Ciência Rural, Santa Maria, v.54:09, e20230123, 2024 http://doi.org/10.1590/0103-8478cr20230123
ISSNe 1678-4596
CROP PRODUCTION

Bacterial consortium of *Azospirillum brasilense* and *Pseudomonas fluorescens* on the stimulation of growth of corn culture

Itacir Eloi Sandini[1*] **Fabiano Pacentchuk**[1] **Daniel Andrade de Siqueira Franco**[2] **Anthony Hasegawa Sandini**[3]

[1]Universidade Estadual do Centro-Oeste (UNICENTRO), 85040-080, Guarapuava, PR, Brasil. E-mail: isandini@hotmail.com. *Corresponding author.
[2]Centro Avançado de Pesquisa e Desenvolvimento em Sanidade Agropecuária, Instituto Biológico/APTA/SAA, Campinas, SP, Brasil.
[3]Universidade Federal de Santa Catarina (UFSC), Curitibanos, SC, Brasil.

ABSTRACT: The evaluation of plant growth-promoting bacteria in order to increase corn productivity is a biotechnology of global interest. This study evaluated the efficiency of the consortium between *P. fluorescens* CNPSo 2719(=CCTB03) and *A. brasilense* Ab-V06 in corn. Four experiments were carried out in four different locations, with a randomized block design and four replications, with the treatments: (1) without inoculation and without application of N coverage; (2) without inoculation and application of 100% of the recommended dose of N in coverage (200 kg ha^{-1} of N); (3) *Azospirillum brasilense* + application of 75% of the recommended dose of N in coverage (150 kg ha^{-1} of N); (4) bacterial consortium of *Azospirillum brasilense* + *Pseudomonas fluorescens* + application of 75% of the recommended dose of N in coverage (150 kg ha^{-1} of N). In all locations, with the use of the bacterial consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N in coverage, it was possible to reduce the dose of N in coverage by up to 25% without compromising the productivity of the crop. In this way, it is concluded that the use of the consortium of the two bacteria promotes savings of up to 25% of N fertilization in top dressing and the possibility of using two microorganisms with the capacity to stimulation plant growth with a single application.
Key words: *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Zea mays*, inoculant, plant growth-promoting bacteria, nitrogen.

Consórcio bacteriano de *Azospirillum brasilense* e *Pseudomonas fluorescens* na estimulação do crescimento da cultura do milho

RESUMO: A avaliação de bactérias promotoras de crescimento de plantas visando aumentar a produtividade da cultura de milho constitui uma biotecnologia de interesse global. O objetivo deste estudo foi avaliar eficiência do consórcio entre *P. fluorescens* CNPSo 2719(=CCTB03) e *A. brasilense* Ab-V06 na cultura do milho. Foram realizados quatro experimentos em diferentes localidades, com o delineamento em blocos ao acaso e quatro repetições, com os seguintes tratamentos: (1) sem inoculação e sem aplicação de N de cobertura; (2) sem inoculação e aplicação de 100% da dose recomendada de N em cobertura (200 kg ha^{-1} de N); (3) *Azospirillum brasilense* + aplicação de 75% da dose recomendada de N em cobertura (150 kg ha^{-1} de N); (4) consórcio bacteriano de *Azospirillum brasilense* + *Pseudomonas fluorescens* + aplicação de 75% da dose recomendada de N em cobertura (150 kg ha^{-1} de N). Em todos os locais, o uso do consórcio bacteriano (*A. brasilense* + *P. fluorescens*) + 75% da dose recomendada de N em cobertura, foi possível reduzir a dose de N em cobertura em até 25% sem comprometer a produtividade da cultura. Desta forma, conclui-se que a utilização do consorcio das duas bactérias promove economia de até 25% da adubação de N em cobertura e a possibilidade de usar dois microrganismos com capacidade de estimular o crescimento de plantas em uma única aplicação.
Palavras-chave: *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Zea mays,* bactérias promotoras de crescimento de plantas, nitrogênio.

INTRODUCTION

Sustainable agricultural production is increasingly addressed in discussions about the future of planet Earth. Thus, alternatives to increase productivity while using inputs that do not harm the environment and/or alternatives that replace, even partially, inputs that are currently used in agriculture, are constantly sought.

Among crops of economic interest, corn (*Zea mays* L.) is one of the most important crops worldwide, due to its importance, mainly for animal feed (ARF et al., 2018). According to data from FAOSTAT (2022), the harvested area for this crop worldwide and in Brazil in 2020 was 201.9 million ha and 18.2 million ha, respectively. Therefore, any improvement in the production system of this cereal will have a great impact at both the national and international level.

In this context, microorganism-based products represent an emerging technology designed to improve long-term system productivity. They can be seen as a technology that is in line with sustainable agriculture principles, and that opposes the increased

Received 03.03.23 Approved 11.21.23 Returned by the author 04.10.24
CR-2023-0123.R2
Editors: Leandro Souza da Silva Anderson Nunes

use of pesticides and fertilizers (NAIMAN et al., 2009). Among these products, those composed of plant growth-promoting bacteria (PGPB) stand out.

PGPB are beneficial microorganisms that colonize plant roots, which increases crop productivity and offers an efficient way to replace chemical fertilizers, pesticides and supplements (TORIBIO-JIMENEZ et al., 2017). PGPB can benefit plants in many ways, e.g., by increasing seed germination, root and lateral root branching development, branch and root weight, or by inducing leaf area growth, stem elongation or higher chlorophyll and protein content in the plant (VACHERON et al., 2013).

As for corn, there are many reports of microorganisms that promote the growth of this crop, with emphasis on *Klebsiella*, *Bacillus*, *Azospirillum*, *Herbaspirillum*, *Burkholderia* and *Pseudomonas* (ARRUDA et al., 2013). However, despite literature reports on the benefits of a series of microorganisms for the corn crop, to date, there are few microorganisms with proven effective gains in productivity; and therefore, potential for their use.

Among the aforementioned microorganisms, those that show the best results for corn are *Azospirillum brasilense* and *Pseudomonas fluorescens*. These bacteria are capable of producing and secreting plant hormones that are used as growth-promoting substances by the host plant (ABBAS-ZADEH et al., 2009).

A. brasilense is a nitrogen-fixing microorganism that colonizes the root. This bacterium also produces growth-promoting substances such as indoleacetic acid (IAA), gibberellins, pantothenic acid, thiamine and niacin, in addition to increasing root density and branching (TAMILSELVI et al., 2016). Positive results with the inoculation of *A. brasilense* on maize were reported by QUADROS et al. (2014) and HUNGRIA et al. (2010). Currently, *A. brasilense* has been successfully employed as a growth promoting microorganism in rice (MATTOS, 2010), brachiaria (HUNGRIA et al., 2016), corn and wheat (HUNGRIA et al., 2010). Additionally, this bacterium has gained great prominence in the co-inoculation of soybeans and beans (HUNGRIA et al., 2013), where it exerts synergic action with *Bradyrhizobium sp*. in the process of biological nitrogen fixation. HUNGRIA et al. (2022) found an increased productivity with the inoculation of *A. brasilense* in corn and a reduction in the dose of N, supporting the results obtained by BARBOSA et al. (2022) and GALINDO et al. (2022).

Pseudomonas fluorescens, in turn, is a very common gram-negative bacterium (TAMILSELVI et al., 2016). The bacterial genus *Pseudomonas* includes several species with activity recognized as PGPB (ANTOUN & PRÉVOST, 2005). The use of this bacterium in corn has been highlighted by OLIVEIRA et al. (2015). In 2017, the efficacy of a *Pseudomonas fluorescens*-based inoculant was proven in corn, which led to its approval as a growth promoter for this crop (Product Registration PR-93923 10104-7).

Even in the face of positive reports on the use of PGPB in agriculture, in most situations the use of these microorganisms alone is recommended (PRASAD & BABU, 2017). Therefore, according to the same authors, farmers are subject to applying these microorganisms separately and several times during the crop growth stage. The above, in addition to increasing production costs, in some cases may even discourage farmers from adopting this technology in the field.

Thus, it is desirable that microorganisms that have beneficial effects on the corn crop can be applied in combination. The use of beneficial microorganisms intercrops is based on the principles of natural ecosystems that are sustained by their constituents (PRASAD & BABU, 2017). Compatibility between different PGPB is a pre-requisite for them to be used together in the field. In this sense, the results obtained by PRASAD & BABU (2017) reveal the existence of positive interactions between *P. fluorescens* and *A. brasilense* in promoting growth in peanut plants, indicating the compatibility between these bacterial species. Additionally, literature data show that the combination of *P. fluorescens* and *A. brasilense* significantly promotes lettuce leaf growth, reinforcing that this bacterial association acts synergistically (APONTE et al., 2017).

Thus, this study evaluated the agronomic efficiency and feasibility of using the consortium between *P. fluorescens* CNPSo2719(=CCTB03) and *A. brasilense* Ab-V06 in corn.

METHODS

Four experiments were carried out in locations with different edaphoclimatic conditions, namely: Candói (Paraná), Guarapuava (Paraná), Lapa (Paraná) and Sertão (Rio Grande do Sul). All tests were carried out in the 2017/18 growing season. Information related to each location is provided in table 1.

The experiment was conducted in a randomized block design (RBD) with six replications. Test treatments were as follows:

Treatment 1 - Absolute Control - without inoculation and without application of N coverage;

Table 1 - Description of the locations and management carried out in the tests. Guarapuava, Paraná, Brazil, 2019.

Description	-----Location-----			
	Candói (PR)	Guarapuava (PR)	Lapa (PR)	Sertão (RS)
Previous Crop Summer	Corn	Soy	Perennial Pasture	Soy
Previous Crop Winter	Oat	Oat	Perennial Pasture	Rye Grass
Hybrid	AG 9025 PRO3	AG 9025 PRO3	AG 9025 PRO3	AG 9025 PRO3
-----Location-----				
Latitude	25º 31`56.9" S	25º 22' 59" S	25º 50` 52.3" S	28º 01` 37" S
Longitude	51º 48` 0.8" W	51º 33' 14.5" W	49º 39` 8.4" W	52º 15` 52" W
Altitude Meters	920	995	855	720
Seeding	09/20/2017	10/05/2017	09/15/2017	10/15/2017
V4	10/18/2017	11/05/2017	10/15/2017	11/05/2017
V6	11/04/2017	11/15/2017	11/03/2017	11/15/2017
Harvest	03/15/2018	03/20/2018	03/11/2018	03/30/2018
-----Soil Texture-----				
Clay (g/kg)	550	590	220	560
Silt (g/kg)	290	290	310	270
Sand (g/kg)	160	120	470	170
Texture Class	Clayey	Clayey	Average	Clayey
-----Soil Chemical Analysis (0 to 20 cm deep)-----				
pH (CaCl)	5.35	5.14	5.42	5.06
O.M. (g/dm^3)	26.51	46.55	21.75	45.94
P - Mehlich (mg/dm^3)	3.22	9.44	4.89	18.14
K (cmol/dm^3)	0.46	0.61	0.12	0.23
Ca (cmol/dm^3)	4.56	6.3	4.06	7.18
Mg (cmol/dm^3)	1.95	2.01	1.35	2.4
Al (cmol/dm^3)	0	0.03	0	0.05
H+Al (cmol/dm^3)	3.88	5.91	3.06	6.03
SB (cmol/dm^3)	6.97	8.92	5.63	9.81
CTC- pH 7.0 (cmol/dm^3)	10.85	14.8	8.69	16.1

Treatment 2 - Nitrogen Control - without inoculation and application of 100% of the recommended dose of N in coverage (200 kg ha^{-1} of N);

Treatment 3 – *Azospirillum brasilense* + application of 75% of the recommended dose of N in coverage (150kg ha^{-1} of N);

Treatment 4 – Bacterial Consortium- (*Azospirillum brasilense* + *Pseudomonas fluorescens*) + application of 75% of the recommended dose of N in coverage (150 kg ha^{-1} of N);

Bacteria from treatments 3 and 4 were applied via seed treatment (ST). ST was carried out in a shaded place, shortly before sowing, at a dose of 100 mL for 60,000 seeds in the treatment with *Azospirillum brasiliense* and 150 mL for 60,000 seeds in the treatment with the evaluated bacterial consortium.

Each experimental unit consisted of 10 rows with a spacing of 0.45 m between rows and a length of 6.0 m, which totaled an area of 27 m². The hybrid corn used was the AG 9025 PRO3 commercialized by Agroceres, in a population of 75,000 plants per hectare. Sowing dates, as well as other management information, are provided in table 1.

Sowing was carried out in a no-tillage system. The experimental area of each location was desiccated with glyphosate (720 g ha^{-1} i.a.) 15 days before sowing. In the background fertilization, 28 kg ha^{-1} of N, 105 kg ha^{-1} of phosphorus (P_2O_5) and 70 kg ha^{-1} of potassium (K_2O) were used, respectively, for all locations. During the crop cycle, agrochemicals were applied in order to manage pests, diseases and weeds following standard pratices. Top dressing nitrogen fertilization was carried out in plots at stages V4 and V6 (table 1).

The variables studied were: productivity, mass of a thousand grains, dry matter production per hectare, N content in the grains and N content in the plant.

To evaluate the dry mass of the aerial part, five plants were collected from the useful area of each plot at the R1 stage. The total fresh mass of the aerial part was weighed and 150 grams were sampled; subsequently, it was placed in a forced air ventilation oven at 65ºC for 72 hours to determine the percentage of dry mass. After this evaluation, the dry mass per plant was estimated. The formula for this determination was as follows:

Dry mass per plant =

$$\frac{\left[total\ fresh\ mass\ x\ \left(\frac{150\ g}{dry\ mass}\right) x\ 100\right]}{5}$$

For productivity, after maturation, the three central lines of the plot were harvested, 0.50 meters of each headland were discarded, and the material was subsequently threshed and dried; grain yield at 14% humidity was then determined.

Subsequently, from a subsample of this material, 300 grains were counted and weighed for each plot, and from these values, the mass of one thousand grains was calculated.

For the N content in the grains and in the aerial part of the plant, the methodology described by EMBRAPA (2009) was followed.

Seeds from treatment 3 were inoculated with Total Biotecnologia's (Brazil) inoculant, with a guaranteed minimum concentration of 2 x 10^8 CFU/mL on the expiration date. The inoculant contains *Azospirillum brasilense* Ab-V5 (=CNPSo 2083) and Ab-V6 (=CNPSo 2084) strains. Treatment 4 inoculation used the bacterial consortium of the inoculant containing *Azospirillum brasilense* (Ab-V6=CNPSo 2084 strain) + *Pseudomonas fluorescens* (CNPSo 2719=CCTB 03 strain). The CCTB03 strain was isolated by Total Biotecnologia Ind. e Com. S.A. and is deposited in the Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja (WFCC Collection # 1213, WDCM Collection # 1054).

The pre-marketing inoculant was formulated with a guarantee of total microorganisms = 1 x 10^8 CFU (colony forming units)/mL, on the expiration date, in a 1,500 mL package.

The concentration and purity analysis of the inoculant containing *Azospirillum brasilense* and *Pseudomonas fluorescens* was carried out by the laboratory of Total Biotecnologia Ind. e Com. S.A., by the method of scattering in RC and King B medium, and observation under the microscope. The results of the analysis of the inoculant used in the experiments are provided in table 2.

The results were submitted to analysis of variance, and the averages were then compared by the Duncan Test at 5% using the ASSISTAT software (Universidade Federal de Campina Grande).

RESULTS AND DISCUSSION

The results of the first experiment, conducted in Candói, are shown in table 3. According to the summary of the analysis of variance, it was found that the treatment source of variation significantly influenced the variables productivity and DM production per hectare. There was no significant difference for the other variables.

Figure 1 shows that the Absolute Control treatment was statistically inferior to all other tested treatments. Maximum productivity (16,734 kg ha^{-1}) was obtained through treatment with the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% N, which showed a productivity increase of 2,004 kg ha^{-1} when compared to the Absolute Control treatment. Furthermore, it is worthy noting that the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N showed a productivity that was statistically similar to the Nitrogen Control treatment, which received 100% of the recommended dose of N. Based on the results obtained, it can be inferred that when using the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) it is possible to reduce 25% of the N dose (50 kg ha^{-1} of N) applied in the corn crop without causing any losses in productivity.

The Nitrogen Control treatment showed the highest DM production per hectare (11,981 kg ha^{-1}) and significantly differed from the Absolute Control, which showed the lowest value for this

Table 2 - Total microbial count, in colony forming units (CFU) of *Azospirillum brasilense* Ab-V6 =CNPSo 2084 and *Pseudomonas fluorescens* CCTB03=CNPSo2719, in the inoculant used in the experiments and presence or absence of contaminants in the 10^5 dilution, as provided for in Brazilian legislation.

Inoculant	-------Guarantee (CFU/mL)-------	----Concentration (CFU/mL)----	-------Contaminant (10^{-5})-------
------------Harvest – 2017/2018------------			
Total microbial count	1.0 x 10^8	1.2 x 10^9	Zero

Table 3 - Summary of the analysis of variance with mean square values of the variables productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Candói, Paraná.

FV	--------GL--------	---Mean Square---				
		Productivity	TGM	DM/ha	N plant	N grains
Block	5	312,295.50 ns	176.11 ns	1581744.64 ns	35.20 **	4.47 ns
Treatment	3	5,394,916.78 *	568.35 ns	7879707.15 *	16.85 ns	1.45 ns
Error	15	1,047,805.61	473.99	1823120.15	7.02	4.87
CV (%)	-	6.34	4.94	12.13	9.64	12.73
Average	-	16,144	440.86	11,129	27.48	17.33

CV (%) – coefficient of variation; GL – degrees of freedom; ** and * - significant at 1 and 5%, respectively; ns – not significant.

variable (9,446 kg ha^{-1}) to table 4. Treatments with *A. brasilense* + 75% of the recommended dose of N and Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N showed intermediate values and did not differ from the other treatments.

The results of the second experiment, conducted in Guarapuava, Paraná, are provided in table 5. According to the summary of the analysis of variance, the treatment source of variation significantly influenced the variables productivity, thousand grain weight and N content in the plant. There was no significant difference for the other variables.

According to figure 2 and table 6 the Absolute Control treatment had the lowest productivity (13,726 kg ha^{-1}) and statistically differed from the other treatments. Also, it was found that treatments with Nitrogen Control and *A. brasilense* + 75% of the dose of N showed higher yields than the Absolute Control and did not differ statistically from each other. Finally, the highest productivity (16,757 kg ha^{-1}) was obtained with the application of the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N, and this treatment significantly differed from the others.

Treatment with the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N showed a productivity increase of 3,031 kg ha^{-1} when compared to the Absolute Control. Treatment with the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N provided an increase of 1,045 kg ha^{-1} in the corn crop productivity when compared to the Nitrogen Control. The results of this study suggest that the inoculation of *A. brasilense* + *P. fluorescens* makes it possible to reduce the recommended dose of N by 25% for the corn crop, in addition to increasing the crop's productivity. Finally, it was observed that there was an increase of 1,133 kg ha^{-1} in the corn crop productivity when comparing the application of *A. brasilense* + *P. fluorescens* + 75% of the recommended dose of N with the application of *A. brasilense* + 75% of the recommended dose of N. The results of our study indicated that the Bacterial Consortium is more beneficial for the corn crop than the single species inoculation, as demonstrated through the significant increase in the corn crop productivity.

Table 4 - Productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Candói, Paraná.

Treatment	-Productivity-	------TGM------	-----DM/ha-----	----N Plant----	---N Grains---
	kg ha^{-1}	g	kg ha^{-1}	---mg kg^{-1}---	
Absolute Control (0% N)	14,730 b	430.38 ns	9,446 b	25.02 ns	16.72 ns
Nitrogen Control (100% N)	16,625 a	451.73	11,981 a	28.04	17.61
A. brasilense + 75% N	16,489 a	445.96	11,676 ab	28.79	17.83
A. brasilense + *P. fluorescens* + 75% N	16,734 a	435.37	11,413 ab	28.08	17.17
Average	16,145	440.86	11,129	27.48	17.33

Means followed by the same letter do not differ from each other by Duncan's test at 5%. Ns – not significant.

Table 5 - Summary of the analysis of variance with mean square values of the variables productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Guarapuava, Paraná.

FV	-GL-	-----Mean Square-----				
		Productivity	TGM	DM/ha	N plant	N grains
Block	5	2,284,645.17 *	421.87 ns	2,272,087.60 ns	16.74 ns	16.04 ns
Treatment	3	9,563,945.50 **	558.49 *	4,488,479.72 ns	116.71 **	14.15 ns
Error	15	719,868.37	160.25	2,183,877.69	8.21	5.69
CV (%)	-	5.49	3.11	10.83	11.6	10.46
Average	-	15,454.58	406.88	13,650	24.71	22.79

CV (%) – coefficient of variation; GL – degrees of freedom; ** and * - significant at 1 and 5%, respectively; ns – not significant.

These results corroborated those obtained by PRASAD & BABU (2017), who demonstrated that the bacterial consortium *A. brasilense* + *P. fluorescens* was more efficient in promoting the growth of the aerial part of the plants than single species inoculation.

The results of the third experiment, conducted in Lapa, are provided in table 7. According to the summary of the analysis of variance, it was found that the treatment source of variation significantly influenced the variable productivity only.

According to figure 3 and table 8, the lowest productivity (16,263 kg ha^{-1}) was observed in the Absolute Control, which differed statistically from the other treatments. The treatments with application of *A. brasilense* + 75% N (17,703 kg ha^{-1}) or Bacterial Consortium + 75% N (17,584 kg ha^{-1}) did not differ from the Nitrogen Control. Therefore, it was found that the use of growth-promoting bacteria contained in the Consortium makes it possible to reduce the N rates by 25% without compromising the productivity of the corn crop.

The results of the fourth experiment, conducted in Sertão, Rio Grande do Sul, are provided in table 9. According to the summary of the analysis of variance, the treatment source of variation significantly influenced the variables productivity, thousand grain weight, dry mass/hectare, N content in the plant and N content in the grains.

By observing figure 4, it was verified that all treatments differed from the Absolute Control, which showed the lowest productivity (5,506 kg ha^{-1}). It was also found that the treatments with application of *A. brasilense* + 75% of N (11,845 kg ha^{-1}) or Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% N (11,704 kg ha^{-1}) did not differ from the Nitrogen Control. As verified in the experiments in the four areas, the result obtained in this study suggests that it is possible to reduce the dose of N applied in coverage by up to 25% without damaging the corn crop productivity.

According to table 10, the same trend observed for the productivity variable was found for the variables TGM, DM production/hectare and N content in the grains, so that the absolute control was statistically inferior to the other treatments, which in turn did not differ from each other. The trend observed

Table 6 - Productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Guarapuava, Paraná.

Treatment	Productivity	TGM	DM/ha	N Plant	N Grains
	kg ha^{-1}	g	kg ha^{-1}	-----mg kg^{-1}-----	
Absolute Control (0% N)	13,726 c	392.48 ns	12,368 ns	18.10 b	20.63 ns
Nitrogen Control (100% N)	15,712 b	412.61	14,015	27.60 a	24.52
A. brasilense + 75% N	15,624 b	410.22	13,955	27.60 a	23.00
A. brasilense + *P. fluorescens* + 75% N	16,757 a	412.06	14,263	25.00 a	22.87
Average	15,455	406.84	13,650	24.60	22.75

Means followed by the same letter within columns do not differ from each other by Duncan's test at 5%. Ns – not significant.

Table 7 - Summary of the analysis of variance with mean square values of the variables productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Lapa, Paraná.

FV	GL	---Mean Square---				
		Productivity	TGM	DM/ha	N plant	N grains
Block	5	3,451,810.97 *	194.62 ns	3,250,647.77 ns	37.43 ns	4.33 ns
Treatment	3	2,812,683.17 *	173.01 ns	9,027,249.93 ns	44.34 ns	2.70 ns
Error	15	85,849.70	230.96	323,6298.8	15.41	6.00
CV (%)	-	5.34	3.29	12.96	13.8	13.48
Average	-	17,287	462	13,886	28.44	18.18

CV (%) – coefficient of variation; GL – degrees of freedom; ** and * - significant at 1 and 5%, respectively; ns – not significant.

for these variables allowed us to infer that with the use of *A. brasilense* + 75% N or the bacterial consortium + 75% N, the 25% reduction in topdressing N dose does not cause losses in the yield components, DM production per hectare and N content in the corn grains.

By observing all the studied locations, it was found that there was a significant difference in productivity between the Absolute Control and Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N in coverage, as the latter showed higher values than the first one. Additionally, in all locations, the use of the bacterial consortium + 75% N has shown to make it possible to reduce the dose of N in coverage by up to 25% without compromising the crop productivity, since, in all locations the yields were statistically equal to or greater than those obtained in the Nitrogen Control treatment (100% of the recommended N fertilization) (Figures 1, 2, 3 and 4). Therefore, the results of this study allowed us to infer that there was a positive effect of the use of PGPB in the corn crop. According to ABBAS-ZADEH et al. (2009) *A. brasilense and P. fluorescens*, are capable of producing and secreting plant hormones (secondary metabolites) that are used as growth-promoting substances by the host plant.

Plant growth-promoting bacteria can facilitate plant growth and development, both directly and indirectly (GLICK, 2014). Direct plant growth promotion by plant growth-promoting bacteria typically facilitates the acquisition of nutrients from the environment, including nitrogen, iron and phosphate that is fixed in the soil, or specifically modulates plant growth by altering levels of hormones such as auxin, cytokinin and ethylene. Indirect growth promotion occurs when these bacteria decrease or prevent some of the deleterious effects of a plant pathogen (usually a fungus) through one or more mechanisms of action (GLICK, 2012).

According to ARRUDA et al. (2013) PGPB, such as *A. brasilense* and *P. fluorescens,* have characteristics that promote plant growth, including, but not limited to phosphate solubilization, siderophores production, nitrogen fixation, and have potential for use as inoculants aiming crop enhancement. Also, plants inoculated with PGPB induce morphological and biochemical changes,

Table 8 - Productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Lapa, Paraná.

Treatment	Productivity	TGM	DM/ha	N Plant	N Grains
	kg ha^{-1}	g	kg ha^{-1}	---mg kg^{-1}---	
Absolute Control (0% N)	16,263 b	464.30 ns	12,154 ns	24.40 ns	17.20 ns
Nitrogen Control (100% N)	17,597 a	454.61	14,357	29.20	18.70
A. brasilense + 75% N	17,703 a	467.16	14,018	30.20	18.60
A. brasilense + *P. fluorescens* + 75% N	17,584 a	461.92	15,014	29.90	18.20
Average	17,287	462.00	13,886	28.40	18.20

Means followed by the same letter do not differ from each other by Duncan's test at 5%. ns – not significant.

Table 9 - Summary of the analysis of variance with mean square values of the variables productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Sertão, Rio Grande do Sul.

FV	GL	Mean Square				
		Productivity	TGM	DM/ha	N plant	N grains
Block	5	5,630,719 **	1267 *	1,593,148.07 ns	3.62 ns	2.49 ns
Treatment	3	63,930,455 **	9160 **	27,666,832.61 **	39.21 *	76.79 **
Error	15	98,9717	371	1,578,045.84 ns	11.26	2.74
CV (%)	-	9.59	5.25	11.86	14.52	7.24
Average	-	10,378	367.08	10,592	23.11	22.9

CV (%) – coefficient of variation; GL – degrees of freedom; ** and * - significant at 1 and 5%, respectively; ns – not significant.

resulting in increased tolerance to abiotic stresses defined as IST (induced systemic tolerance) (ETESAMI & MAHESHWARI, 2018).

The results obtained also demonstrated the possibility of reaching high production ceilings and reducing the use of chemical fertilizers through the use of PGPB. According to ETESAMI & ALIKHANI (2016), several studies have demonstrated the possibility of reducing the use of chemical fertilizers by inoculating plants with PGPB. Fertilizers rate reductions through the use of PGPB was also discussed by MANCILLA et al. (2017). In addition to the benefits in optimizing the use of nitrogen fertilization, PGPB inoculation can increase the absorption of several other nutrients, such as Ca, K, Fe, Cu, Mn and Zn (PEREZ-MONTANO et al., 2014).

The aforementioned growth promotion mechanisms may have contributed alone or in combination to the positive results reported in this study, in which it was possible to reduce the dose of N in coverage by up to 25%, in all the studied locations, without damaging to corn crop productivity.

Field effectiveness and feasibility of using *A. brasilense* and *Pseudomonas fluorescens* alone as growth promoters has already been proven in corn, which resulted in the products being registered in Ministério da Agricultura, Pecuária e Abastecimento (Brazil). Thus evaluated the use of a bacterial consortium containing both *Azospirillum brasilense* and *Pseudomonas fluorescens* in corn. The positive results obtained in this study with the application of this consortium are similar to those observed in lettuce by APONTE et al. (2017), peanut by PRASAD & BABU (2017) and corn (BARBOSA et al., 2022, GALINDO et al., 2022 and HUNGRIA et al., 2022). The use of a bacterial consortium (*A. brasilense* and *P. fluorescens*) may increase the chance of synergistic effects, as it combines microorganisms with different growth-promotion mechanisms. In addition, by using this type of approach, we mimic the conditions found in natural ecosystems, where different microorganisms coexist in balance and provide positive results for plants.

Based on the results obtained, it is evident that the use of the Bacterial Consortium *Azospirillum*

Table 10 - Productivity, thousand-grain mass (TGM), dry matter (DM) production per hectare, N content in the plant and N content in the grain, in the test carried out in the city of Sertão, Rio Grande do Sul.

Treatment	Productivity	TGM	DM/ha	N Plant	N Grains
	kg ha^{-1}	g	kg ha^{-1}	mg kg^{-1}	
Absolute Control	5,506 b	310.66 b	7,409 b	19.40 b	17.70 b
Nitrogen Control	12,456 a	400.49 a	11,911 a	25.20 a	25.80 a
A. brasilense	11,845 a	375.78 a	11,858 a	23.50 ab	24.60 a
A. brasilense + P. fluorescens	11,704 a	381.40 a	11,189 a	24.20 ab	23.40 a
Average	10,378	367.08	10,592	23.10	22.90

Means followed by the same letter do not differ from each other by Duncan's test at 5%. ns – not significant.

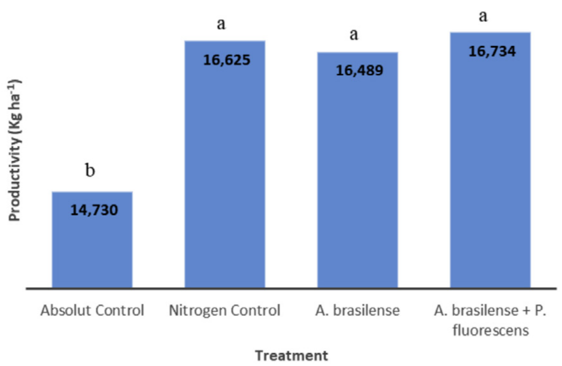

Figure 1 - Productivity (kg ha^{-1}) as a function of the application of different treatments in corn crop in the city of Candói, Paraná. 2019. Bacterial Consortium = *Azospirillum brasilense* + *Pseudomonas fluorescens*. Means followed by the same lowercase letter do not differ by 5% by Duncan's test.

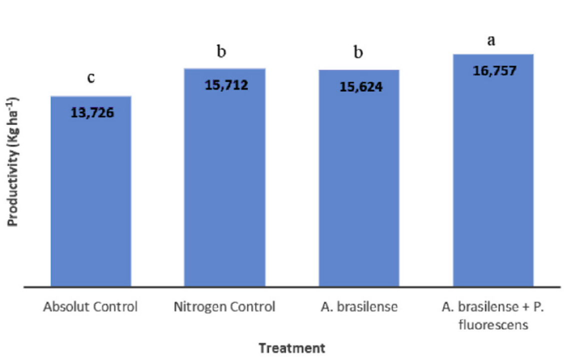

Figure 2 - Productivity (kg ha^{-1}) as a function of the application of different treatments in corn crop in the city of Guarapuava, PR. 2019. Bacterial Consortium = *Azospirillum brasilense* + *Pseudomonas fluorescens*. Means followed by the same lowercase letter do not differ by 5% by Duncan's test.

brasilense (Ab-V6) and *Pseudomonas fluorescens* (CCTB03) makes it possible to reduce production costs in two ways: 1) through savings of up to 25% in top dressing N fertilization; 2) possibility of using two microorganisms capable of promoting plant growth with a single application.

CONCLUSION

In all the studied locations, the corn crop grain yield with the application of the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N was statistically superior to the yield of the Absolute Control treatment.

With the application of the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) + 75% of the recommended dose of N, it is possible to reduce the dose of N applied in coverage by up to 25% without damaging corn crop productivity, reaching productivity levels similar to and even higher than those obtained with the application of the recommended dose of N in coverage for the crop (Nitrogen Control), thus being a viable alternative to reduce the production cost and the environmental impact caused by the application of nitrogen-based fertilizers.

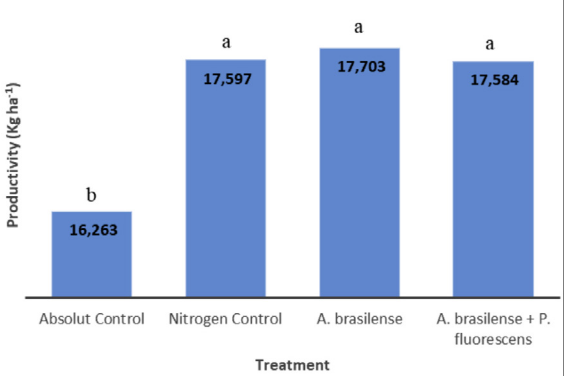

Figure 3 - Productivity (kg ha^{-1}) as a function of the application of different treatments in corn crop in the city of Lapa, Paraná. 2019. Bacterial Consortium = *Azospirillum brasilense* + *Pseudomonas fluorescens*. Means followed by the same lowercase letter do not differ by 5% by Duncan's test.

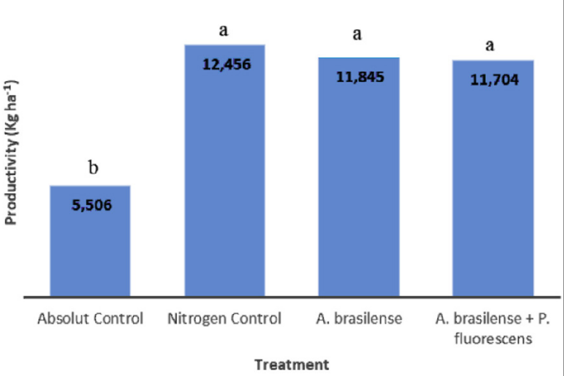

Figure 4 - Productivity (kg ha^{-1}) as a function of the application of different treatments in corn crop in the city of Sertão, Rio Grande do Sul. 2019. Bacterial Consortium = *Azospirillum brasilense* + *Pseudomonas fluorescens*. Means followed by the same lowercase letter do not differ by 5% by Duncan's test.

Through the Bacterial Consortium (*A. brasilense* + *P. fluorescens*) it is possible to apply, concomitantly, two microorganisms that may promote plant growth in the corn crop and reach high production levels.

The results obtained are consistent and support the agronomic efficiency in the field, as well as the feasibility of recommending the use of Bacterial Consortium containing *Azospirillum brasilense* (Ab-V6) and *Pseudomonas fluorescens* (CCTB03) as a growth promoter for corn.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

ABBAS-ZADEH, P. et al. Plant growth-promoting activities of fluorescent pseudomonads, isolated from the Iranian soils. **Acta Physiologiae Plantarum**, v.32, n.2, p.281-288, 2009. Available from: <https://link.springer.com/content/pdf/10.1007/s11738-009-0405-1.pdf>. Accessed: Dec. 01, 2022. doi: 10.1007/s11738-009-0405-1.

ANTOUN, H.; PRÉVOST, D. Ecology of Plant Growth Promoting Rhizobacteria. In: **PGPR: Biocontrol and Biofertilization**. Z. A. Siddiqui. Springer, Dordrecht. 2005. Available from: <https://link.springer.com/chapter/10.1007/1-4020-4152-7_1>. Accessed: Dec. 01, 2022. doi: 10.1007/1-4020-4152-7_1.

APONTE, A. et al. Rhizobacteria *Pseudomonas fluorescens* and *Azospirillum* sp. association enhances growth of *Lactuca sativa* L. under tropical conditions. **Journal of Central European Agriculture**, v.18, n.2, p.424-440, 2017. Available from: <https://jcea.agr.hr/articles/773891_Rhizobacteria_Pseudomonas_fluorescens_and_Azospirillum_sp_association_enhances_growth_of_Lactuca_sativa_L_under_tropical_condit_en.pdf (agr.hr)>. Accessed: Dec. 01, 2022. doi: 10.5513/JCEA01/18.2.1916.

ARF, O. et al. Benefícios do milho consorciado com gramínea e leguminosas e seus efeitos na produtividade em sistema plantio direto. **Revista Brasileira de Milho e Sorgo**, v.17, n.3, p.431-444, 2018. Available from: <http://www.researchgate.net/publication/329855512_BENEFICIOS_DO_MILHO_CONSORCIADO_COM_GRAMINEA_E_LEGUMINOSAS_E_SEUS_EFEITOS_NA_PRODUTIVIDADE_EM_SISTEMA_PLANTIO_DIRETO>. Accessed: Dec. 01, 2022. doi: 10.18512/1980-6477/rbms.v17n3p431-444.

ARRUDA, L. et al. Screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. **Applied Soil Ecology**, v.63, n.15-22, 2013. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0929139312002247?via%3Dihub>. Accessed: Dec. 01, 2022. doi: 10.1016/j.apsoil.2012.09.001.

BARBOSA, J. Z. et al. Meta-analysis of maize responses to *Azospirillum brasilense* inoculation in Brazil: Benefits and lessons to improve inoculation efficiency. **Applied Soil Ecology**, v.170, p.104276, 2022. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0929139321003991>. Accessed: Dec. 01, 2022. doi: 10.1016/j.apsoil.2021.104276.

EMBRAPA. **Manual de Análises Químicas do Solo, Plantas e Fertilizantes**. 2 ed. Brasilia – DF, 2009.

ETESAMI, H.; ALIKHANI, H. A. Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant. **Rhizosphere**, v.2, n.5-12, 2016. Available from: <https://www.researchgate.net/publication/308718963_Co-inoculation_with_endophytic_and_rhizosphere_bacteria_allows_reduced_application_rates_of_N-fertilizer_for_rice_plant_Oryza_sativa_L>. Accessed: Dec. 01, 2022. doi: 10.1016/j.rhisph.2016.09.003.

ETESAMI, H.; MAHESHWARI, D. K. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. **Ecotoxicol Environ Saf**, v.156, n.225-246, 2018. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0147651318301921?via%3Dihub>. Accessed: Dec. 01, 2022. doi: 10.1016/j.ecoenv.2018.03.013.

FAOSTAT. **Crops - area harvested**. 2022. Available from: <https://www.fao.org/faostat/en/#data/QCL>. Accessed: Dec. 03, 2022.

GALINDO, F. S. et al. Enhancing agronomic efficiency and maize grain yield with *Azospirillum brasilense* inoculation under Brazilian savanah conditions. **European Journal of Agronomy**, v.134, 126471, 2022. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1161030122000193>. Accessed: Dec. 01, 2022. doi: 10.1016/j.eja.2022.126471.

GLICK, B. R. Plant growth-promoting bacteria: mechanisms and applications. **Scientifica (Cairo)**, v.2012, n.1–15, 2012. Available from: <https://www.researchgate.net/publication/258925236_Plant_Growth-Promoting_Bacteria_Mechanisms_and_Applications>. Accessed: Dec. 01, 2022. doi: 10.6064/2012/963401.

GLICK, B. R. Bacteria with ACC deaminase can promote plant growth and help to feed the world. **Microbiological Research**, v.169, n.1, p.30-39, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S094450131300150X>. Accessed: Dec. 01, 2022. doi: 10.1016/j.micres.2013.09.009.

HUNGRIA, M. et al. Improving maize sustainability with partial replacement of N fertilizers by inoculation with *Azospirillum brasilense*. **Agronomy Journal**. v.1-12, 2022. Available from:<https://www.researchgate.net/publication/362739679_Improving_maize_sustainability_with_partial_replacement_of_N_fertilizers_by_inoculation_with_Azospirillum_brasilense#fullTextFileContent>. Accessed: Dec. 01, 2022. doi: 10.1002/agj2.21150.

HUNGRIA, M. et al. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. **Plant and Soil**, v.331, n.1, p.413–425, 2010. Available from: <https://link.springer.com/article/10.1007/s11104-009-0262-0>. Accessed: Dec. 01, 2022. doi 10.1007/s11104-009-0262-0.

HUNGRIA, M. et al. Co-inoculation of soybeans and common beans with rhizobia and azospirilla: Strategies to improve sustainability. **Biology and Fertility of Soils**, v.49, n.7, p.791–801, 2013. Available from: <https://link.springer.com/article/10.1007/s00374-012-0771-5>. Accessed: Dec. 01, 2022. doi: 10.1007/s00374-012-0771-5.

HUNGRIA, M. et al. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics. **Agriculture, Ecosystems and Environment**, v.221, p.125–131, 2016. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0167880916300366>. Accessed: Dec. 01, 2022. doi: 10.1016/j.agee.2016.01.024.

MANCILLA, A. G. et al. Characterization and selection of rizobacterias growth promoters in plant of Chile poblano (*Capsicum annuum* L.). **Revista Internacional De Contaminacion Ambiental**, v.33, n.3, p.463-474, 2017. Available from: <https://www.revistascca.unam.mx/rica/index.php/rica/article/view/RICA.2017.33.03.09>. Accessed: Dec. 01, 2022. doi: 10.20937/RICA.2017.33.03.09.

MATTOS, M. L. T. Parte I: Bactérias Endofíticas Diazotróficas Isoladas das Cultivares BRS 7 "Taim" e BRS Pelota. In: **Fixação Biológica de Nitrogênio na Cultura do Arroz Irrigado por Inundação**. 1a ed. [s.l.] Embrapa, 2010. v. Documentos.

NAIMAN, A. D. et al. Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens*: Impact on the production and culturable rhizosphere microflora. **European Journal of Soil Biology**, v.45, n.1, p.44-51, 2009. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1164556308001416>. Accessed: Dec. 01, 2022. doi: 10.1016/j.ejsobi.2008.11.001.

OLIVEIRA, M. A. et al. Adubação fosfatada associada à inoculação com *Pseudomonas fluorescens* no desempenho agronómico do milho. **Revista de Ciências Agrárias**, v.38, n.1, p.18-25, 2015. Available from: <https://revistas.rcaap.pt/rca/article/view/16864/13746>. Accessed: Dec. 01, 2022. doi:10.19084/rca.16864.

PEREZ-MONTANO, F. et al. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. **Microbiological Research**, v.169, n.5-6, p.325-336, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S094450131300164X>. Accessed: Dec. 01, 2022. doi: 10.1016/j.micres.2013.09.011.

PRASAD, A. A.; BABU, S. Compatibility of *Azospirillum brasilense* and *Pseudomonas fluorescens* in growth promotion of groundnut (*Arachis hypogea* L.). **Annals of the Brazilian Academy of Sciences**, v.89, n.2, p.1027-1040, 2017. Available from: <https://www.scielo.br/j/aabc/a/vwf8qtx8SWvGfxGB7WgSqDv>. Accessed: Dec. 01, 2022. doi: 10.1590/0001-3765201720160617.

QUADROS, P. D. et al. Desempenho agronômico a campo de híbridos de milho inoculados com *Azospirillum*. **Revista Ceres**, v.61, n.2, p.209-2018, 2014. Available from: <https://www.scielo.br/j/rceres/a/v4RGgXhxKtJzkKRtGTh7RDj>. Accessed: Dec. 01, 2022. doi: 10.1590/S0034-737X2014000200008.

TAMILSELVI, S. et al. Development and formulation of *Azospirillum* and *Pseudomonas fluorescens* as effective biological agents for enhanced agro-productivity. **Bioscience Journal**, v.32, n.3, p.670-683, 2016. Available from: <https://www.researchgate.net/publication/303777035_Development_and_formulation_of_Azospirillum_lipoferum_and_Pseudomonas_fluorescens_as_effective_biological_agents_for_enhanced_agro-productivity#fullTextFileContent>. Accessed: Dec. 01, 2022. doi: 10.14393/BJ-v32n3a2016-32289.

TORIBIO-JIMENEZ, J. et al. Isolation and screening of bacteria from *Zea mays* plant growth promoters. **Revista Internacional de Contaminación Ambiental**, v.33, n.143-150, 2017. Available from: <https://www.revistascca.unam.mx/rica/index.php/rica/article/view/RICA.2017.33.esp01.13>. Accessed: Dec. 01, 2022. doi: 10.20937/RICA.2017.33.esp01.13.

VACHERON, J. et al. Plant growth-promoting rhizobacteria and root system functioning. **Front Plant Sci**, v.4, n.356, 2013. Available from: <https://www.researchgate.net/publication/256980374_Plant_growth-promoting_rhizobacteria_and_root_system_functioning>. Accessed: Dec. 01, 2022. doi: 10.3389/fpls.2013.00356.