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MOLECULAR ECOLOGY BASED ON 16S rDNA OF BACTERIAL COMMUNITIES FROM RHIZOSPHERES OF SENSITIVE AND TOLERANT MAIZE VARIETIES UNDER ALUMINUM-STRESSING AND NON-STRESSING CONDITIONS THIRTY AND NINETY DAYS AFTER SOWING

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Introduction

Acid soils cover about 40% of the Earth's arable land and represent a major limitation to plant production [1]. The main constraint to plant growth on these soils is the aluminum (Al) that is solubilized by the acidity into the toxic Al^{3+} cation. Al toxicity results in the inhibition of root growth within minutes or hours resulting in poor uptake of water and nutrients [2] and the subsequent effects are poor growth and productivity. Plant production on acid soils can be maintained by neutralizing the soil acidity with lime ($CaCO_3$) and through the use of Al-tolerant plant varieties. However, the lime can take decades to correct acidity at depth, and many important crop and pasture varieties lack sufficient Al tolerance to allow effective breeding [3]. Therefore, the development of Al-tolerant maize cultivars may help improve food production in developing countries. Many strategies for Al tolerance have been reported. Among them, the exudation of Al-chelating organic acids, such as malate, oxalate, or citrate, in the rhizosphere has been proposed as the most effective tolerance mechanism to avoid Al toxicity in many plants [4]. The secreted anion organic acids bind Al^{3+} into a nontoxic form and protect the root apex from damage.

Root exudates selectively influence the growth of bacteria that colonize the rhizosphere by altering the chemistry of soil in the vicinity of the plant roots and by serving as selective growth substrates for soil microorganisms. Microorganisms in turn influence the composition and quantity of root exudate components through their effects on root cell leakage, cell metabolism, and plant nutrition. Based on differences in root exudation and rhizodeposition in different root zones, rhizosphere microbial communities can vary in structure and species composition in different root locations or in relation to soil type, plant species, nutritional status, age, stress and other environmental factors [5, 6, 7, 8].

Therefore, the aim of this study was to compare bacterial communities from rhizospheres of sensitive and tolerant maize varieties cultivated on aluminum-stressing and non-stressing conditions thirty and ninety days after sowing.

Materials and Methods

Maize varieties and experimental conditions: Two tolerant (Cateto 237 and L3) and one sensitive (L16) varieties of *Zea mays* have been chosen based on their Al-tolerance and they were cultivated in Cerrado soil (EMBRAPA-CNPMS, Sete Lagoas, MG) on different conditions of aluminum saturation (0 and 30%). Before sowing soil pH values were adjusted to 5.0 (30% Al-saturation) and 6.3 (0% Al-saturation). Thirty and ninety days after sowing samples of rhizosphere and bulk soils were collected. Samples were kept at -20°C before DNA extraction.

DNA extraction and PCR-DGGE: DNA Samples were extracted by Fastprep DNA Spin Kit for soil (Qbiogene, BIO 101 Systems, Carlsbad, CA, USA) and bacterial community profiles were obtained by PCR-DGGE analysis of 16S rDNA with U968-GC clamp and L1401 primers, as previously described by Peixoto et al. [9].

Comparing community profiles: After DGGE, Unweighted group with mathematical averages (UPGMA) and DICE coefficient were used in NT-SYS software package (version 2.02, Exeter Software, Setauket, NY) to compare the banding patterns obtained.

Identification of DGGE bands: Bands were excised from DGGE gels, reamplified using U986 or L1401 primers, cloned in pGEM-T vector and then sequenced with M13 primers in an ABI Prism 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were identified using BlastN (www.ncbi.nlm.nih.gov/blast) and Seqmatch program with RDPII Database (http://rdp.cme.msu.edu/).

Results and Discussion

Although aluminum-tolerant and sensitive maize varieties had their growth and grain production differently affected under the same aluminum condition (data not shown), their rhizospheres showed similar DGGE profiles (Figure 1).

Thirty days after sowing, rhizospheres of Al-tolerant and sensitive varieties obtained from aluminum-stressing condition showed profiles more similar to bulk soils profiles than to profiles from non-stressing condition (Figure 1. I). Those profiles showed many bands including Actinobacteria (bands 3, 4 and 7) and Rizobiales (bands 6, 11 and 18) populations (Figure 1. I and Table 1). On the other hand, rhizosphere profiles obtained under non-stressing aluminum conditions showed a prevalent band identified as Burkholderiales (band 5).

Ninety days after sowing only bulk soils patterns showed a prevalent population (Figure1. II, band 11) identified as Rhizobiales (Table 1). DGGE profiles obtained from bulk soils and rhizospheres profiles obtained under Al-stressing conditions showed a band, which is still being sequenced. A population of Actinobacteria (Figure1. II, band 6) was observed only in 30% Al-saturation bulk soils. Ninety days after sowing, rhizospheres obtained under 30% aluminum saturation showed DGGE profiles more similar to rhizospheres obtained from 0% aluminum condition than to bulk soils profiles (Figure1. II).

Conclusion

Bacterial communities from rhizospheres of maize were more affected by aluminum conditions of soil than by the varieties of maize cultivated (Al-sensitive and Al-tolerant). Moreover, differences were observed between the sampling times (30 and 90 days after sowing). Thirty days after sowing, rhizospheres under Al-stressing showed DGGE profiles more similar to bulk soil than non-stressed rhizospheres, showing that plant metabolic stress could affect the rhizospheric bacterial community.

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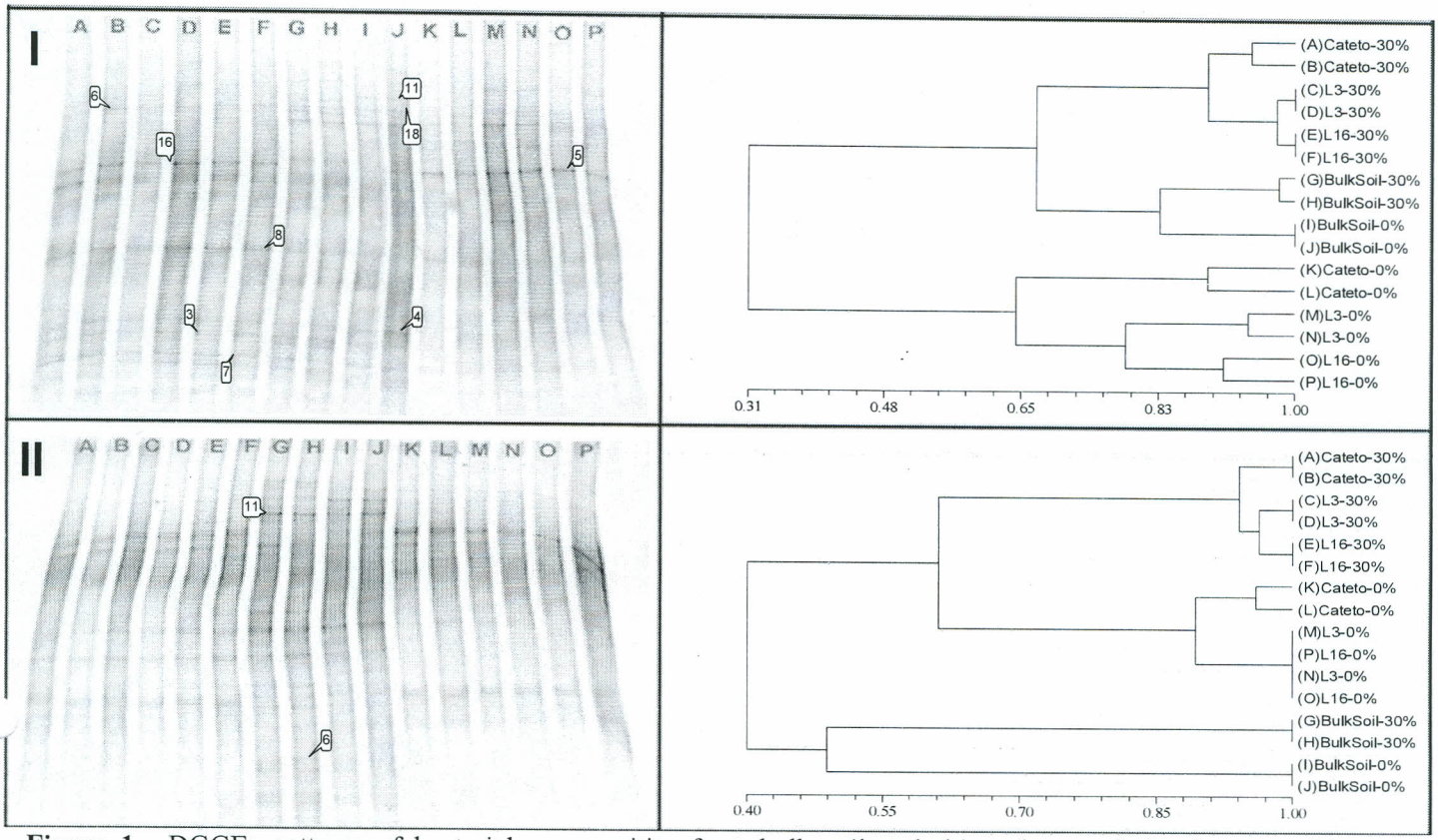

Figure 1. DGGEs patterns of bacterial communities from bulk soil and rhizospheres of aluminum-sensitive and tolerant maize varieties obtained 30 days (I) and 90 days (II) after sowing under aluminum-stressing and non-stressing conditions and their respective dendrograms constructed by DICE coefficient and UPGMA method. Indicated bands were sequenced and their identifications are show in Table 1.

Table 1. Identification of DGGE bands obtained 30 days (I) and 90 days (II) after sowing by RDP SeqMatch (Order) and NCBI BlastN (first hit).

BANDS	ORDER RDP ISOLATES	BLASTN NCBI 05/03/06
I-6	Rhizobiales	AY917421.1| Uncultured bacterium clone 1700a-25
I-18	Rhizobiales	AJ863369.1| Uncultured bacterium associated with poplar *(Populus* sp.) trees
I-11	Rhizobiales	AY391680.1| Uncultured soil bacterium clone M64 from manure-treated agroecosystem
I-5	Burkholderiales	AY278883.1| Beta proteobacterium T2-17 isolate T2-17
I-16	Burkholderiales	AF297697.1| *Telluria mixta* from endophytic bacterial communities of potato
I-8	Acidobacteriales	AY930313.1| Uncultured soil bacterium clone Y8-18 from Australian soil
I-4	unclassified_Actinobacteria	AY326627.1| Uncultured soil bacterium clone 1202-2 from Amazon soil
I-7	Actinomycetales	AY917754.1| Uncultured bacterium clone 1969b-35
I-3	Actinomycetales	AY360165.1| Micromonospora sp. I19
II-6	Rubrobacterales	AY321277.1| Uncultured bacterium clone SM-OTU59
II-11	Rhizobiales	AJ863369.1| Uncultured bacterium associated with poplar *(Populus* sp.) trees