BRAZILIAN JOURNAL OF MICROBIOLOGY

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Environmental Microbiology

Effect of plant growth-promoting bacteria on the growth and fructan production of *Agave americana* L.

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ARTICLE INFO

Article history:
Received 4 June 2015
Accepted 11 February 2016
Available online 22 April 2016

Associate Editor: Iêda de Carvalho Mendes

Keywords:
Agave
Inoculation
Bacteria
Inulin
16S rRNA

ABSTRACT

The effect of plant growth-promoting bacteria inoculation on plant growth and the sugar content in *Agave americana* was assessed. The bacterial strains ACO-34A, ACO-40, and ACO-140, isolated from the *A. americana* rhizosphere, were selected for this study to evaluate their phenotypic and genotypic characteristics. The three bacterial strains were evaluated *via* plant inoculation assays, and *Azospirillum brasilense* Cd served as a control strain. Phylogenetic analysis based on the 16S rRNA gene showed that strains ACO-34A, ACO-40 and ACO-140 were *Rhizobium daejeonense*, *Acinetobacter calcoaceticus* and *Pseudomonas mosselii*, respectively. All of the strains were able to synthesize indole-3-acetic acid (IAA), solubilize phosphate, and had nitrogenase activity. Inoculation using the plant growth-promoting bacteria strains had a significant effect ($p < 0.05$) on plant growth and the sugar content of *A. americana*, showing that these native plant growth-promoting bacteria are a practical, simple, and efficient alternative to promote the growth of agave plants with proper biological characteristics for agroindustrial and biotechnological use and to increase the sugar content in this agave species.

Introduction

The use of nitrogen-fixing microorganisms and plant growth-promoting bacteria (PGPB) is an important alternative to replace chemical fertilizers for the cultivation of agricultural plants.

The search for PGPB as well as research on their biological properties are increasing at a rapid pace because efforts are being made to exploit them commercially as inoculants.

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http://dx.doi.org/10.1016/j.bjm.2016.04.010

Significant improvement on the growth and yield of crops in response to microbial inoculation has been reported by many workers.[1–3] Studies confirm that inoculants formulated with PGPB have shown positive effects on the agricultural yield and crop quality.[2,4] Regarding the effect of PGPB on plant growth, it has been reported that *Glycine max* L. Merrill seedlings inoculated with PGPB (*Pseudomonas* sp. strain AK-1, and *Bacillus* sp. strain SJ-5) demonstrated enhanced plant biomass and that the plants had a higher proline content than control plants.[5] In another study, the efficiency of *Mesorhizobium*, *Azotobacter*, and *Pseudomonas* on the growth, yield, and disease suppression in chickpea plants (*Cicer arietinum* L.) was evaluated. *Pseudomonas* showed positive IAA production, phosphate solubilization, and antagonistic activities against *Fusarium oxysporum* and *Rhizoctonia solani* compared to other strains.[6] Martínez-Rodriguez et al.[7] also reported that cultivable endophytic bacteria from the leaf base of *Agave tequilana* Weber var. Blue have the potential to enhance plant growth.

Scientific evidence supports that the agave genus includes several species of economic, social and cultural importance for people around the world.[8] Agave plants are greatly relevant to Mexico because this country is considered to be the point of origin of the evolution and diversification of this genus.[9] Approximately 163 species grow in Mexico, and 123 species are endemic.[10]

Agave americana L. has successfully adapted to climatic and edaphic conditions and proliferated in the highlands of Chiapas, Mexico, where it is an important source of natural fibre, medicine, fructans, and traditional alcoholic beverages for the local community. Due to the economic significance of this plant, several commercial plantations have been established in the state of Chiapas to produce sufficient raw materials for agro-industrial use. However, when the plantlets are transplanted to the field, their growth and development is slow, and consequently, 5–7 years are required to obtain mature plants for industrial use.[11] An alternative for obtaining mature plants for industrial use is the application of plant growth-promoting bacteria, but it is necessary to assess the possible effects of PGPB on *A. americana* to increase the survival and growth of plantlets. PGPB are rhizosphere bacteria that enhance plant growth by a wide variety of mechanisms, such as phosphate solubilization, siderophore production, biological nitrogen fixation, phytohormone production, antifungal activity, induction of systemic resistance, promotion of beneficial plant-microbe symbioses, and so on.[12,13]

Many aspects of the microbial community associated with agaves are still unknown and only a manuscript related to[14] suggests that the hypothesis that PGPB inoculation significantly increases the growth and sugar content (mainly inulin) in *A. americana* is true. Therefore, the objective of this study was to evaluate the effect of PGPB inoculation on plant growth and sugar accumulation in *A. americana*.

Materials and methods

Bacterial strains

The bacterial strains ACO-34A, ACO-40, and ACO-140 were chosen subsequent to a study of approximately 235 strains previously isolated from the rhizosphere of *A. americana*. These three strains were selected based on their capacity for nitrogen fixation, auxin production, P-solubilization and biosynthesis of IAA (Table S1) and were provided by the Instituto Tecnológico de Tuxtla Gutiérrez, while the reference strain *Azospirillum brasilense* Cd was provided by the Centro de Ciencias Genómicas, Cuernavaca, México. All strains were grown in yeast extract-mannitol (YMA) medium[15] at 28 °C and preserved at 4 °C until use.

Phenotypic and genotypic analysis of strains

The cell morphologies of the strains isolated from *A. americana* were examined by light microscopy (Zeiss® PS7, Germany). The Gram reaction was determined using a kit (Merck®, Germany), according to the manufacturer's procedure, and colony morphology was determined with cells grown on YMA plates at 28 °C for 5 days.[16]

Bacteriological and physiological characterization of strains ACO-34A, ACO-40, and ACO-140 were performed with isolates from YMA medium. Salt tolerance was evaluated at 28 °C with 0.5, 1.0, 2.0, 3.0 and 5.0% (w/v) NaCl and pH levels of 4.0, 5.0, 9.0 and 11.0. Acid or alkali production was determined on the same medium supplemented with 25 mg mL^{-1} bromothymol blue as a pH indicator.[16] Antibiotic resistance was tested on YMA plates following the process recommended by Martínez-Romero et al.[17]. In addition, the Al and Cu tolerance of the strains were determined on solid YMA medium.[18]

16S rRNA gene sequencing and phylogenetic analysis

The strains were grown in 2.0 mL of YMA medium overnight. Total genomic DNA was extracted using a DNA Isolation Kit for Cells and Tissues (Roche®, Switzerland), according to the manufacturer's specifications. PCR was performed with the bacterial universal 16S rRNA primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGATCCAGCC-3′), which amplified products of approximately 1500 bases, and procedures were performed as described by Weisburg et al.[19]. The PCR products were purified using the PCR Product Purification System Kit from Roche® and sequenced (Macrogen®, Korea). All sequences were compared with the reference sequences obtained by a BLAST search.[20] The sequences were aligned using the CLUSTAL X (2.0) software with the default settings.[21] Minor modifications in the alignment were made using the BIOEDIT sequence editor. Phylogenetic and molecular evolutionary analyses were performed with MEGA v5.2.[22] The phylogenetic tree of the 16S rRNA gene sequences from type strains was constructed by Neighbour-Joining[23] and a Bootstrap analysis with 1000 pseudoreplicates using the Tamura-Nei model.[24] The 16S rRNA gene sequence of strains ACO-34A, ACO-40, and ACO-140 were deposited in the GenBank database under the accession numbers KM349967, KM349968, and KM349969, respectively. Additionally, strains ACO-34A, ACO-40, and ACO-140 were deposited in DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), Germany as an open collection under the deposit numbers DSM 101606, DSM 01771 and DSM 01784, respectively.