Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga
ISSN 1983-2125 (online)

http://dx.doi.org/10.1590/1983-21252024v3711724rc

Scientific Article

Screening of bacterial isolates antagonists and suppressors of blast in rice plants

Seleção de isolados bacterianos antagonistas e supressores de brusone em plantas de arroz

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ABSTRACT - Grain yields of rice (*Oryza sativa*) are affected globally by rice blast (*Magnaporthe oryzae*). The main objective of this study was to identify isolates of rhizobacterial antagonists of *M. oryzae* (BRM10781) and screen the most effective isolates for suppressing rice blast under greenhouse conditions. Two assays (E1 and E2) were performed with 22 treatments in a completely randomized design with three replicates. E1 investigated *in vitro* antagonism between 21 isolates and *M. oryzae* under laboratory conditions. The E2 experiments were conducted under greenhouse conditions, with rice cultivar BRS Primavera seeds in plastic trays containing 3 kg of fertilized soil. After 21 days, the rice leaves were spray-inoculated with a bacterial cell suspension (1×10^8 CFU) and *M. oryzae* (3×10^5 conidia.mL^{-1}) or with water (absolute control). Seven isolates, *Serratia marcescens* (BRM65918, BRM65923, BRM65926, and BRM63532), *Bacillus cereus* (BRM65919), *Stenotrophomonas nitritireducens* (BRM65917), and *Priestia megaterium* (BRM65929), reduced radial growth of *M. oryzae* colonies from 80.26 to 77.33%. The best leaf blast severity reducers were *Pseudomonas nitroreducens* (BRM32112), *B. thuringiensis* (BRM65928), *P. megaterium* (BRM65916), *S. marcescens* (BRM65918), *S. nematodiphila* (BRM63522), and *Enterobacter hormaechei* (BRM65925), varying from 97 to 95% respectively. The isolate BRM65918 (*S. marcescens*) showed the best efficiency for both antagonism and disease suppression, indicating its potential as a bioproduct for the biocontrol of rice blast in rice plants.

Keywords: Bioagents. Biocontrol. Inoculation. *Magnaporthe oryzae*. *Oryza sativa*.

RESUMO - A produtividade do arroz (*Oryza sativa*) é afetado mundialmente pela brusone do arroz (*Magnaporthe oryzae*). O principal objetivo deste estudo foi identificar isolados de rizobactérias antagonistas de *M. oryzae* (BRM10781) e selecionar os isolados mais eficazes para suprimir a brusone do arroz em condições de casa de vegetação. Foram realizados dois ensaios (E1 e E2) com 22 tratamentos em um delineamento inteiramente casualizado com três repetições. E1 investigou o antagonismo *in vitro* entre 21 isolados e *M. oryzae* em condições de laboratório. O experimento E2 foi conduzido em condições de casa de vegetação, com sementes de arroz da cultivar BRS Primavera em bandejas plásticas contendo 3 kg de solo adubado. Após 21 dias, as folhas de arroz foram inoculadas por aspersão com uma suspensão de células bacterianas (1×10^8 UFC) e *M. oryzae* (3×10^5 conídios.mL^{-1}) ou com água (controle absoluto). Sete isolados, *Serratia marcescens* (BRM65918, BRM65923, BRM65926 e BRM63532), *Bacillus cereus* (BRM65919), *Stenotrophomonas nitritireducens* (BRM65917) e *Priestia megaterium* (BRM65929), reduziram o crescimento radial de colônias de *M. oryzae* de 80,26 para 77,33%. Os melhores supressores da severidade da brusone foliar foram *Pseudomonas nitroreducens* (BRM32112), *B. thuringiensis* (BRM65928), *P. megaterium* (BRM65916), *S. marcescens* (BRM65918), *S. nematodiphila* (BRM63522) e *Enterobacter hormaechei* (BRM65925), variando de 97 a 95%, respectivamente. O isolado BRM65918 (*S. marcescens*) apresentou a melhor eficiência tanto para o antagonismo quanto para a supressão da doença, indicando seu potencial como bioproduto para o biocontrole da brusone em plantas de arroz.

Palavras-chave: Bioagentes. Biocontrole. Inoculação. *Magnaporthe oryzae*. *Oryza sativa*.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: February 4, 2023.
Accepted in: November 28, 2023.

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INTRODUCTION

Upland rice cultivation systems are affected by both biotic and abiotic factors. Biotic stresses, such as blast caused by the pathogen *Magnaporthe oryzae* (Herbet) Barr. [anamorph *Pyricularia oryzae* (Cook) Sacc] can lead to losses, low performance, and negative effects on production (ENEBE; BABALOLA, 2018). Rice blast disease (*M. oryzae*) is the most destructive disease. Its distribution is geographically wide and occurs in practically all regions where rice is grown. The disease can infect leaves, stalks, panicles, and consequently, seeds, which can lead to a 100% loss in yield, destroying approximately 10–30% of the world's rice harvested (FILIPPI et al., 2011; BEZERRA et al., 2021). Disease control depends on integrated management, including planting resistant cultivars, cultural practices, and fungicide applications.

Genetically improved cultivars are primarily selected for their vertical resistance to the most common pathogens in the population. Despite the sequential release of genetically improved cultivars for blast resistance, variability in pathogen populations renders genetic resistance fragile (FILIPPI et al., 2011; SOUZA et al., 2015). Thus, the instability of the vertical resistance of improved

cultivars, combined with the size of the planted area and excess nitrogen fertilizers, leads farmers to use fungicides indiscriminately, even on the eve of grain harvest, increasing the chances of grains containing fungicide residues (FILIPPI et al., 2011). Chemical control is one of the most harmful methods of disease control. If not part of sustainable management, it can negatively affect the environment by leaving residues and harming the environment and human health (WIRASWATI et al., 2019).

Nevertheless, given the projections of population growth, in contrast to societal concerns about the impacts of the indiscriminate use of pesticides, biological control is considered an attractive contribution to the reduction of environmental and social impacts (PRATHAP; RANJITHA KUMARI, 2017). Plant Growth Promoter Rhizobacteria (PGPR) is an effective biocontrol agent to combat economically important crop pathogens. They are considered an eco-friendly and sustainable approach to managing plant diseases.

Many studies have been conducted on rice plant endophytes and rhizosphere microorganisms that exhibit antagonistic activity against rice pathogens (MARTINS et al., 2020). Some research teams have shown successful examples. Rais et al. (2018) selected *Bacillus spp*. as an antagonist of *M. oryzae*, which reduced the blast incidence in rice and increased grain yield under field conditions. Oliveira et al. (2020) evaluated the potential of four liquid formulations containing *Burkholderia cepacia* (BRM32111) and *Serratia* sp. (BRM32113) in rice fields. All treatments were efficient in suppressing leaf and panicle blast and promoting biomass increase and grain yield, and could be included in the integrated management of blast control in rice fields.

Among the rice diseases, sheath blight (*Rhizoctonia solani*) causes significant yield losses in rice (*Oryza sativa* L.). Looking for its sustainable management, Ajulo et al. (2023) taxonomically identified and evaluated 21 isolates of rhizobacteria. The authors identified BRM32112 (*Pseudomonas nitroreducens*), BRM65929 (*Priestia megaterium*), and BRM65919 (*Bacillus cereus*) as antagonists of *R. solani*, and BRM63523 (*Serratia marcescens*), BRM65923, BRM65916 (*P. megaterium*), and BRM65919 (*B. cereus*) as sheath blight suppressors under greenhouse conditions. The main objective of this study was to evaluate 21 isolates as potential antagonists of *M. oryzae* and leaf blast suppressors.

MATERIAL AND METHODS

The experimental area is located at Fazenda Capivara, Embrapa Rice and Beans, in the municipality of Santo Antônio de Goiás, GO, Brazil (16°28'00" S and 49°17"00" W). The region has an altitude of 823 m, and the predominant climate is tropical, with two well-defined seasons: a rainy season (October–April) and a dry season (May–September). Soil chemical analysis of the experimental area (0–20 cm depth) was performed as described by Claessen (1997). The experiment was conducted in a greenhouse between November 2021 and November 2022.

Microorganisms

The bacterial isolates used in this study belonged to the Multifunctional Collection of Microorganisms from Embrapa Rice and Beans and were taxonomically identified by Ajulo et al. (2023). The NCBI and local codes, taxonomic identification, and treatment descriptions are presented in Table 1. All bacterial isolates were obtained from the roots of cv. BRS Primavera cultivated in the agroecological systems. The strains were preserved using the Castellani method (CAPRILES; MATA; MIDDELVEEN, 1989) and deep freezing. The bacterial isolates were transferred and cultured in Petri plates containing Nutrient Agar (NA), which were then incubated for 48 h at 28°C.

Table 1. Treatments, local and NCBI codes, and Taxonomic identification of the bacterial isolates applied for testing leaf Blast suppression.

Local Code	NCBI ID	Treatment order	Taxonomic identification
BRM65919	PP025212	T1	*Bacillus cereus*
BRM65924	PP025213	T2	*Bacillus cereus*
BRM65921	PP025214	T3	*Bacillus cereus*
BRM65922	PP025215	T4	*Bacillus cereus*
BRM65927	PP025216	T5	*Bacillus cereus*
BRM65915	PP025217	T6	*Priestia megaterium*
BRM65916	PP025218	T7	*Prestia megaterium*
BRM65929	PP025219	T8	*Priestia megaterium*
BRM65928	PP025320	T9	*Bacillus thuringiensis*
BRM65918	PP025321	T10	*Serratia marcescens*
BRM65923	PP025322	T11	*Serratia marcescens*
BRM65920	PP025393	T12	*Serratia marcescens*
BRM65926	PP025394	T13	*Serratia marcescens*
BRM63523	PP025395	T14	*Serratia marcescens*
BRM63522	PP025401	T15	*Serratia nematodiphila*
BRM63521	PP025400	T16	*Serratia marcescens*
BRM65930	PP025402	T17	*Stenotrophomonas maltophilia*
BRM65917	PP025421	T18	*Stenotrophomonas nitritireducens*
BRM63525	PP025422	T19	*Enterobacter hormaechei*
BRM65925	PP025423	T20	*Enterobacter hormaechei*
BRM32112	MT188712	T21	*Pseudomonas nitroreducens*
BRM10781	-	T22	*Magnaporthe oryzae*

Antagonism between bacterial isolates and *M. oryzae in vitro*

M. oryzae (BRM10781) was grown in Petri dishes containing PDA medium for nine days at 28ºC. The assays were conducted in a completely randomized design with 21 treatments and a control (*M. oryzae*), with five repetitions each. Five mm mycelium discs from colonies of *M. oryzae* were placed in the center of the Petri dish, and 20 µL of bacterial suspensions were distributed at four equidistant points (MARTINS et al., 2020). The plates of *M. oryzae* were incubated at 28ºC under continuous fluorescent light. Plates containing only *M. oryzae* mycelium discs were used as positive controls. After nine days of incubation, the evaluation was performed, measuring the diameter of the pathogen colonies compared to the control treatment.

Rice blast suppression under greenhouse conditions

Seeds of the cultivar BRS Primavera were disinfected with sodium hypochlorite (1 min), 70% alcohol (1 min), and distilled water before planting. Plastic trays measuring 15 cm × 30 cm × 10 cm were filled with approximately 3 kg of unsterilized soil. A fertility analyses was performed: pH in H_2O 5.8, pH in $CaCl_2$ 0.01 M 4.5, Ca 27.7 $mmol_c$ dm^{-3}, Mg 11.2 $mmol_c$ dm^{-3}, Al 0 $mmol_c$ dm^{-3}, H + Al 17 $mmol_c$ dm^{-3}, P 1.6 $mmol_c$ dm^{-3}, K 100 $mmol_c$ dm^{-3}, Cu 1.1 mg dm^{-3}, Zn 1.8 mg dm^{-3}, Fe 11.3 mg dm^{-3}, Mn 27.2 mg dm^{-3}, and soil organic matter 42.7 g dm^{-3}. Soil fertilization was performed by applying 5 g NPK (5-30-15) + 1.5 g ammonium sulfate at the sowing time and 3 g ammonium sulfate 17 days after planting. Seeds were sown in eight furrows of approximately 4 cm in length. The experimental design was completely randomized, with 22 trials (21 rhizobacterial isolates and one control) and three repetitions. The treatments consisted of mixing *M. oryzae* conidial suspension and rhizobacterial cells.

Bacterial suspension

Twenty-one rhizobacterial isolates were transferred and cultured in Petri plates containing Nutrient Agar (NA), which were then incubated for 48 h at 28°C. The bacteria were cultivated in Petri dishes containing nutrient agar medium at 28°C for 24 h. Then, with the aid of a platinum loop, a portion of the bacteria grown on the plates was collected and placed in an autoclaved Erlenmeyer flask containing nutrient broth (100 mL) to prepare bacterial suspensions, which were left for 24 h with stirring at 140 rpm. The concentration of the bacterial suspension was adjusted to an absorbance of 0.5 at a wavelength of 540 nm, corresponding to a concentration of 1×10^8 CFU.mL^{-1} (FILIPPI et al., 2011; MARTINS et al., 2020).

Conidia suspension of *M. oryzae*

Mycelial fragments of isolate BRM10781 were transferred to sterile Petri dishes containing PDA medium (potato agar). Plates were incubated under continuous fluorescent light at 25ºC ± 2 and humidity above 80% for seven days. After two days, using distilled water and a brush, the conidia were removed and filtered with sterelized tissue. Conidium counting was performed in a Neubauer chamber and optical microscope, and the concentration was adjusted to 3×10^5 conidia.mL^{-1}, according to Filippi and Prabhu (2001).

Rice leaves spray inoculation

At 21 days after planting, the rice plants were sprayed with 15 mL of a bacterial suspension (1×10^8 CFU.mL^{-1}), mixed with 15 mL of *M. oryzae* conidial suspension (final concentration 3×10^5 conidia.mL^{-1}) (ARRIEL-ELIAS et al., 2023). The rice trays were separated into plastic boxes to isolate the treatments.

Following inoculation, the challenged plants were subjected to temperatures that fluctuated between 28 and 30ºC and up to 90% humidity in the greenhouse, inside the plastic boxes covered with a transparent plastic top to favor the infection development. Eight days after inoculation, based on the percentage of leaf area affected by the disease, 10 plants per treatment were evaluated, with three leaves per plant for a total of 1,320. Severity assessments of blast on the leaves were performed eight days after inoculation, through the percentage of the leaf area affected by the disease on the first open leaf, using a scale of 10 degrees (0, 0.5, 1, 2, 4, 8, 16, 32 and 82%) according to Notteghem (1981), determining the percentage of leaf area affected by the disease.

Statistical analysis

The averages of each test were calculated, the variances analyzed, and the proportions were compared using the Scott-Knott at 5% significance using the *R* platform (R CORE TEAM, 2023).

RESULTS AND DISCUSSION

Among the 21 isolates tested, five belong to the species *Bacillus cereus*, one to *B. thuringiensis*, three to *Priestia megaterium*, six to *Serratia marcescens*, one to *S. nematodiphila*, two to *Stenotrophomonas spp*., two to *Enterobacter spp*. and one to *Pseudomonas nitroreducens*, according to Ajulo et al. (2023).

Analysis of the antagonistic data revealed a statistically significant difference between treatments. The comparison between the means organized the treatments into four groups (Figures 1 and 2). Group 1 was the most efficient at reducing the area of the *M. oryzae* colony, from 80.26 to 77.33%. Group 1 comprised four isolates of *S. marcescens*, one of *B. cereus*, one of *P. megaterium*, and one of *S. nitritireduncens*. Group 2 comprised 12 isolates, reducing *M. oryzae* colonies' area from 76.69 to 74.52%. In group 3, isolate BRM65920 (*S. marcescens*) reduced by 72%, and BRM65916 (*P. megaterium*) represented group 4 with 66% of colony reduction area (Figures 1 and 2).

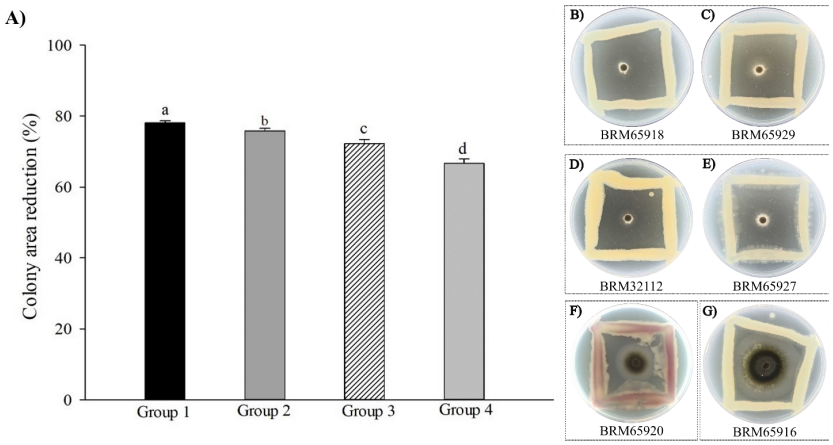

Figure 1. Antagonism assay between *M. oryzae* and 21 Rhizobacteria in vitro. A) The *M. oryzae* colony area was reduced by four groups of rhizobacteria isolates. Different letters are statistically different by the Scott-Knott test ($p < 0.05$). Group 1 (BRM65919, BRM65918, BRM65917, BRM65923, BRM65926, BRM65929 and BRM63523); Group 2 (BRM65915, BRM65925, BRM65921, BRM63525, BRM65930, BRM65927, BRM65928, BRM65922, BRM63521, BRM65924, BRM63522 and BRM32112); Group 3 (BRM65920); Group 4 (BRM65916). Petri dishes containing PDA with *M. oryzae* colony surrounded by different isolates of Rhizobacteria: B) *S. marcescens* (BRM65918); C) *P. megaterium* (BRM65929); D) *P. nitroreducens* (BRM32112); E) *B. cereus* (BRM65927); F) *S. marcescens* (BRM65920); G) *P. megaterium* (BRM65916).

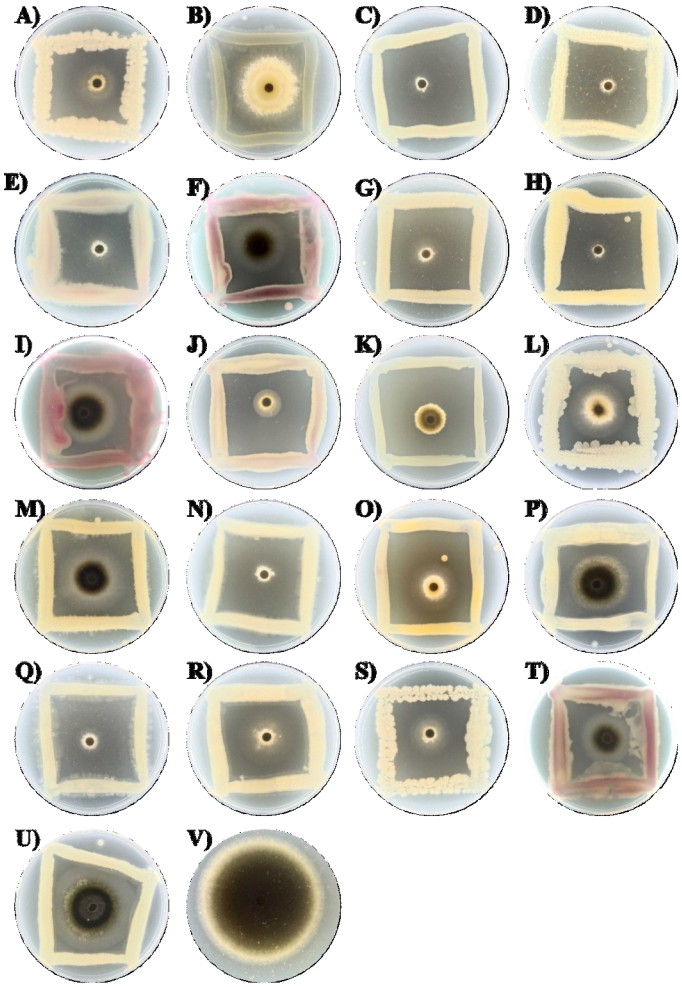

Figure 2. In vitro antagonism between 21 rhizobacteria isolates and control and rice pathogens was evaluated by the pairing method with *Magnaporthe oryzae*. Group 1: A) BRM63523; B) BRM65917; C) BRM65918; D) BRM65919; E) BRM65923; F) BRM65926; G) BRM65929. Group 2: H) BRM32112; I) BRM63521; J) BRM63522; K) BRM63525; L) BRM65915; M) BRM65921; N) BRM65922; O) BRM65924; P) BRM65925; Q) BRM65927; R) BRM65928; S) BRM65930. Group 3: T) BRM65920. Group 4: U) BRM65916. V) Control. For details on the isolates' species, see Table 1.

After identification as a good antagonist, the next step for bioagent selection should be testing in the greenhouse, evaluating the efficiency of the same isolates on disease suppression in interaction among the host, pathogen, and beneficial microorganisms. The greenhouse assay revealed that, among the 21 treatments, all bacterial isolates reduced leaf blast severity, differing from the control treatment. The lowest leaf blast severity was observed following treatment T22, BRM32112 (*Pseudomonas nitroreducens*). Leaf blast reduction was approximately 97% (Figure 3, Table 2).

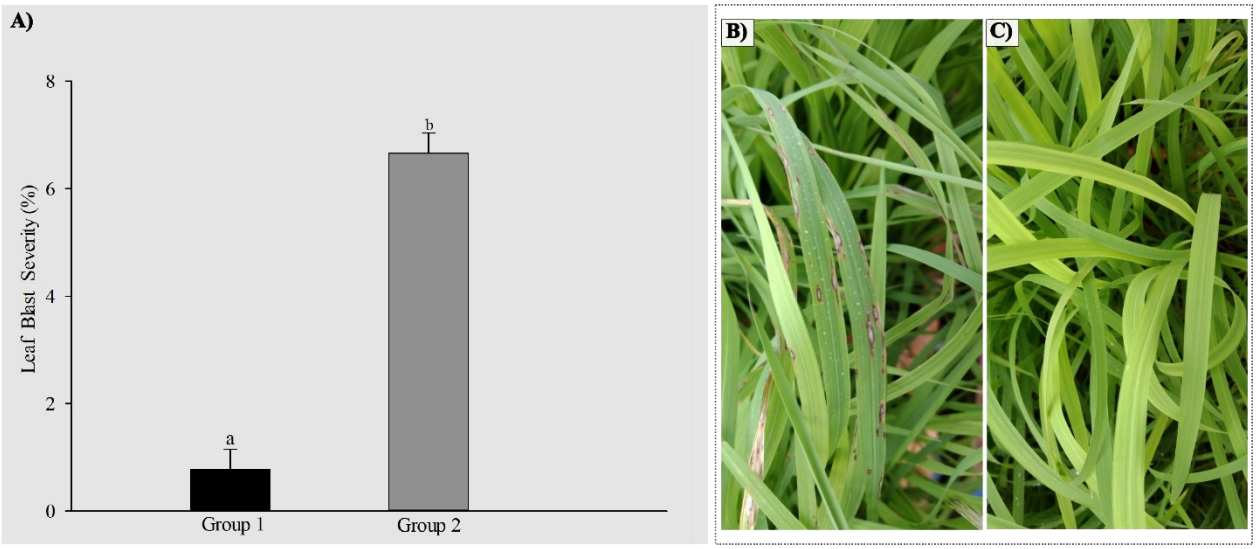

Figure 3. Suppression of leaf blast severity. A) Leaf blast severity has two groups: Group 1 (BRM65919, BRM65918, BRM65917, BRM65923, BRM65926, BRM65929, BRM63523, BRM65915, BRM65925, BRM65921, BRM63525, BRM65930, BRM65927, BRM65928, BRM65922, BRM63521, BRM65924, BRM63522, BRM32112, BRM65920 and BRM65916); Group 2 (CONTROL). Different letters are statistically different ($p < 0.05$) by the Scott-Knott test. Rice Blast symptoms, sporulating typical lesions on control (B), contrasting with rice leaves with no symptoms (C).

Table 2. Efficiency of 21 bacterial isolates on reducing *Magnaporthe oryzae* colony growth and rice blast severity[1].

Collection code	NCBI	Treatment order	Taxonomic identification	Rice Blast Severity (%)	*M. oryzae* colony reduction (%)
BRM65919	PP025212	T1	*Bacillus cereus*	0.50b	77.33a
BRM65924	PP025213	T2	*Bacillus cereus*	0.50b	76.56b
BRM65921	PP025214	T3	*Bacillus cereus*	0.83b	75.26b
BRM65922	PP025215	T4	*Bacillus cereus*	0.83b	76.24b
BRM65927	PP025216	T5	*Bacillus cereus*	0.83b	75.51b
BRM65915	PP025217	T6	*Priestia megaterium*	1.50b	74.52b
BRM65916	PP025218	T7	*Prestia megaterium*	0.33b	66.67d
BRM65929	PP025219	T8	*Priestia megaterium*	2.33b	78.88a
BRM65928	PP025320	T9	*Bacillus thuringiensis*	0.33b	75.98b
BRM65918	PP025321	T10	*Serratia marcescens*	0.33b	77.42a
BRM65923	PP025322	T11	*Serratia marcescens*	0.50b	77.42a
BRM65920	PP025393	T12	*Serratia marcescens*	1.50b	72.21c
BRM65926	PP025394	T13	*Serratia marcescens*	0.50b	78.18a
BRM63523	PP025395	T14	*Serratia marcescens*	0.83b	80.26a
BRM63522	PP025401	T15	*Serratia nematodiphila*	0.33b	76.67b
BRM63521	PP025400	T16	*Serratia marcescens*	0.83b	76.38b
BRM65930	PP025402	T17	*Stenotrophomonas maltophilia*	0.50b	75.47b
BRM65917	PP025421	T18	*Stenotrophomonas nitritireducens*	0.50b	77.42a
BRM63525	PP025422	T19	*Enterobacter hormaechei*	2.00b	75.37b
BRM65925	PP025423	T20	*Enterobacter hormaechei*	0.33b	74.64b
BRM32112	MT188712	T21	*Pseudomonas nitroreducens*	0.16b	76.69b
BRM10781	-	T22	*Magnaporthe oryzae*	6.66a	-

[1]Means followed by the same letter do not statistically differ from each other but differ in the Scott-Knott test ($p < 0.05$). The severity of the rice blast was assessed using a diagrammatic scale (NOTTEGHEM, 1981).

Pseudomonas spp. isolates are efficient biological control agents against plant diseases, probably because they can produce cellulose, siderophores, and phosphatase, in addition to inducing resistance in rice plants (FILIPPI et al., 2011; AJULO et al., 2023), which work together to limit *M. oryzae* tissue plant colonization. The leaf blast cycle begins when the conidia germinate and form a thin germ tube as the spores adhere to the hydrophobic cuticle of the rice leaf. The germ tube develops into an appressorium, and a small penetration peg forms that pierces the cuticle and allows access to the rice epidermis. Bulbous, invasive hyphae penetrating the rice plasma membrane and entering epidermal cells are responsible for plant tissue invasion and symptom development (BODDY, 2016). As shown in Figure 3, the control plants presented typical sporulative lesions, indicating that *M. oryzae* conidia germinated and colonized the rice leaves, and plasmodesmata migrated cell-to-cell, covering the rice leaves with typical symptoms. In contrast, plants treated with the isolate BRM32112 (*P. nitroreducens*) had no lesions, indicating that *M. oryzae* conidia did not produce symptoms, although we cannot confirm that antagonism only occurs.

Other isolates also presented a very high rice blast suppression, such as 95, 92, and 70% (Figure 2). It is important to highlight the diversity between the results obtained for isolates of the same species (Table 2), which can be observed among isolates of *P. megaterium* (formerly *B. megaterium*) (LIU et al., 2023), *S. marcescens*, *Bacillus spp*., and *Enterobacter spp*.

In recent years, many bacterial genera have been studied, including more frequently the genera *Bacillus*, *Stenotrophomonas*, *Burkholderia,* and *Pseudomonas* (LIU et al., 2023). *Bacillus* is one of the main genera, and its taxonomy has recently been revisited using comprehensive phylogenomic and comparative genomic approaches. *Priestia megaterium* is a new genus separate from *Bacillus* and is considered a potential biological control agent with antimicrobial activities and various control effects on plant diseases. Several mechanisms allow *P. megaterium* to act as a biopesticide or biocontrol agent. Ajulo et al. (2023) also demonstrated that *P. megaterium* could solubilize phosphorus and zinc and produce lytic enzymes, such as lipase, laccase, amylase, protease, siderophores, and the phytohormone IAA, indicating that some of the mechanisms applied by this isolate are efficient antagonists of *M. oryzae*.

Serratia spp. is a rod-shaped bacterium that has been proposed as a plant growth-promoting rhizobacterium due to its phosphate solubilization properties, IAA, siderophores, and biofilm production, besides enzyme activities such as ACC deaminase, cellulase, β-1-3 glucanase, chitinase activity, and antifungal metabolites, such as pyrrolnitrin, carbapenem, prodigiosin, haterumalide, and siderophores (LEVENFORS et al., 2004). Compared to synthetic compounds, these natural products offer greater structural diversity and synergism between molecules and are considered exceptional sources of new agrochemicals (TREMACOLDI; SOUZA FILHO, 2006). These special traits perfectly fit the requirements for the biological control of phytopathogens (ARRIEL-ELIAS et al., 2023). In our study, *S. marcescens* (BRM63523) stood out in reducing the area of *M. oryzae* colonies.

Pseudomonas spp. belongs to the family Pseudomonadaceae and comprises 191 described species. Members of this genus exhibit high levels of metabolic diversity, allowing them to colonize a wide range of niches (KOEHORST et al., 2016). Numerous studies have focused on the effects of bacteria of the genus *Pseudomonas* on fungal growth (BAJPAI et al., 2018). When investigating the antifungal activity against major rice pathogens and discovering an isolate capable of inhibiting mycelial growth when isolated and purified, they discovered that 2,4-diacetylfloroglucinol (DAPG) could potentially inhibit *M. oryzae* growth and suppress rice plant colonization.

Bacillus is another important genus, endospore producers that is highly resistant to adverse environmental conditions (CLAUS; BERKELEY, 1986). *Bacillus cereus* is a spore-forming soil bacterium proven to be an effective biological control agent against plant diseases. However, it is uncommon to have *B. thuringiensis* as a plant disease suppressor (Figures 1 and 2). *B. thuringiensis* and its metabolites efficiently reduced the *M. oryzae* colony area and suppressed the severity of rice blast. The mechanisms applied by *B. thuringiensis* could limit pathogen growth and tissue colonization or improve plant defense systems (FILIPPI et al., 2011; ARRIEL-ELIAS et al., 2023). A good example of *Bacillus spp.* as a bioagent was described by Zhu et al. (2021). The group found that *B. velezensis* either directly or indirectly defends the plant against rice blast by generating antibiotics against plant pathogenic bacteria and triggering a rice PAMP-triggered immune (PTI) response.

Other studies carried out under controlled conditions demonstrated the potential of the rhizobacteria *Burkholderia cepacia* (BRM32111) and *S. marcescens* (BRM32113) in plant growth promotion, resistance induction, leaf blast suppression, dry matter gain, promote positive changes in physiological parameters and induce positives morpho-anatomic in rice roots, increase in root length, root cortex expansion, and increase spaces aerenchyma (FILIPPI et al., 2011; PRATHAP; RANJITHA KUMARI, 2017; SOUZA et al., 2021).

Among the 21 rhizobacterial isolates investigated here, originating from an agroecological agricultural environment, seven were classified as belonging to the genus *Serratia*, six as *Bacillus*, three as *Priestia*, two as *Stenotrophomonas*, two as *Enterobacter,* and one as *Pseudomonas*. These isolates were identified by sequencing amplicons from the 16S rRNA region (AJULO et al., 2023). The taxonomic classification of prokaryotes has been carried out using techniques that have evolved and become more robust over the years, thus making it possible to adjust interest rates and their components. Expectations are that prokaryotic taxonomy will acquire a more stable status in the genomic era (HELENE; KLEPA; HUNGRIA, 2022) and thus improve our understanding of microbiota populations and their constituents.

These results provide relevant food and nutritional options. The environmentally friendly control of plant diseases fits perfectly into the ONU 2030 Agenda. It is aligned with the Concept of One Health System, an integrated and unifying approach that aims to sustainably balance, improve, and optimize the health of people, animals, and ecosystems (HOFFMANN et al., 2022).

CONCLUSION

Seven isolates, classified as *S. marcescens* (BRM65918, BRM65923, BRM65926, and BRM63523), *B. cereus* (BRM65919), *Stenotrophomonas nitritireducens*

(BRM65917), and *P. megaterium* (BRM65929) reduced radial growth of *M. oryzae* from 80.26 to 77.33%.

The best leaf blast severity reducers were *P. nitroreducens* (BRM32112), *B. thuringiensis* (BRM65928), *P. megaterium* (BRM65916), *S. marcescens* (BRM65918), *S. nematodiphila* (BRM63522), and *E. hormaechei* (BRM65925), varying from 97 to 95% respectively. BRM65918 presented the best efficiency for both antagonism and disease suppression, breaking the disease cycle throughout the infection process, in which the bacteria operate by direct antagonism with *M. oryzae*. However, these 14 isolates should be tested separately under field conditions and mixed to determine their synergistic effects on rice disease management.

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