Revista Ciência Agronômica, v. 55, e20207793, 2024
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Bacterial biocontrol of Rice disease: Compatibility with Pesticides and effects on the rhizosphere microbiota[1]

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ABSTRACT - The aim of this study was to determine the behaviour of bacterial biocontrol agents (BBAs) selected to control disease in irrigated rice, in the presence of pesticides, and to study changes in the soil microbiota. We assessed the compatibility between these BBAs and pesticides using different doses of each compound, besides the impact of the BBAs on the quality and diversity of the soil microbiota under field conditions. For the microbial evaluations, rice seeds were treated with the same BBAs, in addition to the following combinations: DFs-C6 (DFs185 *(Pseudomonas synxantha)*/DFs306 (/unidentified)); DFs-C7 (DFs306/DFs416 (*Bacillus* sp.)) and DFs-C8 (DFs185/DFs306/DFs416). Saline solution was used for the control treatment. Soil samples were collected close to the roots to evaluate basal respiration, organic matter, carbon, and microbial biomass. EcoPlate™ microplates were used to determine the activity and diversity of the microbial metabolic profile. All the BBAs (DFs185, DFs223 (*P. fluorescens*), DFs306 and DFs416) were tolerant to the fungicides and herbicides regardless of the dose, whereas tolerance to the insecticides occurred only in the DFs416 isolate. Resistance, however, was only seen in the DFs185 (pyraclostrobin + epoxiconazole) and DFs223 (azoxystrobin) isolates. There were significant changes to the soil microbiota, especially with the DFsC06 treatment, which was the only treatment to increase the microbial biomass and quotient. Little or no difference was found in microbial metabolic activity or diversity. These results suggest the potential use of these bacterial treatments in integrated pest management, as they are resistant/tolerant to many pesticides used in rice cultivation, and some of them had a positive impact on the soil microbiota.

Key words: Environmental impact. Integrated disease control. Microbial activity. *Bacillus. Pseudomonas*.

DOI: 10.5935/1806-6690.20240015
Editor-in-Chief: Eng. Agrônomo, Manoel Barbosa Filho - manoel.filho@ufc.br
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Received for publication 16/12/2020; approved on 25/07/2023
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INTRODUCTION

Rice is an important crop worldwide, with Brazil the tenth largest producer. The state of Rio Grande do Sul is responsible for more than half of all Brazilian production. Increased productivity is achieved using high-yield cultivars, balanced fertilisation and pesticides. The excessive use of pesticides is a concern due to the high environmental impact of these products. Chemical control also has many other drawbacks, such as the high cost, phytotoxicity to rice plants, stringent time requirements for correctly applying the fungicide, the risk of resistance, and toxicity to non-target organisms (LIU *et al.*, 2018). Despite these problems, chemical control is still the most important way of controlling pests (PENG *et al*., 2014).

Biological control strategies, which are more environmentally friendly and generally of low cost, not only ensure disease is controlled, but also promote plant growth. This would seem to be an ideal option, and is gradually being applied on a large scale in rice production (LIU *et al*., 2018). However, the effects of biological control are slow and relatively poor (PENG *et al*., 2014). Therefore, to overcome the limitations of both types of control, and to take advantage of their positive aspects, biological control is often combined with chemical control. There have been positive results when fungicides are compatible with some isolates of the bacterial genera used in biological control and/or to promote plant growth (ANAND *et al*., 2010; GOPALAKRISHNAN *et al*., 2012; PENG *et al*., 2014; PALAZZINI; TORRES; CHULZE, 2018). Knowledge of which pesticides can be used together with these microorganisms can help in an integrated strategy for disease and pest management (LIU *et al*., 2018; PALAZZINI; TORRES; CHULZE, 2018).

Introducing microorganisms to the soil can promote changes in the native soil community (TRABELSI; MHAMDI, 2013) and affect the quality of the soil. The soil microbial biomass (SMB) is considered the living and active part of the soil organic matter (GONZALEZ-QUIÑONES *et al*., 2011), and is responsible for the decomposition and accumulation of organic matter in the environment, controlling the dynamics of the available mineral nutrients (ROSCOE; MERCANTE; SALTON, 2006). Soil quality can therefore be measured by changes in the SMB and in the basal respiration of the microbial community (GONZALEZ-QUIÑONES *et al*., 2011).

Microorganisms added to the soil also cause changes in the activity and diversity of soil microbes by altering the organisation, physiology and metabolic profile of the native soil microbiota (ROSCOE; MERCANTE; SALTON, 2006).

Earlier studies involving *Pseudomonas* and *Bacillus* isolates (DFs185, DFs223 and DFs416) demonstrated their potential use in rice as bacterial biocontrol agents (BBAs) and to promote plant growth (LUDWIG *et al*., 2009; SOUZA-JUNIOR *et al*., 2010). However, the interaction between these bacteria and the soil microbiota, including their behaviour in the presence of pesticides, has not yet been clarified.

The aims of this study were: i - to determine the behaviour of these bacteria in the presence of pesticides; and ii - to evaluate in the field, the impact of treating rice seeds with BBAs on the microbiological quality of the soil, as well as the diversity and activity of the soil microbiota.

MATERIAL AND METHODS

Pesticide Compatibility

The interaction between BBAs and pesticides was initially assessed by comparing the effects of different active ingredients (a.i.) approved for use in irrigated rice: fungicides (azoxystrobin, pyraclostrobin + epoxyconazole; trifloxystrobin + tebuconazole), herbicides (cyhalofop-p-butyl; clomazone; imazapic + imazethapyr; propanil; clefoxydim), and insecticides (cypermethrin; λ-cyhalothrin and thiamethoxam).

The BBAs used in the study are from the collection of the Plant Bacteriology Laboratory, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. These bacteria were selected by Ludwig *et al*. (2009) to control various diseases in rice, and were combined as described by Souza-Junior *et al*. (2010). The bacterial isolates used were DFs185 (*Pseudomonas synxantha*), DFs223 (*P. fluorescens*), DFs306 (unidentified) and DFs416 (*Bacillus* sp.).

Two experiments were conducted in a completely randomised design with four replications. The first experiment evaluated the tolerance of bacteria grown in the presence of one active ingredient (a.i.) in a culture medium with different doses of that a.i.

The second experiment evaluated the resistance of the isolates to the a.i. in different fungicides (used as the sole source of carbon and nitrogen). Tolerance was evaluated in sterile Petri dishes containing 10 mL of Medium 523 (KADO; HESKETT, 1970). After solidification, 100 µL of the bacterial culture with 24 hours of growth was spread onto the medium. Five sterile filter paper discs were then distributed on the medium, each containing 10 µL of the product in different dilutions (0%, 50%, 100%, 150%, and 200% (v/v) of the dose recommended by the producer). The dishes were sealed and incubated at 28 °C.

The evaluations were made following 24 hours incubation, observing the occurrence or absence of zones

of growth inhibition around the discs, indicating bacterial tolerance or intolerance to the pesticides.

Resistance was evaluated in Petri dishes containing 10 mL of minimal medium (MM) (2 g K_2PO_4, 0.75 g $MgPO_4$, and 8 g NaCl) (ORTIZ-HERNÁNDEZ; SÁNCHEZ-SALINAS, 2010) (modified) mixed with 10 µL of the different fungicide dilutions (10% 5%, 1%, 0.5%, 0.1%, and 0% MM (w/v)). After solidification, 100 µL of the bacterial culture previously grown in modified Medium 523 (with only 50% of the recommended sucrose concentration) was spread onto the MM. The positive control consisted of dishes containing Medium 523 with no added pesticides. The dishes were sealed and incubated at 28 °C. The number of colony-forming units (CFUs) were evaluated from 24 to 96 hours after incubation.

Impact on the soil microbiology

The changes in soil microbiology following seed inoculation with the BBAs were assessed by a field trial in the experimental area of the Instituto Rio Grandense de Arroz–IRGA, Areia Grande, Rio Grande do Sul, Brazil (29°20'07'' S, 49°43'37''W). The soil in the region is classified as a eutrophic Melanic Gleisol. The average temperature during the experiment was 22.3 °C, with a relative humidity of 82.8% and rainfall of 208.3 mm.

For the experiment, the seeds were treated with DFs185, DFs223, DFs306, and the following combinations: DFs-C6 (DFs185/DFs306), DFs-C7 (DFs306/DFs416) and DFs-C8 (DFs185/DFs306/DFs416). A 0.85% saline solution was used as the control. The bacterial combinations used were based on Souza-Junior *et al*. (2010), who selected non-antagonistic bacterial isolates which gave satisfactory results as BBAs.

Each experimental plot measured 1.20 m in length and 0.60 m in width, with four rows spaced 0.15 m apart. The seeding density was equal to 110 kg of rice seeds per hectare. The experimental design was of randomised blocks with three replications.

The seeds (IRGA 420) were inoculated with the bacterial treatments, which had been grown in Medium 523, 24 hours prior to the procedure. The suspensions were prepared, and the concentration adjusted to 10^4 CFU/mL using the McFarland scale. Subsequently, 100 mL of saline or single isolate suspension, 50 mL of each double combination of bacterial isolates, or 35 mL of each triple combination of bacterial isolates were transferred into plastic bags containing the seeds, and shaken for 10 minutes. After mixing, the excess suspension was removed, and the seeds were left to dry in the shade for sowing.

Soil samples (roots and rhizosphere) were collected when the rice plants were at stage V6 (fully formed collar on the sixth leaf of the main stem). Three subsamples of soil were collected between the centrelines, close to the roots of the plants (Dutch auger, 0–20 cm) to form a 1-kg composite sample from each statistical plot. The samples were kept on ice. One part of the soil sample was used to determine the moisture content and weight corrections in the laboratory, and the other was stored in a freezer for later use.

Soil quality was determined by quantifying the carbon dioxide (CO_2) released by microbial respiration over a period of 30 days following the methodology described by Stotzky (1965).

To determine the organic matter and organic carbon content of the samples, the Walkeley Black test was used (TEDESCO *et al*., 1995), where 0.5 g of soil were transferred to a 250-mL Erlenmeyer flask to which 10 mL of $K_2Cr_2O_7$ solution (1N) were added. The flask was lightly agitated while 20 mL H_2SO_4 (1N) were introduced. The samples were allowed to stand for 10 minutes, then 100 mL distilled water and three drops of ferro complex solution (0.025N) were added and titrated with $FeSO_4$ (0.5N). The white test was done using an empty bottle. The organic carbon content was calculated as per the equation: %C = [(me $K_2Cr_2O_7$ – me $FeSO_4$)/SDM] x 0.4, where me $K_2Cr_2O_7$ is the milliequivalent of $K_2Cr_2O_7$ added to the sample; me $FeSO_4$ is the milliequivalent of $FeSO_4$ added to the sample, and SDM is the soil dry matter of the sample.

The soil microbial biomass was determined by irradiation-extraction (VENCE; BROOKES; JENKINSON, 1987). Soil samples were irradiated for four minutes and transferred to snap-cap flasks containing a K_2SO_4 solution (0.5 mol L^{-1}). These were then stirred for 30 minutes, and a 25 mL aliquot of the supernatant (microbial carbon) was removed and placed in a 250 mL Erlenmeyer flask. The samples were then titrated with $FeSO_4$ (0.25 mol L^{-1}). The irradiated and non-irradiated carbon was calculated as per the equation: C (mg Kg^{-1}) = [(m mol L^{-1} Cr_2O_7 – m mol L^{-1} Fe^{2+})]/g of sample x 300. The carbonate content of the biomass was calculated using the equation: C biomass = (C irradiated sample – C non-irradiated sample)/Kc, where Kc is the carbon mineralisation rate constant (0.33).

The metabolic quotient was calculated from the ratio between the basal respiration and the microbial biomass carbon; the microbial quotient was obtained from the ratio of microbial carbon to total carbon. The impact on the soil microbiota was evaluated by calculating the metabolic activity and metabolic profiles of the microbial communities using EcoPlate™ microplates (Biolog, Inc. Hayward, CA, USA) (ZAK *et al*., 1994). Rhizosphere soil (1 g of the soil adhering to the roots of the rice plants) from each experimental plot was suspended in 30 mL sterile saline (NaCl 0.85%) under manual stirring. Immediately afterwards, 120 µL of the rhizosphere

soil suspension from each experimental plot were distributed over each of the 32 EcoPlate™ wells (corresponding to 31 different substrates and the control with no substrate). The plates were incubated at 28 °C, and the metabolic activity and metabolic profile were determined by measuring the absorbance at 590 nm using a spectrophotometer microplate reader (Thermo-Plate, TP-Reader) after 18, 24, 48, 72 and 144 hours. These data were used to calculate the mean bacterial activity, $[\sum$ (*Abs* Sample-*Abs* control*)]/31*, where: *Abs* = absorbance for each period of evaluation, used to construct the respective curve. The readings were taken after 72 hours, and used to calculate the metabolic diversity of the microbial community. Substrate richness (*R*) was calculated as the number of substrates utilised, and the Shannon evenness index (E) was calculated as per Zak *et al.* (1994).

The response variables were submitted to analysis of variance using the F-test ($p \leq 0.05$). When significant, the mean values of each treatment were compared by the Scott-Knott test at 5% significance using the R software (R CORE TEAM, 2015).

RESULTS AND DISCUSSION

Pesticide Compatibility

Analysis of the compatibility between the fungicides used in the irrigated rice and the bacteria used as biological controllers showed that all of the BBAs were tolerant (i.e. grew normally in the culture medium in the presence of each of the products) regardless of the dose employed (Figure 1A). Similarly, all the isolates developed normally when the herbicides were added to the Medium 523 (Figure 1C). However, with the exception of DFs416, each of the isolates was susceptible (intolerant) when cultured in the medium containing the insecticides (Figure 1B).

In the second bioassay, we sought to identify which bacterial isolate was resistant, i.e. which grew in MM containing only fungicides as the source of carbon and nitrogen. Different results were seen for each BBA. Isolate DFs306 did not grow in any concentration, including the absence, of the three fungicides added to the MM, although growth was adequate in Medium 523 (positive control). Isolate DFs416 grew in the presence of azoxystrobin only, but growth was limited (Table 1). Isolate DFs185 showed different growth for each fungicide added to the MM as the source of nutrients, developing normally in the presence of azoxystrobin, with no significant difference between the positive control (Medium 523) and the different doses of the compound; this isolate used pyraclostrobin + epoxiconazole as the source of carbon and nitrogen, the number of CFUs increasing to 44 at the 0.5% dose compared to the control (4.6), and was therefore considered resistant.

Tolerance was seen in the presence of trifloxystrobin + tebuconazole, but the number of CFUs decreased at the higher doses. This decrease indicates the possibility of tolerance to the compound being dose-dependent, however, this aspect was not studied in the present work (Table 1). On the other hand, isolate DFs223, showed tolerance to each a.i. for all the dilutions under evaluation. Additionally, isolate DFs223 was resistant to azoxystrobin at concentrations between 1% and 5%, since the increase in the number of CFUs was consistent (Table 1). There are few reports showing resistance of the BBAs to fungicides, as seen in our data for both of the *Pseudomonas* isolates (DFs185 and DFs223). Similar behaviour was seen in *P. fluorescens* growing in media supplemented with azoxystrobin, metalaxyl-M or pyraclostrobin (SALMAN; ABUAMSHA, 2012). Liu *et al*. (2018) also showed that growth in *B. subtilis* could be stimulated in media supplemented with certain concentrations of strobilurins.

The search for control measures that can be used together is the basis of integrated pest management, and methods of identifying compatibility between biocontrollers and different pesticides are seen as additional tools to enhance the effectiveness of both types of control (PALAZZINI; TORRES; CHULZE, 2018). In this respect, the BBAs under evaluation are able to adjust to the presence of most pesticides that are registered for use in rice cultivation, as they are tolerant to both the fungicides and herbicides.

Many earlier studies that evaluated the effects of pesticides on beneficial microorganisms used in agriculture showed similar results. Myresiotis, Vrysas and Mourkidou (2012) found that the presence of thiamethoxam in the soil did not affect the development of *Bacillus* species. They also noted that these species showed adequate growth in the presence of the herbicides metribuzin and propenamide, and of the fungicides acibenzolar-S-methyl and propamocarb hydrochloride.

However, high doses of metribuzin and thiamethoxam (twice the dose recommended by the manufacturer) inhibited the development of *Rhisobium* sp. in liquid medium (AHEMAD; KHAN, 2011). These same authors evaluated the effect of four herbicides on the growth of *P. putida* and found that the highest dose reduced the growth of the bacterium despite its tolerance to quisalafop-p-ethyl, clodinafop, metribuzin and glyphosate. Moreover, they found that high concentrations of the same herbicides lowered the production of indole acetic acid and siderophores in *P. putida*, hampering plant growth (AHEMAD; KHAN, 2012).

On the other hand, the interaction between BBAs and fungicides can have a synergistic effect on disease control. Many authors have found promising results in the

greenhouse and in field trials when spraying the leaves or treating the seeds. For instance, the use of *B. subtilis* H158 and the fungicide azoxystrobin (LIU *et al.*, 2018), or *B. subtilis* NJ-18 and jinggangmycin (PENG *et al.*, 2014), increased the control of rice sheath blight compared to fungicide used alone. Synergic effects were also seen when combining *P. fluorescens* Pf1 and azoxystrobin (ANAND *et al*., 2010).

The ability of microorganisms to avoid the effect of chemical compounds can occur via two mechanisms: tolerance and resistance. Resistance occurs when bacterial isolates are able to degrade the pesticide (ORTIZ-HERNANDEZ; SANCHEZ-SALINAS, 2010). The results of the second bioassay showed that resistance was used by the *Pseudomonas* isolate DFs185 against pyraclostrobin + epoxiconazole, and by DFs223 against azoxystrobin. These isolates grew in the presence of carbon and nitrogen supplied solely by the fungicides. There are various reports that show compatibility between pesticides and BBAs, especially the *Bacillus*

Figure 1 - Tolerance of the bacterial biocontrol agents in the presence of different dilutions (0%, 50%, 100%, 150% and 200% (v/v)) of fungicides (A), insecticides (B) and herbicides (C), in Medium 523 and incubated at 28°C. *Pseudomonas synxantha* (DFs185)*; P. fluorescens* (DFs223)*;* unidentified (DFs306); *Bacillus* sp. (DFs416)

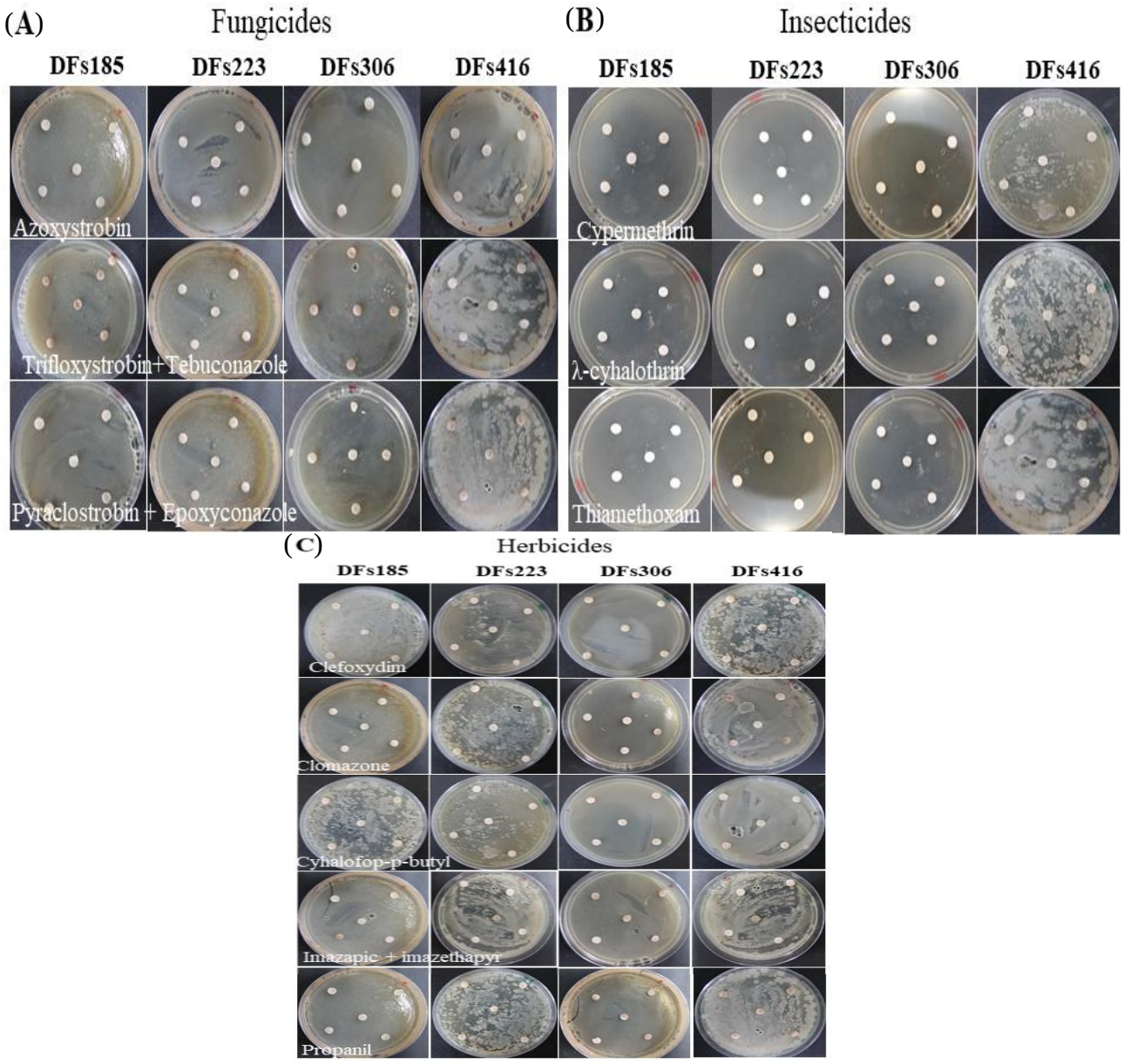


Table 1 - Resistance, tolerance and intolerance to fungicides recommended for rice cultivation, expressed by the number of colony-forming units (per Petri dish) of bacterial biocontrol agents in medium containing different active ingredients in increasing concentrations as the only source of carbon and nitrogen

Fungicide dosage[1]	Bacterial isolates			
	[2]DFs185	[3]DFs223	[4]DFs306	[5]DFs416
	Azoxystrobin			
0%	+100	60	0	21
0.1%	+100	53	0	8
0.5%	+100	56	0	2
1%	+100	+100	0	2
5%	+100	+100	0	5
10%	+100	60	0	4
	Pyraclostrobin + Epoxiconazole			
0%	4,6	55	0	0
0.1%	0	22	0	0
0.5%	44	40	0	0
1%	6.5	22	0	0
5%	12.24	54	0	0
10%	2	63	0	0
	Trifloxystrobin + Tebuconazole			
0%	+100	30	0	0
0.1%	+100	58	0	0
0.5%	+100	39	0	0
1%	59	68	0	0
5%	36	25	0	0
10%	63	56	0	0
	Kado& Heskett 523			
	+100	+100	+100	+100

[1]Calculated dosage as recommended by the producer; [2]*Pseudomonas synxantha;* [3]*P. fluorescens;* [4]undentified; [5]*Bacillus* sp

and *Pseudomonas* isolates (ANAND *et al*., 2010; DEVI; PRAKASAM, 2013; LIU *et al*., 2018; PALAZZINI; TORRES; CHULZE, 2018; PENG *et al*., 2014). There are also some studies showing that *Pseudomonas* has a strong ability to degrade such fungicides as tebuconazole and such herbicides as atrazine (OBANDA; SHUPE, 2009). However, as far as we know, this is the first time that degradation by *Pseudomonas* has been reported when these fungicides are the only source of carbon or nitrogen for bacterial growth.

These results show the possibility of using these BBAs and pesticides together. Additionally, the *Pseudomonas* isolates have two positive characteristics: a competitive advantage from using unusual sources of carbon and nitrogen, and the ability to reduce the environmental impact of these fungicides by degrading them.

Impact on the soil microbiology

An analysis of the microbial biomass showed that DFs-C6 was the only bacterial treatment to show an increase in microbial biomass carbon compared to the control treatment (with no inoculation). This amount was 17% greater than the amount found in the control, and 97% greater than that seen for isolate DFs223 (the lowest value). The microbial quotient followed the same trend, where combination DFs-C6 had the highest value (1.20), which was 19% higher than the control and 120% higher than the lowest value obtained for isolate DFs185 (Table 2).

Basal respiration ($C\text{-}CO_2$) increased by 36% and 53% with isolate DFs185 and combination DFsC07, respectively. Two levels of increase were seen in the

metabolic quotient (qCO_2): an intermediate increase for isolate DFs306 (71%) and for combination DFs-C7 (92%), and a high increase for isolate DF185 (171%). The DFs-C6 combination, which had the highest values for the other variables, had a basal respiration and metabolic quotient similar to those seen with the control (0.35 and 0.75, respectively) (Table 2).

Such soil analyses are commonly used to evaluate soil that is being restructured, or to assess the quality of soils used under different cropping systems. Only a few reports have tried to determine microbial changes in the rhizosphere following the inoculation of seeds with microorganisms. Mengual *et al.* (2014) found that inoculating the seeds of *Pinus halenpensis* with *Azospirillum brasiliensis* and *Pantoea dispersa* increased microbial biomass and respiration, corroborating the results of the present study. However, these authors saw an increase in total carbon, which contrasted with the present results, where total carbon did not significantly affect growth in the microbial biomass, since only one production cycle was evaluated in this study, and it takes several years to observe significant changes in the total carbon content of the soil.

Combination DFs-C6 was the only treatment to increase the soil microbial biomass (462.54 mg kg^{-1}). There is no ideal value for this variable, so the microbial quotient is used, which is the percentage of microbial carbon in relation to the total organic carbon in the soil. In this case, the microbial quotient of DFs-C6 followed the trend of the microbial biomass, showing that the DFs-C6 treatment may be more stable under stress conditions (in this case, the introduction of microorganisms), since under these conditions, the carbon sequestration capacity of soil microorganisms is reduced, with a consequent reduction in the metabolic quotient (WARDLE, 1994).

The stability of the DFs-C6 treatment was confirmed by the low basal respiration (0.35 mg $C\text{-}CO_2$ h^{-1} g^{-1}) using the USDA guidelines (2001), which establish low ranges of basal respiration of between 0.23 and 0.37 mg $C\text{-}CO_2$ h^{-1} g^{-1}. This was a positive point that shows there was no effect from the use of this bacterial treatment, keeping the microbial environment stable, as shown by the low metabolic quotient (0.75).

An efficient soil environment with quality soil is one where less carbon is lost through respiration in the form of CO_2 and more carbon is incorporated into microbial tissue (ROSCOE; MERCANTE; SALTON, 2006). Both of these factors were seen in the DFs-C6 treatment, where the environment remained stable following the introduction of BBAs into the soil. This hypothesis was also considered in a study to assess the impact of different variables on the microbiota of soil cultivated with cowpea. The authors saw an increase in the number of spores of arbuscular mycorrhizal fungi, with a reduction in basal soil respiration and the metabolic quotient under high salinity, showing that the soil microbiota was stable and there was no change in the microbiological quality of the soil (BEZERRA *et al*., 2010). On the other hand, treating the maize seeds with *Azospirillum lipoferum* CRT1 had an impact on the soil nitrogen cycling process. The authors found that in soils where carbon was limited, treating the seeds stimulated the roots to exude carbon, favouring the population of denitrifying bacteria and the denitrification process. In contrast, in soils with no carbon limitation, carbon was not exuded by the roots, resulting in competition for nitrate by the inoculated denitrifying bacteria (FLORIO *et al*., 2017).

Table 2 - Impact of the introduction of bacterial biocontrol agents on the microbial quality of the rhizosphere of rice plants in a field trial

Treatment	Microbial biomass*	Organic carbon	Basal respiration*	Microbial quotient*	Metabolic quotient*
	mg kg^{-1}		mg $C\text{-}CO_2/h^{-1}$ kg^{-1}	mg kg^{-1}	qCO_2 x 10^{-3}
1DFs185	266.63 d	3.792^{ns}	0.41 a	0.54 d	2.06 a
2DFs223	235.86 d	3.922^{ns}	0.26 b	0.60 d	0.84 c
3DFs306	281.79 d	3.915^{ns}	0.32 b	0.72 c	1.30 b
4DFs-C6	462.54 a	3.955^{ns}	0.35 b	1.20 a	0.75 c
5DFs-C7	324.77 c	3.916^{ns}	0.46 a	0.97 b	1.46 b
6DFs-C8	341.28 c	3.955^{ns}	0.36 b	0.86 b	0.82 c
Control	395.03 b	3.683^{ns}	0.30 b	1.01 b	0.76 c
CV(%)	9.46	3.64	11.49	8.11	15.56

^{ns} Mean values do not differ by the F-test;* Mean values with the same letter do not differ significantly by the Scott-Knott test (α = 0.05); [1]*Pseudomonas synxantha;* [2]*P. fluorescens;* [3]undentified; [4]combination DFs185/306; [5]combination DFs306/DFs416; [6]combination DFs185/DFs306/DFs416. CV: coefficient of variation

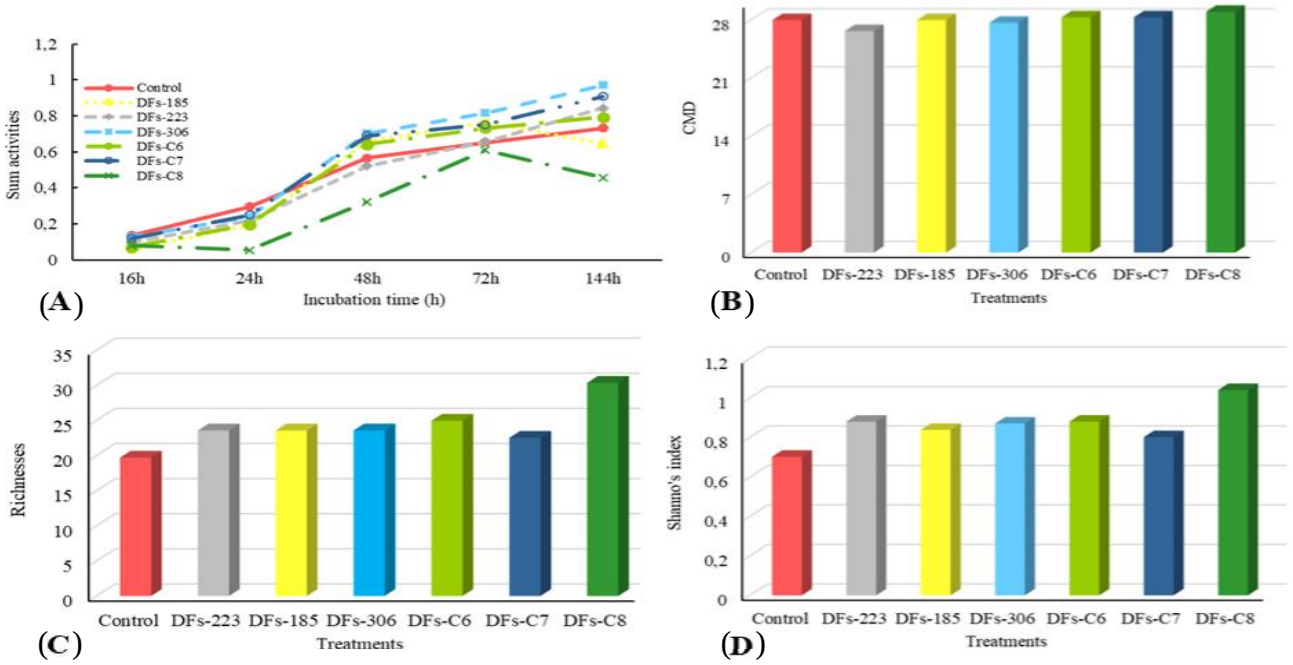
Previous studies have shown that the use of microbial inoculants can affect the number and composition of the taxonomic groups in the native microbiota (JU *et al.*, 2019; PELLEGRINO *et al.*, 2012; TRABELSI; MHAMDI, 2013). The use of bioinoculants can promote taxon colonisation in the soil, increasing the availability of resources and improving performance under stress conditions (IRSHAD *et al.*, 2020; SINGH *et al.*, 2019). A study that evaluated the rhizosphere of wheat inoculated with *Azospirillum* sp. and *Pseudomonas* sp. reported that these isolates reduced the number of fungi and distinct subgroups of native bacteria in the rhizosphere of the inoculated plants, and changed the utilisation profile of carbon sources by the native soil microbiota (NAIMAN; LATRÓNICO; SALAMONE, 2009).

A study of the metabolic activity and diversity profile of the microbiota showed little or no difference between the treatments and the control. The sum of activities increased throughout the incubation period, and showed the highest values at the end of the evaluation (144 h), except for DFs185 and DFsC08. These values increased under all of the BBA treatments, except for DFs-C8 (Figure 2A). The metabolic diversity of the microbial community (MDC) ranged between 24.3 (DFs223 and DFs-C8) and 26.3 (DFs-C7) (Figure 2B). The lowest values for richness and the Shannon index were found in the rhizosphere of the control, and the highest values in the DFs-C8 treatment; the values of the remaining treatments were similar to each other (Figure 2C and 2D, respectively).

It can be seen that the treatments increased the use of different carbon sources compared to those used by the native microbiota, especially the triple combination (DFs-C8). This result is in partial contrast to the results of Salomone *et al*. (2012), where inoculating rice seeds with *Azospirillum* sp. changed the metabolic profile of the microbial community and reduced the use of different carbon sources. These authors concluded that this one isolate reduced the functionality of the rhizosphere microbiota. Moreover, the use of commercial products (*A. brasiliense* and *P. fluorescens*) in rice seeds resulted in competition between the inoculated microorganisms and the native strains of microbiota, temporarily affecting the native community (SALAMONE *et al*., 2012).

It should be noted that manipulating the rhizosphere is regarded as a key mechanism for critical environmental problems, including the sustainability of agricultural and forestry systems (TRABELSI; MHAMDI, 2013). However, considering the evidence that the complexity of microbial communities affects the physiology of the plants (LAU; LENNON, 2011), the rhizobacteria x rhizosphere x plant interaction needs to be clarified.

Figure 2 - Impact of treating rice seeds with bacterial biocontrol agents on microbial metabolic activity and the diversity of the rhizosphere soil profiles in a field trial evaluated using EcoplateBiolog® microplates. A: Sum of activities; B: Metabolic diversity of the microbial community (MDC); C: Shanon index; D: Richness, calculated as the number of substrates utilised. Saline 0.85% (control); *Pseudomonas synxantha*(DFs185)*; P. fluorescens* (DFs223)*;* unidentified (DFs306); *Bacillus* sp. (DFs416); DFs185/DFs306 (DFs-C6); DFs306/DFs416 (DFs-C7) and DFs185/DFs306/DFs416 (DFs-C8)



Under the current system of rice production, it is more feasible and effective to reduce the rate of pesticide application than eliminate their use. Combining BBAs and chemical treatments (whether together or by alternating their use) is therefore a good way of reducing the use of pesticides, especially if lower doses of fungicides can be employed, thereby reducing the impact on the environment and the risk of pesticide resistance.

CONCLUSION

The present study suggests the potential use of bacterial treatments for integrated pest management, since: i) the *Bacillus* sp. and *Pseudomonas* sp. isolates under study are resistant/tolerant to azoxystrobin, and those of *Pseudomonas* sp., to pyraclostrobin + epoxiconazole and trifloxystrobin + tebuconazole; ii) treating rice seeds with these bacteria had no negative effects on the microbial quality of the rhizosphere, nor on the diversity or activity of the microbiota.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code AUXPE-PNPD 61623/2010.

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