

TITLE: NITROGEN FIXING BACTERIA COMMUNITY IN FERRUGINOUS AND QUARTZITIC RUPESTRIAN FIELDS

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ABSTRACT:

Mining activities generate impacts on the environment that can be minimized in the long term with the application of revegetation techniques. Plant growth promoting microorganisms, such as nitrogen fixing bacteria (NFB), play an important role in the management programs aimed at reestablishing native vegetation in degraded areas. The Rupestrian ecosystem presents considerable species richness and high rate of endemism. Some of these fields have been used for iron ore extraction activities and the knowledge of the microbiota associated with these environments are essential for the attempt to recover the area after the mining. In this work, we compare the NFB community of ferruginous and quartzitic field, by PCR-DGGE. Three samples of 1 kg of soil were collected per area. From each sampling, 250 mg were used to extract the total DNA, as established in the Nucleo Spin Soil kit, according to the manufacturer's recommendations. For amplification *nifH* gen, the primers used were 19F and 407R, followed by Nested-PCR with the primers 19F-GC and 278R. The PCR reaction consisted of 20 ng of total DNA, 0.2 μ M of each primer, 200 μ M dNTP, 2 mM MgCl₂, 0.5 mg mL⁻¹ of BSA and 1.25 U GO Taq DNA polymerase in one final volume of 50 μ L. Twenty μ L of each Nested-PCR product were subjected to DGGE analysis on 8% polyacrylamide gel (w/v) in 1X TAE buffer. The DGGE gel was prepared with a gradient ranging from 50-65% (where 100% denaturation showed the concentration of 7M urea and 40% formamide). This gel was subjected to vertical electrophoresis for 12 h at 100 V at 60 °C. After this period, the gel was stained with SYBR Gold (1x) for 40 min. The bands profile was analyzed by BioNumerics software (Version 5.1, Applied Maths NV). Although the evaluated areas have the same climatic characteristics and are in the same region, the NFB communities showed only 60% similarity. This indicates that in order to reestablish some of these fields it is necessary to carry out the microorganism prospection in areas with the same formation in order to try to reestablish more quickly the microbial community closer to the natural conditions of the environment.

Keywords: DGGE, Nitrogen Fixing Bacteria, degraded areas

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