Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Orginal Article

Microorganisms from corn stigma with biocontrol potential of *Fusarium verticillioides*

Microrganismos do estigma do milho com potencial de biocontrole de *Fusarium verticillioides*

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Abstract

The mycotoxigenic fungus *Fusarium verticillioides* is the primary maize pathogen and causes the maize stalk and ear rot diseases with significant economic losses. Furthermore, the excessive use of fungicides to control *F. verticillioides* constitutes threats to the environment and human health. Thus, sustainable alternatives such as biological control are needed to minimize the hazards associated with the current method. Although much is known about the vulnerability of the maize silks as a gateway for several fungal pathogens invading the developing grains, studies on the chemical properties of silk extracts and their resident microbiota are scarce. This study isolated and characterized bacteria and fungi that colonize the maize stigma to assess new potential biocontrol agents. The samples were collected from maize fields in the Brazilian localities of Sete Lagoas-MG, Sidrolândia-MS, Sertaneja-PR, and Goiânia-GO. One hundred sixty-seven microorganisms were isolated, 46% endophytic and 54% epiphytic. First, the antagonist activity was evaluated by the agar disc diffusion method performed in triplicate, and 83% of the isolates showed antagonist activity against *F. verticillioides*. Then, the 42 most efficient isolates were identified based on the partial sequencing of the bacterial 16S rRNA gene and fungi ITS region. The bacteria belong to the genera *Bacillus* (57.1%), *Burkholderia* (23.8%), *Achromobacter* (7.1%), *Pseudomonas* (2.4%), and *Serratia* (2.4%), while the fungi are *Penicillium* (2.4%), *Candida* (2.4), and *Aspergillus* (2.4%). The results showed that microorganisms from maize stigma might represent new promising agents for *F. verticillioides* control.

Keywords: microorganisms, antagonists, *Fusarium moniliforme*, biofungicide, phytopathogen, maize.

Resumo

O fungo micotoxigênico *Fusarium verticillioides* é o principal patógeno do milho e causa doenças do colmo e da podridão da espiga com perdas econômicas significativas. Além disso, o uso excessivo de fungicidas no controle de *F. verticillioides* constitui uma ameaça ao meio ambiente e à saúde humana. Assim, alternativas sustentáveis, como o controle biológico, são necessárias para minimizar os riscos associados ao método atual. Este estudo isolou e caracterizou bactérias e fungos que colonizam o estigma do milho para avaliar novos agentes de biocontrole em potencial. As amostras foram coletadas em campos de milho nas localidades brasileiras de Sete Lagoas-MG, Sidrolândia-MS, Sertaneja-PR e Goiânia-GO. Cento e sessenta e sete microrganismos foram isolados, 46% endofíticos e 54% epifíticos. O teste de antagonismo empregando a técnica de disco de difusão em meio sólido, mostrou que 83% dos isolados apresentaram atividade antagonista contra *F. verticillioides*. Em seguida, 42 isolados mais eficientes foram identificados a partir do sequenciamento parcial do gene 16S rRNA bacteriano e da região ITS de fungos. Os isolados bacterianos pertencem ao gênero *Bacillus* (57,1%), *Burkholderia* (23,8%), *Achromobacter* (7,1%), *Pseudomonas* (2,4%) e *Serratia* (2,4%), enquanto os fungos são *Penicillium* (2,4%), *Candida* (2.4), e *Aspergillus* (2,4%). Os resultados mostraram que microrganismos do estigma do milho podem representar novos agentes promissores para o controle de *F. verticillioides*.

Palavras-chave: microrganismos, antagonistas, *Fusarium moniliforme*, biofungicida, fitopatógeno, milho.

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Received: March 29, 2022 – Accepted: August 1, 2022

1. Introduction

Fusarium verticillioides (Sacc.) Nirenberg (sinônimo, *Fusarium moniliforme*, Sheldon) is considered one of the primary pathogens of maize, being found both in temperate and humid tropical to subtropical climate regions (Gomes et al., 2020). This pathogen can cause disease symptoms at all stages of a plant's development, causing seed losses, seedling death, stem rot, root and ear rot, and damages to stored grains (Samsudin and Magan, 2016). In addition, *F. verticillioides* produces fumonisins, mainly fumonisin B1, which can cause various health problems for humans and animals (Blacutt et al., 2018). *Fusarium verticillioides* can infect maize through the seeds, plant wounds, or stigma (Munkvold et al., 1997).

In maize, the style is called silk to refer to the female maize flowers (Rosli et al., 2008). Therefore, the silk emerging at the tips of corn cobs is critically important, not only for seed establishment but also because it is the gateway of entrance of carcinogenic mycotoxin-producing fungal pathogens that devastate millions of maize farmers (Johnson et al., 2007; Thompson and Raizada, 2018). Thus, the maize stigma has been described as the most efficient form of plant contamination by fungal pathogens invading the developing grain using the silk channels and causing disruption of the seeds and severe ear rot (Warfield and Davis, 1996; Khalaf et al., 2021).

The maize silk is an herb used traditionally as a therapeutic remedy to treat various diseases in many parts of the world (Alam, 2011; Hasanudin et al., 2012). The potential antioxidant and healthcare applications of maize silk are due to the properties of its bioactive constituents, such as flavonoids and terpenoids. In addition, many microorganisms and natural compounds derived from plants have fungistatic or fungicidal effects and can be used to manage plant diseases (Dias et al., 2020; Ahmadu et al., 2021; Matrose et al., 2021). However, despite the importance of maize silks to both reproduction and global grain disease, there are few studies in the literature about the effect of maize silk extracts and their microbiome for controlling plant diseases (Khalaf et al., 2021). Alam (2011), evaluating the antioxidant and antibacterial activities of ethanolic extract of Egyptian Maydis stigma against human pathogenic bacterial species, found an antibacterial activity of ethanolic extract of both upper and lower parts of maize silk. Also, Feng et al. (2012) reported that the antimicrobial activity of ethanolic extract of maize silk could inhibit the microbial activity of several human pathogenic species of bacteria and fungi.

Recent data from scientific literature highlight the potential of bacteria and fungi isolated from maize silks as biocontrol agents for plant pathogens. In the first report on maize silk-associated microbial communities, Diniz et al. (2017) found that the antagonistic activity of 174 bacteria and fungi against *F. verticillioides* was highly variable with 20% to up 60% activity. Fifty seven isolates reduced the radial growth of *Fusarium verticillioides* between 40% and 60%, and twelve isolates stood out for antagonistic activity by reducing above 60% the growth of the pathogen.

In another study, Diniz et al. (2021) tested four endophytic and epiphytic bacteria from maize silks in vitro and greenhouse against *F. verticillioides* and hydrolytic enzyme production. The authors found that all strains showed antifungal activity in vitro with inhibition values from 58.5 to 100%; they changed hyphae morphology and inhibited conidial germination up to 100% (strain IPR45) by producing at least one enzyme with antifungal activity. In addition, the microbiolized seeds reduced the fungal development in stored grains and stalk rot severity in the greenhouse by approximately 73% (strain ISD04).

Khalaf et al. (2021), applying MiSeq 16S rRNA gene sequencing of 328 open-pollinated silk samples (healthy/fungi-infected), reported that maize silks possess complex and dynamic microbiomes with more than 5,000 taxa spanning the prokaryotic tree of life (47 phyla/1,300 genera).

In tropical Africa and Latin America, silk-invaders include the mycotoxin-producing fungal pathogens *Aspergillus flavus* and *Fusarium verticillioides* that devastate tens of millions of maize farmers, where they produce carcinogens (Patriarca and Pinto, 2017; Thompson and Raizada, 2018). These fungi produce mycotoxins with toxic effects on gastrointestinal, hematic, and reproductive systems, affecting livestock and human health (Patriarca and Pinto, 2017). Altered temperature, rainfall, and humidity associated with climate change are expected to increase the fungus-based mycotoxins in maize (Magan and Medina, 2016).

Alternative control methods of disease control for minimizing the harmful effects of chemical fungicides and increasing food production and quality have been studied and tested since the early twentieth century (Baker, 1987; Adetunji et al., 2019; Ons et al., 2020; Awuchi et al., 2021). Among the methods, the use of microorganisms with potential to control plant pathogens has been increasingly recognized as a promising environmentally friendly and low-cost approach compared to conventional methods (van Lenteren et al., 2018). Thus, biological control using antagonistic microorganisms may be a powerful alternative to inhibit the development of phytopathogens in different cultures (Tariq et al., 2020). Furthermore, the isolation and characterization of native microorganisms increase the efficiency of the biological control since they have a great capacity to compete and survive in their original environment (Figueroa-López et al., 2016). Therefore, in this work, native endophytic and epiphytic microorganisms were isolated from the maize stigma collected from different locations aiming to obtain greater diversity and efficiency for *F. verticillioides* control. The direct isolation of microorganisms from the maize stigma, the main entry points for *F. verticillioides* into the plants, was carried out for the first time and may contribute to providing information about the microbial diversity in this organ of the maize plant and lead to the discovery of new species with biotechnological potential.

2. Material and Methods

2.1. Sample collection and isolation of microorganisms

Stigma of maize plant (*Zea mays*) cultivated in the cities of Sete Lagoas-MG (Embrapa Maize and Sorghum,

experimental plots), Sidrolândia-MS (field maize), Goiânia-GO (Embrapa Rice and bean, experimental plots) and Sertaneja-PR (field maize) in the 2016 harvest were randomly collected.

Endophytic microorganisms candidates for biocontrol were isolated from corn stigmas washed and disinfected as Bulgari et al. (2009). Then, they were macerated with the aid of a pistil and suspended in sterile saline solution (0.9%) to obtain dilutions (10^{-4} to 10^{-6}) that were plated on Potato Dextrose Agar (BDA) medium. In half of the plates, a streptomycin solution and the other half of cycloheximide (20mg/L) were added to select fungi and bacteria, respectively. Epiphytic microorganisms were isolated using the same technique but without initial disinfestation of stigmas. The plates were incubated for seven days at 28 °C and visualized every 48 h for the isolation of microorganisms based on morphological differences such as colony shape and appearance, size, color, border type, and growth rate. The 167 isolated morphotypes were preserved in glycerol (20%) and stored at -4 °C (bacteria) and eight 8 °C (fungi) for further studies.

2.2. Selection of biocontrol agents against Fusarium verticillioides

One hundred and sixty-seven isolates were tested for their potential to inhibit the growth of *F. verticillioides* by the confrontation test of cultures in a solid medium. The *F. verticillioides* isolate (CML2743) used in the antagonism tests was obtained from the Phytopathology Laboratory of Embrapa Milho e Sorghum in Sete Lagoas-MG.

A 5 mm disk from the edge of the phytopathogen culture grown in PDA medium for five days at 28°C was removed and transferred to the center of a new plate containing the same culture medium. A 5mm mycelial disk of each fungal isolate or 10 µL of bacterial suspension (10^8 CFU/mL) was added at four equidistant points. Incubation was carried out at 28°C and 12 h photoperiod. In the negative control, measurements were performed after the entire growth of the pathogen in the plate.

Percent inhibition was calculated by PI= [(radius of mycelium in control - radius of mycelium with antagonist) / radius of mycelium in control)] × 100.

The analysis of variance (ANOVA) was used to check the obtained data, followed by the Scott-Knott means comparison test at $p < 0.05$. All treatments were performed in triplicate and the results were expressed as mean of the repetitions.

2.3. Molecular identification of the most efficient isolates

Forty-two isolates that showed greater antagonist activities were identified through partial sequencing of the 16S rRNA gene and the ITS region. DNA extraction from the isolated bacteria and fungi was performed using the Wizard® Genomic DNA Purification Kit (Promega, USA) and the UltraClean™ Microbial DNA Isolation Kit (MoBio, USA) respectively. PCR amplification of the 16S rRNA region of bacterial isolates was performed with 8F primers: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-GGTTACCTTGTTACGACTT-3 (Turner et al., 1999). For the ITS region of the isolated fungi, the primers ITS4R:5'-TCCTCCGCTTATTGATATGC-3' and ITS5F: 5'-GGAAGTAAAAGTCGTAACAAGG-3' were used (White et al., 1990). Products were analyzed by 1.0% agarose gel electrophoresis and documented using a Gel Logic 200 system (KODAK Company, USA). The PCR products were sequenced on the ABI PRISM 3500xL Genetic Analyzer sequencer (Applied Biosystem, USA) and the sequences obtained aligned with the Sequencer 4.1.4 program (Genes Codes Corporation). To identify the isolates, the alignment search tool (BLAST) was used to find similarities with sequences deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/).

3. Results

3.1. Isolation and selection of biocontrol agents against Fusarium verticillioides

Based on their morphological characteristics, 167 microorganisms were isolated from maize stigmas collected in different producing regions of Brazil, being 46 isolated from the cities of Goiânia, 43 from Sidrolândia, 42 from Sertaneja, and 36 from Sete Lagoas. Endophytic and epiphytic microorganisms represented 46% and 54% of the total isolates, respectively.

Variations in the inhibition capacity of the isolates were observed according to the region of each microorganism (Figure 1). The most significant number of isolates that inhibited the growth of *F. verticillioides* (0 and 20%) were collected in Sete Lagoas. The inhibition index between 20.01 and 40% was obtained mainly by the isolates from Goiânia-GO. The isolates from Sidrolândia-MS showed the highest inhibition potential among all the microorganisms evaluated. In this region, the most significant number of microorganisms showed inhibition potential ranging from 40.01 to 60%, and it was the only region that contained an isolate that inhibited 100% of the phytopathogen growth (ISD04). The development of new, sustainable technologies based on the search aims for genetic resistance of plants, chemical substances, and biological control to reduce environmental and health problems generated by fungicides in agriculture. In this work, the search for microorganisms

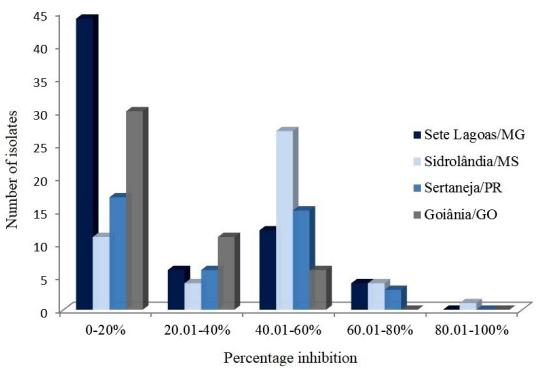

Figure 1. Percentage of mycelial growth inhibition of *Fusarium verticillioides* by endophytic and epiphytic microorganisms from maize silks collected in different Brazilian regions.

from different regions aimed to obtain a more significant number of isolates that could have potential as biocontrol agents. The antagonist agent selection test showed that in all four regions, microorganisms were found that showed great potential for inhibiting *F. verticillioides*.

All isolates were tested for antagonist activity by measuring the growth of *F. verticillioides* in the direct challenge test. Of these, 28 showed no difference in growth reduction compared to the control (Group A, Table 1). Another 139 isolates that significantly reduced the growth of *F. verticillioides* were separated into fifteen groups according to the statistical test concerning the percentage of inhibition. The most significant number of isolates (group K) showed an inhibition potential that ranged from 48.7 to 52.1% (Table 1).

3.2. Molecular identification of selected isolates

Forty-two isolates with inhibition values of *F. verticillioides* growth above 48.7% were identified based on partial sequencing of the 16S rRNA bacterial gene and ITS region of fungi. The molecular identification showed that these isolates belong to eight different genera: *Bacillus* sp. (57.1%), *Burkholderia* sp. (23.8%), *Achromobacter* sp. (7.1%), *Pseudomonas* sp. (2.4%), *Serratia* sp. (2.4%), *Penicillium* sp. (2.4%), *Candida* sp. (2.4%) and *Aspergillus* sp. (2.4%).

The most efficient bacteria were *Bacillus subtilis, Bacillus velezensis, Burkholderia cepacia, Pseudomonas aeruginosa, Serratia nematodiphila, Achromobacter insuavis, Achromobacter xylosoxidans, and Burkholderia gladioli*. The fungi that stood out in the control of *F. verticillioides* in vitro were *Aspergillus flavus* (IPR24) and *Penicillium* sp. (CT01) (Table 2).

4. Discussion

Screening is a critical step in biological control, as the success of all subsequent steps depends on the ability of a procedure to identify candidates with antagonist potential (Cavaglieri et al., 2004). In this work, the in vitro confrontation test was appropriate to evaluate many antagonistic microorganisms faster at a low cost. Of all tested isolates, 83% showed inhibitory activity against *F. verticillioides*. The microorganisms were isolated from various Brazilian maize-producing areas to obtain isolates to screen their potential as biocontrol agents against *Fusarium verticillioides*.

Considering that the maize silk is one of the main entry points of *F. verticillioides* into the plant (Munkvold et al., 1997), isolating native microorganisms from the maize silk was the main criteria used in this work. Due to the isolates' adaptation, they are presumably more suitable for biological control of *F. verticillioides* entering thorough maize stigma. Furthermore, adopting this strategy may allow the identification of microorganisms coexisting with the natural microbiota. Thus, they may present more competitive advantages than introduced exotic species (Figueroa-López et al., 2016). The tests of antagonistic activity against *F. verticillioides* showed that the isolates from the four regions showed great potential for inhibiting *F. verticillioides*. In addition, these microorganisms, more adapted to their original site, may have a more remarkable ability to survive under field conditions and colonize the maize plants, which may increase the chances of successful biological control. Despite the widespread use of maize silk in traditional medicine and the knowledge of its role as one of the main entries of diseases in maize plants, studies on its chemical constituents and microbiota are scarce (Alam, 2011; Hasanudin et al., 2012; Žilić et al., 2016; Thompson and Raizada, 2018).

The maize silk is an herb used traditionally as a therapeutic remedy to treat various diseases in many parts of the world (Alam, 2011; Hasanudin et al., 2012). Furthermore, the maize stigma has been described as the most efficient form of plant contamination by fungal pathogens invading the developing grain using the silk channels and causing disruption of the seeds and severe ear rot (Warfield and Davis, 1996; Khalaf et al., 2021). Thus, despite the widespread use of maize silk in traditional medicine and the knowledge of its role as one of the main entries of diseases in maize plants, studies on its chemical constituents and microbiota are scarce recent (Alam, 2011; Hasanudin et al., 2012; Žilić et al., 2016; Thompson and Raizada, 2018). The potential antioxidant and healthcare applications of maize silk are due to the properties of its bioactive constituents, such as flavonoids and terpenoids

Table 1. Groupings of isolates concerning the percentage of inhibition of *F. verticillioides* growth in a direct challenge test.

Group	Number of isolates	PI*	Group	Number of isolates	PI*
A	28	0-1.7	I	7	35.8-39.3
B	20	2.5-7.6	J	20	43.5-48.3
C	9	8.5-10.2	K	30	48.7-52.1
D	11	11.6-16.6	L	4	53.2-57.1
E	10	17.0-21.3	M	5	58.4-62.5
F	8	22.0-25.0	N	1	71.6
G	5	26.4-28.5	O	1	75.8
H	7	30.7-35.0	P	1	100.0

*Percent inhibition (PI) values were obtained from measuring the radius of *F. verticillioides* colonies compared with the control. The groups formed by different letters are significantly different based on the Scott-Knott test with $p<0.05$.

Table 2. Isolates, collection site, type (lifestyle), percentage of inhibition, molecular identification and Genbank accession number of the 42 isolates that showed inhibition of the growth of *Fusarium verticillioides* ≥ 48.7%.

Isolate	Collection place	Type	Inhibition (%)	Identification	Genbank accession number
ISD04	Sidrolândia	Endophytic	100	*Achromobacter xylosoxians*	MK461853
IPR24	Sertaneja	Epiphytic	75	*Aspergillus flavus*	MK461562
CT01	Sete Lagoas	Epiphytic	71	*Penicillium* sp.	MK461563
CT02	Sete Lagoas	Epiphytic	62	*Bacillus velezensis*	MK461847
IPR45	Sertaneja	Endophytic	61	*Pseudomonas aeruginosa*	MK461572
IPR23	Sertaneja	Endophytic	60	*Achromobacter xylosoxians*	MK461842
IM14	Sete Lagoas	Epiphytic	58	*Bacillus velezensis*	MK461831
IPR06	Sertaneja	Endophytic	58	*Bacillus velezensis*	MK461833
2080	Sete Lagoas	Epiphytic	57	*Bacillus subtilis*	MK461830
IGN23	Goiânia	Endophytic	55	*Bacillus velezensis*	MK461855
1919	Sete Lagoas	Epiphytic	53	*Burkholderia cepacia*	MK461832
IGN01	Goiânia	Endophytic	53	*Bacillus velezensis*	MK461844
IGN36	Goiânia	Epiphytic	52	*Candida intermedia*	MK461561
IGN14	Goiânia	Endophytic	51	*Bacillus velezensis*	MK461867
IPR09	Sertaneja	Epiphytic	50	*Bacillus velezensis*	MK461835
ISD23	Sidrolândia	Endophytic	50	*Bacillus velezensis*	MK461837
CT04	Sete Lagoas	Epiphytic	50	*Bacillus velezensis*	MK461849
CT03	Sete Lagoas	Epiphytic	50	*Bacillus velezensis*	MK461850
IM25	Sete Lagoas	Epiphytic	50	*Bacillus velezensis*	MK461851
ISD03	Sidrolândia	Epiphytic	50	*Bacillus velezensis*	MK461856
ISD01	Sidrolândia	Endophytic	50	*Bacillus velezensis*	MK461860
ISD41	Sidrolândia	Endophytic	50	*Bacillus velezensis*	MK461854
CT05	Sete Lagoas	Endophytic	50	*Bacillus velezensis*	MK461845
ISD36	Sidrolândia	Epiphytic	50	*Bacillus velezensis*	MK461846
IM45	Sete Lagoas	Endophytic	50	*Serratia marcescens*	MK461848
ISD31	Sidrolândia	Epiphytic	50	*Burkholderia gladioli*	MK461861
ISD12	Sidrolândia	Epiphytic	50	*Burkholderia gladioli*	MK461862
ISD40	Sidrolândia	Endophytic	50	*Burkholderia gladioli*	MK461863
ISD34	Sidrolândia	Endophytic	50	*Burkholderia gladioli*	MK461864
ISD46	Sidrolândia	Epiphytic	50	*Burkholderia gladioli*	MK461866
ISD15	Sidrolândia	Endophytic	50	*Burkholderia gladioli*	MK461859
IPR16	Sertaneja	Epiphytic	50	*Burkholderia gladioli*	MK461841
ISD11	Sidrolândia	Epiphytic	50	*Burkholderia gladioli*	MK461865
ISD49	Sidrolândia	Endophytic	49	*Achromobacter xylosoxians*	MK461857
ISD05	Sidrolândia	Endophytic	49	*Bacillus velezensis*	MK461858
ISD07	Sidrolândia	Endophytic	49	*Bacillus velezensis*	MK461843
ISD18	Sidrolândia	Epiphytic	49	*Bacillus velezensis*	MK461852
IPR04	Sertaneja	Epiphytic	48	*Burkholderia gladioli*	MK461834
IPR05	Sertaneja	Epiphytic	48	*Bacillus subtilis*	MK461836
IPR03	Sertaneja	Epiphytic	48	*Bacillus velezensis*	MK461838
IPR41	Sertaneja	Endophytic	48	*Bacillus velezensis*	MK461839
IPR01	Sertaneja	Endophytic	48	*Bacillus velezensis*	MK461840

(Alam, 2011). In addition, many microorganisms and natural compounds derived from plants have fungistatic or fungicidal effects and can be used to manage plant diseases (Dias et al., 2020; Ahmadu et al., 2021; Matrose et al., 2021). Feng et al. (2012) reported that the ethanolic extract of maize silk could inhibit the microbial activity of many human pathogenic bacteria and fungi.

In the first report on maize silk-associated microbial communities, Diniz et al. (2017) found that the antagonistic activity of 174 bacteria and fungi against *F. verticillioides* was highly variable with 20% to up 60% activity.

The isolates from Sidrolândia-MS showed the highest inhibition potential among all the microorganisms evaluated. Probably, this find may be related to the wetter climate of this region, which is more favorable to the development of *Fusarium*. Also, this result may be due to higher disease pressure since the area in which the isolates were collected correspond to a large commercial farming area (Sidrolândia) and not a restricted experimental area, as in SL and Goiânia. However, these factors need to be investigated. Nevertheless, this result revealed that sampling at different sites is more efficient than sampling at a single site when searching for more antagonistic microorganisms in the control of *Fusarium verticillioides*.

In another study, Diniz et al. (2021), analyzing the in vitro antifungal activity of four endophytic (ISD04 and IPR45) and epiphytic (CT02 and IM14) bacterial strains from maize silks, found inhibition values from 58.5 to 100%. In addition, the four isolates changed the hyphae morphology and inhibited the conidial germination by up to 100% (strain IPR45). Also, the four strains produced at least one enzyme with antifungal activity, and the microbiolized seeds reduced the fungal development in stored grains and stalk rot severity in the greenhouse by 72.6% (strain ISD04). Recently, Khalaf et al. (2021), applying MiSeq 16S rRNA gene sequencing of 328 open-pollinated silk samples (healthy/fungi-infected), reported that maize silks possess complex and dynamic microbiomes with more than 5,000 taxa spanning the prokaryotic tree of life (47 phyla/1,300 genera). These results highlight the high complexity and the antifungal activity potential of silk-associated microbial communities as new biocontrol agents against *F. verticillioides* entering the maize silks.

Endophytic microorganisms live, at least part of their life cycle, within plant tissues, such as leaves, stems (Johnston-Monje and Raizada, 2011), and mainly roots (Miliute et al., 2015). However, microorganisms can enter the plant tissue through emergence sites of lateral roots, lesions, natural openings such as stomata, and germinating radicles. Endophytic microorganisms are asymptomatic and, unlike pathogenic microorganisms, do not cause damage to the host plant. Furthermore, they can protect plants against phytopathogens by producing antimicrobial compounds, competition for space and nutrients, and induction of host plant resistance (Souza, 2001).

The great diversity of endophytic and epiphytic microorganisms in the maize silks (Khalaf et al., 2021) and the high silk vulnerability as one of the main gateway for *Fusarium verticillioides* entering into the maize plants and colonizing the grains (Thompson and Raizada, 2018) point out the existence of intense niche competition for space and food between species (Senghor et al., 2020). Thus, it is reasonable to presume the occurrence of potential *Fusarium* antagonists among the maize-friendly microorganisms.

The molecular identification of the isolates revealed that both endophytic and epiphytic *Bacillus* spp. were found in more significant numbers. The prevalence of the genus *Bacillus* in different environments can be explained by the ability of this genus to form endospores that give them advantages over other microorganisms, such as greater tolerance to temperature fluctuations and nutrient shortages, among other environmental stresses (Sonenshein et al., 2002). In addition, *Bacillus* isolates stood out as *F. verticillioides* control agents. This result agrees with other works that have shown the potential of this genus in the control of different fungal phytopathogens, including *Fusarium* (Figueroa-López et al. 2016; Raaijmakers and Mazzola, 2012).

In a study by Passari et al. (2018), *Bacillus* was also the dominant genus among rhizosphere isolates with the potential to inhibit mainly *Fusarium* species. This genus represented 56.2% of the total of isolates, followed by *Staphylococcus* (15.6%), *Pseudomonas* (12.5%), *Sphingomonas* (9.3%), and *Achromobacter* (3.1%). The prevalence of *Bacillus* species was also found in the maize rhizosphere by Figueroa-López et al. (2016), looking for native microorganisms with the potential to control *F. verticillioides*. These authors identified as the primary bacterial genera *Bacillus* (341 isolated), *Enterobacter* (38), *Pseudomonas* (23), and *Lysinibacillus* (13), which exhibited percentage of inhibition ranging from 53 to 99%.

In the present study *Bacillus* represented 56.2% of the total of isolates, followed by *Staphylococcus* (15.6%), *Pseudomonas* (12.5%), *Sphingomonas* (9.3%), and *Achromobacter* (3.1%). The high prevalence of *Bacillus* species (82.17%) with the potential to control *F. verticillioides* was also found in the maize rhizosphere by Figueroa-López et al. (2016). The *Bacillus* species in this work have been described as biocontrol agents against different phytopathogens of important crops. *Bacillus subtilis* and *Bacillus velezensis*, are two closely related species (Dunlap et al., 2016). Indeed, recently many *B. subtilis* was reclassified as *B. velezensis* based in phylogenetic analysis (Palazzini et al., 2016). These authors also demonstrated the antagonist activity of *B. velezensis* against *Fusarium graminearum*, by reducing disease severity and decreasing mycotoxin accumulation under field conditions. The antifungal activity was attributed to antimicrobial lipopeptides such as surfactin, phengycin, and iturine, produced by *B. velezensis* (Palazzini et al., 2016). Martínez-Raudales et al. (2017), analyzing the genome of *B. velezensis*, found several putative genes involved in the biosynthesis of antifungal compounds with toxic effects on fungi cell growth and morphology that resulted in cell death, and genes related to systemic induced resistance in plants. Chung et al. (2016) demonstrated that species of the genus *Bacillus*, as well as species from *Paenibacillus*, *Serratia*, and *Pseudomonas* produce volatile compounds (e.g., 2,3-butanediol) that elicit the host immune response. These results may explains the characteristics of this species as fungal antagonist agent (Khalaf et al., 2021).

Burkholderia cepacia is also an antagonistic of phytopathogenic fungi, including *Fusarium*. *B. cepacia* produce an antifungal compound (CF66I) that inhibit the growth of *F. solani* (Li et al., 2009). High concentrations of CF66I caused the death of the phytopathogen, and low concentrations changed the hyphae structure, such as swelling and abnormal deposition of chitin, which also inhibited the growth of the fungus. *Pseudomonas aeruginosa*, another species identified in this work, is an efficient biocontrol agent against *F. verticillioides* (Borah et al., 2016). *P. aeruginosa* secret biosurfactant, identified as rhamnolipid, with antifungal activity through damaging the mycelial structure of the fungus. In addition, *P. aeruginosa* supernatants disperse sulfate-reducing bacterium biofilms, by producing four proteins (putative phospholipases) that influence biofilm formation (Wood et al., 2018). Seed treatment with *P. aeruginosa* efficiently suppressed the disease symptoms and controlled stem and ear rot, increased plant biomass, and improved the fruiting of corn plants.

The species *Serratia nematodiphila* can inhibit the growth and aflatoxins levels of *Aspergillus parasiticus* in peanut seeds through chitinase production, which degrades the fungal cell wall (Wang et al., 2013). Thus, in addition to its potential as a biological control agent, this species is a plant growth promoter. In work by Khan et al. (2017), the complete genome of *Serratia nematodiphila* revealed the existence of genes involved in the biosynthesis of indoleacetic acid (IAA), phosphate solubilization, and enzymatic genes to oxidative stress. Likewise, *Burkholderia gladioli* specie is a biological control agent against different phytopathogenic fungi (Elshafie et al., 2012). The antifungal activity of *B. gladioli* is due to extracellular hydrolytic enzymes, such as chitinase, protease, and glucanase, besides diffusible and volatile secondary metabolites that reduced the growth rate of *F. oxysporum* and *Rhizoctonia solani*. *Burkholderia gladioli* were also identified as corn growth promoter due to its activity of phosphorus solubilizer, phytohormone producers such as indoleacetic acid (IAA), gibberellin, and other compounds (Gunjal and Kapadnis, 2013).

Achromobacter xylosoxidans, frequently found in the rhizosphere zone, has been identified as a biocontrol agent of *Fusarium* spp. in tomato plants due to siderophore and chitinase production, and induction of systemic resistance in plants (Vaidya et al., 2001; Moretti et al., 2008).

5. Conclusions

The present study successfully evaluated the antifungal activity of endophytic and epiphytic microbiota associated with maize silks.

The maize silk microbiota is highly variable, highlighting its potential for selecting bacterial antagonists against fungal diseases in maize.

The study highlights the potential of epiphytic and endophytic microorganisms as an effective biocontrol strategy against colonization of maize plants by *F. verticillioides*.

Sustainable alternatives such as biological control are critical to minimize the hazards associated with the current chemical methods for fungal disease control.

Acknowledgements

The authors would like to thank the CNPq and Embrapa Milho e Sorgo for financial support.

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