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Original article

Diversity of *Paenibacillus durus* strains isolated from soil and different plant rhizospheres evaluated by ARDRA and *gyrB*-RFLP analysis

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Abstract

Strains belonging to *Paenibacillus durus* isolated from the rhizosphere of various grasses and from bulk soil were previously divided into five phenotypic groups (A1–A5) based on the fermentation pattern of six carbohydrates (A1: sorbitol (+), A2: dulcitol and tagatose (+), A3: starch and glycogen (+), A4: starch, glycogen and D-arabitol (+) and A5: negative for these carbohydrates). This study aimed to assess whether plant types select for specific *P. durus* phenotypic groups. For that purpose, polymerase chain reaction-restriction fragment length polymorphism analysis of part of genes encoding 16S rRNA (ARDRA) and DNA gyrase subunit B (*gyrB*-RFLP) were used to produce genetic fingerprints. ARDRA and *gyrB*-RFLP data were clustered together to generate a dendrogram and two main clusters were observed. Cluster I showed a predominance of strains isolated from wheat, maize and sugarcane rhizospheres. Strains isolated from maize were distributed among the five patterns of carbohydrate metabolism, while strains isolated from sugarcane showed to be predominantly able to metabolize starch and glycogen. Neither sorbitol- nor arabitol-metabolizing strains were found in cluster II, which consisted of strains isolated from soil and from all plant species used. Our results suggest that the plants influenced the diversity of *P. durus* in their rhizospheres.

Keywords: *Paenibacillus durus*; Diversity; ARDRA; *gyrB*-RFLP; Rhizospheres

1. Introduction

Plant growth-promoting rhizobacteria (PGPR) establish positive interactions with plant roots and can affect plant growth by different ways, as antagonizing and repressing soil-borne pathogens, producing phytohormones, reducing nitrate, fixing atmospheric nitrogen and disponibilizing nutrients to the plant or inducing plant resistance to diseases after root colonization by PGPR [7,30,31]. Due to these properties, a high diversity of bacteria have been already identified and categorized as economically important for a wide range of crops in different soils and climatic regions [30].

Strains belonging to the species *Paenibacillus durus* were shown to be efficient nitrogen fixers found in the rhizospheres of maize, sorghum, sugarcane, wheat and forage grasses [18,21,23,26]. Some strains are also able to produce antimicrobial substances against different

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Gram-positive and Gram-negative bacteria [24] and solubilize calcium phytate [18]. These characteristics can be considered to be important for the establishment of *P. durus* strains in the plant rhizospheres. Furthermore, because strains of this species can form spores, they can resist a range of environmental stress conditions like high temperatures, dryness or heavy rainfalls, usually present in tropical countries.

The diversity among populations of *P. durus* associated with different gramineous plants has already been investigated by the assessment of homology with a *nifKDH* probe in hybridization experiments, the use of a selected primer to produce RAPD profiles and of BOX-PCR to generate genomic fingerprints and by phenotypic tests using the API50CH system [18]; and the results showed that the plants studied did not select a specific subpopulation of *P. durus*. On the other hand, other studies showed that the type and age of plants and/or the type of soil where the plants are grown can influence the communities of *Paenibacillus* species, in particular *P. durus* and *P. polymyxa*, in different rhizospheres [12,17,27,32]. This might be due to differences in the bacterial responses to different root exudates and, therefore, studies are still granted to elucidate whether arbitrary diversity exists among strains or whether plants select specific bacterial populations to coexist with them.

When Rosado et al. [18] studied the diversity of different *P. durus* strains isolated from the rhizoplane and rhizospheres of different grasses and from bulk soil by genetic and phenotypic approaches, all the strains produced acid from 15 carbohydrates in the API50CH test, and presented variable results with six carbohydrates (sorbitol, dulcitol, tagatose, starch, glycogen and D-arabitol). Using these six carbohydrates, they could be divided into five groups. A previous study had already suggested that sorbitol metabolism may play a role in the establishment of rhizobacteria in the vicinity of wheat roots [11], and other monosaccharides which are metabolized by *P. durus* strains are usually found in the exudates of several plants. Therefore, the aim of this study was to investigate whether there is a genetic link between carbohydrate metabolism and the plant/soil where the strains were isolated. For that, polymerase chain reaction-restriction fragment length polymorphism analysis of part of genes encoding 16S rRNA (ARDRA) and DNA gyrase subunit B (*gyrB*-RFLP) were used to produce genetic fingerprints. Although the gene *gyrB* has already been used for taxonomic and phylogenetic studies in different bacterial species [4,9,34], it is the first time it is used within *P. durus* strains.

2. Materials and methods

2.1. Bacterial strains

The *P. durus* strains used in this study, their sources and their phenotypic groups based on the fermentation patterns of six carbohydrates (sorbitol, dulcitol, tagatose, starch, glycogen and D-arabitol) are listed in Table 1. All strains were stored aerobically at room temperature on GB agar slants [25] supplemented with 1% $CaCO_3$ (w/v). For growth of *P. durus* cells, cultures were inoculated for 24–48 h in TBN medium [23] and incubated still at 32 °C.

2.2. Preparation of genomic DNA

Total DNA was extracted from all strains by the method described by Seldin and Dubnau [22]. All DNA preparations were dissolved in 300–500 μl of TE buffer [20] and quantified spectrophotometrically (Gene Quant apparatus, Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.3. Multiplex PCR

To confirm the identification of all strains used in this study, a multiplex PCR was developed based on two set of primers specific for *P. durus*: BAZO1 (5′GAGTTGT GATGGAGCT3′) and BAZO2 (5′AGGAGCC CATGGTT3′) and NHA1 (5′TCCACTCGTCT GATCCTG3′) and NHA2 (5′CTCGCGGATTGG CATTGCG3′), both systems described by Rosado et al. [17,19]. The reaction buffer contained 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 3.75 mM $MgCl_2$. The mixture of dNTPs was added to a concentration of 2 mM of each deoxyribonucleoside, whereas 0.2 μM (NHA) and 1 μM (BAZO) of each of the two sets of primers was used. *Taq* polymerase (1.25 U) (Invitrogen, São Paulo, Brazil) was used to amplify 100 ng of template DNA. The cycle applied was: 35 × (1 min, 94 °C; 1 min 30 s, 55 °C, 1 min 30 s, 72 °C); 1 × (10 min, 72 °C).

2.4. PCR amplification of 16S rRNA and gyrB gene fragments

For amplification of *Paenibacillus* 16S rRNA gene fragments, a primer set consisting of the specific forward primer PAEN515F and the universal reverse primer 1377 [28] was used. The amplification conditions were: 35 cycles of 94 °C (1 min), 58 °C (1 min) and 72 °C