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Exploring The Rhizobacterial Potential In In Vitro Control Of Pestalotiopsis

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Resumo:
In this study, the aim was to explore the potential of biological control, specifically utilizing rhizobacteria, for the in vitro biocontrol of Pestalotiopsis sp. The experiments were carried out in the Forest Protection Laboratory at UNICENTRO, Irati Campus, Paraná. Pestalotiopsis sp. was isolated from symptomatic leaves of a specific species of eucalyptus (if known), which were placed on Petri dishes containing 20% agar-water medium (w/v). The rhizobacteria, whose isolation details are (if available), were streaked longitudinally at each end of a Petri dish containing Potato Dextrose Agar (PDA) medium. In the center of the dish, 2mm mycelium discs of Pestalotiopsis sp. were placed and incubated at 25 °C for 10 days under a 12-hour photoperiod. The effect on fungal growth inhibition was quantified by measuring the growth radius of the mycelium towards each rhizobacteria isolate compared to the control setup (details of control), yielding the percentage of inhibition for each isolate relative to the control. Among the six isolates tested, isolate 8E demonstrated the most substantial control potential, while isolate 10E showed the least, highlighting important variations in the efficacy of different rhizobacteria isolates for pathogen control in eucalyptus.

Keywords*: Phytopathology, Forestry, Biocontrol.*

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I. Introduçtion

The Brazilian agribusiness is heavily reliant on its forestry sector, renowned for its highly productive forests. However, the sector faces significant challenges, primarily due to the impact of pathogenic attacks, which lead to considerable economic losses in crop production. A notable example of such a pathogen is *Pestalotiopsis* sp., responsible for a variety of plant diseases (Alfenas et al., 2009). This pathogen adversely affects several economically valuable botanical species, such as the guava tree (Psidium guajava L.), causing stem rot (Cardoso & Maia, 2002), the mangosteen (Garcinia mangostana) with leaf spots and fruit lesions (Bastos & Bezerra, 2001), and various Eucalyptus species, leading to leaf spots and lesions on branches and stems, notably in E. benthamii, a species crucial to Paraná's economy (Schultz, 2011).

In light of these challenges, various control methods have been explored to mitigate the losses caused by *Pestalotiopsis* sp. However, to date, no method has demonstrated high effectiveness. Ongoing research seeks to identify more effective strategies for fungal control. For instance, Oliveira et al. (2011) reported promising results using thermal and osmotic treatments in the storage of Eugenia spp. seeds, reducing their infestation rates. Additionally, investigations into disease resistance (Padua et al., 2011) and cultural practices (Alfenas et al., 2009) are being evaluated for their potential in managing this issue.

This study focuses on the prospecting of biological control, specifically employing a microbial population to manage the phytopathogen. A particular emphasis is placed on a special group of bacteria, known as rhizobacteria, as potential biocontrol agents. The use of rhizobacteria in agricultural practices has shown promising results in various crops and is extensively implemented in countries like Brazil, China, India, Israel, EUA and others.

The biocontrol of plant diseases using rhizobacteria can be approached through direct antagonism or by inducing resistance in plants (Coelho et al., 2007; Santiago, 2010). Particularly, the method of antibiosis in

biocontrol has garnered attention due to its efficacy and ease of implementation, as well as its effectiveness in controlling the pathogen, contingent upon the pathogen population's sensitivity (Brunetta, 2006).

The primary objective of this study is to select rhizobacteria based on their effectiveness in the biocontrol of *Pestalotiopsis* sp. This involves assessing the interactions between rhizobacteria and *Pestalotiopsis* spp., as well as estimating the degree of in vitro control exerted by each rhizobacteria isolate. By doing so, the study aims to understand the dynamic relationship between these organisms and identify the most effective strains of rhizobacteria in suppressing the growth and spread of *Pestalotiopsis* sp. This selection process is critical in developing a biological control strategy that could offer a sustainable and environmentally friendly alternative to traditional pest management methods.

II. Material and methods

Test location

The experiments were conducted at the Forest Protection Laboratory of the State University of the Midwest (UNICENTRO), Irati Campus, Paraná, Brazil.

Isolation of *Pestalotiopsis* sp.

Pestalotiopsis sp. was isolated from symptomatic eucalyptus leaves, following the method described by Oliveira et al. (2012). Selected plant tissue fragments were superficially disinfected through sequential immersions in a 70% (v/v) aqueous alcohol solution for 30 seconds, followed by a 2% (v/v) sodium hypochlorite solution for 1 minute, and then rinsed with sterilized distilled water. These fragments were then placed on Petri dishes containing 20% (w/v) agar-water medium and incubated in a B.O.D. incubator at 28 ± 2 °C with a 12-hour photoperiod. After 3 days of incubation, 5 mm diameter mycelial discs were transferred to the center of Petri dishes containing Potato Dextrose Agar (PDA) medium and incubated for 7 days in a B.O.D. incubator at 28 ± 2 °C with a 12-hour photoperiod.

Rhizobacteria

The rhizobacteria used in this study belong to the collection of the Forest Pathology Laboratory at UNICENTRO. The cultures were preserved in a refrigerator and then transferred to Petri dishes containing medium 523 (Kado & Heskett, 1970), and incubated in a B.O.D. incubator at 28 °C for 48 hours without a photoperiod.

Antibiosis Test by Culture Pairing

The test was conducted according to the methodology proposed by Romeiro (2007). Rhizobacteria were streaked longitudinally at the edge of a Petri dish containing PDA medium, with one isolate at each end. In the center of the dish, 2 mm mycelial discs of *Pestalotiopsis* sp. were placed and incubated at 25 °C for 10 days with a 12-hour photoperiod. The inhibition effect on fungal growth was evaluated by measuring the mycelial growth radius towards each rhizobacteria isolate compared to a control area on the dish without rhizobacteria isolates. The percentage inhibition for each isolate was calculated relative to the control.

III. Results and Discussion

Five strains of *Pestalotiopsis* sp. were isolated, with the fastest-growing strain in PDA medium being selected for the biocontrol assay. In the biocontrol test, it was observed that all six evaluated rhizobacteria isolates reduced the mycelial growth of *Pestalotiopsis* sp. compared to the control (Figure 1). Due to the nature of the culture pairing assay, it was not possible to determine the exact mechanism of control involved; however, antibiosis and/or competition are believed to be the likely processes (Romeiro, 2007). The percentage of mycelial growth inhibition varied from 21% to 36% relative to the control.

Given the frequent occurrence of *Pestalotiopsis* sp. in Eucalyptus cultivations in southern Brazil (Schultz, 2011), the limited number of studies on this pathosystem, and the absence of registered fungicides for controlling this fungus in eucalyptus cultivation, biological control holds significant potential.

In vitro biocontrol using the culture pairing test on *Pestalotiopsis* theae was observed with six bacterial isolates out of 320 tested. These isolates demonstrated considerable potential, particularly as they also showed resistance to the fungicides used for controlling the fungus in India (Pallavi et al., 2012).

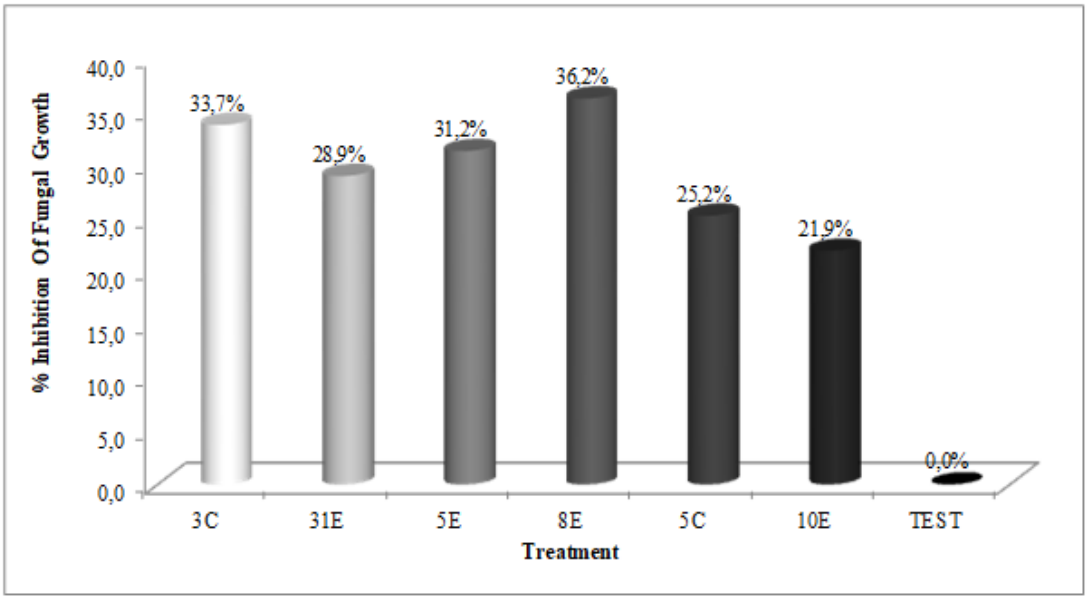
In a study comparing the effectiveness of fungal control agents, bacterial agents, aqueous plant extracts, and fungicides in controlling *Pestalotiopsis* sp., biocontrol agents were reported to have a significant effect on the pathogen (Saju et al., 2011).

The genetics of bacterial isolates is a crucial factor in understanding their ability to inhibit the growth of pathogens such as *Pestalotiopsis* sp. Genomic studies can identify genes responsible for the production of antimicrobial substances or other mechanisms of action, such as competition for resources or induction of

resistance in plants. Identifying these genes can allow not only an understanding of the mechanism of action but also the enhancement of biocontrollers through biotechnology techniques.

The effectiveness of biocontrollers can also be influenced by the genetics of the host, that is, the varieties of eucalyptus that are more resistant or susceptible to the pathogen (Fernandez et al., 2012). The interaction between the plant's genetics and the soil microbiota, including biocontrollers, can result in greater or lesser protection against pathogens.

Figure 1: Inhibition of the percentage of mycelial growth of a *Pestalotiopsis* sp isolate by rhizobacteria, by six different strains of rhizobacteria



* Treatment: Rhizobacteria isolates and control; Mycelium inhibition (%) = percentage of inhibition of mycelial growth of each treatment compared to the control.

IV. Conclusion

- The six isolates used reduced the mycelial growth of the pathogen.
- Isolate 8E showed the greatest control potential, while isolate 10E showed the least.
- These data suggest that the use of biocontrollers could be a viable alternative. However, it is important to note that these are results from an in vitro assay. While indicative of the potential of the isolates, further studies, especially in vivo assays, are necessary to confirm the effectiveness and safety of these biocontrollers in field conditions. It will be necessary to evaluate not only the efficacy of these isolates against the pathogen under natural conditions but also their economic viability, ease of application, and the absence of negative effects on other plant species or beneficial microorganisms present in the ecosystem.

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