%), and Parcubacteria (11.4%). Among Proteobacteria, the most abundant reads were assigned to neutrophilic iron-oxidizing taxa, such as *Gallionella*, *Sideroxydans*, and *Mariprofundus*, which are involved in Fe cycling and harbor several metal resistance genes. Functional analysis revealed a large number of genes participating in nitrogen and methane metabolic pathways despite the low concentrations of inorganic nitrogen in the Carrapatos stream. Our findings provide important insights into bacterial community interactions in a mining-impacted environment.

1. Introduction

Freshwater ecosystems are threatened by a myriad of pollutants, especially those generated by mining activities. Among these, iron ores mining is of great importance since iron is the most mined metal worldwide (Edwards et al., 2004). Brazil is one of the world's largest producers of iron ores (Yellishetty et al., 2010), which considerably impact Brazilian ecosystems.

Iron exists in different oxidation states, but in biological systems, it can be found as Fe(II) or Fe(III). Redox cycling of iron in aquatic ecosystems can be closely tied to biogeochemical transformations of other elements, including carbon, nitrogen, and sulfur. In addition, iron cycling is important for pollutant transformation and mobility, meaning great environmental relevance of iron (Ghiorse, 1984; Lovley, 2000; Emerson and Weiss, 2004; Picardal and Cooper, 2005; Roden and Emerson, 2007).

Iron redox reactions are often driven by microorganisms. Fe-reducing bacteria (FeRB), e.g., *Geobacter sulfurreducens*, couple reduction of Fe(III) with oxidation of organic matter or molecular hydrogen, while Fe-oxidizing bacteria (FeOB), which occur in many phyla (e.g., Nitrospirae, Firmicutes, Actinobacteria, Chlorobi, and

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especially Proteobacteria), can use Fe(II) or molecular hydrogen as electron donors (Lovley et al., 1989; Jiao et al., 2005; Emerson et al., 2010; Byrne et al., 2015).

Recent advances in metagenomic technologies allow for more comprehensive assessment of the microbial biodiversity and functioning in aquatic environments. Sediments are important for metal cycling in these ecosystems because they can retain over 90% of metals and metalloid pollutants and have higher microbial diversity than bulk water (Lozupone and Knight, 2007; Mortatti et al., 2010).

To our knowledge, only a few other studies that involved pyrosequencing and denaturing gradient gel electrophoresis have shown the structure of microbial communities in iron-rich environments at circumneutral pH (Gorra et al., 2012; Wang et al., 2014; Emerson et al., 2015). Although FeOB are present under oxic and anoxic conditions, from extremely acidic to circumneutral pH, only a few genera of neutrophilic FeOB have been identified to date, e.g., *Gallionella*, *Sideroxydans*, *Ferriphaselus*, *Ferritrophicum*, and *Mariprofundus* (Emerson et al., 2010; Kato et al., 2014). Therefore, iron-rich sediments of tropical freshwater streams are not sufficiently explored.

In this study, we performed a taxonomical and functional analysis of a microbial community from mining-impacted stream sediment by means of metagenomics and compared our results with different datasets in order to improve the current understanding of the role of bacteria in iron-rich environments.

2. Materials and methods

2.1. The study site

The Carrapatos stream (CS; 19°58′15.4″S and 43°27′50.7″W) is located in the Iron Quadrangle (Minas Gerais state, Brazil), a region rich in ores, especially iron ones, explored by mining since the 19th century. One study (Reis et al., 2014) showed that CS can be classified as a mesotrophic, circumneutral (pH 7.9), and mesothermal (17 °C) environment. Furthermore, its sediment contains high metal concentrations, including magnesium (1416 mg kg^{-1}), manganese (2319 mg kg^{-1}) and especially iron (21,850 mg kg^{-1}) (Reis et al., 2014).

2.2. DNA extraction

A composite sample from CS sediment (100 g) was collected, and DNA was extracted using the PowerSoil DNA Extraction Kit (MoBio Laboratories, USA). Quantification of total DNA was performed using a Qubit fluorometer (Invitrogen–Life Technologies, USA).

2.3. Sequencing and analysis of the V3 and V4 regions of the 16S rRNA gene

For taxonomic profiling, primers S-D-Bact-0341-b-S-17 (5′CCTACGGGNGGCWGCAG3′) and S-D-Bact-0785-a-A-21 (5′GACTACHVGG-GTATCTAATCC3′) (Klindworth et al., 2013), targeting the V3 and V4 hypervariable regions of the bacterial 16S rRNA gene were used, with Illumina adapters added. The amplification step was performed with the KAPA HiFi HotStart ReadyMix (KAPA, Woburn, MA, USA), followed by purification with AMPure XP beads (Agencourt Bioscience, Beverley, MA, USA). Library construction was performed according to the manufacturer's instructions for paired-end sequencing on the MiSeq platform (Illumina, Inc., USA).

Bioinformatic analysis was carried out in the Mothur software, version 1.33.0 (http://www.mothur.org). Sequences of low quality ($Q \leq 20$, with ambiguities and homopolymers above eight) or outside the range of 422–466 bp were discarded. Filtered reads were aligned and classified against the Silva v.223 16S rRNA database (Quast et al., 2013). Mitochondrial and chloroplast reads and reads that did not match any reference sequence were discarded. Chimeric reads were identified and removed by means of Uchime (http://drive5.com/uchime). The remaining reads were grouped into operational taxonomic units (OTUs), with 97% similarity, by the average neighbor method. Construction of the rarefaction curve, calculated for the evolutionary distance of 0.03, was conducted according to the Mothur pipeline (Schloss et al., 2009). The nucleotide sequences were deposited to GenBank [GenBank ID: SRR2087753].

2.4. Shotgun sequencing and analysis

To obtain a functional profile of the metagenome, whole-community shotgun sequencing from the CS sediment was performed on a SOLiD™ v.4 sequencer (Applied Biosystems), according to the manufacturer's protocol. Briefly, 10 μg of total DNA was randomly fragmented using the Covaris™ S2 system (Life Technologies, USA). DNA fragment library (200–250 bp long) was then constructed. Emulsion PCR was carried out to clonally amplify fragments on sequencing beads, followed by enrichment, deposition in a plate, and sequencing. The DNA fragment size of the library was verified using the Agilent 2100 Bioanalyzer.

Bioinformatic analysis comprised an initial step of error screening in the SOLiD™ Accuracy Enhancer Tool (SAET) software (http://solidsoftwaretools.com/gf) and conversion of sequences from *color space* to *letter space* format by means of the encodeFasta.py software (http://gnome.googlecode.com/svn/trunk/pyGenotypeLearning/src/pytools/encodeFasta.py). Filtered reads were assembled in the Metavelvet software, as described by Namiki et al. (2012). Contigs were analyzed by means of the Metagenomics RAST Server (MG-RAST v3.3) (Meyer et al., 2008), which provides quality control (duplicate removal and size or base quality screening) prior to annotation. Functional analysis was conducted using SEED and KEEG subsystems (available on the MG-RAST Server), with an e-value of 10^{-5} and identity of 60% (Mitra et al., 2011; Costa et al., 2015). Sequencing data of the present study are available at MG-RAST (code 4520540.3).

The metagenome from the CS sediment was compared with two other metagenomes, one from an arsenic-rich sediment (Mina stream sediment [MSS]: MG-RAST ID 4519449.3) and another from a low-metal-concentration freshwater sediment (Férin [FER]: NCBI Bioproject ID 246439), described by Costa et al. (2015) and Gillan et al. (2014), respectively. These metagenomes were chosen for the comparative analysis on the basis of the study region similarity with CS (MSS) and availability of pristine-environment data (FER). To identify possible significant differences with CS in the functional composition of each metagenome, we performed two-sided Fisher's exact test (Fisher, 1958), with the p value > 0.05, using the Statistical Analysis of Metagenomic Profiles (STAMP; Parks and Beiko, 2010).

Metagenomic recruitment plots were used to identify the abundant species in the CS metagenome, by comparing the CS contigs with individual bacterial genomes available in the National Center for Biotechnology Information (NCBI) database, as reported by Costa et al. (2015).

2.5. Quantitative real-time PCR (qPCR)

qPCR was used to estimate the absolute number of copies of bacterial 16S rDNA in the sediment. The primers were 338F (5′TACGGGAGGCAGCAG3′) (Raskin et al., 1994) and 518R