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Polyphasic characterization of bacteria obtained from upland rice cultivated in Cerrado soil☆

Lívia Fabiana Braga[a], *Fênix Araújo de Oliveira*[a], *Eva Aparecida Prado do Couto*[a], *Karina Freire d'Eça Nogueira Santos*[a], *Enderson Petrônio de Brito Ferreira*[b,*], *Claudia Cristina Garcia Martin-Didonet*[a]

[a] *Universidade Estadual de Goiás (UEG), Anápolis, Goiás, Brazil*
[b] *Embrapa Arroz e Feijão, Santo Antônio de Goiás, Goiás, Brazil*

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ABSTRACT

This work aimed to characterize 20 isolates obtained from upland rice plants, based on phenotypic (morphology, enzymatic activity, inorganic phosphate solubilization, carbon source use, antagonism), genotypic assays (16S rRNA sequencing) and plant growth promotion. Results showed a great morphological, metabolic and genetic variability among bacterial isolates. All isolates showed positive activity for catalase and protease enzymes and, 90% of the isolates showed positive activity for amylase, catalase and, nitrogenase. All isolates were able to metabolize sucrose and malic acid in contrast with mannitol, which was metabolized only by one isolate. For the other carbon sources, we observed a great variability in its use by the isolates. Most isolates showed antibiosis against *Rhizoctonia solani* (75%) and *Sclerotinia sclerotiorum* (55%) and, 50% of them showed antibiosis against both pathogens. Six isolates showed simultaneous ability of antibiosis, inorganic phosphate solubilization and protease activity. Based on phylogenetic analysis of the 16S rRNA gene all the isolates belong to *Bacillus* genus. Under greenhouse conditions, two isolates (S4 and S22) improved to about 24%, 25%, 30% and 31% the Total N, leaf area, shoot dry weight and root dry weight, respectively, of rice plants, indicating that they should be tested for this ability under field conditions.

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* *Corresponding author.*
E-mail: enderson.ferreira@embrapa.br (E.P. Ferreira).

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Introduction

Beneficial endophytic microorganisms can live inside plants without causing damage to the host. Among them, many bacteria colonize the intercellular spaces throughout the whole plant, including seeds.[1] Intercellular spaces contain sources of carbohydrates and minerals, such as: nitrogen, phosphorus, calcium, potassium, and chlorine, as well as other metabolites as organic acids, facilitating bacterial development and colonizing.[2]

Among several microorganisms colonizing plant tissues, plant growth-promoting rhizobacteria (PGPR) were also found.[3] These PGPRs provide beneficial effects, such as: mineral nutrition improvement, increase on the tolerance of biotic and abiotic stresses, root development promotion, and suppression of soil borne diseases.[2]

Also, these bacteria can be involved on nitrogen fixation, inorganic phosphate solubilization, iron complexation, phytohormones synthesis and plant pathogen control, involving many enzymes on these processes.[2,4] Thus, plants under influence of PGPR can be greater, stronger, more productive and healthier.[3,5,6] Besides, the assessment of biochemical characteristics in bacteria allows inferences about their adaptive capacity to the environment and about the plant–bacteria interaction, since it involves several genes and mechanisms, such as chemoattraction and biofilm formation, among other activities.[3]

Association between PGPRs and root of rice plants has been studied and many isolates showing effects on rice growth were found.[5] The application of this technology on rice chain production can provide benefits for crop growth and reduce production costs, as well as helping on the reduction of environmental risks.[5,7]

Furthermore, the identification and characterization of bacteria as PGPR provide insights for understanding the composition of bacterial communities associated with rice plants grown under Cerrado conditions. However, studies with focus on the isolation and characterization of bacteria from Cerrado soils able to promote plant growth of upland rice are still scarce as well as, necessary for this crop. Thus, this study aimed to characterize bacterial isolates obtained from rice roots based on biochemical and genetic characteristics and, to determine their ability to promote plant growth aiming their use as inoculant for upland rice.

Material and methods

Bacterial isolates

The isolates evaluated in this study were obtained from rice roots by Rezende[8] and are available at the Collection of Microorganisms and Multifunctional Fungi of Embrapa Rice and Beans.

Morphological characterization and Gram coloring assays

Morphological characterization was performed according to Vermelho et al.[9] based on shape, border, surface, consistence and elevation of the colonies. Gram coloring was performed according to Louvet et al.[10]

Biochemical assays

All biochemical trials were performed on a completely randomized design in triplicate under laboratory conditions. For the evaluation of carbon sources use (maleic acid, malic acid, nicotinic acid, D-arabinose, D-fructose, D-glucose, mannitol, mio-inositol, sucrose and sorbitol), each isolate was inoculated in Petri dishes containing solid King B medium added with each different carbon sources and incubated (28 °C; 2 d). After incubation, we checked out the growth of the isolates according to Hungria et al.[11]

The activity of the enzymes citrate lyase and urease was measured through qualitative assays by inoculating the isolates on solid Simmons citrate and urea medium and observing the blue and red colors of the culture medium, respectively.[9] Nitrate reductase and nitrogenase enzymes were qualitatively assayed in semi-solid medium.[12] Catalase activity was assayed according to Vermelho et al.[9] For amylase and protease activities, we observed the formation of a translucid halo around the colonies, formed by the isolates inoculated on M9 solid medium containing 1% of starch and 2% of milk protein, respectively.[13] Cellulase activity was determined according to Cattelan et al.[14]

Solubilization of inorganic phosphate assay was performed using three replicates. Solubilization ability was confirmed based on the observation of a translucid halo around the colonies formed by the isolates inoculated on NBRI-P[15] and Pikovskaya medium.[16]

Antagonistic test

The antagonistic ability of the isolates was performed using three replicates, under *in vitro* conditions against the fungi *Sclerotinia sclerotiorum* and *Rhizoctonia solani* on Petri dishes containing BDA medium according to Hammami et al.[17]

Clustering analysis of the isolates based on morpho-physiological data

Morphological, enzymatic activity, carbon source use and antibiosis data were transformed into a binary matrix and submitted to cluster analysis performed by the software NTSYSpc®[23] using UPGMA as grouping algorithm and Jaccard as similarity coefficient.

16S rRNA gene sequencing analysis

Based on the clustering analysis of the isolates based on morpho-physiological data 11 isolates (S2, S4, S6A, S17, S22, S26, S29, S37, S41, S63 and S105) were selected for 16S rRNA gene sequencing. The DNA of the isolates was obtained according to Laranjo et al.[18] The 16S rRNA gene was amplified by PCR using the primers Y1 and Y3.[18,19] The amplicons were purified and used on the sequencing reaction according to Laranjo et al.[18] Sequences coding for the partial 16S rRNA gene of the isolates S2, S4, S6A, S17, S22, S26, S29, S37, S41, S63 and S105 was obtained and when submitted to the

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