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ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Biotechnological potential of bacteria from genera *Bacillus Paraburkholderia* and *Pseudomonas* to control seed fungal pathogens

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Abstract

Fungal pathogens are important determinants of plant dynamics in the environment. These pathogens can cause plant death and occasionally yield losses in crops, even at low initial densities in the soil. The objective of this study was to select and evaluate fungal antagonistic bacteria and to determine their biological control capacity in soybean seedlings. A total of 877 strains from the genera *Pseudomonas*, *Bacillus*, and *Paraburkholderia*/*Burkholderia* were screened, and their antagonistic effects on fungi frequently found in seeds were evaluated using four methods: quadruple plating, paired culture confrontation, strain containment, and inoculation of soybean seeds. The experimental design was completely randomized, with three replications for the first three methods and five replications in a 3 × 9 factorial scheme for the fourth treatment. The strains with the highest biotechnological potential were inoculated into soybean seeds to evaluate the biological control of fungi that attack this crop at germination. Seventy-nine strains presented some type of antagonistic effect on the tested fungi, with two strains presenting a broader antagonistic action spectrum in the seed test. In addition to the antagonistic potential, strains BR 10788 and BR 11793, when simultaneously inoculated or alone, significantly increased the seedling dry matter mass, and promoted the growth of soybean seedlings even in the presence of most fungi. Thus, this study demonstrated the efficiency of the antagonistic activity of these strains in relation to the target fungi, which proved to be potential agents for biological control.

Keywords *Bacillus* · Biocontrol · Bioprospecting · *Paraburkholderia* · Screening

Introduction

Fungal pathogens are determinants of plant population dynamics in agricultural environments. For soybeans, examples of economically important pathogens include *Colletotrichum* spp., *Fusarium* spp., *Aspergillus* spp., *Cercospora* spp., *Penicillium* sp., *Alternaria* spp., *Cladosporium* sp., *Sclerotinia* spp., and *Rhizoctonia* spp., which can cause plant death and occasionally total yield loss even at low initial soil inoculum densities [1–3].

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The use of biological agents to reduce pathogens has been shown to be an effective, promising, and widely studied method [4–6]. However, although the number of diseases that can be controlled biologically in practical terms is significant, few biological products have actually been used. This is because of the limitations imposed by the pathogens and failure of the selection procedure, environmental factors, technical challenges, and strategies in the experimental system [7].

The process of selecting microorganisms for use in new commercial products for the biocontrol of plant fungal pathogens is complex because there are several criteria to be analyzed that are crucial for the success of subsequent steps. Among these, the antagonistic efficacy, mechanisms of antagonism, growth in a vehicle that maintains an adequate population, and even procedures of legal property rights, and market insertion are crucial [8].

Cultural collections currently provide services to the scientific community, ensuring the considerable diversity of pure

and authentic microorganisms already available. Microorganisms from these institutions have beneficial characteristics and can be used in various programs of agricultural interest [9]. Thus, the use of microorganisms belonging to the collections, isolated from native cultures, and already identified facilitates the development of processes and products of economic interest.

Different bacteria isolated from various hosts that have the ability to promote plant growth, and may also be antagonists to plant pathogens, have been studied for the development of commercial products [10]. Despite their high biotechnological potential, there are still a few products registered on the market for this purpose. However, the use of microorganisms as biocontrol agents is growing worldwide, albeit slowly. *Trichoderma* spp. is an example used to control soil-dwelling fungi [11].

Alternatively, other species and genera of bacteria are important groups of biological control agents, such as *Pseudomonas*, *Bacillus*, and *Paraburkholderia*. These have been shown to have the potential to control fungal and/or bacterial diseases because they occupy an ecological niche similar to that occupied by the pathogens [12–14]. In addition, bacteria of the genus *Bacillus* can produce a wide variety of antimicrobial compounds, such as iturine, surfactin, subtilosin, fengycin, and bacillomycin [15–17], giving it the ability to protect plants from phytopathogenic fungi [18–21]. Additionally, lipopeptide biosurfactants produced by *Pseudomonas* and *Bacillus* are effective in biocontrol because of their positive potential for competitive interactions with other organisms, including bacteria, fungi, protozoa, nematodes, and plants [22–24].

Similarly, the *Paraburkholderia* species mainly stand out due to their biocontrol and bioremediation properties [25–27]. This genus was recently reclassified as belonging to the genus *Burkholderia* based on molecular markers [25]. It comprises a versatile group consisting of taxonomic members with beneficial characteristics to the environment and associated plants [28]. In addition to promoting plant growth, it can improve nutrient absorption, increase tolerance to stress, induce systemic resistance, and confer resistance to plant pathogens [29]. For example, Glick et al. [30] revealed that a *Paraburkholderia phytofirmans* strain decreased the level of ethylene in host plants by producing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Similarly, it was shown that *Burkholderia* strains could contribute to plant nutrition by producing plant hormones, indirectly leading to reduced disease susceptibility [31, 32].

Thus, the goals of this study were to select and evaluate bacteria antagonistic to phytopathogenic fungi and to determine their biological control capacity in soybean seedlings. The strategy used in this study was to screen bacteria isolated previously from healthy plants and deposited in culture collections.

Material and methods

Bacterial and fungal strains

The study was based on bacterial strains already deposited in the culture collection of the Embrapa Agrobiologia Johanna Dobereiner Center for Biological Resources (CRB-JD). Eight hundred and seventy-seven strains previously characterized by 16S rRNA in the genera *Pseudomonas*, *Bacillus*, and *Paraburkholderia*/*Burkholderia* were subjected to a phylogenetic analysis using the MEGA (version 7.0) program [33] based on the neighbor-joining method and the Tamura 3-parameter, which was the best substitution model. Subsequently, representative strains of specific phylogenetic groups within each genus were defined (Table S1). We used the name *Paraburkholderia*/*Burkholderia* throughout because of the uncertain taxonomic position of some strains related to these genera. The 16S rRNA of all bacteria was amplified with the primers 27F and 1492, as recommended elsewhere.

The fungi used have the potential to attack soybean, bean, rice, and cotton seeds during germination. These included *Aspergillus flavus* (F5), *Rhizoctonia solani* (F4), *Corynespora cassiicola* (F3), *Fusarium piperis* (F7), *Fusarium semitectum* (F1), *Phomopsis sojae* (F6), *Sclerotinia sclerotiorium* (F2), *Cladosporium* sp. (A104), and an isolate of the order *Pleosporales* (A103 and A105). The fungal strains originated from the Culture Collection of the Embrapa Agropecuária Oeste Seed Laboratory and from CRB-JD. The strains of the order *Pleosporales* (A103 and A105) and *Cladosporium* sp. (A104) can colonize plant roots, such as rice and tomato [34].

Phylogeny of the bacteria strains

Strain affiliation with different species groups within each genus was obtained by phylogenetic analysis based on the 16S rRNA gene sequences of the strains and type strains. The neighbor-joining method was used for the analysis and the models that best fit model for each genus (*Pseudomonas*: Kimura two parameters; *Bacillus*: Tamura-Nei; *Paraburkholderia*/*Burkholderia*: Tamura three parameters) using the MEGA program (version 7.0). The closest type strains within each genus described in the bacterio.net platform (http://www.bacterio.net/methylophilus.html) were considered. Based on clusters, species were placed within the appropriate groups.

Initial screening of antagonistic bacteria

The 101 strains selected were analyzed for antagonism against phytopathogenic fungi. As a first approach, a quadruple plating method was used, in which plates containing PDA medium were divided into quadrants. Fungi also grown in PDA (10

days at 28 °C in a growth chamber) were used as inoculant. Seven-millimeter discs of this culture medium containing the grown fungi were arranged in the center of a new plate containing BDA medium, and in each quadrant, a distinct strain was inoculated, totaling four in each Petri dish. This was followed by incubation (15 days at 28 °C in a growth chamber). At 7 and 15 days, visual evaluations were performed to identify the antagonism (absence of fungal growth and/or an inhibition halo) promoted by the bacterial strains. From this test, the strains were selected within each bacterial genus for further tests.

Specific antagonism tests for the five strains within each genus

The paired culture confrontation technique proposed by Mariano [35] was used, with minor adaptations to evaluate the antagonism of 15 (five for each of the three genera) strains. Briefly, the strains were plated on PDA medium in two bands spaced 40 mm apart. Seven-millimeter discs of the culture medium containing fungi, as mentioned above, were arranged in the center of the plate such that they were equidistant between the bacterial bands, followed by incubation at 28 °C for 15 days. Control plaques consisted of fungal discs in the absence of bacteria. A completely randomized design with three replications was used, and the data were analyzed by the Scott-Knott test at 5% probability.

In this second stage, antagonism was interpreted by analyzing three variables: the inhibition zone, colony area, and percentage of inhibition. The inhibition zone was evaluated at 15 days by measuring the distance between the fungal colony's edges and the bacterial strip [35, 36]. The area of the fungal colony was obtained by measuring its radial growth on the orthogonal axes. The mean between these two measurements was calculated and assumed to be the value of the radius (r) for area calculation using the formula $2\pi r^2$. The results were expressed in mm^2. The percentage inhibition was determined by the relationship between the area of growth of the pathogen in the presence of each bacterium and the area occupied by the pathogen in the control treatment. Using these values, the percent inhibition was calculated using formula (1) adapted from Tullio [36]: (% In = 100 − [(treatment area mm) × 100)/(control area mm)]. In addition, three levels of antagonism (below 40%, 40–80%, and above 80%) were defined for the obtained values.

In the third stage, a technique for fungus containment was adapted from Mariano [35]. A 7-mm disc of fungus on PDA medium (as described above) was arranged in the center of a Petri dish containing the same medium. Then, two bacterial strains that presented the best square-shaped results around the fungus disc were inoculated on the same plate. For the control treatment, the fungus was used without the presence of bacteria. The plates were then incubated for growth (28 °C), and each treatment consisted of three replicates. At 15 days, the fungus containment caused by the bacteria in the plaques was visually verified.

Bioassay to assess the bacterial antagonism to the fungus during soybean germination

Bacterial strains identified with higher antagonistic potential from the previous tests were selected and cultured in BP culture medium, optimized using glycerol as a carbon source [37], for 24 h at 28 °C, and under orbital agitation at 150 rpm. Individual growth curves were generated to determine the maximum cell production potential. Initially, each bacterial strain was grown in a test tube with 5 mL of medium (20 h; 28 °C; with shaking at 1500 rpm). Then, the growth broth was transferred to a 250-mL Erlenmeyer flask, and the volume was adjusted to 50 mL with the same medium, followed by a new growth cycle (18 h, 28 °C, and 1500 rpm agitation). After that, the growth broth was transferred to another Erlenmeyer flask, where the volume was adjusted to 250 mL and incubated (28 °C; with 1500 rpm agitation). Every 2 h, aliquots were taken to determine the optical density and also plated on "spiral plate" equipment at dilutions 10^{-5}, 10^{-6}, and 10^{-7} CFU/ml. After determining the point of maximum cell production, each strain (BR 10788 and BR 11793) and the mixture of the two were formulated according to Scheidt et al. [37] for further use in seed treatments.

The bioassay was conducted in a completely randomized design with five replications in a 3 × 9 factorial scheme, corresponding to three strains (BR 10788, BR 11793, and a mixture of both) and nine fungi (*F. piperis*, *Pleosporales*, *Cladosporium* sp., *Ph. sojae*, *A. flavus*, *C. cassiicola*, *R. solani*, *F. semitectum*, and *S. sclerotiorum*). Each plot corresponded to a Petri dish consisting of eight seeds, half of which were inoculated with one of the three inoculants, and the other half (four seeds) was not inoculated. All seeds were initially disinfected: immersion in 70% ethanol for 30 s and in 4% (v/v) H_2O_2 for 3 min and then rinsed three times in sterile distilled water [38] before inoculation.

For bacterial inoculation, 0.2 mL of each product was applied to 180 seeds (after being distributed in the plates), resulting in a cell concentration of approximately 10^8 bacterial cells per seed. Fungi inoculum discs (8 mm) were placed in the center of the plate, been the inoculated seeds on one side of the plate and the control without inoculant on the other side. After sowing the plates, they were incubated (28 °C; 12 h photoperiod; 7 days).

The variable for antagonistic potential (PA) was evaluated by using formula (2): PA = [(number of injured control treatment seedlings-number of injured inoculated seedlings)/number of injured control treatment seedlings) × 100]. For the fresh mass (g), dry mass (g), number of germinated seeds (NSG), and number of injured seedlings (NPL) of all four

seedlings for each treatment in the plate were joined as a plot, and an analysis of variance (ANOVA) was performed. A P value ≤ 0.05 was defined as statistically significant.

Results and discussion

Evaluation of bacterial antagonism to fungi in Petri dishes

A total of 877 strains were phylogenetically studied based on the 16S rRNA within the genera *Pseudomonas*, *Bacillus*, and *Paraburkholderia*/*Burkholderia*. The strains were isolated in Brazil and originated from both hosts and substrates, such as roots, legume nodules, and the plant rhizosphere (Table S1). From the groupings, 32 strains were selected from the genera *Pseudomonas* and *Bacillus* and 37 from the genus *Paraburkholderia*/*Burkholderia* to represent distinct phylogenetic groups, totaling 101 strains.

The formation of six major phylogenetic groups was observed for *Pseudomonas*, seven for *Bacillus*, and ten groups for *Paraburkholderia*-type strains, wherein new strains were distributed (Fig. 1). The *Pseudomonas* and *Bacillus* species comprised of 272 and 380 species, respectively. The genus *Paraburkholderia*, recently defined by a reclassification of the genus *Burkholderia*, had 74 recognized species. *Burkholderia* is a large and complex group containing pathogenic, non-pathogenic, symbiotic, and non-symbiotic strains from a wide variety of habitats. Thus, its taxonomy has been reevaluated and divided into six genera: *Burkholderia*, *Caballeronia*, *Mycetohabitans*, *Paraburkholderia*, *Robbisia*, and *Trinickia* [39].

The *Pseudomonas* and *Paraburkholderia/Burkholderia* groups' strains showed biological control potential for almost all fungal isolates tested. For *Pseudomonas*, 26 strains show some type of antagonism, similar to all isolates of the *Paraburkholderia*/*Burkholderia* group (Fig. 1). Sixteen isolates of *Bacillus* controlled the fungi evaluated. Although the number of isolates with control capacity was smaller than that of the other genera, they show a greater antagonistic capacity to the fungi (Fig. 1). Thus, the results showed that *Pseudomonas* and the *Paraburkholderia*/*Burkholderia* group had a broader spectrum of control, but *Bacillus* spp. had more pronounced antagonistic effects.

After the initial screening for visual identification of bacterial control ability of fungi and identification of different groups of strains, five strains from each genus showed greater antagonism and were studied in more detail using the paired culture challenge test. In this test, it is observed that the control-efficient *Pseudomonas* strains were mostly grouped with species groups 1, 3, 4, and 6, in which those belonging to the type 1 and 4 strain groups presented inhibition potential higher than 80% for Pleosporales, *Cladosporium* sp., and *S. sclerotiorum* (Fig. 1). It was also observed that the only strain among the 101 selected (considered for this test), capable of inhibiting the growth of the phytopathogen *A. flavus*, was BR 10843 belonging to group 3, represented by *Pseudomonas* species, *Ps. baetica* and *Ps. helmanticensis*. This inhibitory effect may have been expressed more intensely because of this strain's growth rate, resulting in a greater ability to inhibit sporulation.

Within the five *Bacillus* strains, those showing antagonism are those represented in groups 3, 5, 6, and 7 (Fig. 1). Strains of this genus showed a narrower spectrum of control than the *Paraburkholderia*/*Burkholderia* genera; however, strains with higher antagonism (> 80%) were obtained from *Pleosporales*, *Cladosporium* sp., *C. cassiicola*, *Ph. sojae*, and *S. sclerotiorum*.

The strains of the genus *Paraburkholderia*/*Burkholderia* had the broadest spectrum of antagonism, controlling a large number of fungi, ranging from 4 to 7. Most strains presented antagonistic levels of 40–80% (groups 1, 4, and 10), highlighting the species groups *P. caballeronis*, *P. kururiensis* (group 4), and *P. andropogonis* and *B. vietnamiensis* (group 10), which presented levels above 80% for *S. sclerotiorum* (Fig. 1).

The ability to produce and release one or more compounds; for example, lytic enzymes against compounds, such as chitin, proteins, cellulose, hemicellulose, and DNA, may contribute to the suppression of pathogen activity [40–42]. In addition, when a bacterial strain is in contact with the pathogen, competition for space and nutrients may occur, which may have occurred in this situation.

From the analysis of the paired culture challenge test data, two strains were selected, one from the genus *Bacillus* (BR 10788) and one from the *Paraburkholderia*/*Burkholderia* group (BR 11793), which presented the broadest spectrum of action and the highest level of antagonism against the fungi.

In the challenge test, strain BR 11793 performed better, as it restricted the development of all phytopathogens within the square and was even able to significantly inhibit the growth of *A. flavus* (Fig. 2; C1). On the other hand, BR 10788 inhibits all fungi, except *A. flavus* (Fig. 2; B1) and *F. semitectum* (Fig. 2; B8).

Both strains show high antagonistic potential for *S. sclerotiorum* (Fig. 2; B5 and C5), especially when compared to other fungi tested (Fig. 2). The ability of bacteria to parasitize and degrade spores and hyphae and produce compounds that inhibit pathogenic fungi's development is widely described in the literature [43]. The action of bacteria leads to an inhibition of fungal growth and can range from simply fixing cells to hyphae to the complete breakdown and degradation of the fungi. Bacterial cell adherence to hyphae often occurs because of biofilm production. Zucchi [44] demonstrated that strains of *Bacillus subtilis* and *Paenibacillus lentimorbus*, chitinase producers, are capable of parasitizing *Aspergillus parasiticus*. Due to their ability to degrade the cell

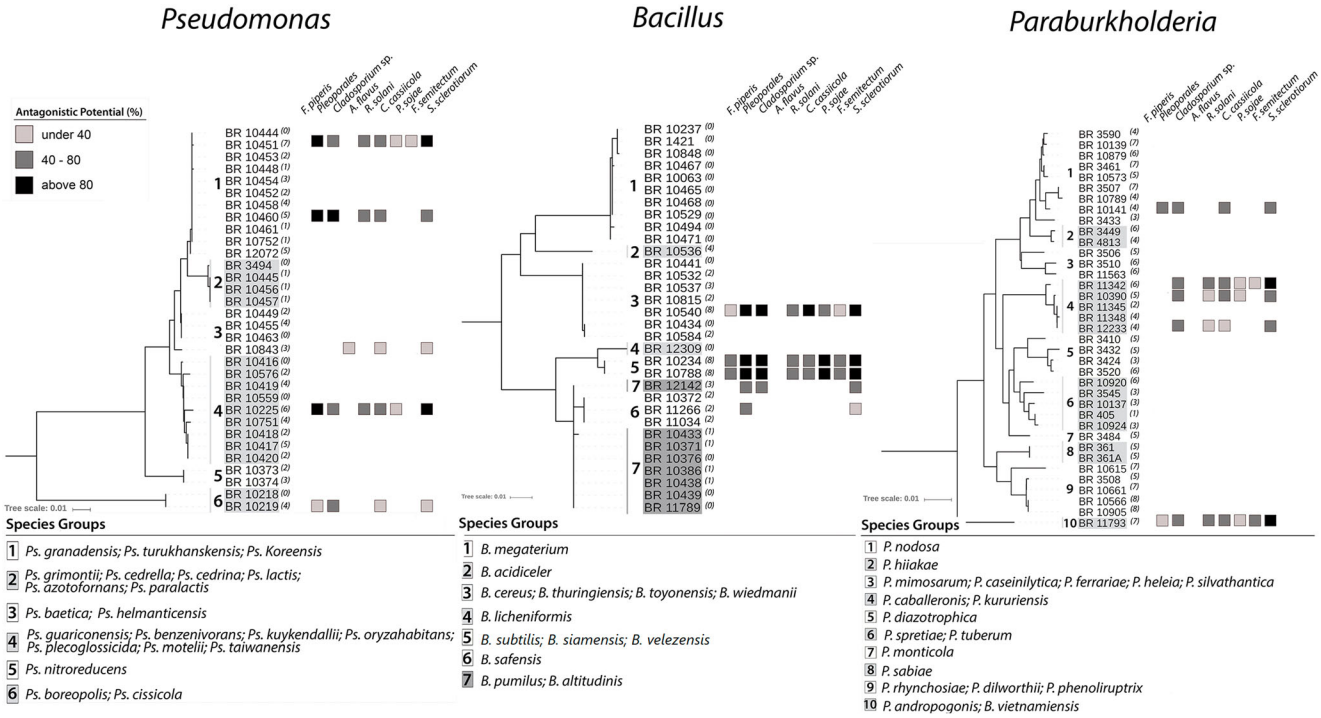

Fig. 1 Phylogenetic tree of isolates based on 16S rRNA (1250 nt) of the genera *Pseudomonas*, *Bacillus*, and *Burkholderia*/*Paraburkholderia* tested for the biological control of the fungi *Fusarium piperis*, *Fusarium semitectum*, *Pleosporales*, *Cladosporium* sp., *Phomopsis sojae*, *Rhizoctonia solani*, *Corynespora cassiicola*, *Sclerotinia sclerotiorum*, and *Aspergillus flavus*. The numbers shown before the strains indicate the type of strain groups to which they belong and the number in parentheses indicates the number of fungi controlled by the strain and the scale

wall of filamentous fungi, including *Aspergillus, Penicillium*, *Rhizoctonia*, and *Colletotrichum* species—chitinases are considered an important ally in the control of phytopathogenic fungi.

Lima et al. [45] evaluated the antagonistic effect of ten isolates of *Bacillus* spp., on *Fusarium oxysporum*, the causative agent of tomato fusariosis, using the containment technique, observed that a large number of isolates had an inhibitory effect on the pathogen, indicating that the characteristic of fungal inhibition is frequent within certain groups of *Bacillus* species. *B. subilis* isolates were also effective in inhibiting mycelial growth of pathogenic fungi *Fusarium subglutinans*, *Curvularia lunata*, and *Bipolaris* when evaluated by circular (or containment) methods with a high percentage of mycelial growth inhibition [3].

Therefore, the results presented here corroborate the results of previous studies, especially because the phylogenetically isolated isolates close to *B. subtilis* presented a high level of antagonism. In addition, the challenge test indicated that the selected strains did not show preferential action on a specific fungus, which allows their use for different plant-pathogen systems.

Bioassay for fungal strain antagonism evaluation on soybean seed germination

In the bioassay to evaluate the antagonism of strains against fungi on seeds, it is observed that strain BR 10788 was able to effectively control the fungi in the genus *Fusarium* and *S. sclerotiorum* compared to that of the other fungi with an antagonistic potential from 80 to 100%, which is highly efficient in the control of the latter (Table 1). Strain BR 10788 also presented antagonistic potential superior to 50% for *Ph. sojae* and *C. cassiicola*. On the other hand, BR 11793 significantly controlled the fungi of the genera *Fusarium* and *Ph. sojae*, *A. flavus*, and *S. sclerotiorum*, ranging from 66 to 100%. In particular, it exhibited strong control of pathogens in the genus *Fusarium*, with a potential above 90%. There was variation in the antagonistic potential of other fungi, between 5 and 44%, with no significant differences between them for the different bacterial compositions. When the bacterial mixture was used, the observed results are similar to the individualized bacteria, except for *S. sclerotiorum*, for which the antagonistic potential was lower (Table 1).

Considering the 100% antagonistic potential presented by BR 10788 for the fungus *S. sclerotiorum*, it can be concluded that it has the potential to control this fungus on seeds effectively. It is also noteworthy that treatment with this bacterium led to better seed germination because it controlled the development of the fungus (Table 2).

Bacillus subtilis, one of the species in the group to which BR 10788 belongs, is classified as an efficient biological disease control agent in plants and has been widely studied and used in agriculture for soil phytopathogen control [46–48].

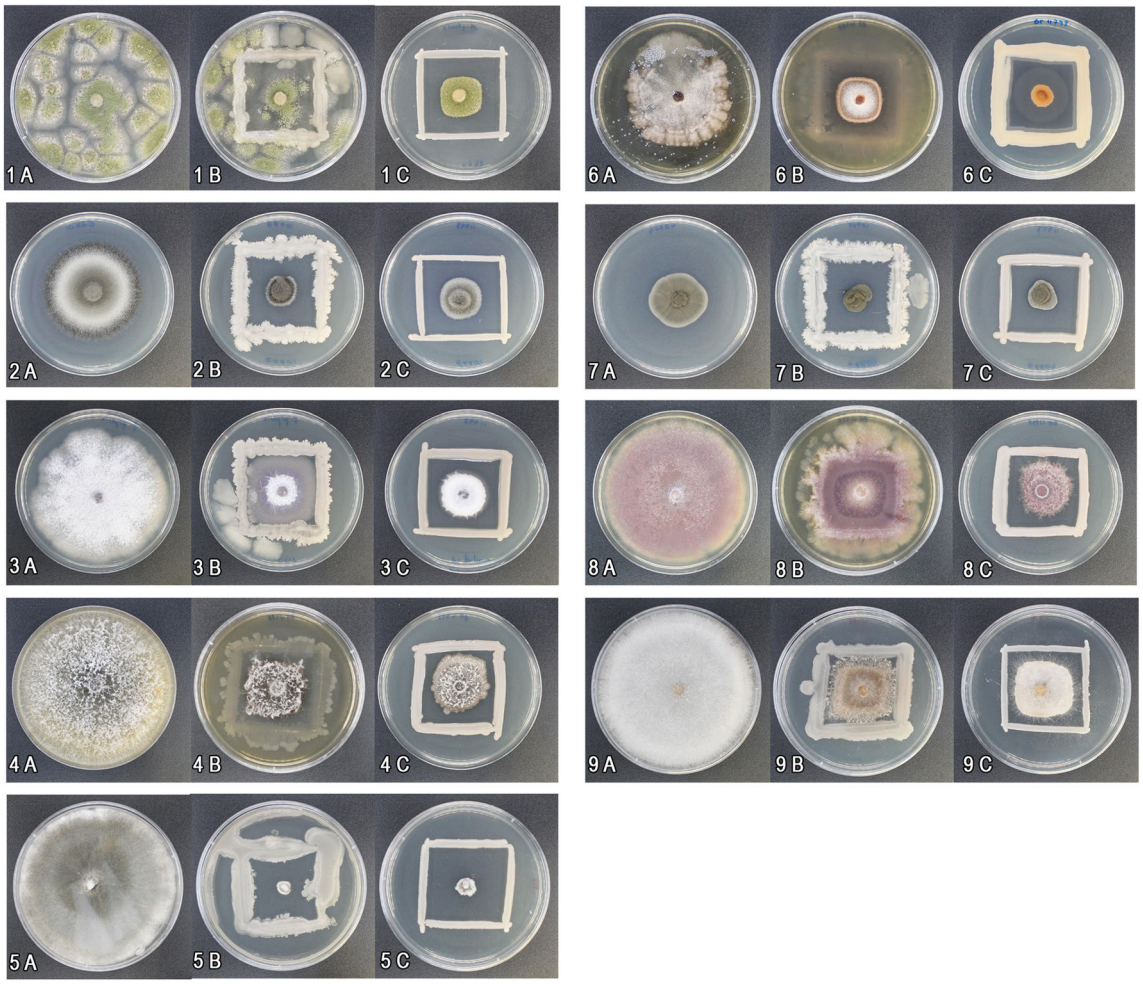

Fig. 2 Containment test for the control evaluation of the BR 10788 and BR 11793 strains with the fungal pathogens *Aspergillus flavus*, *Pleosporales*, *Fusarium semitectum*, *Phomopsis*. *sojae*, *Sclerotinia sclerotiorum*, *Corynespora cassiicola*, *Cladosporium* sp., *Fusarium piperis*, and *Rhizoctonia solani*. Column (A) Control; column (B) BR 10788; column (C) BR 11793. Row 1, *Aspergillus flavus*; row 2, Pleosporales; row 3, *Fusarium semitectum*; row 4, *Phomopsis. sojae*; row 5, *Sclerotinia sclerotiorum*; row 6, *Corynespora cassiicola*; row 7, *Cladosporium* sp.; row 8, *Fusarium piperis*; row 9, *Rhizoctonia solani*

Table 1 Antagonistic potential of bioassay inoculants against phytopathogens in the presence of soybean seeds

Fungi	BR 10788	BR 11793	Mixture	Means
	Antagonism (%)*			
F. semitectum	86.7 aA	93.3 aA	100.0 aA	93.3 a
F. piperis	80.8 aA	100.0 aA	89.6 aA	90.2 a
P. sojae	57.8 bA	85.6 aA	68.3 aA	70.6 b
C. cassiicola	52.6 bA	27.2 bA	55.9 bA	45.2 c
A.flavus	21.1 cB	86.5 aA	58.3 bA	55.3 c
S. sclerotiorum	100.0 aA	66,.7 aB	35.6 bB	67.4 b
Pleosporales	36.5 cA	44.4 bA	50.0 bA	43.7 c
R. solani	5.0 cA	5.0 bA	5.0 cA	5.0 d
Cladosporium sp.	22.2 cA	36.3 bA	51.2 bA	36.6 c
Means	51.4 A	60.6 A	57.1 A	-
CV (%)	-	-	-	37.7

*Means followed by the same letters, lowercase letters (a, b, c, d—between fungi) and uppercase letters (A, B—between bacteria) do not differ from each other by the Scott–Knott method at 5% probability

For example, in the USA, products formulated from *B. subtilis* have been used since 1983 for peanut seed treatment and foliar and soil applications. Moreover, *B. subtilis* is also used as an active seed treatment against *Fusarium* wilt, *P.* damping-off, and leaf blotch caused by *Cercospora*, *Colletotrichum*, *Alternaria*, *Ascochyta*, *Myrothecium*, *Ramularia*, *Xanthomonas*, and *Erysiphe polygoni* in cotton, cereals, vegetables, fruit, and ornamentals in India. Its action is mainly due to the production of antibiotics, competition for space and nutrients, antibiosis, and cell wall degradation. Thus, products based on antagonistic microorganisms serve as a tool for the control of phytopathogens.

Studies by Araújo [49] also showed that the treatment of soybean seeds with *Bacillus* spp. reduced the incidence of fungi in the seeds and improved the plants' nodulation and development in the presence of *Bradyrhizobium japonicum*. These additional effects of *B. subtilis* strains may be caused by the bacteria's ability to act on the synthesis of auxins,

Table 2 Treatment of inoculated seeds against phytopathogenic fungi

Bacteria strain	Number of seed germination*								
	1	2	3	4	5	6	7	8	9
Control	18.6a	18.0a	20.0a	17,3a	17,3b	19.6a	19.6a	17.0a	18.6a
BR 10788	19.6a	18.0a	19,6a	20.0a	19,6a	19.6a	19.6a	19.3a	19.6a
Control	18,6a	19,33a	17,6b	16,6b	19,3a	18.6a	19.6a	19.0a	20.0a
BR 11793	19,6a	20.0a	19,6a	19,6a	19.0a	19.0a	19.6a	19.3a	19.0a
Control	19,6a	18,33a	18,6a	18,6a	17,3a	19.6a	18.0a	19.6a	19.6a
Mixture	20.0a	18,33a	19,67a	19,3a	19.0a	19.6a	19.0a	19.0a	18.3a
CV (%)	4.78								

*Means followed by the same letter vertically (between bacteria and control) do not differentiate each other by F test at 5% probability; 1, *F. piperis*; 2, Pleosporales; 3, *Cladosporium* sp.; 4, *A. flavus*; 5, *R. solani*; 6, *C. cassiicola*; 7, *Ph. sojae*; 8, *F. semitectum*; 9, *S. sclerotiorum*

gibberellins, and cytokines leading to better root system development [50, 51].

Regarding germination, higher NSG is observed compared with the control, which did not receive the bacterial treatment for seeds inoculated with strain BR 11793 against *A. flavus* and *Cladosporium* sp. (Table 2). In the case of strain BR 10788, the NSG was significantly higher when tested against *R. solani*. The other treatments did not differ statistically from each other ($P < 0.05$) (Table 2).

Rhizoctonia solani is an optional phytopathogenic fungus, naturally inhabiting the soil and lives saprophytically, which, although not specific to a host, deserves attention. It is an aggressive pathogen that, through enzyme production, degrades the cell wall and rapidly kills the plant, promoting decomposition and reproducing rapidly at the expense of available nutrients [52]. Thus, the seeds are attacked soon after absorbing water and starting germination. Given the softened integument and soaked interior tissues, the seeds favor pathogen action. In this sense, BR 10788 was efficient against *R. solani* germination by providing rapid germination and emergence of seedlings, which led to an acceleration in the differentiation and maturation of plant tissues, thereby increasing their resistance to both penetration and colonization by this pathogen. Studies by Kondoh et al. [53], also using a *B.*

Table 3 Treatment of inoculated seeds against phytopathogenic fungi

Bacteria strain	Treatments with fungi								
	1	2	3	4	5	6	7	8	9
Plant dry matter (mg planta^{-1})*									
Control	8.5b	9.8b	11.0a	8.5a	11.0a	9.9a	9.6b	8.1a	10.2b
BR 10788	10.6a	11.9a	12.9a	10.1a	12.0a	10.7a	11.3a	10.7a	13.8a
Control	10.4b	8.7a	12.8a	9.9b	9.6b	7.7b	9.6b	11.4a	9.7b
BR 11793	13.4a	10.5a	16.8a	11.9a	11.0a	8.5a	11.3a	12.7a	12.4a
Controle	11.5b	14.5a	12.1b	9.3a	9.2a	9.9a	7.4b	9.6b	12.4a
Mixture	14.3a	16.1a	15.8a	11.0a	10.0a	11.4a	9.8a	11.7a	14.6a
CV (%)	10.60								
Number of injured seedlings*									
Control	5.6a	5.6a	16.6a	19.3a	20.0a	16.3a	17.3a	5.0a	5.6a
BR 10788	1.0b	3.3a	13.0a	15.3a	19.0a	7.6b	7.3b	0.6b	0.0b
Controle	5.3a	3.0a	7.3a	19.3a	20.0a	14.3a	17.3a	5.3a	3.0a
BR 11793	0.0b	1.6a	4.6b	2.6b	19.0a	10.3a	7.3b	0.3b	0.3a
Control	5.6a	1.0a	10.3a	18.0a	20.0a	11.3a	20.0a	6.6a	5.6a
Mistura	0.6b	0.6a	5.0b	7.0b	19.0a	4.3a	6.3b	0.0b	3.0a
CV (%)	31.56								

*Means followed by the same letter vertically (a). Between bacteria and control. Do not differentiate each other by *F* test at 5% probability at 5% probability; 1, *F. piperis*; 2, *Pleosporales*; 3, *Cladosporium* sp.; 4, *A. flavus*; 5, *R. solani*; 6, *C. cassiicola*; 7, *Ph. sojae*; 8, *F. semitectum*; 9, S. sclerotiorum

subtilis isolate plus the fungicide flutolanil, found a synergistic effect for *R. solani* control in tomatoes. Therefore, growth promotion provided by *B. subtilis* or other *Bacillus* species can lead to rapid germination.

Regarding the biomass accumulation of soybean seedlings, strain BR 10788 also provided higher values occurring prominently in the presence of the fungi *F. piperis*, *F. semitectum*, *Ph. sojae*, and *S. sclerotiorum*. This further highlights the bacteria's ability to control fungi and have an additive effect on the promotion of plant growth (Table 3).

Regarding BR 11793, seedlings originating from seeds inoculated with this strain accumulated greater biomass than the control without bacteria in most treatments, as occurred for strain BR 10788. This effect is observed for all fungi, except *Cladosporium* and *F. semitectum* (Table 3). For the mixture of the two bacterial strains, an increase in seedling biomass is also observed, mainly for *F. piperis*, *Cladosporium*, *Ph. sojae*, and *F. semitectum* representing an additional effect on *Cladosporium* sp., not observed with the individualized strains (Table 3).

In the NPL evaluation, when inoculated with BR 10788, there was a reduction in lesions in the presence of the fungi *F. piperis*, *Cladosporium* sp., *A. flavus*, *Ph. sojae*, and *F. semitectum*, with a tendency of reduction in the lesions for the other treatments.

The success of *Bacillus* and *Paraburkholderia* in promoting plant growth and controlling fungal attacks on seeds is intrinsically related to the biological characteristics of these microorganisms. Thus, treatment of seeds before planting with biofungicides is an additional guarantee for the establishment of plants in the field, because they protect seedlings from pathogen attack early in seedling development.

BR 11793, including the *P. andropogonis* species group, showed a broad spectrum of action, presenting biocontrol capacity above 60% for most of the pathogens tested. Recently, comparative genomic analyses of the genome of *P. kururiensis* strain KP23T, M130, and ATSSB13T revealed important traits, such as genes involved in plant growth, including ACC deaminase, genes for AIA biosynthesis, and genes involved in the breakdown of aromatic compounds. These findings indicate important mechanisms that require further investigation of these strains for environmentally strategic applications, bioremediation, biofertilization, and biocontrol of plant pathogens [28]. However, although they have shown positive results in the applied tests, little is known about the molecular mechanisms involved in the bacterium-plant relationship, and further research is needed for their elucidation.

Conclusions

Strains BR 10788 and BR 11793 showed efficiency in the treatment of soybean seeds against phytopathogenic fungi. The combination of bacteria BR 10788 and BR 11793 showed fungal control ability equal to or superior to individual strains. The studied bioagents can reduce the use of fungicides in the treatment of seeds of agricultural crops.

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Code availability Not applicable

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Data Availability The data are stored and available in our institution following the own rules

Declarations

Ethics approval Not applicable

Consent to participate All authors consent to participate in the paper.

Consent for publication All authors consent to participate in the paper publication.

Conflicts of interest/Competing interests Not applicable

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