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Bacterial endophytes of sweet potato tuberous roots affected by the plant genotype and growth stage

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ABSTRACT

This study aimed to characterize the endophytic bacterial communities in the tuberous roots of three sweet potato genotypes, IPB-149, IPB-137 and IPB-052. The hypothesis is that plant genotype and growth stage influence bacterial composition. Using cultivation-independent (Polymerase chain reaction-denaturing gradient gel electrophoresis–PCR-DGGE–based on 16S rRNA gene fragments and DNA sequencing) and cultivation-dependent (bacterial isolation) methods it was shown that plant growth stage influenced the endophytic bacterial communities, and that plant genotypes also significantly influenced these communities, but only at the first sampling (three months after planting). A total of 93 strains were isolated from the roots of the different genotypes, and 59 groups were revealed by BOX-PCR. Bacteria from the *Proteobacteria*,*Actinobacteria* and *Firmicutes* phyla were found, and *Bacillus* strains predominated in the roots of IPB-052 and IPB-149 (72% and 79%, respectively). Almost half of the strains isolated from IPB-137 roots (47%) were *Gammaproteobacteria*. In addition, the isolates were tested for antimicrobial substance and indole acetic acid production, organic and inorganic phosphate solubilization, siderophore production and for the presence of nitrogenase gene. All isolates with antagonistic activity against the sweet potato-pathogenic fungus *Plenodomus destruens* belong to the genus *Bacillus*. Indole acetic acid production was predominantly found among the strains isolated from IPB-137; once again suggesting an influence of the sweet potato genotype on its endophytes.

1. Introduction

Endophytic bacteria can be simply defined as those occurring inside of a plant or, in a scientific concept, the bacteria that can be isolated from surface sterilized plant tissues and do not visibly harm host plants (Hallmann et al., 1997). Inside of the plant roots, these bacteria may find less competition for nutrients and protection from the adverse conditions of external environment. The close interaction between bacteria and plant host can be advantageous also for the plant (Asis and Adachi, 2003; Barka, 2006).

Endophytes can improve plant development (Babalola, 2010). They are called 'Plant Growth-Promoting Bacteria' (PGPB) and use a variety of mechanisms to promote plant growth. Some of these mechanisms include antimicrobial activity against phytopathogenic fungi, siderophores and indole acetic acid production, phosphate solubilization and biological nitrogen fixation (Glick, 1995). This plant growth-promoting potential has already been described in a variety of bacterial species, and PGPB in association with plants of economic importance can lead to a significant development and yield enhancement of these crops in the field (Babalola, 2010).

Sweet potato is a subsistence crop and has a huge economic and social importance in developing countries (Souza and Lorenzi, 2008). In Brazil, it comes sixth in vegetable consumption and is one of the main crops in the Northeast Region of the country. The sweet potato plant (*Ipomoea batatas* L., Convolvulaceae family) has a complex root system composed of fibrous roots (thin roots used for nutrient uptake) and storage or tuberous roots (thick roots used for

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starch storage) (Echer et al., 2009). The sweet potato tuberous roots have a high nutritional content and are largely used for food. The plant is highly efficient in nutrient absorption and soil exploitation, which results in a rapid depletion of soil. Poor nutrition of sweet potato plants may reduce starch and glucose storage and cause small tuberous roots of low market acceptance (Santos et al., 2006).

Pests can also jeopardize sweet potato production. The occurrence of the fungus *Plenodomus destruens*, the phytopathogenic agent of the foot rot disease, can totally destroy sweet potato crops, as previously reported from the Northeast Region of Brazil (Pereira et al., 2008). Fungicides (such as benomyl, thiabendazole and captan) are typically applied to combat this disease which, however, can have adverse environmental effects and lead to fungus resistance (Lopes and Silva, 1993; Hayes et al., 2014). Different studies have already described bacterial strains that can produce antimicrobial substances against phytopathogenic agents associated with different crops (Berg et al., 2006; Köberl et al., 2013). However, the presence of bacteria able to produce antimicrobial substances against *P. destruens* associated with sweet potato has not been studied so far.

In a previous study, Marques et al. (2014) studied the bacterial communities present in the rhizosphere of the tuberous roots (tuber rhizosphere) of three different sweet potato genotypes (*Ipomoea batatas* genotype 149 [IPB-149], *Ipomoea batatas* genotype 137 [IPB-137] and *Ipomoea batatas* genotype 052 [IPB-052]) originated from the Northeast Region of Brazil. The results showed that the composition of bacterial communities in the tuber rhizosphere was correlated to the sweet potato genotypes and that the genus *Bacillus* was surprisingly abundant in the tuber rhizosphere of all genotypes. However, the endophytic bacteria present in the tuberous roots of these genotypes were not studied yet. Therefore, in this study, we hypothesize that plant genotype and growth stage also influence the composition of the endophytes. Using traditional cultivation-dependent and -independent methods, we aimed to analyze the structure of the endophytic bacterial communities and to isolate bacterial strains for a comparison of their distribution among the sweet potato genotypes.

2. Material and methods

2.1. Sweet potato genotypes

Three different sweet potato genotypes from the Active Germplasm Bank of the Federal University of Sergipe ("Universidade Federal do Sergipe", UFS) were used in the field experiments. The genotypes IPB-149, IPB-137 and IPB-052 were previously described by Marques et al. (2014). Briefly, IPB-052 and IPB-149 showed similar characteristics as to surface color of the tuberous roots (white), starch content, and strong resistance to insect attack. IPB-149 is the sweet potato genotype predominant in the northeast of the country. The IPB-137 genotype, presenting a pink tuberous root surface, had a significant lower number of tuberous roots, also lower starch content when compared to the other genotypes, and was more susceptible to insect attack.

2.2. Field experiment and sampling conditions

The field experiment was performed in 2011 in the Research Farm "Campus Rural da UFS", located in 'São Cristóvão' municipality (10°55′27″S/37°12′01″W), Sergipe State, northeast of Brazil. The experimental farm soil characteristics and the experimental field design were described recently by Marques et al. (2014). In brief, the experimental farm soil had a slightly acidic pH (5.4) and was composed of 73.82% sand, 20.72% silt, 5.46% clay and 0.86% of organic carbon (C_{org}). The experimental plot consisted of rows with spaces of 0.8 m between the rows and 0.35 m between plants of different genotypes randomly planted. Sweet potato plants showing comparable plant growth developmental stages were collected from the research farm three (t1) and six (t2) months after planting, in order to analyze the genotypes before and during the harvesting period of the crop. At each sampling, five plants/replicates per sweet potato genotype were randomly harvested from the rows. Then, the tuberous roots of each genotype replicate were washed in running tap water for 1 h and the root surface was disinfected using 70% ethanol and ultraviolet light exposure for 15 min in a laminar flow hood. After that, the ends of the tuberous roots were cut, the core was removed using a sterile sampler and cut in small pieces. Five replicates per genotype were used for the extraction of endophytic total community DNA (TC-DNA). In order to isolate endophytic bacterial strains, pieces of core from each genotype harvested at the first sampling were pooled and considered as one sample per genotype. Finally, the core samples were kept at 4 °C before bacterial isolation and at −20 °C before endophytic TC-DNA extraction.

2.3. Endophytic TC-DNA extraction

The endophytic TC-DNA was extracted from the core of the tuberous roots of five replicate samples per genotype (0.5 g of each) using the Fast DNA Spin Kit for soil (Qbiogene, BIO 101 Systems, Carlsbad, CA, USA) according to the manufacturer's instructions. TC-DNA preparations were visualized after electrophoresis in a 0.8% agarose gel in 1X TBE buffer (Tris base 89 mM, EDTA 2.5 mM, H_3BO_3 89 mM, pH 8.2) to assess their integrity and then stored at −20 °C prior to PCR amplification.

2.4. PCR amplification of 16S rRNA gene fragments

Fragments of the 16S rRNA encoding gene were amplified from the endophytic bacterial community using TC-DNA from the tuberous roots (1–5 ng) as a template for PCR amplification. A nested-PCR approach was chosen to minimize the amplification of chloroplast DNA. Briefly, the primers F799/R1492 were used for the first round of PCR for the amplification of the 16S rRNA gene of the endophytic bacterial community. The reaction conditions for the first-round PCR were those as previously described by Chelius and Triplett (2001). A 1:100 dilution of the first-round PCR product was used as a template for the second round with the primers F984-GC/R1378 using the same reaction conditions as described by Heuer et al. (1997). Fragments of 16S rRNA gene were visualized after electrophoresis in a 1.0% agarose gel in 1X TBE buffer to assess their integrity and then stored at −20 °C until DGGE analyses.

2.5. DGGE and statistical analyses

DGGEs were performed using the INGENY phorU-2 system (INGENY International BV, Goes, The Netherlands). PCR products (5 μl) were applied directly to DGGEs containing a denaturing gradient of urea and formamide varying from 46.5–60%. Moreover, a standard bacterial marker composed of 11 bacterial strains (GC-clamped 16S rRNA gene fragments) with different electrophoretic mobilities (Heuer et al., 1997) was loaded in the gel. The electrophoresis conditions were those described in Gomes et al. (2005). After electrophoresis, the gels were silver-stained according to Heuer et al. (2001). The cluster analysis was performed by the unweighted pair group method with average linkages (UPGMA) using the software package GelCompare II 4.5 (Applied Maths, Ghent, Belgium) and dendrograms were constructed based on Pearson correlation index. Significant differences ($P < 0.05$) between DGGE profiles were determined using PERMTEST