EVALUATION OF THE INHIBITORY EFFECT OF BACILLUS AMYLOLIQUEFACIENS ON THE GROWTH OF CRYPTOSPORIOPSIS SSP FUNGUS

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ABSTRACT

The global population has been demanding healthier products free from agrochemicals, leading to an increased interest in more biological, self-sustainable, and clean agriculture. In this context, several studies involving microorganisms have been conducted in agroecological crops. Among the most studied microorganisms are the Bacillus genus Rhizobacteria, which have demonstrated significant potential for use as biological control agents. Bacillus amyloliquefaciens, a bacterium known for its ability to produce amylolytic enzymes, is relevant in various industrial sectors. However, its potential for inhibiting fungi has not been fully explored. In this study, a fungus from sugarcane culture was isolated, and a confrontation test was conducted using a strain of Bacillus amyloliquefaciens isolated from the company GI Innovar. The microorganisms were inoculated on potato dextrose agar (PDA) before the antagonism experiment. The antagonistic effect of Bacillus *Amyloliquefaciens* against the fungus *Cryptosporiopsis ssp* was measured on PDA plates. After 7 days of experimentation, it was observed that Bacillus amyloliquefaciens significantly inhibited the growth of the *Cryptosporiopsis spp* fungus when both microorganisms were simultaneously inoculated on PDA plates. This demonstrates that it is a highly promising strain with robust antifungal capabilities against pathogenic fungi.

Keywords: ***A****myloliquefaciens* 1. Bacillus 2. Biológico 3. *Cryptosporiopsis* 4. Fungo 5.

1 INTRODUCTION

Numerous strategies have been developed to combat and control fungal spoilage. These methods mainly cover physical and chemical techniques such as pulsed light treatment, UV-C light-emitting diodes, chlorine dioxide, and chemical fungicide. However, the impact of the excessive use of chemical fungicides on the environment, animals and human health is of concern, making the use of biological control strategies extremely interesting as an ecological alternative that aims to promote environmentally responsible agricultural and food production practices, while effectively mitigating fungal damage (Hu et al., 2024). The extensive use of agrochemicals has also gradually degraded productive soils, reducing the carbon and associated nutrient content and altering the physicochemical quality of the soil, with consequent effects on soil microbial diversity.

Biocontrol strains have witnessed significant advancements in recent years, driven by progress in various scientific and technological fields. B. amyloliquefaciens stands out as a versatile candidate in the ever-evolving domain of biocontrol research. However, there remains a crucial need for a comprehensive mechanistic understanding of their interactions with diverse plant pathogens at the molecular and cellular levels (Hu et al., 2024).

Bacillus species are widely recognized for their substantial biocontrol potential, due to their ability to synthesize a diverse range of antimicrobial compounds and insecticides, which positions them at the forefront of biocontrol strategies (Gong et al., 2014). For example, Bacillus atrophaeus and B. mojavensis exhibit broad-spectrum antifungal efficacy against pathogens such as Verticillium dahlia and Fusarium graminearum (Hu et al., 2024). B. subtilis and B. velezensis have an inhibitory effect against *Fusarium oxysporum* (Gao et al., 2022).

In view of the above, this work aims to evaluate the ability of Bacillus *Amyloliquefaciens* to confront a species of fungi related to agriculture, under controlled laboratory conditions.

2 MATERIAL & METHODS

The microorganisms used in this work were Bacillus *Amyloliquefaciens* isolated by GI Innovar and the fungus *Cryptosporiopsis spp*, isolated from a sugarcane crop in the Triângulo Mineiro region. The preserved ones were inoculated and activated in potato dextrose agar (BDA) medium before the antagonism experiment. The antagonistic effect of Bacillus *Amyloliquefaciens* against the fungus *Cryptosporiopsis ssp* was measured in PDA plates. Five-millimeter buffers of the fungus *Cryptosporiopsis ssp*, taken from an actively growing culture were used and inoculated at the four corners of the plate equidistant from the center, and the culture of the strain of Bacillus *Amyloliquefaciens* was inoculated at the center of the plate. The cultures were kept in an incubator at 26°C to observe the growth and antagonism between the fungus *Cryptosporiopsis* and Bacillus *Amyloliquefaciens.* The antagonistic experiment included three biological replicates, each containing three plates.

3 RESULTS & DISCUSSION

To confirm the antagonistic effect of Bacillus *Amyloliquefaciens* and the fungus *Cryptosporiopsis spp,* a PDA plaque confrontation experiment was performed. In Figure 1, we can observe the inoculation of Bacillus *Amyloliquefaciens* and the fungus *Cryptosporiopsis spp* with 3 days of inoculation, and in Figure 2 only the fungus *Cryptosporiopsis spp* in the same growth period. The results showed that when the fungus *Cryptosporiopsis spp* was inoculated alone, white mycelia covered the entire plaque at 7 days after inoculation, and sclerotia were also observed, indicating their rapid growth rate (Figure 4).

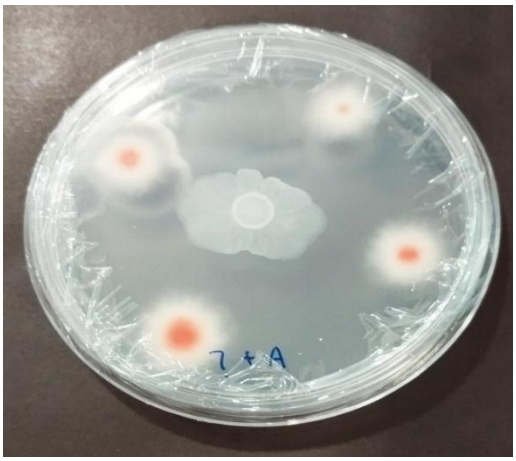

Figure 1: Third day of the confrontation test between Bacillus *Amyloliquefaciens* x *Cryptosporiopsis SPP.*

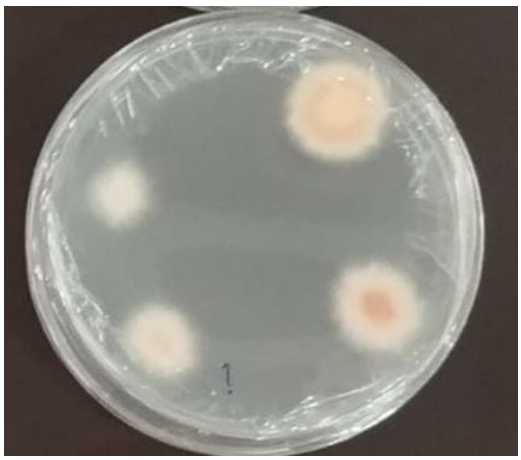

Figure 2: Third day of inoculation of the fungus *Cryptosporiopsis SPP*..

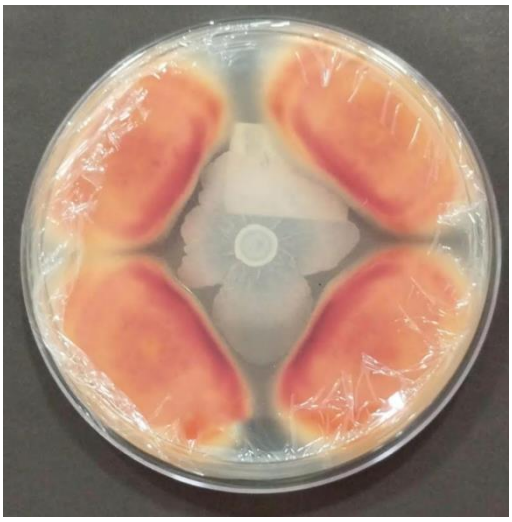

Figure 3: Seventh day of the confrontation test between Bacillus *Amyloliquefaciens* x *Cryptosporiopsis SPP.*

In contrast, when the fungus *Cryptosporiopsis spp* was inoculated around the plate and Bacillus *Amyloliquefaciens* was inoculated in the center of the plate (Figure 3), the results showed that the diameter of the colony of Bacillus *Amyloliquefaciens* cultured for 7 days was significantly larger than that of Bacillus *Amyloliquefaciens* cultured for 3 days, suggesting that Bacillus *Amyloliquefaciens* also grew rapidly. Based on the above observations, we conclude that Bacillus *Amyloliquefaciens* significantly inhibited the growth of the fungus *Cryptosporiopsis spp* when the two microorganisms were inoculated simultaneously into PDA plates.

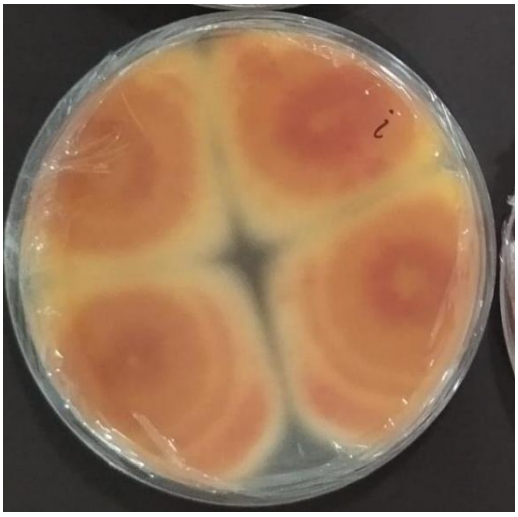

Figure 4: Seventh day of inoculation of the fungus *Cryptosporiopsis SPP.*

4 CONCLUSION

Bacillus *Amyloliquefaciens* used in this work has been shown to be a promising source of antimicrobial agents against pathogenic fungi. Its potential as an alternative to conventional fungicides deserves further research for development and application in agriculture, as well as in different sectors.

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