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## **Environmental Microbiology**

# Bacterial communities in mining soils and surrounding areas under regeneration process in a former ore mine $\stackrel{\mbox{\tiny $\infty$}}{}$

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#### ABSTRACT

Human activities on the Earth's surface change the landscape of natural ecosystems. Mining practices are one of the most severe human activities, drastically altering the chemical, physical and biological properties of the soil environment. Bacterial communities in soil play an important role in the maintenance of ecological relationships. This work shows bacterial diversity, metabolic repertoire and physiological behavior in five ecosystems samples with different levels of impact. These ecosystems belong to a historical area in Iron Quadrangle, Minas Gerais, Brazil, which suffered mining activities until its total depletion without recovery since today. The results revealed Proteobacteria as the most predominant phylum followed by Acidobacteria, Verrucomicrobia, Planctomycetes, and Bacteroidetes. Soils that have not undergone anthropological actions exhibit an increase ability to degrade carbon sources. The richest soil with the high diversity was found in ecosystems that have suffered anthropogenic action. Our study shows profile of diversity inferring metabolic profile, which may elucidate the mechanisms underlying changes in community structure in situ mining sites in Brazil. Our data comes from contributing to know the bacterial diversity, relationship between these bacteria and can explore strategies for natural bioremediation in mining areas or adjacent areas under regeneration process in iron mining areas.

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2

## **ARTICLE IN PRESS**

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#### Introduction

Brazil is one of the largest producers of iron ore along with China and Australia. Iron ore is a raw material used in the manufacturing of steel. A metal supplies the steel industry and is used in a variety of consumer products. Brazil has extracted 320 million tons of iron ore accounting for almost 10% of world production and feature extraction occurs in rocks and further processing of the ore because this material is mixed with other elements.<sup>1</sup> Increasingly, this feature has been exploited in the wild because of global demand, which is, in turn, controlled by population growth and intensification of industrial sectors. Mining activity is causing environmental change in the landscape, particularly by changing the vegetation cover and affecting soil and water from the mine boundary region.<sup>2</sup> Furthermore, the mining activities require an additional area to be used for disposal of waste, and years of intensification affect the quality of surrounding soils and aquifers and threaten human health and ecosystems with serious environmental impact.3-5

Many mining companies are aware of the detrimental effects of mining. Companies have invested research and technology into how to practice sustainable exploitation and environmental protection, but these efforts have been associated with an economically less costly production chain. In mining-contaminated regions, soil plays an important role in the transport and storing of contaminants. These characteristics determine the ecological balance and biodiversity of the ecosystem.<sup>6</sup> There is a consensus in the literature that metal-contaminated soil exhibits an extremely complex and well-adapted microbial community.<sup>7,8</sup> It should be noted that these communities play an important role in biogeochemical cycling and are involved in the transformation of nutrients such as N and C.<sup>8</sup>

From an environmental standpoint, mining practices are considered unsustainable due to the removal of natural resources until its total depletion (i.e., mineral resources are nonrenewable) and the high negative impact on ecosystems, especially on soil habitats, with the removal of soil layers.<sup>9</sup> Procedures to minimize the impacts of mining practices might include the maintenance of vegetation cover as well as preservation of soil macro and microfauna. Therefore, the use of practical tools (i.e., bioindicators) becomes crucial when evaluating biological processes and patterns that may indicate the current environmental condition of soil systems previously subjected to mining activities.<sup>10</sup>

Considering that microorganisms play an essential role in environmental biogeochemical cycling and may influence the speciation and bioavailability of metals, it is relevant to obtain more comprehensive knowledge of the taxonomic qualities and diversity of the prokaryotic community in metal-contaminated soil.<sup>11,12</sup> Although previous studies of the microbial community in metal-contaminated soil have been performed,<sup>3,13</sup> none of them have assessed the microbial community of a preserved mine area with five different soils, through taxonomic diversity and functional inference evaluation.

In this study, we assess the current environmental status of an area (Córrego do Meio Mine) subjected to several decades

(1946–2005) of mining activities. During this period, deeper layers of soil were removed for the extraction of iron in the area. The removed subterranean soil was accumulated on the surface forming piles of soil next to the open-pit (Fig. 1). In 2006, however, the Center for Research and Biodiversity Conservation of the Iron Quadrangle (CeBio) was established and the area is now under recovery. The CeBio comprises three different types of natural vegetation (i.e., rainforest, savannah and ironstone outcrops). Additionally, two impacted areas are currently under recovery with the introduction of input vegetation.

Monitoring soil quality is key to improving strategies for the recovery of mining areas. In this study, we based on 16S rRNA gene sequences using high-throughput DNA sequencing and a metabolic analysis to examine the taxonomic and infer functional composition of the bacterial community in an off mining area, which currently is in recovery, but during 30 years suffered with mining activities without regeneration process. The issue of this work is showing the bacterial diversity of fundamental importance to deepen our current understanding of microbial interactions in mining ecosystems in regeneration. In addition, the metabolic diversity of bacterial community present in mining sites makes these environments ideal for studies of genomes, ecology, evolution, tolerance mechanisms, and the interactions between bacteria and environmental factors.

### Materials and methods

#### Area of study

The study was conducted in the Córrego do Meio Mine area (CeBio), located near the city of Sabará, Minas Gerais State, in the southeast of Brazil (Fig. 1). The mean annual temperature in the region is 20.8 °C with a mean rainfall of 1500 mm. High yielding iron (Fe) deposits were extracted until mining activities ceased in 2005 due to low production. Based on the vegetation and soil substrate, the mining area (approximately 711 ha) is roughly categorized into five ecosystems: Atlantic semi-deciduous forest, neotropical savannah, ironstone outcrops, soil covered with eucalyptus trees and soil covered with grasses.

#### Soil sampling and identification

Sampling was carried out in September of 2013. Three replicate sampling plots were randomly established in the five ecosystems for the collection of soil samples (Fig. 1). These included three natural ecosystems with no level or low level of disturbance: Atlantic Semi-deciduous Forest (ASF), Neotropical Savannah (BCS) and Ironstone outcrops (SIC); and two disturbed areas: Eucalyptus planted area (EFS) and Grass landscape former Iron-tailing Mound (GIM), with medium/high and high impact, respectively (Table 1). The total sampled surface soil (0–20 cm in depth) was collected (Fig. 1) and packed in sterile plastic bags. Each bag was sealed and transported on ice to the laboratory for further study. Fractions of the samples were divided in two: one was used to study metabolic diversity and the other was stored at  $-80\,^\circ\text{C}$  for molecular analyses.

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