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Rhizobial characterization in revegetated areas after bauxite mining

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ABSTRACT

Little is known regarding how the increased diversity of nitrogen-fixing bacteria contributes to the productivity and diversity of plants in complex communities. However, some authors have shown that the presence of a diverse group of nodulating bacteria is required for different plant species to coexist. A better understanding of the plant symbiotic organism diversity role in natural ecosystems can be extremely useful to define recovery strategies of environments that were degraded by human activities. This study used ARDRA, BOX-PCR fingerprinting and sequencing of the 16S rDNA gene to assess the diversity of root nodule nitrogen-fixing bacteria in former bauxite mining areas that were replanted in 1981, 1985, 1993, 1998, 2004 and 2006 and in a native forest. Among the 12 isolates for which the 16S rDNA gene was partially sequenced, eight, three and one isolate(s) presented similarity with sequences of the genera *Bradyrhizobium*, *Rhizobium* and *Mesorhizobium*, respectively. The richness, Shannon and evenness indices were the highest in the area that was replanted the earliest (1981) and the lowest in the area that was replanted most recently (2006).

Introduction

Nitrogen-fixing bacteria are an extremely important group of microorganisms for various ecosystems because they promote the entry of nitrogen into the soil. The capacity to fix atmospheric nitrogen is widely distributed among microorganisms with different levels of phylogenetic relationships, including representatives of Archaea and Eubacteria. However, the capacity to fix atmospheric nitrogen and induce nodule formation in leguminous plants is restricted to members of the proteobacteria phylum.[1–4] Legume nodulating nitrogen-fixing bacteria, which are commonly known as rhizobia, are abundant in the soil of many ecosystems[5] and have a high diversity and variability regarding symbiotic efficiency.[6–8]

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The importance of symbiotic biological nitrogen fixation (BNF) in agricultural systems is well-documented in plant species such as soybeans, common beans and peanuts.[6,9,10] However, the role of this group of microorganisms in natural ecosystems is poorly understood.[11,12] Little is known about the contribution of the increased diversity of nitrogen-fixing bacteria to the productivity and diversity of plants living in natural communities. Melloni et al.[13] reported that a greater diversity of bacteria in the soil results in greater resilience of the system and that a higher diversity of legume nodulating bacteria can favor symbiosis with various leguminous plant species and maximize the biological fixation of nitrogen in degraded areas. Previous research by van der Heijden et al.[11] demonstrated that symbiotic nitrogen-fixing bacteria promote evenness, productivity and nitrogen capture in systems that are rich in leguminous species, which suggests that the presence of nodulating bacteria is necessary for different species of leguminous and non-leguminous plants to coexist.

Although mining activities generally alter a proportionally smaller area than other human activities, such as farming and planting pastures for livestock, the level of environmental degradation is very high because of the intense disturbance of the soil. This makes it necessary to take measures to restore these degraded areas at the end of the mining operations. In Brazil, to promote the rapid revegetation of highly degraded mined areas, the planting of leguminous species inoculated with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi has been successfully employed.[14] The planting of leguminous species with selected isolates of these microorganisms enables the initial colonization of substrates that have been subjected to high chemical, physical and biological degradation.[15] The colonization with legumes leads to the deposition of litter and increases the concentrations of nutrients in the soil surface, enabling the replanted sites to enter the initial stages of plant succession.[15]

Within this context and in this study, we assessed the diversity of these bacteria in areas that were revegetated after bauxite mining to better understand their role in degraded ecosystems under the recovery process. The areas studied were revegetated between 1981 and 2006 on soil consisting of overburden or tailings and used mixes of native species and inoculated leguminous species.

Materials and methods

Study area and collection procedure

The company Mineração Rio do Norte (MRN) operates the Saracá, Almeidas and Avisos mines (all within the Saracá-Taqüera National Forest, which is located in the municipality of Oriximiná, Pará state/Brazil, at 1°21′S – 56°22′W, 180 m elevation).[16] In these mines, ore is found at an average depth of 8 m and is covered by dense vegetation and a layer called overburden, which is composed of organic soil, nodular bauxite and ferruginous laterite. To mine the reserves, it is necessary to remove the overburden to reveal the economically exploitable bauxite ore. This operation is conducted sequentially in which the overburden is deposited in an adjacent pit that was previously mined. In these areas, the replanting is performed on the overburden. The bauxite ore is crushed, cycloned and filtered. At the end of this process, 27% of the solid mass is tailings, which are deposited in ponds.[16] These tailings pond areas are then revegetated when they become full.

The replanting of the overburden areas investigated in this study was conducted by a company using available seeds of various species (*Parkia multijiga*, *Parkia pendula*, *Parkia oppositifolia*, *Ormosia holerythra*, *Ormosia excelsa*, *Sclerolobium paniculatum* and *Acosmium nitens*) in 1981, 1985, 1993, 1998, 2004 and 2006. The revegetation of the two tailings ponds was conducted using the species *Acacia mangium* in 1993. In part of this area, the planting was conducted without rhizobia inoculation (Tailings Waste 1), and the other part with the rhizobia inoculation consisted of a mixture of all of the recommended rhizobial strains from Embrapa Agrobiologia (Tailings Waste 2).

In each area, twenty simple samples (0–0.20 m) were harvested to compose a compound sample in plots measuring 250 m^2. Nine compound samples were collected in 2007: six in plots that had been revegetated on overburden, two in tailings plots and one in a native forest plot (Table 1). The chemical analysis of the soil samples was conducted according to the Manual on Soil Analysis Methods.[17]

Obtaining the nodules and bacterial isolation

The experiment was conducted using sterilized Leonard jars containing sand and vermiculite at a proportion of 1:1 (v/v) and in a randomized block design with three repetitions. The treatments consisted of the inoculation of a suspension of soil from each plot on the host trap plants *Macroptilium atropurpureum* (siratro) and *Mimosa acutistipula*.

The seeds were treated with concentrated sulfuric acid (H_2SO_4) for 10 min to break the dormancy and with 30% hydrogen peroxide (H_2O_2) for 3 min for surface disinfection. They were then germinated in Petri dishes containing moistened filter paper and cotton. Three seedlings of each species were transplanted to each jar, and each seedling was inoculated with 1 mL of the soil suspension in saline solution. The suspension was prepared using 10 g of soil in 90 mL of NaCl solution (0.145 M) and kept under orbital agitation for 30 min.

The experiment lasted 90 days, during which the plants received water and a nutrient solution[18] intercalated every 15 days. To isolate the bacteria present in the nodules, they were washed in ethanol (70%, v/v – 1 min), externally disinfected with 30% hydrogen peroxide for 3 min, washed five times in sterile distilled water and crushed in Petri dishes containing YMA medium.[19]

Molecular characterization

The restriction analysis of the 16S rDNA gene was conducted according to Laguerre et al.[20] and Teixeira et al.[8] using *Hinf*I, *Msp*I and *Dde*I endonucleases. The DNA was extracted according to Doyle and Doyle[21] using the detergent CTAB. The 16S rDNA gene was amplified using the universal primers Y1 (5′-TGGCTCAGAACGAACGCTGGCGGC-3′) and Y3 (5′-CTGACCCCACTTCAGCATTGTTCCAT-3′).[22]

We selected isolates representing 12 distinct clusters in the ARDRA dendrogram for partial sequencing of the 16S