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ISOLATION AND IDENTIFICATION OF BROAD-SPECTRUM ANTAGONIST BACTERIA AGAINST PATHOGENIC FUNGI OF MAIZE CROP

ABSTRACT – Fungal diseases may cause significant damage to crops worldwide, generating yield losses, poor grain quality, and health risks to humans and animals. Biological control using antagonistic bacteria offers innovative solutions for sustainable management aiming at plant protection. However, beneficial plant-microorganism interactions are particular, and few antagonists with broad-spectrum activity have been reported. In this work, two bacteria isolated from sorghum seeds were identified by partial sequencing of the 16S rRNA gene and tested *in vitro* for their capacity to control six important pathogenic fungi of maize: *Fusarium verticillioides*, *Macrophomina phaseolina*, *Stenocarpella* sp., *Fusarium graminearum*, *Colletotrichum graminicola*, and *Bipolaris* sp. The molecular identification revealed that the bacterial isolates belong to the genera *Bacillus* (strain LIS05) and *Paenibacillus* (strain LIS04). Both bacterial isolates inhibited the growth of all six phytopathogens by at least 49%. The isolate LIS05 showed the most significant antagonistic potential against the fungal pathogens tested, at an average of 73% inhibition. The highest antagonist activity (86.1% inhibition) was observed in the confrontation test between the isolate LIS05 and *C. graminicola*. In addition to the mycelial growth inhibition, the isolate LIS04 blocked the production of dark pigments by *Bipolaris* sp. This study showed that LIS05 and LIS04 are promising alternatives for developing integrated management strategies to control fungal diseases in maize and sorghum.

Keywords: *Zea mays*, phytophatogenic fungi, biocontrol, *Bacillus* sp., *Paenibacillus* sp.

RESUMO - ISOLAMENTO E IDENTIFICAÇÃO DE BACTÉRIAS ANTAGONISTAS DE AMPLO ESPECTRO CONTRA FUNGOS PATOGÊNICOS DO MILHO

RESUMO - As doenças fúngicas podem causar danos significativos às culturas em todo o mundo, gerando perdas de rendimento, má qualidade dos grãos e riscos à saúde de seres humanos e animais. O controle biológico utilizando bactérias antagonistas oferece soluções inovadoras para um manejo sustentável visando a proteção das plantas. No entanto, as interações benéficas planta-microrganismo são particulares, e poucos antagonistas com atividade de amplo espectro foram relatados. Neste trabalho, duas bactérias isoladas de sementes de sorgo foram identificadas por sequenciamento parcial do gene 16S rRNA e testadas *in vitro* quanto à sua capacidade de controlar seis fungos patogênicos relevantes na cultura do milho: *Fusarium verticillioides, Macrophomina phaseolina, Stenocarpella* sp., *Fusarium graminearum, Colletotrichum graminicola* e *Bipolaris* sp. A identificação molecular revelou que os isolados pertencem aos gêneros *Bacillus* (cepa LIS05) e *Paenibacillus* (cepa LIS04). Ambos os isolados inibiram em pelo menos 49% o crescimento de todos os seis fitopatógenos testados. O isolado LIS05 apresentou o maior potencial antagonista contra os fitopatógenos, com média geral de 73% de inibição. A maior atividade antagonista (86,1% de inibição) foi observada no teste de pareamento entre o isolado LIS05 e o fungo *C. graminicola*. Além de inibir o crescimento micelial, o isolado de LIS04 inibiu a produção de pigmentos escuros pelo fitopatógeno *Bipolaris* sp. As bactérias *Bacillus* sp. (cepa LIS05) e *Paenibacillus* sp. (cepa LIS04) são promissoras para o desenvolvimento de novos bioprodutos para uso na agricultura. Esse estudo mostrou que LIS05 e LIS04 são alternativas promissoras para o desenvolvimento de estratégias de manejo integrado de doenças fúngicas nas culturas do milho e do sorgo.

Palavras-chave: *Zea mays*, fungos fitopatogênicos, biocontrole, *Bacillus* sp., *Paenibacillus* sp.

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Maize is the second most important crop in the Brazilian agricultural scenario, surpassed by soybean (CONAB, 2022). Although Brazil is one of the world's largest maize producers, its average productivity is still lower than the leading producer countries. This scenario has been attributed to factors affecting maize yield, including soil type, low fertility, water stress, sowing period, hybrid yield potential, weeds competition, insect attack, and diseases (Barroso et al., 2017). Crop damages and yield losses caused by fungal diseases have raised concerns for producers and technicians (Lanza et al., 2012), affecting the quantity and quality of the grains produced (Nguyen et al., 2017).

It is estimated that more than 15% of all losses in agricultural production worldwide are due to plant diseases, with more than 70% being caused by fungi (Dobrzyński et al., 2023; Asad, 2022; Liu et al., 2017). Diseases are the second cause of grain deterioration in maize, bypassed only by insect attacks. Contamination of grains by fungi can occur at any stage of the productivity chain. It may be influenced mainly by factors such as the physical and sanitary condition of the grains, storage conditions, presence of insects and mites, climatic conditions, water availability, the chemical composition of grains, and agricultural practices, among others (Silva et al., 2017).

Symptoms of maize diseases in the field are often observed in plants and are caused by various phytopathogens. Stem rot caused by *Fusarium verticillioides*, *Fusarium graminearum*, *Stenocarpella maydis*, and *Colletotrichum graminicola* are currently considered the most important diseases of maize due to the damages, yield losses, and reduction of grain size and quality they cause to the crop (Reis & Casa, 1996; Wordell Filho & Casa, 2010; Sabato & Fernandes, 2014; Pfordt et al., 2020). In addition, these phytopathogens also cause ear rot with the formation of burnt kernels, resulting in a reduction of the product's value in the market since a percentage referring to the presence of these grains is deducted from the sale value. (Silva et al., 2015). In addition, burned grains have a high reduction in their nutritional value, besides lighter than healthy grains, leading to a reduction in productivity (Costa et al., 2011).

Fusarium verticillioides and F. *graminearum* cause seedling death and ear-, root- and stem rot, leading to loss of productivity and grain quality worldwide (Munkvold, 2003). Both species produce mycotoxins, toxic metabolites harmful to humans and animals that consume the contaminated grains (Deepa & Sreenivasa, 2017; Blacutt et al., 2018). Fumonisins are the most common mycotoxins associated with *F. verticillioides* (Lanza et al., 2014; Pitt, 2014; Blacutt et al., 2018), while deoxynivalenol (DON) and nivalenol (NIV) of the type B trichothecene group are frequent in *F. graminearum* (Munkvold & Desjardins, 1997). Grain contamination by mycotoxins may constitute an obstacle to international trade due to phytosanitary barriers imposed by consumer countries with strict control

of the maximum permitted limits of mycotoxins in imported products (Mulunda et al., 2013).

Along with rot diseases caused by *Fusarium* species, the white ear rot caused by *Stenocarpella maydis* is the primary disease affecting maize ears in Brazil, with losses greater than 70% (Costa, 2013). *Stenocarpella maydis* causes ear rot in almost all countries where maize is cultivated, thus, a growing concern worldwide (Rossouw et al., 2009). This phytopathogen is also responsible for leaf spots seed- and stem rot. Stem rot impairs the plant's normal development and weakens the base of the stem, resulting in lodging and premature death of the plants (Shurtleff, 1992; Reis & Casa, 1996; Sabato & Fernandes, 2014; White, 1999;). When the plant is lodged, the ear comes into direct contact with the soil, favoring contamination by storing fungi and compromising grain quality (Casa et al., 2006).

Macrophomina phaseolina is also a critical phytopathogen of maize causing the disease known as macrophomina rot or gray rot, whose symptoms are characterized by black lesions on the roots and stems of infected plants, which can progress to wilting, falling plants and until an early death. When death does not occur, the plants wilt, drop leaves and reduce productivity. (Singh et al., 1990, Costa et al., 2019). *Colletotrichum graminicola* is another permanent fungus species in the maize crop, causing the stem rot known as stem anthracnose. This phytopathogen can infect all plant parts, resulting in different symptoms on the leaves, stem, ear, roots, and tassel (White, 1999). The fungus colonizes the stem tissues, reducing the absorption of water and nutrients, thus reducing weight and compromising the regular filling of the grains, which results in significant yield losses (Parreira et al., 2016). *Bipolaris maydis* affect maize crops in hot and humid regions around the world, causing the foliar disease known as bipolaris spot (Costa et al., 2014). The increase of lesions on the leaf surface consequently impairs the photosynthetic area of the plants, generating losses or reductions in crop productivity (Agrios, 2005). Losses were more significant than 70% reported in temperate regions and hot and humid tropical areas, where bipolaris spot occurs more severely. In Brazil, high disease severity was detected in some states, such as Rondônia, Mato Grosso, Goiás, and Tocantins (Costa et al., 2014).

Biological control involves using organisms that interfere with developing pathogens and plant pests. Biological control is a natural tool and an eco-friendly alternative to overcome the hazards of chemical methods for plant protection (Saito et al., 2009; Brito et al., 2013). Thus, microorganisms with antagonistic activity may depict a promising approach for fungal disease management besides their simple cultivation, environmental safety, and low-cost compared to conventional methods (Silva et al., 2003; Brito et al., 2013). Antagonist organisms can prevent the growth of phytopathogenic fungi through different modes of action, such as releasing toxic substances into the environment, such as antibiotics and extracellular hydrolytic enzymes (chitinases, cellulases, glucanases, proteases, and

lipases (Mabood et al., 2014). In addition, many microbial biological control agents induce the plant defense mechanisms against pathogens by acting as elicitors that induce a signal to stimulate the plant defense mechanism against pathogens (Zehra et al., 2021), or controlling phytopathogens by mycoparasitism (Deacon, 1980). Currently, biological control using antagonistic microorganisms has been consolidating as a viable alternative to control the development of phytopathogens in different crops (Tariq et al., 2020).

Antagonistic microorganisms can be isolated from soil (rhizosphere) or different parts of plants (epiphytic and endophytic) of different species. On the other hand, isolating native microorganisms increases the efficiency of biological control, as they present adaptations to compete and survive in their original environment (Figueroa-López et al., 2016). Therefore, in this work, epiphytic bacteria isolated from sorghum seeds without apparent fungal growth were selected for in vitro tests against F. verticillioides since it constitutes one of the primary source of grain contamination.

Many strategies have been proposed to control these phytopathogens (Khan et al., 2017), including a promising alternative to biological control (Rahman, 2018). This strategy has the advantages of being environmentally safe and inexpensive compared to conventional methods for controlling fungal diseases (Brito et al., 2013).

Among the bacterial genera used as biocontrol agents, many species of *Bacillus* and *Paenibacillus* stand out (O'Brien, 2017; Padda et al., 2017; Bonaterra et al., 2022). Bacteria from these groups have unique traits, such as producing secondary bioactive metabolites with antagonistic activity against pathogenic fungi and bacteria, giving them a valuable advantage as biological control agents (Saharan and Nehra, 2011). In addition, producing spores resistant to heat and drying is another crucial advantage for choosing *Bacillus* and *Paenibacillus* in commercial formulations. This trait ensures more remarkable survival of the strains under field conditions and extends the shelf life of the inoculants compared with the agrochemicals lifetime (Wu et al. 2015).

Most studies involving biological control have been characterized by evaluating the effectiveness of a single biocontrol agent against a single phytopathogen (Wilson & Backman, 1999; Bressan, 2003; Domenech et al., 2006; Bressan & Figueiredo, 2010; Li et al. 2011; Köberl et al. 2013; Prabhukarthikeyan et al., 2014; Abdullah et al., 2017). However, under natural conditions, several diseases often occur in the same culture during its life cycle. Therefore, tests with at least the most relevant phytopathogens of a culture can increase the chance of success of a bioformulation. Thus, in this study, two bacterial isolates from sorghum seeds were tested *in vitro* for antagonistic activity against six phytopathogenic fungi of different genera frequently infesting maize crops.

Material and Methods

Microorganisms and culture conditions

In the study, the phytopathogenic strain CML 2778 of *Fusarium verticillioides* from the Mycological Collection of the Federal University of Lavras/MG (CML), and the phytopathogens *Macrophomina phaseolina*, *Stenocarpella* sp., *Fusarium graminearum*, *Colletotrichum graminicola*, and *Bipolaris* sp. from the Phytopathology Laboratory of Embrapa Maize and Sorghum (CNPMS) were tested.

Seed samples of sorghum (*Sorghum bicolor* L.) were collected in the experimental field of Embrapa Milho e Sorgo, located in the Cerrado region in Sete Lagoas/MG. In the laboratory, the hundred seeds were disinfected by immersiong in a 3% (v/v) hypochlorite solution for 5 minutes, followed by three washes with deionized water. After this procedure, the seeds were placed in gerbox plastic boxes, measuring 11 x 11 x 3 cm, containing three layers of filter paper moistened with sterile deionized water. For each treatment, 100 seeds were distributed in four replicates of 25 seeds per box. The boxes were incubated at 22 ± 2 °C, with a 12-h light photoperiod. Seed with visible bacterial mass without mycelial growth were used for bacterial isolation (Figure 1). Samples of bacterial mass were collected using sterile platinum loops and inoculated through streaks on the surface of plates containing TSA Soy Triptone Agar culture medium (Kasvi, Brazil). Colonies with different macromorphological characteristics were collected and purified until pure cultures were obtained. Afterward, the isolated colonies were stored at -20 ºC and -80 oC in TSB Tryptone Soy Broth with 20% glycerol, and deposited in the Embrapa Maize and Sorghum Microorganism Collection (CMMF).

In vitro antagonist test of bacterial isolates against phytopathogenic fungi

The *in vitro* antagonism against phytopathogens was evaluated by the Direct Confrontation (DC) test using a solid medium according to methodology adapted from Sagahón et al. (2011). Initially, a 5 mm disk from the edge of the pure culture of the pathogen, grown for seven days in Potato Dextrose Agar (PDA) medium, was transferred to the center of a Petri dish (90 mm in diameter) containing the same medium. At four points equidistant from each other and from the center, 10 µL of bacterial suspension was added at an approximate concentration of 108 CFU/mL, grown for 24 h at 28 °C. The test was performed in triplicate, and the control was composed of plates containing only the phytopathogen. The measurement of the radius of the phytopathogen colony was carried out in the presence and absence of the antagonist microorganisms when the entire surface of the medium was colonized by the pathogen in the control treatment, approximately seven days after incubation at 28°C with a photoperiod of 12 hours of light. The inhibition by the isolates was calculated concerning the mycelial growth in the control plate according to the formula:

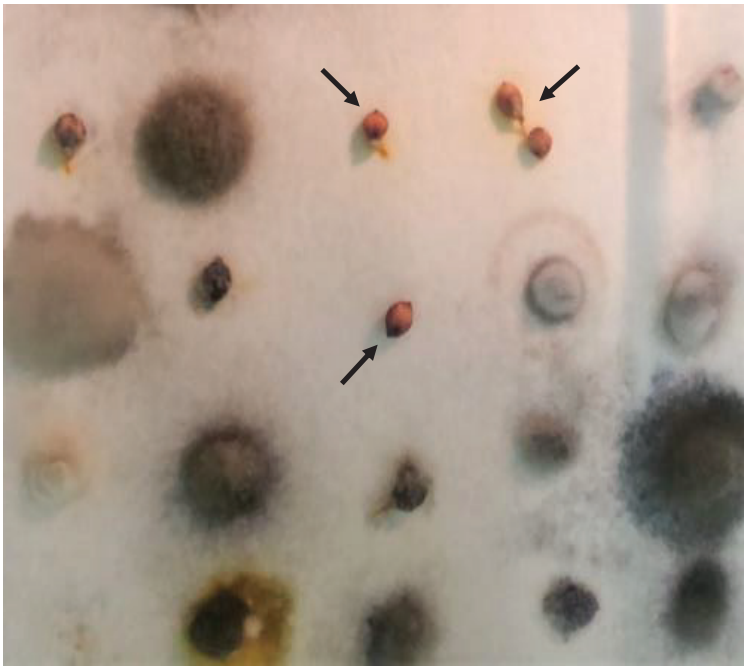

Figure 1. Sorghum seeds collected from plants growing in the experimental area of Embrapa Maize and Sorghum Research Center showing different degrees of colonization by bacteria and filamentous fungi. The arrows show the seeds with bacterial mass without apparent fungus infestation.

$$IZ(\%) = \frac{(N1 - N2)\ x\ 100}{N1}$$

Where: |IZ| = Inhibition Zone; N1= mycelial radius in the absence of antagonist; N2= mycelial radius in the presence of antagonist (Campanile et al., 2007).

Molecular identification of the bacterial isolates

DNA extraction

The DNA extraction 16S rRNA amplifications of the bacterial isolates were performed according to Diniz et al. (2022), using pure cultures grown in Luria Bertani liquid medium at 28 °C for 24 h under agitation at 150 rpm. After this period, the culture was centrifuged at 16,000 rpm, and the supernatant was discarded. Genomic DNA extraction was performed with the kit Wizard® Genomic DNA Purification (Promega, EUA), following the manufacturer's recommendations. DNA integrity was assessed by electrophoresis on a 1% (w/v) agarose gel in 1X TAE buffer (10 mM Tris, 20 mM acetic acid, and one mM EDTA; pH 8.0), stained with GelRed solution (Biotium, USA). The DNA samples were visualized in a Transilluminator under UV light and photographed in the Gel Logic 200 equipment (KODAK Company, USA). DNA quantification was performed in an ND-1000 UV/VIS spectrophotometer (NanoDrop Technologies, USA), and the samples were diluted to a

concentration of 20 ng/µL.

16S rRNA gene amplification

PCR reactions were made with the universal primers 8F and 1492R (Lane et al., 1985; Turner et al., 1999). DNA amplification was performed with a final reaction volume of 20 µl, containing 2 µL of DNA (20 ng/µL); 2 µL 10X reaction buffer (Invitrogen); 1.0 µL of deoxynucleotide triphosphate (dNTP) 2.5 mM each; 4 µL of Dimethylsulfoxide (DMSO) 20% (v/v); 0.8 µL of each primer (10 µM); 0.2 µL of Taq DNA Polymerase 5 U/ µL-1 (Invitrogen), 0.6 µL of 50mM MgCl2, and 8.6 µL of ultrapure water. Amplification was performed in a Veriti thermocycler (Applied Biosystems, USA) with initial denaturation at 95 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 59 °C for 30 seconds, extension at 72 °C for 1 minute and 30 seconds and a final extension of 10 min at 72 °C. The PCR product was subjected to electrophoresis on a 1% agarose gel (w/v) for one hour at 100 V in 1X TAE buffer. The gel was stained with Gel Red solution (Biotium, USA), visualized under UV light, and photographed in the Gel Logic 200 equipment (KODAK Company, USA).

DNA sequencing

The amplified DNA fragments were purified with 4 µL of Exo-Sap enzyme (GE HealthCare, USA) in a 15 µL product of the PCR reaction. Then, the samples were incubated at 37°C for 30 minutes and 80°C for 15 minutes. Sequencing reactions were performed with 4 µL of purified PCR product, 1 µL of primers 1492R, 8F, 902R, or 515F (Lane et al., 1985; Turner et al., 1999), 0.5 µL Big Dye V3.1 (Applied Biosystems, USA), 1.75 µL of reaction buffer (Applied Biosystems, USA) and 2.75 µL of ultrapure water. Then, the plates were incubated in a thermocycler under the following conditions: 96 °C for 1 minute, 96 °C for 2 seconds, 50 °C for 15 seconds, 60 °C for 4 minutes, repeated 30 times. After the end of the reaction, 60 µL of absolute ethanol plus 5 µL of EDTA (125 mM) were added. Then, the samples were homogenized and incubated in the dark for 15 minutes at room temperature. After this time, the samples were centrifuged at 4.000 rpm for 45 minutes. The supernatant was discarded, and 100 µL of 70% (v/v) ethanol was added to each sample. Again, the samples were centrifuged at 4.000 rpm for 10 minutes. Then, the ethanol was discarded, and the samples were centrifuged upside down at 300 rpm with low acceleration and deceleration. Subsequently, they were placed in an oven at 65 °C for 3 minutes or until complete drying. Finally, 10 µL of HI-DI formamide (Applied Biosystems, USA) were added to each sample, followed by denaturation in a thermocycler at 95 °C for 5 minutes. The samples were analyzed in the DNA sequencer model ABI PRISM 3500xL Genetic Analyzer (Applied Biosystem, USA), and the sequences obtained were aligned using the Sequencher 4.1.4 program (Genes Codes Corporation).

The isolates were identified by sequence comparison to the National Center for

Biotechnology Information (NCBI) database using the BLASTN program (http://blast.ncbi.nlm.nih.gov). The sequences were deposited in Genbank and received the following access codes: OR542589.1 and OR548005.1.

Results and Discussion

Identification of bacteria isolated from sorghum seeds and antifungal activity

During an experiment aiming to isolate pathogenic fungi from sorghum seeds, the observation of seeds completely colonized by fungi and seeds without fungi but colonized by bacteria indicated that those bacteria were antagonists of seed-colonizing or saprophytic fungi. Thus, the seeds without fungi were selected for further experiments. After the bacterial colonies grew in the medium, two colonies were replicated until pure colonies isolation.

The two bacterial isolates showed a broad spectrum and high growth inhibition activity against the six phytopathogenic fungi relevant to the maize crop: *Fusarium verticillioides*, *Bipolaris* sp., *C. graminicola*, *F. graminearum*, *M. phaseolina* and *Stenorcapella maydis* (Figure 2, Table 1.). Both bacterial isolates showed inhibition values above 49% (Table 1). As expected, the inhibition zone was not observed in plates used as a negative control, where fungal mycelia covered the entire surface of the culture medium. The *Bacillus* isolate LIS05 statistically showed the highest percentages of inhibition of the five phytopathogens (overall average of 73% inhibition). The highest overall inhibition percentage was also observed with the isolate (LIS05) against the fungus *C. graminicola* (86.1%), which causes anthracnose disease in maize.

Plants face constant challenges against fungal diseases in their natural environment, and several diseases caused by different phytopathogens may occur in the same crop throughout its life cycle (Fernandes et al., 2021). However, most studies involving biological control start by evaluating the effectiveness of a single biocontrol agent against a single phytopathogen in a single host-single disease system (Wilson & Backman, 1999; Bressan, 2003; Domenech et al., 2006; Bressan & Figueiredo, 2010; Li et al. 2011; Köberl et al. 2013; Prabhukarthikeyan et al., 2014; Abdullah et al., 2017). However, the rhizosphere community shelters abundant microorganisms and small animals living in complex interactions with surrounding plants (Praeg & Illmer, 2020; Liu et al., 2023). Environmental conditions, soil types, plant species, and even cultivars of crops modulate the microbial community, making it a highly dynamic micro-ecossistem (Jiang et al., 2017). In addition, almost every time, plants in natural environments face attacks of multiple pathogens. Unfortunately, in vitro studies in laboratories cannot reproduce the myriad of parameters defining the complex rhizosphere environment. Therefore, evaluating the isolates selected in this work regarding the potential to inhibit different pathogens can contribute to

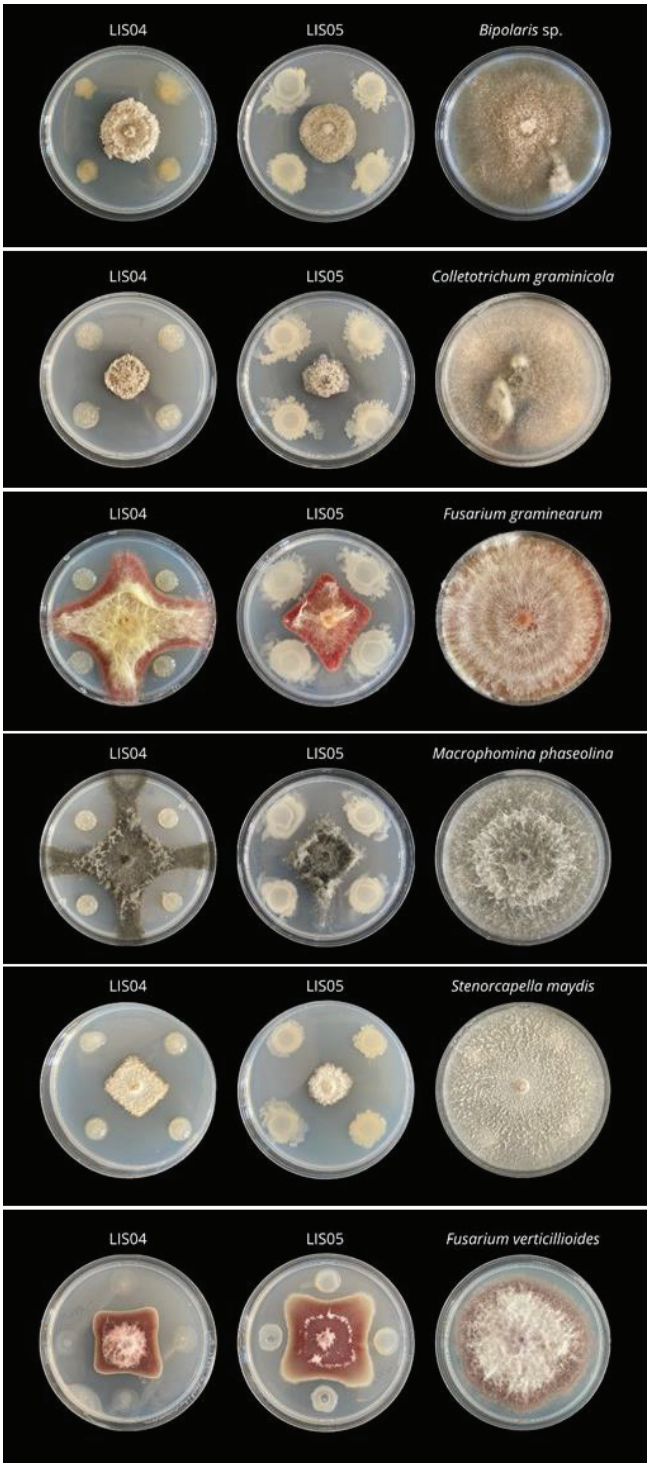

Figure 2. Antagonistic activity of *Paenibacillus* sp. LIS04 and *Bacillus* sp. LIS05 against phytopathogenic fungi relevant to the maize crop: *Bipolaris* sp., *Colletotrichum graminicola*, *Fusarium graminearum*, *Macrophomina phaseolina*, *Stenorcapella maydis*, and *Fusarium verticillioides*.

Table 1. *In vitro* Inhibition Zone (IZ %) of the growth of six phytopathogens by antagonist bacteria compared to control plates containing only a phytopathogen.

Isolate	*Fusarium verticillioides*	*Bipolaris* sp.	*Colletotrichum graminicula*	*Fusarium graminearum*	*Macrophomina phaseolina*	*Stenorcapella maydis*
			ZI* (%)			
LIS04	67.5 aB	62.7 bC	69.1 bB	49.0 bE	54.5 bD	71.6 bA
LIS05	57.5 bD	75.8 aB	86.1 aA	73.9 aB	66.6 aC	78.3 aB

* The Inhibition Zone was calculated by the formula IZ % = (N1-N2) x 100/N1, where N1= radius of the mycelium in the absence of the antagonist; N2= radius of the mycelium in the presence of the antagonist. Means followed by the same letters in the column (lower case letters) or row (upper case letters) do not differ statistically by the Scott-Knott Test at 5% probability ($p < 0.05$).

selecting broad-spectrum agents effective against different pathogens that cause plant diseases.

The two bacterial isolates with antagonist activity were identified by partially sequencing the 16S rRNA gene and compared with nucleotide sequences deposited in Genbank. The result revealed that the isolates belong to the genera *Bacillus* (LIS05) and *Paenibacillus* (LIS04), with similarity values above 98%. Bacteria of these two genera are non-pathogenic, gram-positive, aerobic, form endospores, are widely found in the most diverse agricultural systems, and form symbiotic interactions with plants (Tsotetsi et al., 2022). Several *Bacillus* strains are being used as biological pesticides (Domínguez-Arrizabalaga et al., 2020), plant growth-promoter (Blake et al., 2021; Gohil et al., 2022), abiotic stress (Lastochkina, 2019), biofungicides (Lastochkina et al., 2019; Bonaterra et al., 2022; Nievierowski et al., 2023), and nematicidal (Hussain et al., 2020; Zhou et al., 2022). The antagonistic potential of *Paenibacillus* for a wide range of pathogenic fungi, such as *Fusarium* sp., is well documented (Hsu et al., 2017; Luo et al., 2018; Li & Chen, 2019). In addition to their biocontrol activity, representatives of the genera Bacillus and Paenibacillus also directly promote plant growth and health through phytostimulatory and biofertilizer characteristics (Kavamura et al., 2013; Abdel-Rahman et al., 2017; Vurukonda et al., 2018), and Its ability to form durable, heat-resistant endospores allows for easy formulation (Emmert & Handelsman, 1999; Adesemoye et al., 2009).

A notable effect observed in this study was the inhibition of the production of dark pigments by the hyphae of *Bipolaris* sp. by metabolites secreted by the isolate (LIS04) of *Paenibacillus* sp. (Figure 3).

Melanin production is related to fungi pathogenicity (Butler et al., 2001; Aranda et al., 2023) and confers survival and adaptation advantages in extreme environmental conditions (Nosanchuk & Casadevall, 2003). Melanized fungi are more resistant to lysis caused by cell wall-degrading enzymes released by antagonistic microorganisms, especially in soil. As demonstrated by Butler et al. (1989), the cellular integrity of a melanized fungus was maintained in the presence of high concentrations of lytic enzymes for several days, while its albino mutants (without melanin) were destroyed in a matter of minutes. Controlling a pathogenic fungus by interfering with its infection mechanism is an exciting strategy, as it does not involve interference in biological processes common to other organisms and causes fewer effects on the host plant. (Jennings et al., 2000). Thus, the inhibition of melanin synthesis by LIS04 is a vital control mechanism that must be considered when selecting antagonists to develop new biofungicides.

Diseases caused by fungi in maize crops have been a serious problem worldwide, and different strategies have been proposed to control these pathogens (Khan et al., 2017). Biological control has been presented as a

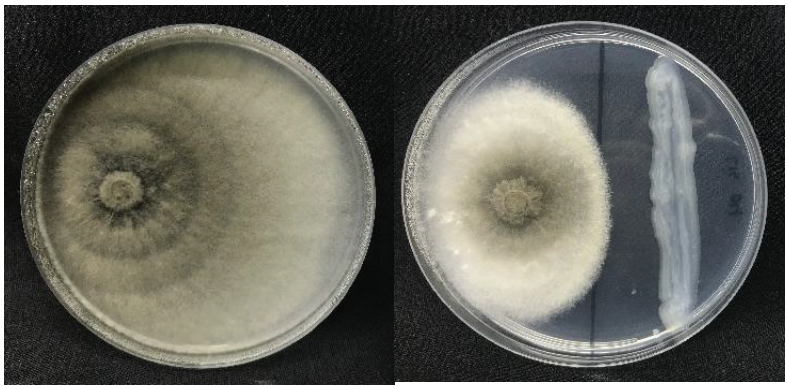

Figure 3. Inhibition of melanin production by the phytopathogen *Bipolaris* sp. by the antagonist bacterium LIS04 of *Paenibacillus* sp. Growth on the control plate of the phytopathogen *Bipolaris* sp. with the production of dark pigments (A). Inhibition of fungus and pigment production by *Paenibacillus* sp. LIS04 (B).

promising alternative in this case (Rahman, 2018), as it has the advantages of being safe for the environment and low cost compared to conventional treatment methods (Brito et al., 2013). In this work, the *in vitro* selection method made it possible to evaluate bacteria of the genera *Bacillus* and *Paenibacillus* with broad-spectrum antagonistic potential more quickly and at a lower cost. Furthermore, it allowed us to identify another possible mechanism of action used by antagonists: the inhibition of the production of dark pigments by the phytopathogen. These results open new perspectives for using LIS04 and LIS05 strains to develop multifunctional biofungicides.

REFERENCES

ABDEL-RAHMAN, H. M.; SALEM, A. A.; MOUSTAFA, M. M. A.; EL-GARHY, H. A. S. A novice *Achromobacter* sp. EMCC1936 strain acts as a plant-growth-promoting agent. **Acta Physiologiae Plantarum**, v. 39, n. 61, 2017. DOI: https://doi.org/10.1007/s11738-017-2360-6.

ABDULLAH, A. S.; MOFFAT, C. S.; LOPEZ-RUIZ, F. J.; GIBBERD, M. R.; HAMBLIN, J.; ZERIHUN, A. Host–Multi-Pathogen Warfare: Pathogen Interactions in Co-infected Plants. Front. Plant Sci. 8:1806. 2017. DOI http://doi.org/10.3389/fpls.2017.01806.

ADESEMOYE, A. O.; TORBERT, H. A.; KLOEPPER, J. W. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. **Microbial Ecology**, v. 58, p. 921-929, 2009. DOI https://doi.org/10.1007/s00248-009-9531-y.

AGRIOS, G. N. Foliar diseases caused by *Ascomycetes* and *Deuteromycetes* (Mitosporic Fungi). In: G. AGRIOS, ed. Plant pathology. 5th ed. Burlington: Elsevier Academic Press,

2005. p. 466-468.

ARANDA, C.; MÉNDEZ, I.; BARRA, P. J.; HERNÁNDEZ-MONTIEL, L.; FALLARD, A.; TORTELLA, G.; BRIONES, E.; DURÁN, P. Melanin Induction Restores the Pathogenicity of *Gaeumannomyces graminis* var. *tritici* in Wheat Plants. **Journal of Fungi**, 9(3):350, 2023. DOI: https://doi.org/10.3390/jof9030350

ASAD, S. A. Mechanisms of action and biocontrol potential of Trichoderma against fungal plant diseases-a review. **Ecological Complexity**, v. 49:100978, 2022. DOI: https://10.1016/j.ecocom.2021.100978

BARROSO, V. M.; ROCHA, L. O.; REIS, T. A.; REIS, G. M.; DUARTE, A. P.; MICHELOTTO, M. D.; CORREA, B. *Fusarium verticillioides* and fumonisin contamination in Bt and non-Bt maize cultivated in Brazil. **Mycotoxin Research**, v. 33, p. 121-127, 2017.DOI: https://doi.org/10.1007/s12550-017-0271-4

BLACUTT, A. A.; GOLD, S. E.; VOSS, K. A.; GAO, M.; GLENN, A. E. *Fusarium verticillioides*: Advancements in understanding the toxicity, virulence, and niche adaptations of a model mycotoxigenic pathogen of maize. **Phytopatology**, v. 108, p. 312-326, 2018. DOI: https://doi.org/10.1094/PHYTO-06-17-0203-RVW

BLAKE, C.; CHRISTENSEN, M. N.; KOVÁCS, A.T. Molecular aspects of plant growth promotion and protection by *Bacillus subtilis*. **Molecular Plant Microbe Interactions**. v. 34, n. 1, p. 15-25, 2021. DOI: https://doi.org/10.1094/MPMI-08-20-0225-CR

BONATERRA, A.; BADOSA, E.; DARANAS, N.; FRANCÉS, J.; ROSELLÓ, G.; MONTESINOS, E. Bacteria as Biological Control Agents of Plant Diseases. **Microorganisms**, 10(9):1759, 2022. DOI:https://doi.org/10.3390/microorganisms10091759.

BRESSAN, W. Biological control of maize seed pathogenic fungi by use of actinomycetes. BioControl, 48: 233-240, 2003.

BRESSAN, W.; FIGUEIREDO, J. E. F. chitinolytic bacillus spp. isolates antagonistic to *fusarium moniliforme* in maize. Journal of Plant Pathology, 92 (2), 343-347, 2010.

BRITO, A. H.; PINHO, R. G. V.; PEREIRA, J. L. A. R.; BALESTRE, M. Controle químico da cercosporiose, mancha-branca e grãos ardidos em milho. **Revista Ceres**, v. 60, n.5, p. 629-635, 2013. DOI: https://doi.org/10.1590/S0034-737X2013000500005

BUTLER, M. J.; DAY, A. W.; HENSON, J. M.; MONEY, N. P. Pathogenic Properties of Fungal Melanins, **Mycologia**, v. 93, n.1, p. 1-8, 2001. DOI: https://doi.org/10.1080/00275514.2001.12061273

BUTLER, M. J.; LAZAROVITS G.; HIGGINS, V. J.; LACHANCE, M-A. Identification of a black yeast isolated from oak bark as belonging to genus *Phaeococcomyces* sp: Analysis of melanin

produced by the yeast. **Canadian Journal of Microbiology**, v. 35, n.7, p.728-734, 1989. DOI: https://doi.org/10.1139/m89-118

CAMPANILE, G.; RUSCELLI, A.; LUISI, N. Antagonistc activity of endophytic fungi towards *Diplodia corticola* assessed by *in vitro* and in planta test. **European Journal of Plant Pathology**, v.117, p. 237-246, 2007. DOI: https://doi.org/10.1007/s10658-006-9089-1

CASA, R. T.; REIS, E. M.; ZAMBOLIM, L. Doenças do milho causadas por fungos do Gênero *Stenocarpella*. **Fitopatologia Brasileira**, v. 31, p. 427-439, 2006. DOI: https://doi.org/10.1590/S0100-41582006000500001

CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO. 2022. Acompanhamento da Safra Brasileira de Grãos, Brasília, DF, v.10, safra 2022/23, n.3. terceiro levantamento. Disponível em: https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de- graos. Acesso em dez/2022.

COSTA, R. V. da; COTA, L. V.; CRUZ, J. C.; SILVA, D. D.; QUEIROZ, V. A. V.; GUIMARÃES, L. J. M.; MENDES, S. M. **Recomendações para a redução da incidência de grãos ardidos em milho**. Sete Lagoas: Embrapa-CNPMS, 22 p. (Boletim de Pesquisa e Desenvolvimento/Embrapa Milho e Sorgo Nº 38), 2011. ISSN 1679-0154

COSTA, R. V. da; COTA, L. V.; SILVA, D. D. da. **Doenças causadas por fungos do gênero *Stenocarpella* spp. (*Diplodia* spp.) em milho**. Sete Lagoas: Embrapa-CNPMS, (Circular Técnica/Embrapa Milho e Sorgo Nº 197), 2013. ISSN 1679-1150

COSTA, R. V. da; SILVA, D. D. da; COTA, L. V. **Mancha-de-Bipolaris do Milho.** Sete Lagoas: Embrapa-CNPMS, (Circular Técnica/Embrapa Milho e Sorgo Nº 207), 2014. ISSN 1679-1150

COSTA, R. V. da; SILVA, D. D. da; COTA, L. V.; CAMPOS, L. J. M.; ALMEIDA, R. E. M. de; TUBIANA, D.; EVANGELISTA, B. A.; RIBEIRO, I. L. ***Macrophomina phaseolina* em milho safrinha: levantamento da incidência e perdas na produtividade no Estado do Tocantins**. Sete Lagoas: Embrapa-CNPMS, 19 p. (Boletim de Pesquisa e Desenvolvimento/ Embrapa Milho e Sorgo Nº 202), 2019. ISSN 1679-0154

DEEPA, N.; SREENIVASA, M. Y. *Fusarium verticillioides*, a globally important pathogen of agriculture and livestock: A Review. **Journal of Veterinary Medicine and Research**, v. 4(4): 1084, 2017. DOI: https://doi.org/10.47739/2378-931X/1084

DINIZ, G.F.D.; COTA, L.V.; FIGUEIREDO, J. E. F.; AGUIAR, F. M.; SILVA, D. D.; LANA, U. G. P.; SANTOS, V. L.; MARRIEL, I. E.; OLIVEIRA-PAIVA, C. A. Antifungal activity of bacterial strains from maize silks against *Fusarium verticillioides*. **Archives of Microbiology**, v. 204, n. 89, 2022. DOI: https://doi.org/10.1007/s00203-021-02726-4

DOMÍNGUEZ-ARRIZABALAGA, M.; VILLANUEVA, M.; ESCRICHE, B.; ANCÍN-AZPILICUETA, C.; CABALLERO, P. Insecticidal Activity of *Bacillus thuringiensis* Proteins Against Coleopteran Pests. **Toxins** (Basel), 12(7):430, 2020. DOI: http://doi.org/10.3390/toxins12070430

DOBRZYŃSKI, J.; JAKUBOWSKA, Z.; KULKOVA, I.; KOWALCZYK, P.; KRAMKOWSKI, K. Biocontrol of fungal phytopathogens by *Bacillus pumilus*. **Frontiers in Microbiology**, v. 14:1194606, 2023. DOI: http://10.3389/fmicb.2023.1194606.

DOMENECH, J.; REDDY, M. S.; KLOEPPER, J. W.; Ramos, B.; Gutierrez-Mañero, J. Combined Application of the Biological Product LS213 with Bacillus, Pseudomonas or Chryseobacterium for Growth Promotion and Biological Control of Soil-Borne Diseases in Pepper and Tomato. **Biocontrol** 51, 245-258, 2006. DOI https://doi.org/10.1007/s10526-005-2940-z.

EMMERT, E. A. B.; HANDELSMAN, J. Biocontrol of plant disease: a (Gram-) positive perspective, **FEMS Microbiology Letters**, v. 171 (1), p. 1-9, 1999. DOI: https://doi.org/10.1111/j.1574-6968.1999.tb13405.x

FERNANDES, I. V.; CARVALHO, H. P.; SANTOS, A. C. P. D.; CHAGAS, Y. J. G. Inibição do crescimento micelial de *Fusarium oxysporum* f. sp. *Lycopersici* por isolados de *Trichoderma* spp. **Revista Agrogeoambiental**, v. 13, n. 4, p. 762-771, 2021. DOI: http://dx.doi.org/10.18406/2316-1817v13n420211634

GOHIL, R. B.; RAVAL, V. H.; PANCHAL, R. R.; RAJPUT, K. N. Plant growth-promoting activity of *Bacillus* sp. PG-8 Isolated from fermented Panchagavya and its effect on the growth of *Arachis hypogea*. **Frontiers in Agronomy**, v.4:805454, 2022. DOI: http://doi.org/10.3389/fagro.2022.805454

HSU, L. H.; WANG, H. F.; SUN, P. L.; HU, F. R.; CHEN, Y.L. The antibiotic polymyxin B exhibits novel antifungal activity against *Fusarium* species. **International Journal of Antimicrobial Agents**, v. 49(6), p.740-748, 2017. DOI https://doi.org/10.1016/j.ijantimicag.2017.01.029.

HUSSAIN, T.; HARIS, M.; SHAKEEL, A.; AHMAD, G.; KHAN, A. A.; KHAN, M. A. Bio-nematicidal activities by culture filtrate of *Bacillus subtilis* HussainT-AMU: new promising biosurfactant bioagent for the management of Root Galling caused by *Meloidogyne incognita*. **Vegetos**, v. 33, p. 229-238, 2020. DOI https://doi.org/10.1007/s42535-020-00099-5.

JENNINGS, L. D.; RAYNER, D. R; JORDAN, D. B.; OKONYA, J. F.; BASARAB, G. S.; AMOROSE, D. K.; ANACLERIO, B. M.; LEE, J. K.; SCHWARTZ, R S.; WHITMORE, K. A. Cyclobutane Carboxamide Inhibitors of Fungal Melanin: Biosynthesis and their Evaluation as Fungicides. **Bioorganic & Medicinal Chemistry**, v. 8(5), p. 897- 907, 2000. DOI https://doi.org/10.1016/S0968-0896(00)00034-1.

JIANG, Y.; LI, S.; LI, R.; ZHANG, J.; LIU, Y.; LV, L.; ZHU, H.; WU, W.; LI, W. Plant cultivars imprint the rhizosphere bacterial community composition and association networks. **Soil Biology and Biochemistry**, v. 109, p. 145-155, 2017. DOI: https://doi.org/10.1016/j.soilbio.2017.02.010.

KAVAMURA, V. N.; SANTOS, S. N.; SILVA, J. L.; PARMA, M. M.; AVILA, L. A.;VISCONTI, A.; ZUCCHI, T. D.; TAKETANI, R. G.; ANDREOTE, F. D.; MELO, I. S. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. **Microbiological Research**, v. 168(4), p. 183-191, 2013. DOI https://doi.org/10.1016/j.micres.2012.12.002.

KHAN, N.; MAYMON, M.; HIRSCH, A. M. Combating *Fusarium* Infection Using *Bacillus*-Based Antimicrobials. **Microorganisms**, v. 5(4):75, 2017. DOI https://doi.org/10.3390/microorganisms5040075

KÖBERL, M.; RAMADAN, M. E.; ADAM, M.; CARDINALE, M.; HALLMANN, J.; HEUER, H.; SMALLA, K.; BERG, G. *Bacillus* and *Streptomyces* were selected as broad-spectrum antagonists against soilborne pathogens from arid areas in Egypt. **FEMS Microbiology Letters**, v. 342 (2), p. 168-178, 2013. DOI https://doi.org/10.1111/1574-6968.12089.

LANZA, F. E.; FIGUEIREDO, J. E. F.; COSTA, R. V.; MOURA, L. O.; MARCONDES, M. M.; SILVA, D. D.; COTA, L. V.; CORRÊA, C. L.; NICOLI, A. N.; RAMOS, T. C. D. A. Efeito do tratamento de sementes com o biofungicida bacteriano CNPMS22 na sobrevivência e desenvolvimento de plantas de milho em solo infestado com *Fusarium verticillioides*. In: **XXIX Congresso Nacional de Milho e Sorgo**, 2012. Águas de Lindóia. Anais. Campinas: Instituto Agronômico, Sete Lagoas: Embrapa Milho e Sorgo, p. 555-562. https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/933039/1/Efeitotratamento.pdf.

LANE, D. J.; PACE, B.; OLSEN, G. J.; STAHL, D. A.; SOGIN, M. L.; PACE, N. R. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. **Natl**. Acad. Sci. USA, v. 82, p. 6955-6959, 1985. https://www.jstor.org/stable/26740.

LANZA, F. E.; ZAMBOLIM, L.; COSTA, R. V.; QUEIROZ, V. A. V.; COTA, L. V.; SILVA, D. D.; SOUZA, A. G. C.; FIGUEIREDO, J. E. F. Prevalence of fumonisin-producing *Fusarium* species in Brazilian corn grains. **Crop Protection**, v. 65, p. 232-237, 2014. DOI https://doi.org/10.1016/j.cropro.2014.08.003.

LASTOCHKINA, O. *Bacillus subtilis*-Mediated Abiotic Stress Tolerance in Plants. In: ISLAM, M.; RAHMAN, M.; PANDEY, P.; BOEHME, M.; HAESAERT, G. (eds) Bacilli and Agrobiotechnology: Phytostimulation and Biocontrol. Bacilli in Climate Resilient Agriculture and Bioprospecting. **Springer, Cham**, 2019. DOI https://doi.org/10.1007/978-3-030-15175-1_6.

LASTOCHKINA, O.; SEIFIKALHOR, M.; ALINIAEIFARD, S.; BAYMIEV, A.; PUSENKOVA, L.; GARIPOVA, S.; KULABUHOVA, D.; MAKSIMOV, I. *Bacillus* Spp.: Efficient Biotic Strategy to Control Postharvest Diseases of Fruits and Vegetables. **Plants**, v. 8(4)97, 2019. DOI https://doi.org/10.3390/plants8040097.

LI, H.; LI, H.; BAI, Y; WANG, J.; NIE, M.; LI, B.; XIAO, M. The use of *Pseudomonas*

fluorescens P13 to control sclerotinia stem rot (*Sclerotinia sclerotiorum*) of oilseed rape. **The Journal of Microbiology**, v. 49(6), p. 884-889, 2011. DOI https://doi.org/10.1007/s12275-011-1261-4.

LI, Y.; CHEN, S. Fusaricidin Produced by *Paenibacillus polymyxa* WLY78 Induces Systemic Resistance against Fusarium Wilt of Cucumber. **International Journal of Molecular Sciences**, v. 20(20):5240, 2019. DOI https://doi.org/10.3390/ijms20205240.

LIU, K.; NEWMAN, M.; MCINROY, J. A.; HU, C. H.; KLOEPPER, J. W. Selection and assessment of plant growth promoting rhizobacteria for biological control of multiple plant diseases. **Phytopathology**, v.107, n.8, p. 928-936, 2017. DOI https://doi.org/10.1094/phyto-02-17-0051-r.

Liu, L.; Ma, L.; Zhu, M.; Liu, B.; Liu, X.; Shi, Y. Rhizosphere microbial community assembly and association networks strongly differ based on vegetation type at a local environment scale. Front. Microbiol. 14:1129471. 2023. DOI http://doi.org/10.3389/fmicb.2023.1129471.

LUO, Y.; CHENG, Y.; YI, J.; ZHANG, Z.; LUO, Q.; ZHANG, D.; LI, Y. Complete Genome Sequence of Industrial Biocontrol Strain *Paenibacillus polymyxa* HY96-2 and Further Analysis of Its Biocontrol Mechanism. **Frontiers in Microbiology**, v. 9:1520, 2018. DOI https://doi.org/10.3389/fmicb.2018.01520.

MULUNDA, M.; DZOMA, B.; NYIRENDA, M.; BAKUNZI, F. Mycotoxins occurrence in selected staple food in main markets from Lubumbashi, Democratic Republic of Congo. **Journal of Food, Agriculture and Environment**, v. 11(3), p.51-54, 2013. https://www.academia.edu/23508823/Mycotoxins_occurrence_in_selected_staple_food_in_main_markets_from_Lubumbashi_Democratic_Republic_of_Congo.

MUNKVOLD, G. P. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. **European Journal of Plant Pathology,** v. 109, p. 705-713, 2003. DOI https://doi.org/10.1023/A:1026078324268.

MUNKVOLD, G. P.; DESJARDINS, A. E. Fumonisins in maize can we reduce their occurrence? **Plant Disease**, v. 81, n. 6, p. 556-565, 1997. DOI https://doi.org/10.1094/PDIS.1997.81.6.556.

NGUYEN, P. A.; STRUB, C.; DURAND, N.; ALTER, P.; FONTANA, A. SCHORR-GALINDO, S. Biocontrol of *Fusarium verticillioides* using organic amendments and their actinomycete isolates. **Biological control**, v. 118, p. 55-66, 2017. DOI https://doi.org/10.1016/j.biocontrol.2017.12.006.

NIEVIEROWSKI, T. H.; VERAS, F. F.; SILVEIRA, R. D.; GIOCASTRO, B.; ALOISI, I.; TRANCHIDA, P. Q.; DUGO, P.; BRANDELLI, A.; ZINI, C. A.; WELKE, J. E. A *Bacillus*-based biofungicide agent prevents ochratoxins occurrence in grapes and impacts the volatile profile throughout the Chardonnay winemaking stages. **International Journal of Food Microbiology**, v. 389:110107, 2023. DOI http://doi.org/10.1016/j.ijfoodmicro.2023.110107.

NOSANCHUK, J. D.; CASADEVALL, A. The contribution of melanin to microbial pathogenesis. **Cellular Microbiology**, 5(4), p. 203-223, 2003. DOI https://doi.org/10.1046/j.1462-5814.2003.00268.x.

O'BRIEN, P. A. Biological control of plant diseases. **Australasian Plant Pathology**, v. 46, p. 293-304, 2017. https://doi.org/10.1007/s13313-017-0481-4.

PADDA, K. P.; PURI, A.; CHANWAY, C. P. *Paenibacillus polymyxa*: A Prominent Biofertilizer and Biocontrol Agent for Sustainable Agriculture. In: MEENA, V.; MISHRA, P.; BISHT, J.; PATTANAYAK, A. (eds). Agriculturally Important Microbes for Sustainable Agriculture. **Springer,** Singapore, 2017. p. 165-191. https://doi.org/10.1007/978-981-10-5343-6_6.

PARREIRA, D. F.; ZAMBOLIM, L.; COSTA, R. V. da; SILVA, D. D. da; MARCONDES, M. M.; LANZA, F. E.; NEVES, W. S.; FIGUEIREDO, J. E. F.; SOUZA, A. G. C.; COTA, L. V. A method for *Colletotrichum graminicola* inoculation in maize stalks. **Revista Brasileira de Milho e Sorgo**, v.15, n.1, p. 53-64, 2016. DOI https://doi.org/10.18512/1980-6477/rbms.v15n1p53-64.

PFORDT, A.; ROMERO, L. R.; SCHIWEK, S.; KARLOVSKY, P.; von TIEDEMANN, A. Impact of environmental conditions and agronomic practices on the prevalence of *Fusarium* species associated with ear- and stalk rot in maize. **Pathogens**, v. 9(3), 236, 2020. DOI https://doi.org/10.3390/pathogens9030236.

PITT, J. Mycotoxins:
Fumonisins. **Encyclopedia of Food Safety**, v. 2, p. 299-303, 2014. DOI https://doi.org/10.1016/B978-0-12-378612-8.00192-X.

PRABHUKARTHIKEYAN, R.; SARAVANAKUMAR, D.; RAGUCHANDER, T. Combination of endophytic *Bacillus* and *Beauveria* for the management of Fusarium wilt and fruit borer in tomato. **Pest Management Science,** 70(11), 1742-1750. (2014). DOI https://doi.org/10.1002/ps.3719.

PRAEG, N., ILLMER, P. Microbial community composition in the rhizosphere of Larix decidua under different light regimes with additional focus on methane cycling microorganisms. **Sci Rep** 10, 22324. 2020. DOI https://doi.org/10.1038/s41598-020-79143-y.

RAHMAN, S. F. S. AB.; SINGH, E.; PIETERSE, C. M. J.; SCHENK, P. M. Emerging microbial biocontrol strategies for Plant Pathogens. **Plant Science**, v. 267, p. 102-111, 2018. DOI https://doi.org/10.1016/j.plantsci.2017.11.012.

REIS, E. M.; CASA, R. T. Manual de identificação e controle de doenças de milho. Passo Fundo: Aldeia Norte, 1996. 80 p.

ROSSOUW, J. D.; PRETORIUS, Z. A.; SILVA, H. D.; LAMKEY, K. R. Breeding for resistance to *Stenocarpella* ear rot in maize. **Plant Breeding Reviews**, v. 31, p. 223-248, 2009. DOI https://doi.org/10.1002/9780470593783.ch4.

SABATO, E.; FERNANDES, F. T. Doenças do Milho (Zea mays L.) Sociedade Brasileira de Fitopatologia (SBF). 2014. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/107246/1/Doencas-do-milho.pdf.

SAGAHÓN, I. P.; REYES, M. A. A.; ROJAS, H. V. S.; CUENCA, A. A.; JURADO, A. T.; ALVAREZ, I. O. C.; FLORES, Y. M. Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella Maydis* and *Stenocarpella macrospora*. **International Journal of Molecular Sciences**, v.12, n.9, p. 5522-5537, 2011.DOI: https://10.3390/ijms12095522

SAHARAN, B. S.; NEHRA, V. Plant growth promoting rhizobacteria: a critical review. **Life Sciences and Medicine Research**, v. 2011:LSMR-21, p. 1-30, 2011.

SHURTLEFF, M. C. C. Compendium of maize diseases. St. Paul: American Phytopathological society. 1992. 105 p.

SILVA, D. D.; COSTA, R. V.; COTA, L. V.; LANZA, F. E.; GUIMARÃES, E. A. **Micotoxinas em Cadeias Produtivas do Milho: Riscos à Saúde Animal e Humana**. Sete Lagoas: Embrapa-CNPMS, 27p. (Documentos, 193), 2015. ISSN 1518-4277

SILVA, J. J.; VIARO, H. P.; FERRANTI, L. S.; OLIVEIRA, A. L. M.; FERREIRA, J. M.; RUAS, C. F.; ONO, E. Y. S.; FUNGARO, M. H. P. Genetic structure of *Fusarium verticillioides* populations and occurrence of fumonisins in maize grown in Southern Brazil. **Crop Protection**, v. 99, p.160-167, 2017. DOI: https://doi.org/10.1016/j.cropro.2017.05.020

SINGH, S. K.; NENE, Y. L.; REDDY, M. V. Influence of cropping systems on *Macrophomina phaseolina* populations in soil. **Plant Disease**, v. 74, n. 10, p. 812-814, 1990. DOI https://doi.org/10.1094/PD-74-0812.

TSOTETSI, T.; NEPHALI, L.; MALEBE, M.; TUGIZIMANA, F. *Bacillus* for plant growth promotion and stress resilience: What have we

learned? **Plants**, v.11, n. 19, 2482, 2022. DOI https://doi.org/10.3390/plants11192482.

TURNER, S.; PRYER, K. M.; MIAO, V. P. W.; PALMER, J. D. 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. **Journal of Eukaryotic Microbiology** 46: 327–338.

VURUKONDA, S. S. K. P.; GIOVANARDI, D.; STEFANI, E. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. **International Journal of Molecular Sciences**, 19(4):952, 2018. DOI: https://doi.org/10.3390/ijms19040952.

WILSON, M.; Backman, P. A. Biological control of plant pathogens. In: J.R. Ruberson (eds), Handbook of Pest Management. Marcel Dekker Publishers, New York. pp. 125-139, 1999.

WHITE, D. G. Compedium of corn diseases. 3. ed. St. Paul: **The American Phytopathological Society.**1999. 78 p.

WORDELL FILHO, J.; CASA, R. T. Doenças na cultura do milho. A cultura do milho em Santa Catarina, Florianópolis: **Epagri**. 2010. p.480.

WU, L.; WU, H-J.; QIAO, J.; GAO, X.; BORRISS, R. Novel routes for improving biocontrol activity of *Bacillus* based bioinoculants. **Frontiers in Microbiology**, v. 6:1395, 2015. DOI: https://doi.org/10.3389/fmicb.2015.01395.

ZEHRA, A.; RAYTEKAR, N. A.; MEENA, M.; SWAPNIL, P. Efficiency of microbial bio-agents as elicitors in plant defense mechanism under biotic stress: A review. **Current Research in Microbial Sciences.** vol. 2, 100054, 2022. DOI https://doi.org/10.1016/j.crmicr.2021.100054.

ZHOU, B.; YE, L.; WANG, J. Y.; LIU, X. F.; GUAN, Q.; DOU, N. X.; LI, J.; ZHANG, Q.; GAO, Y. M.; WANG, M.; LI, J. S. Nematicidal activity of volatile organic compounds produced by *Bacillus altitudinis* AMCC 1040 against *Meloidogyne incognita*. **Archives of Microbiology**, v. 204, n. 521, 2022. DOI: https://doi.org/10.21203/rs.3.rs-1500056/v1.