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Bioprospecting of Selected *Trichoderma* spp. for Its Plant Growth Promoting and Biocontrol Properties

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Abstract: Bioprospecting for *Trichoderma* strains from peat soil collected at MARDI Sessang Station, Saratok, Sarawak, revealed promising candidates as plant growth-promoting microbes (PGPM) and producers of phytohormones such as indole-3-acetic acid (IAA) and gibberellic acid (GA3). The study aimed to identify *Trichoderma* strains with dual functions in biocontrol and plant growth enhancement. A total of seventeen strains were isolated and screened for their biocontrol activity against significant plant pathogens—*Ralstonia solanacearum* and *Colletotrichum gloeosporioides* and also their potential to produce IAA and GA_3. Among the isolates, one strain exhibited strong antagonistic activity against *R. solanacearum*, a pathogen responsible for bacterial wilt, suggesting its potential for biocontrol applications. Additionally, two strains showed significant inhibitory effects against *C. gloeosporioides*, the causative agent of anthracnose. Four strains demonstrated the ability to produce IAA, an essential auxin for root development and overall plant growth promotion and two strains were identified as GA_3 producers, contributing to plant growth by promoting cell elongation and germination. This study highlights the potential of *Trichoderma* strains from peat soils in Sarawak as valuable resources for sustainable agriculture. The dual functionality of these strains as biocontrol agents and phytohormone producers makes them promising candidates for integrated pest management (IPM) and biofertilizer development. Future research should focus on field trials and further molecular characterization to fully explore the potential of these *Trichoderma* isolates in enhancing crop productivity.

Keywords: Biological control, green technology, organic agriculture, plant pathogens, white agriculture.

1.0 Introduction

The increasing global demand for sustainable agricultural practices has led to a heightened focus on the use of biocontrol agents and plant growth-promoting microbes (PGPM) as alternatives to chemical pesticides and fertilizers. Among the various microbial agents, *Trichoderma* species stand out for their ability to enhance plant growth and combat a wide range of plant pathogens. Recent research has highlighted the potential of *Trichoderma* strains not only as biocontrol agents but also as producers of plant growth-promoting phytohormones such as indole-3-acetic acid (IAA) and gibberellic acid (GA_3) (Tyśkiewicz et al., 2022). Bioprospecting for new strains of *Trichoderma* from underexplored environments, such as peat soils, is essential to discover novel microbes with enhanced or unique functional traits that can address current agricultural challenges.

Peat soils, characterized by their high organic matter content and moisture, serve as a rich reservoir of microbial diversity. In tropical regions like Sarawak, Malaysia, where these soils are abundant, the microbial communities adapted to these environments may hold untapped potential for agricultural applications (Too et al., 2018). The unique properties of peat soil, including its acidic pH and high organic carbon, could foster the development of *Trichoderma* strains with specialized traits that are particularly suited to combating pathogens and promoting plant growth in challenging agricultural conditions.

The current study focuses on isolating *Trichoderma* strains from peat soil to target two critical plant pathogens: *Ralstonia solanacearum* and *Colletotrichum gloeosporioides*. *R. solanacearum* is considered important because it is the world's second most damaging phytopathogen (Wang et al., 2023). This pathogen thrives in warm, moist environments, making it a significant threat to tropical agriculture. On the other hand, *C. gloeosporioides* is responsible for anthracnose,

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a widespread fungal disease that affects fruit, and vegetable globally, leading to severe yield losses (Peralta-Ruiz et al., 2023). Identifying *Trichoderma* strains with strong antagonistic activity against these pathogens could provide a sustainable solution for managing these diseases, especially in regions where chemical controls are either ineffective or environmentally damaging.

In addition to biocontrol properties, certain *Trichoderma* strains are known to produce phytohormones such as indole-3-acetic acid and gibberellic acid, which play pivotal roles in promoting plant growth. IAA, a key auxin, regulates processes such as root elongation, cell division, and differentiation, thereby enhancing nutrient uptake and overall plant health. Gibberellin is crucial for seed germination, stem elongation, and flowering, further contributing to improved crop productivity. The ability of *Trichoderma* to produce these phytohormones highlights its potential as a biofertilizer, offering an eco-friendly alternative to chemical fertilizers that can enhance crop yields and support sustainable farming practices.

This study aims to bioprospect *Trichoderma* strains from peat soil at MARDI Sessang Station, Saratok, Sarawak, to identify strains with dual functions: biocontrol activity against *R.solanacearum* and *C. gloeosporioides*, and the production of IAA and GA3 which might be useful for the development of integrated pest management (IPM) systems.

2.0 Materials and methods

2.1 Isolation of *Trichoderma* spp. From Soil

Trichoderma spp. was isolated from virgin peat soil collected at MARDI Sessang Station, Saratok, Sarawak, with coordinates 1.92632 latitude and 111.23722 longitude. The soil samples were placed in double zip-lock bags for transport to the laboratory. Upon arrival, 10 g of soil was weighed and added to a 250 mL Erlenmeyer flask containing 100 mL of sterile distilled water, then agitated for 1 hour at 250 rpm. After agitation, 150 µL of the soil suspension was pipetted onto a fresh Potato Dextrose Agar (PDA) plate, which was incubated at room temperature (28±2°C) for 7 days. The emerging *Trichoderma* spp. was cut at the edge of the colony and place onto a fresh PDA.

2.2 Screening for antibacterial activity against *Ralstonia solanacearum*

Trichoderma spp. was cultured on PDA for 7 days. *Ralstonia solanacearum* (MMCC10070) was sourced from the MARDI Microbial Culture Collection (MMCC) and grown on Tetrazolium Chloride Agar (TZC) for 24-48 hours. Once the colonies developed, an antimicrobial test was conducted using the agar diffusion method. *R. solanacearum* was spread on fresh TZC agar, and a plug of *Trichoderma* spp. with the size of 5 mm was placed on the agar surface. Test was conducted in triplicate. The appearance of a clear zone after 24 hours indicated the antimicrobial activity of *Trichoderma* spp. (Figure 1). Percentage of inhibition was calculated based on the formula:-

$$PI=[(Iz-Trt)/Iz]\times 100\%$$

PI: Percent inhibition (%),
Iz: Diameter growth of inhibition zone (mm)
Trt: *Trichoderma* spp. plug size (mm)

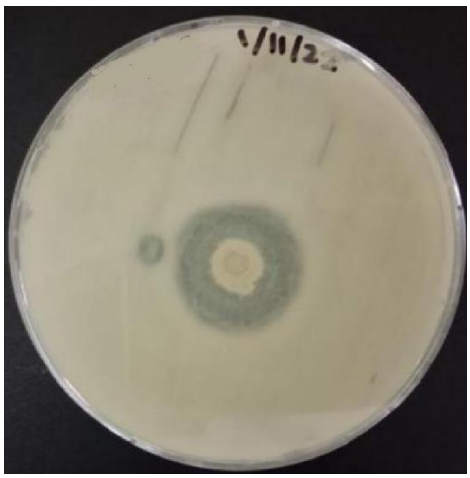

Fig. 1: Antibacterial activity of *Trichoderma* spp. against *Ralstonia solanacearum*

2.3 Screening for antifungal activity againts *Colletotrichum gleosporioides*

Trichoderma spp. was cultivated on PDA for 7 days. *Colletotrichum gleosporioides* (MMCC20013) was sourced from the MARDI Microbial Culture Collection (MMCC) and also grown on PDA for 7 days. Once pure fungal colonies were visible, plugs of *Trichoderma* spp. and *C. gleosporioides* were positioned next to each other in a dual plug antagonist assay (Figure 2). Test was conducted in triplicate. The appearance of a clear zone indicated antifungal activity between *Trichoderma* spp. and *C. gleosporioides*. Percentage of inhibition was calculated based on the formula:-

$$PI=[(Crl-Trt)/Crl]\times 100\%$$

PI: Percent inhibition (%),
Crl: Radial growth of the control *Colletotrichum gleosporioides* (mm),
Trt: Radial growth of *Colletotrichum gleosporioides* with *Trichoderma* spp.(mm)

Fig. 2: Dual culture antagonistic between *Trichoderma* spp. and *Colletotrichum gleosporioides*

2.4 Screening for indole-3-acetic acid (IAA) production

The procedure for producing Indole-3-Acetic Acid (IAA) was adapted from the research of Iqbal and Hasnain (2013) with minor adjustments. Fungal cell suspensions were maintained at a temperature of 30 ± 2°C for 48 hours, and the density of the culture broth was calibrated to 10^6 cfu/mL using a hemacytometer. Then, 2 mL of the culture broth were transferred to a fresh Eppendorf tube and centrifuged at 10,000 rpm for 30 minutes. After centrifugation, the supernatant was combined with Salkowski's reagent in a 2:1 ratio and incubated in the dark for 30 minutes. The appearance of a pink color confirmed the production of IAA by *Trichoderma* spp. A standard curve was created using synthetic IAA (Sigma), samples were analyzed at 520 nm using a Nanodrop spectrophotometer.

2.5 Screening for gibberellic acid (GA_3) production

The screening for GA_3 production by *Trichoderma* spp. was performed following the protocol established by Pandya and Desai (2014). A standard curve was created using synthetic GA_3 (Sigma), samples were analyzed at 254 nm using a Nanodrop spectrophotometer.

2.6 Identification of Trichoderma spp. using molecular method

Genomic DNA from *Trichoderma* spp. was isolated using the QIAamp® DNA Mini Kit, following the protocol provided by Qiagen (Qiagen 2020). Polymerase chain reaction (PCR) was then performed using ITS primers. The reaction mixture had a final volume of 25 µL, containing 0.1 µg of genomic DNA, 10 pM of each primer (ITS4 and ITS5), 1× Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 1 U of Taq DNA polymerase. The thermal cycling conditions included an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 40 seconds, and 72°C for 35 seconds, with a final extension at 72°C for 7 minutes (Lu et al. 2012). The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen 2020) and sent for sequencing to Apical Scientific Sdn. Bhd., Selangor. The sequencing results were then compared with the National Center for Biotechnology Information (NCBI) database.

3. Results

3.1 Isolation of *Trichoderma* spp. and screening of antibacterial and antifungal activities

From the sample of peat soil collected from the virgin peat soil it was observed that 17 isolates of *Trichoderma* spp. were isolated. Each *Trichoderma* spp. was tested for their individual ability to produce antibacterial and antifungal activity. It

was noted from the result that only *Trichoderma* spp. Ps1 inhibited the growth of *Ralstonia solanacearum* while *Trichoderma* spp. Ps 9 and *Trichoderma* spp. Ps 15 inhibited the growth of *Colletotrichum gleosporioides* (Table 1).

3.2 Screening of indole-3-acetic acid (IAA) and gibberellic acid (GA_3)

Screening of phytohormones production indicated that 4 isolates and 2 isolates of *Trichoderma* spp. were able to produce IAA and GA_3, respectively. *Trichoderma* spp. Ps 1, *Trichoderma* spp. Ps 5, *Trichoderma* spp. ps 8 and *Trichoderma* spp. Ps 10 were observed to be IAA producer while *Trichoderma* spp. Ps 3 and *Trichoderma* spp. Ps 4 were identified as GA_3 producer (Table 1).

Table 1: Antibacterial, antifungal activities and phytohormone production of isolated *Trichoderma* spp.

Isolate no.	Antibacterial and antifungal activities (average % inhibition ± SD %)		Phytohormone production (average ± SD µg/mL)	
	Ralstonia solanacearum	***Colletotrichum gleosporioides***	**IAA**	**GA_3**
Ps 1	66.7 ± 0.76	-	9.07 ±0.231	-
Ps 2	-	-	-	-
Ps 3	-	-	-	18.9 ±0.666
Ps 4	-	-	-	-
Ps 5	-	-	4.30 ±0.200	-
Ps 6	-	-	-	-
Ps 7	-	-	-	-
Ps 8	-	-	8.13 ±0.153	-
Ps 9	-	68.5 ± 0.76	-	-
Ps 10	-	-	6.17 ±0.153	-
Ps 11	-	-	-	-
Ps 12	-	-	-	-
Ps 13	-	-	-	-
Ps 14	-	-	-	-
Ps 15	-	81.5 ± 0.29	-	15.4 ±0.321
Ps 16	-	-	-	-
Ps 17	-	-	-	-

3.3 Identification of *Trichoderma* spp.

The 3 *Trichoderma* spp. with antibacterial and antifungal activities were identified using molecular method. Result of the sequencing showed that all the 3 isolates were *Trichoderma harzianum*, *Trichoderma* spp. and *Trichoderma viride* (Table 2).

4.0 Discussion

The isolation of soil *Trichoderma* spp. with biological activities was not something new. The potential of *Trichoderma* species as biological plant protection agents was first described in the early 1930s by Weindling (1932), where he observed that the *T. lignorum* strain having antagonistic activity against. *Rhizoctonia solani*. Researchers around the world had then embark in the research of bioactivity from Trichoderma spp. against other plant pathogens such as Ralstonia solanacearum, *Botrytis cinerea*, *Fusarium graminearum*, *Penicillium expansum, and Ganoderma* (De Zotti et al., 2020; Yendyo et al., 2017; and Supriyanto et al., 2020). In a study conducted by Xue et al. (2020), the researchers observed that *Trichoderma* spp. isolated showed high percentage of inhibition activity against *Colletotrichum gleosporioides* (85 – 97%) compared to our study of only 68 – 81%. Adrian Barbosa et al. (2022), noted that antimicrobial activity between *C. gleosporioides* and *Trichoderma* isolated from yam plantation showed inhibition zones ranging from 81 – 92 % which is not much different if compared to the results we obtained in this study. Satapathy and Beura (2020), showed that *Trichoderma viride* has the potential to produce antifungal activity against *C. gleosporioides* with the percentage of inhibition at 84.9%. In this study we also observed that *T. viride* has the highest percentage of inhibition that is 81%, this might indicate that *T. viride* is a potent antifungal producer. Antimicrobial activity of *Trichoderma* spp. against *Ralstonia solanacearum* was also well studied. A recent study stated that, inhibition of *Trichoderma* versus *R. solanacearum* was observed to be at 71%. This result was not too different from what we observed in our study (66.7%). Study done by Rahman et al. (2023) indicated that *T. harzianum* used in their study was able to exhibit 51% inhibition towards *R. solanacearum*. This showed that *T. harzianum* Ps 1 used in this study have comparable efficacy to *T. harzianum* used in Rahman et al. (2023) study.

Trichoderma spp. isolated by Akbari et al. (2024), showed the highest production of IAA at 63.91 µg/mL, this reading is much higher than the reading that we observed from our *Trichoderma* spp. which is only 9.07 µg/mL. Apart

from that *Trichoderma* TM10 isolated by Akbari et al. (2024) also showed much higher IAA production of 63.91 µg/mL which is about 7 times higher than our finding. For the production of GA_3, Lakhdari et al. (2022) observed that *T. harzianum* S2 gave the highest GA3 content of 83 mg/L. However, Ruikar et al. (2024) noted that *T. harzianum* used in their study only produce 35 µg/mL of GA_3. This showed that the current *Trichoderma* isolated from this study are potential IAA and GA_3 source but might not be as good as Trichoderma isolated by other researchers. However, by combining the potential of biocontrol shown by these *Trichoderma* spp. together with their ability to produce IAA and GA_3, we can produce a potential biopesticide which has the added benefit of plant growth promoting ability.

Table 2: Genetic Sequences and Identification of Three Potential Trichoderma Isolates

Isolate no.	Id	Sequence	Percent identity (%)	E- value
Ps 1	*Trichoderma harzianum*	cgttggtgaa ccagcggagg gatcattacc gagtttacaa ctcccaaacc caatgtgaac gttaccaaac tgttgcctcg gcgggatctc tgccccgggt gcgtcgcagc cccggaccaa ggcgcccgcc ggaggaccaa ccaaaactct tattgtatac ccctcgcgg gttttttact atctgagcca agagccgcgg cctcgtgggc gtttcgaaaa ggaagcaaaa ctttcaacaa cggatctctt ggttctggca tcgatgaaga acgcagcgaa atgcgataag taatgtgaat tgcagaattc aacgcacatt gcgcccgcca gtattctggc gggcatgcct gtccgagcgt catttcaacc ctcgaacccc tccgggggggt cggcgttggg gatcggccct ttacggggcc ggccccgaaa tacagtggcg gtctcgccgc agcctctcct gcgcagtagt ttgcacactc gcatcgggag cgcggcgcgt ccacagccgt aaacttctga aatgttgacc taggaatacc	94.4	0.0
Ps 9	*Trichoderma* spp.	caatccaaac tgttgcctcg gcgggatctc tgccccgggt gcgtcgcagc cccggaccaa ggcgcccgcc ggaggaccaa ccaaaactct tattgtatac cgcctcgcgg gttttttat aatctgagcc ttctcggcgc ctctcgtagg cgtttcgaaa atgaatcaaa actttcaaca acggatctct tggttctggc atcgatgaag aacgcagcga gtaatgtgaa ttgcagaatt cagtgaatca tcgaatcttt gaacgcacat tgcgcccgcc agtattctgg cgggcatgcc tgtccgagcg tcatttcaac cctcgaaccc ctccgggggg tcggcgttgg ggatcggccc tgccttggcg gtggccgtct ccgaaataca gtggcggtct cgccccagcc tctcctgcgc actagtttgc acactcgcat cgggagcgag gcccgtccaa ggccataaat cacccaaatt ctgagatgtg	97.8	0.0
Ps 15	*Trichoderma viride*	gggatcatta ccgagtttac aactcccaaa cccaatgtga accataccaa actgttgcct cggcggggtc acgccccggg tgcgtcgcag ccccggaacc aggcgcccgc cggagggacc aaccaaactc tttctgtagt cccctcgcgg acgttaattt tacagctctg agcaaaaatt caaaatgaat caaaactttc aacaacggat ctcttggttc tggcatcgat gaagaacgca gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcgcc cgccagtatt ctggcgggca tgcctgtccg agcgtcattt caaccctcga acccctccgg ggggtcggcg ttggggactt cgggaacccc taagacggga tcccggcccc gaaatacagt ggcggtctcg ccgcagcctc tcatgcgcag tagtttgcac aactcgcacc gggagcgcgg cgcgtccacg tccgtaaaac acccaacttc tgaaatgttg acctcggatc agg	100.0	0.0

5.0 Conclusion

This study highlights the potential of *Trichoderma* sp isolated from peat soil. *Trichoderma harzianum* Ps1 and *Trichoderma viride* Ps15 isolated from peat soil showed the ability to be used as biological control agents for *Ralstonia solanacearum* and *Colletotrichum gleosporioides* with the percentage of inhibition zone of 66% and 81% respectively. *Trichoderma harzianum* Ps1 and *Trichoderma* sp PS 3 showed the ability to produce IAA and GA_3 at 9.07 and 18.9 µg/mL respectively. Utilization of microbial products for agriculture will help to reduce usage of chemicals which will be detrimental not just for human but also planetary health.

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