

## ***In vitro* antifungal activity of cell-free supernatants from co-cultured *Trichoderma* spp. and *Burkholderia* spp. against *Fusarium oxysporum* f. sp. *cubense* causing banana wilt disease**

Riska<sup>1\*</sup>, Herwita Idris<sup>1</sup>, Jumjunidang<sup>1</sup>, Nurmansyah<sup>1</sup>, Tri Budiyan<sup>1</sup>, Riki Warman<sup>1</sup>, Hermawati Cahyaningrum<sup>1</sup>, Ellina Mansyah<sup>1</sup>, Rasiska Tarigan<sup>1</sup>, Afrizon<sup>2</sup>, Atman<sup>1</sup>

<sup>1</sup>Research Center for Horticultural, National Research and Innovation Agency, Cibinong Science Center, Jl. Raya Jakarta Bogor, Cibinong, Kabupaten Bogor, Indonesia

<sup>2</sup>Research Center for Estate Crops, National Research and Innovation Agency, Cibinong Science Center, Jl. Raya Jakarta Bogor, Cibinong, Kabupaten Bogor, Indonesia

\*Corresponding author's email: risk011@brin.go.id

Received: 16 November 2025 / Revised: 10 February 2026 / Accepted: 16 February 2026 / Published Online: 26 February 2026

### **Abstract**

*Fusarium oxysporum* f. sp. *cubense* (*Foc*) is recognized as one of the most catastrophic pathogens threatening banana production globally. The constrain of chemical method used and limitation of an effective conventional strategies stimulating the use of environmentally responsible biocontrol strategies. This study was purposed to evaluate the efficacy of potential antagonist indigenous *Trichoderma* and *Burkholderia* strains isolated from banana rhizosphere in single- and co-culture approaches. Microbial identification was performed through partial 28SrRNA region amplification (fungi isolates) and 16sRNA region (bacterial isolates) using Polymerase Chain Reaction (PCR). Colony compatibility, antagonist activity test of mycelium and a poisoned food test were assessed. The bioactive compounds produced by single and co-culture cultivation of *Trichoderma* spp. and *Burkholderia* spp. were profiled using GC-MS. Dual plate culture tests showed that *Burkholderia* sp. S10KTR325 and *Trichoderma* sp. PP21 caused significant inhibition of *Foc* growth, at 76.04% and 80.39%, respectively. A poisoned food test through secondary metabolite-based cell-free supernatants dissolved in culture medium revealed that combinations such as *Trichoderma* sp. PP21 and *Burkholderia* sp. S10KTR316 or S10KTR325, as well as co-cultures of *Trichoderma* sp. KPK22 and *Burkholderia* sp. S10KTR25, achieved over 80% inhibition. An exception was found in the combination of *Burkholderia* sp. S10KTR316 and *Trichoderma* sp. KPK22. Those combinations inhibited the percentage of colony growth was only at 2.2%. The single supernatant of *Trichoderma* sp. PP21 showed comparable efficacy to co-culture treatments. All treatments, both in single microbial cultures and co-cultures consistently produced 2-piperidinone, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl). *Trichoderma* sp. PP21 and its co-cultures with *Burkholderia* sp. S10KTR25 are a promising bio-fungicide agent to be developed.

**Keywords:** Banana, *Fusarium oxysporum* f. sp. *cubense*, Secondary metabolite, *Trichoderma* sp., *Burkholderia* sp. co-culture

### **How to cite this article:**

Riska, Idris H, Jumjunidang, Nurmansyah, Budiyan T, Warman R, Cahyaningrum H, Mansyah E, Tarigan R, Afrizon and Atman. *In vitro* antifungal activity of cell-free supernatants from co-cultured *Trichoderma* spp. and *Burkholderia* spp. against *Fusarium oxysporum* f. sp. *cubense* causing banana wilt disease. Asian J. Agric. Biol. 2026: e2025274. DOI: https://doi.org/10.35495/ajab.2025.274

This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License. (<https://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Introduction

Banana (*Musa* spp.) is one of the most popular commodities cultivated on a global scale. According to FAOSTAT (2023), banana production in 2022 reached 135 million metric tons per year globally. Indonesia, with its high favourable agroclimatic environment, offers significant potential for banana cultivation. Data from Badan Pusat Statistik (2024) indicates a consistent annual increase in the total area allocated for banana production. However, the increase of cultivation area yet to directly translate into a substantial increase in national banana yield. In 2024, Indonesia's banana production declined by 0.8% compared to 2023, reaching only 9,260,386.6 metric tons (equivalent to 92,603,866 quintals) (BPS, 2024). One of the major factors affect the fluctuations of banana production in this country, as well as in other major banana-producing nations, is the continuing threat of Panama wilt. The Panama disease was caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) especially *Foc* TR4 or syn *F. odoratissimum*. This soil-borne pathogen can infect almost all commercial cultivated bananas (Kema et al., 2021). Cavendish varieties known as a promising variety due to its resistance to race 1. Now as *Foc* TR4 spread vastly, the variety become a major vulnerability to large-scale and smallholder bananas growers. The continuing damage caused by this pathogen pose a serious obstacle, and to date, cultural practices or chemical treatments are ineffective in controlling the disease. More critically, the use of chemical pesticides can disrupt ecosystems. In addition, it may lead to increased pathogen resistance, environmental contamination, and alterations in the natural equilibrium of soil microflora. Concerns regarding ecological sustainability and health safety have driven the search for more effective and environmentally friendly alternatives for pathogen control (Bouanaka et al., 2021; Dugassa et al., 2021).

The use of microorganisms as biocontrol agents against plant pathogens has been extensively studied. Numerous bacterial and fungal strains have been demonstrated antagonistic activity against phytopathogenic microorganisms such as *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Trichoderma* spp., *Burkholderia* spp. etc (Azeem et al., 2024; Llorens and Agustí-Brisach, 2022). *Bacillus* spp., have been proven effective in inhibiting the growth of *Fusarium* spp. through the production of compounds such as 2,4-diacetylphloroglucinol

(DAPG) and 2,4-di-tert-butylphenol (DTBP) (Ntushelo et al., 2019; Zakqy et al., 2024). Heo et al. (2022) reported that *B. contaminans* AY001 effectively suppresses Fusarium wilt disease in tomato through the production of antimicrobial compounds and the induction of systemic resistance in the host plant. Furthermore, Lim et al. (2023) reported that secondary metabolites produced by *B. gladioli* capable of inhibiting Fusarium growth by disrupting the fungal cell structure and interfering with critical metabolite processes. The Trichoderma group, widely recognized for its biocontrol efficacy, also produces a broad spectrum of antagonistic compounds that significantly impede the development of various fungal pathogen (Li et al., 2020; Stracquadanio et al., 2020).

Several studies have reported that the efficiency of biocontrol products containing single microbial strains is often limited when it is exposed to the complexity of plant pathogens and variable field conditions (Istikorini and Budiman, 2023; Tyagi et al., 2024). According to Karuppiah et al. (2019) that co-cultivating of diverse microbial strains diversifies the spectrum of antagonistic activity, and promote effective colonization. Consequently, the use of microbial consortia composed of compatible strains with diverse active compounds and modes of action has gained considerable interest (Prigigallo et al., 2023). This study aims to evaluate the efficacy of co-cultivating *Trichoderma* spp. and *Burkholderia* spp. in suppressing *Foc*, the causal agent of Fusarium wilt disease in banana, through the application of cell-free supernatants with antagonistic potential.

## Material and Methods

### Sources of microbes

Samples of rhizosphere soil for the collection of antagonistic bacteria and fungi were obtained from banana plantations at several locations in West Sumatra. The samples were collected from roots and adhering soil located 50 cm beneath the soil surface and 30 cm outward from the base of the banana pseudo stem. Bacterial samples were collected from banana plants under the Hort 2018/178 experimental framework.

### Isolation and screening of bacterial strains for antagonistic activity

A total of 5 g of rhizosphere soil was collected from the Kepok Tanjung (ABB genotype) banana roots

grown in soil originating from Kepok banana plantations in Solok and Padang Pariaman, West Sumatra. The soil was thoroughly homogenized and suspended in 45 ml of phosphate-buffered saline (PBS). The soil suspension was thoroughly shaken to achieve homogeneity, and bacteria were isolated from a  $10^5$  cfu dilution. A volume of 0.001 ml from this dilution was inoculated into well plates containing R2 broth medium. After three days of incubation, the resulting bacterial colonies were purified by subculturing onto fresh R2A medium using a 10 mm inoculating loop. The single purified colony was

preserved in 30% glycerol and maintained at  $-20^{\circ}\text{C}$  for short-term storage.

Antagonistic potential screening was conducted by dual culture assay between the bacterial isolates and *Foc* cultured for 15 days (02.02.01.018, BRIN culture collection) as described by Dugassa et al. (2021), using potato dextrose agar (PDA) medium. Five isolates showing antagonistic potential (Table 1) were further tested for their antagonistic activity against *Foc* in triplicate, using five petri dishes per replicate as experimental units.

**Table-1.** Sampling sites of potentially antagonistic bacteria and fungi.

No	Isolate of microbes	Isolate origin; location; elevation (masl)
1.	S10KTR316	<i>Musa</i> sp. cv. Kepok; <u>0 513 1003734 584.4.77</u> , Nagari Selayo, Kec. Kubung, Kab. Solok, West Sumatera; <b>619.0</b>
2.	S10KTR325	<i>Musa</i> sp. cv. Kepok; <u>0 513 1003734 584.4.77</u> , Nagari Selayo, Kec. Kubung, Kab. Solok, West Sumatera; <b>619.0</b>
3.	PuKTBS53	<i>Musa</i> sp. cv. Kepok; <u>0 46 17 56595 S100 1851 97059 E 340 N</u> , Nagari Batang Anai, Kab. Padang Pariaman West Sumatera; <b>5.0</b>
4.	PuKTBS528	<i>Musa</i> sp. cv. Kepok; <u>0 46 17 56595 S100 1851 97059 E 340 N</u> , Nagari Kasang, Batang Anai, Kab. Padang Pariaman West Sumatera; <b>5.0</b>
5.	PuKTBS542	<i>Musa</i> sp. cv. Kepok; <u>0 46 17 56595 S100 1851 97059 E 340 N</u> , Nagari Kasang, Batang Anai, Kab. Padang Pariaman West Sumatera, <b>5.0</b>
6.	KPK22	<i>Musa</i> sp. cv. Kepok; <u>0.77136635S 1003146539E</u> , Nagari Kasang, Kec. Batang Anai, Kab. Padang Pariaman West Sumatera, <b>3.9</b>
7.	PP21	<i>Musa malaccensis</i> ; <u>0.91378133S 101.42639751E</u> , Nagari Selasih, Kec. Pulau Punjung, Kab Dharmasraya, West Sumatera; <b>120.1</b>
8.	PP22	<i>M. malaccensis</i> ; <u>0.91378133S 101.42660008E</u> Nagari Selasih, Kec. Pulau Punjung, Kab Dharmasraya, West Sumatera; <b>121.4</b>
9.	J21	<i>Musa</i> sp. cv Ambon; <u>0.39348134S 100.55926455E</u> , Nagari Kumango, Kec. Sei Tarab, Kab. Tanah Datar, West Sumatera; <b>809.8</b>

10.	J22	<i>Musa</i> sp. cv Ambon; <u>0.39348134S 100.55926455E</u> , Nagari Kumango, Kec. Sei Tarab, Kab. Tanah Datar, West Sumatera; <b>809.8</b>
10.	50K41	<i>M. salaccensis</i> ; <u>0.02336005S 100. 70708004E</u> , Nagari Jalan Sumbar Riau, Kec. Harau, Kab Limapuluh Kota, West Sumatera; <b>782.0</b>
11.	50K6	<i>M. salaccensis</i> ; <u>0.02336005S 100. 70708004E</u> , Nagari Jalan Sumbar Riau, Kec. Harau, Kab Limapuluh Kota, West Sumatera; <b>782.0</b>

### Isolation and antagonistic test of fungal isolates

Fungal isolates were cultured from soil samples collected from both cultivated and wild banana plantations across several locations in West Sumatra (Table 1). At least five root segments with adhering soil were surface sterilized using 70% alcohol and briefly rinsed with sterile water (1 s each). The root segments were air-dried for a few seconds and then transferred onto 1/3 strength Potato Dextrose Agar (PDA) medium containing 50 ppm chloramphenicol. After three days, fungal colonies showing morphological characteristics typical of *Trichoderma* colonies were sub-cultured onto fresh PDA medium. Morphological identification, both macroscopic and microscopic, was conducted following the criteria described by Burnett (1976).

Screening for antagonistic activity was performed using dual culture assays between the fungal isolates and *Foc*, as described by Dugassa et al. (2021), on PDA medium. A total of seven fungal isolates (Table 1) were tested for antagonistic activity, with three replicates per isolate and five Petri dishes per trial unit. As a control, *Foc* was cultured alone on the same medium.

$$\text{Percentage of colony inhibition} = \left(\frac{C-T}{C}\right) \times 100\%$$

Where C refers to the horizontal length of the fungal test (measured from the right to the left edge of the colony) cultured individually on PDA medium, while T denotes the horizontal colony length of *Foc* paired with the antagonist fungal isolate.

### Molecular characterization of selected antagonist microbes

Genomic DNA (gDNA) was extracted from 0.2 g of fungal culture (grown for seven days on PDA medium) and 0.2 g of bacterial culture (incubated for

two days in R2 Broth) using the Quick-DNA Fungal/Bacterial Microprep Kit (Zymo Research, Irvine, California, USA) following the manufacturer's protocol. The quantity and quality of the microbial DNA isolates were assessed using a Nanodrop spectrophotometer based on the absorbance ratio at A260/280 nm.

Molecular identification of fungal isolates was performed through PCR amplification and sequencing of ribosomal DNA targeting the large subunit (LSU) region using the primers LSU pn2 (5'-GTTACCATCTTTCGGGTCC-3') and LSU pn9 (5'-CTTAAGCATATCAATAAGCGGAGG-3'). For bacterial isolates, genomic DNA (gDNA) was amplified using the 16S rRNA primers 799F (5'-AACMGGATTAGATACCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3'). DNA amplification was performed using Promega GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA) on a Bio-Rad C1000TM thermocycler (Hercules, California, USA) under the following conditions: initial denaturation at 94 °C for 1 min, 28 cycles of denaturation at 95 °C for 15 s, annealing at 50–60 °C for 15 s, and extension at 72 °C for 5 s, followed by final extension at 72 °C for 10 min.

PCR fragments were isolated on 1.2% agarose gel by using electrophoresis at 100 V for 30 minutes. The DNA products were purified and sequenced by the Genomics Laboratory of the National Research and Innovation Agency (NRIA), Cibinong, West Java, Indonesia. Nucleotide sequences were aligned using the BioEdit software and pairwise comparisons with reference genes were performed using Emboss Needles. The aligned sequences were then compared with related species using the BLAST algorithm against the GenBank database (Altschul et al., 1990).

### **Antagonistic bacterial and fungal compatibility test**

Antagonistic bacterial and fungal candidates were evaluated for their compatibilities using a dual culture method (Dugassa et al., 2021). Twenty-four-hour-old bacterial isolates grown in R2 broth medium were cultured (a linear streak of about 3 cm in length) on one side of potato dextrose agar (PDA) plates, approximately 2 cm from the edge of the Petri dish. A 5-mm-diameter fungal plug was then placed on the opposite side, at a distance of 2 cm from the bacterial streak. Fungal isolates grown on PDA medium without bacterial presence served as controls. All cultures were incubated at 28 °C for seven days to observe inhibition zones. Fungal mycelial growth that surpassed bacterial colonies was considered compatible. In this assay, compatibility tests were conducted using five bacterial isolates and two selected fungal isolates, with three Petri dishes used as replicates for each combination.

### **The efficacy of bacterial and fungal cell-free supernatants in single and co-culture systems in inhibiting the growth of *Foc* colonies**

Beef peptone (BP) medium (HiMedia Laboratories Pvt. Ltd., Thane, Maharashtra, India) was used for inducing the secondary metabolites of microbial candidates. The inoculum preparation method for single-and co-cultures of bacteria and fungi followed Ajjiah et al. (2023) with modifications. The bacterial candidates S10KTR325 and S10KTR316 were first cultured on R2 agar for 48 hours, then transferred into BP medium at a final density of  $10^{-8}$  CFU. Cultures were incubated at approximately 28 °C with shaking at 120 rpm for seven days. Two selected antagonistic fungal isolates (PP21 and KPK22) and the pathogenic isolate *Foc* TR4 (02.02.01.018, BRIN culture collection) were cultured on PDA medium at  $\pm 28$  °C for seven days. Fungal spores were harvested using sterile water supplemented with 1% (v/v) Tween 20 (Pol-Aura, Morag, Poland), followed by filtration through Whatman No. 1 filter paper. The filtered spore suspension was cultured in BP medium at a final concentration of  $1 \times 10^6$  spores  $\text{mL}^{-1}$  and incubated at  $\pm 28$  °C with shaking at 120 rpm for seven days. To establish the co-culture, 1-day-old bacterial and fungal cultures were mixed at a 1:1 ratio (50%, v/v each) in fresh BP medium and incubated for seven days under the same conditions.

Cell-free supernatants (CFSs) were obtained on day seven by centrifuging each of the eight cultures individually at 10,000 rpm for 10 minutes at 4 °C. Following concentration optimization, each CFS was combined with molten PDA at  $50 \pm 3$  °C to reach 35% (v/v). Sterile water replaced CFS in the PDA medium for the control, and the medium was dispensed into 90-mm Petri plates. A 5-mm mycelial plug of *Foc* was placed at the center of each PDA plate (Dugassa et al., 2021). Cultures were incubated at  $\pm 28$  °C for 7 days until the pathogenic fungal mycelia on control plate colonized the entire petri dish. Inhibition of *Foc* mycelial growth was measured by comparing colony diameters between the CFS-treated and control plates. The percentage of inhibition was quantified according to the method described by Dugassa et al. (2021).

### **Characterization of antifungal metabolites in supernatant based extract of microbes and co cultivation supernatant**

Supernatant based cell of 7-days old single and co-culture microbes in BP were macerated in 96% ethanol for 72 hours in room condition. Organic fraction were extracted using evaporation rotary (IKA-Werke GmbH & Co. KG, Germany). Secondary metabolites of microbes suspended in 96% ethanol and the compounds were separated using gas chromatography–mass spectrometry (GC-MS).

### **Statistical analysis**

Data from the antagonistic tests of bacterial isolates, selected fungal isolates, and cell-free supernatant treatments against *Foc* mycelial growth were statistically analysed using the Minitab 10 software. To assess the differences of each treatment, we performed analysis of variance (ANOVA) and followed by post hoc evaluation using Tukey's test at a significance level of  $P < 0.05$ .

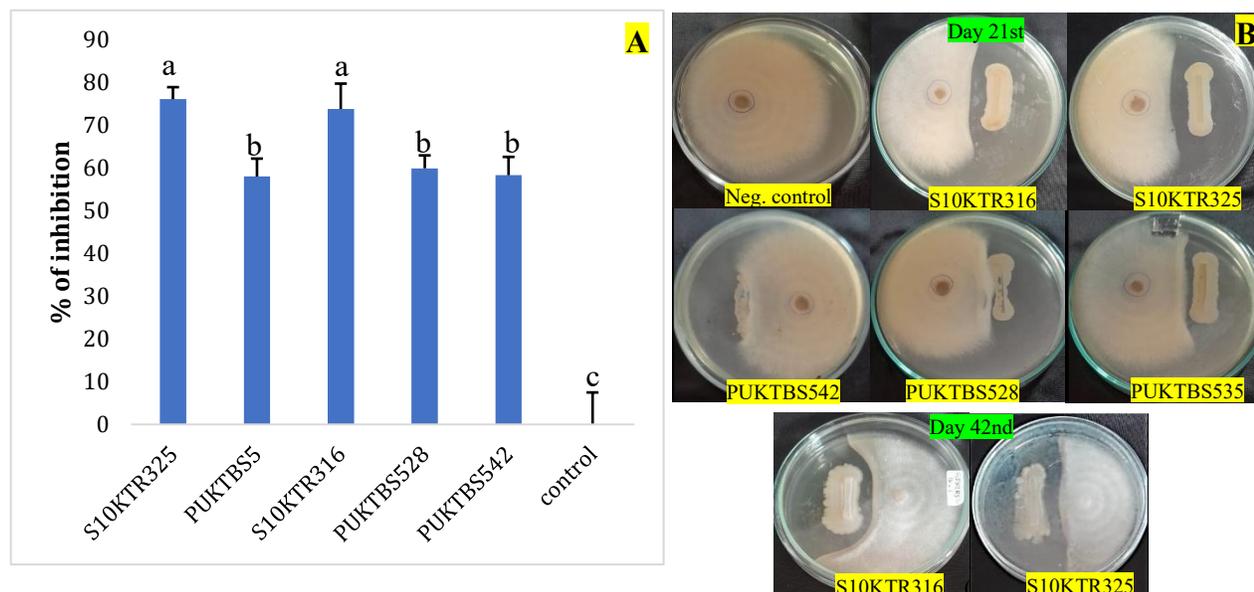
### **Results and Discussion**

Preliminary screening of microbial isolates obtained five selected bacterial isolates and seven fungal isolates with strong inhibitory activity against *Foc*. All selected isolates were subsequently subjected for their antagonistic activities against *Foc*.

### The antagonistic activity of selected fungal isolates and bacterial strains against *Foc*

The Figure 1 showed that all isolates, including both bacterial and fungal isolates, exhibited strong inhibitory activity against *Foc*, with inhibition rates >50%. After nine days of isolation, isolates

S10KTR325 and S10KTR316 demonstrated the highest inhibition percentages against *Foc*, at 76.04% and 73.75%, respectively. These values were significantly different ( $p < 0.05$ ) compared to the control and to isolates PUKTBS542, PUKTBS528, and PUKTBS5.



**Figure-1.** Percentage of bacterial isolate inhibition against *Foc* after nine days of in vitro dual assay (A), and inhibitory performance of bacterial isolates against *Foc* after 21 and 42 days of assay (B). Means that do not share a letter are significantly different based on Tukey's test at  $p=0.01$ .

The percentages of inhibition of isolates PUKTBS5, PUKTBS528, and PUKTBS542 against *Foc* were similar to each other but significantly lower than those of isolates S10KTR316 and S10KTR325, ranging only from 57.95% to 59.86% (Figure 1A). After 21 days of isolation, *Foc* colonies began to overgrow and cover the growth zones of the isolates. In contrast, isolates S10KTR316 and S10KTR325 maintained their inhibitory activities against *Foc* up to day 42, as

evidenced by the formation of halo zones and inhibition of *Foc* hyphal tips development (Figure 1B).

Based on molecular identification targeting the 16S rRNA region, isolates S10KTR316 and S10KTR325 were classified as strains of *Burkholderia* spp., whereas isolates PUKTBS5, PUKTBS528, and PUKTBS542 were grouped as strains of *Bacillus* spp. (Table 2).

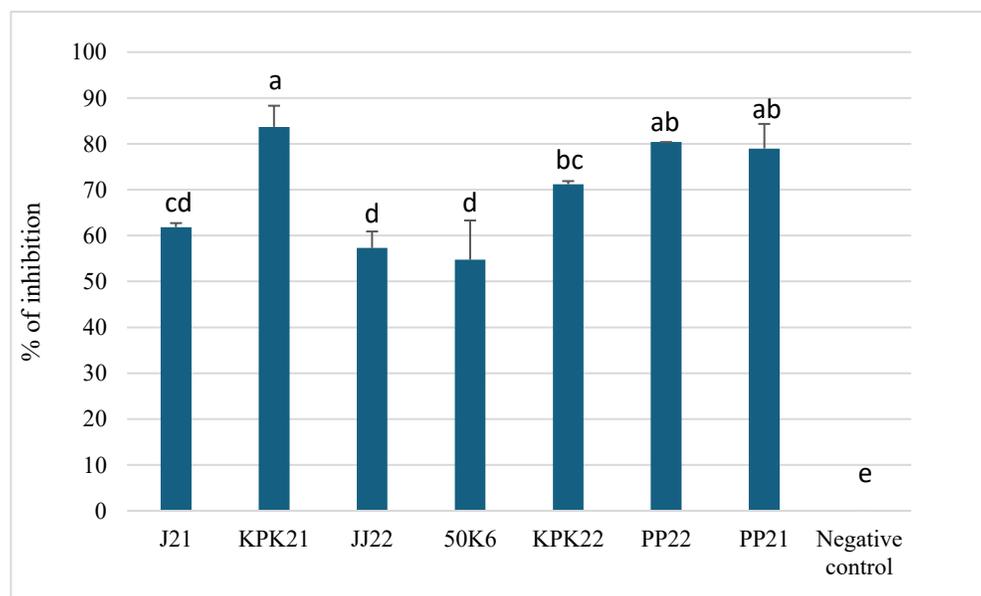
**Table-2.** Molecular characterization of four candidate antagonistic microbes from banana rhizosphere.

Fungal/bacterial code	Species	Reference sequence accession no	Identities between nucleotide (%)
S10KTR316	<i>B. cepacea</i> .	KU318403	96.07
S10KTR325	<i>Burkholderia</i> sp.	KX859146	98.23
PUKTBS5	<i>Bacillus mycoides</i>	MK720178	96.97
PUKTBS528	<i>Bacillus</i> sp	KJ542776	99.41
PUKTBS542	<i>Bacillus</i> sp	Kj534420	93.71
KPK22	<i>T. crissum</i>	PP381086	98.94
PP21	<i>T. lentiformae</i>	OM515076	99.72

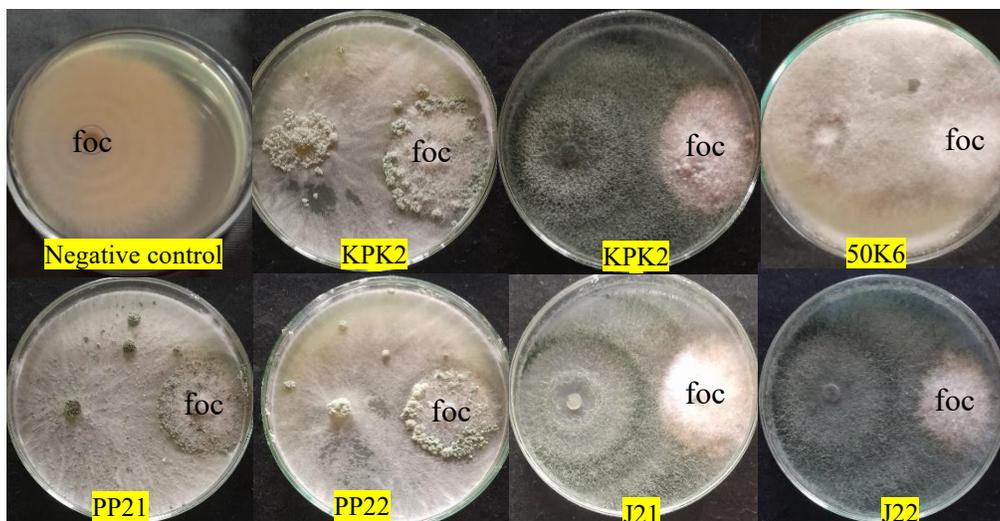
The antagonistic activity of *Burkholderia* in suppressing pathogenic fungal growth has been reported in several studies (Elshafie and Camele, 2021; Elshafie et al., 2017). Members of the *Burkholderia* genus have been reported to produce antibiotics and novel secondary metabolites with promising biotechnological applications (Elshafie and Camele, 2021). Similarly, although the antagonistic activities of the three *Bacillus* strains tested in this study were lower than that of the *Burkholderia* isolates, several species within the order Bacillales produced secondary metabolites indicating antifungal properties (Khan et al., 2018). *Bacillus* species, particularly *B. subtilis*, have been reported to inhibit the growth of soil-borne pathogens including *F. oxysporum*, *Rhizoctonia solani*, and *Ganoderma* spp. (Shao et al., 2025; Khan et al., 2018). Moreover, under in vivo test, the incidence of basal stem rot in oil palm caused by *Ganoderma* spp. was reduced significantly by *B. subtilis* application (Wang et al., 2025). All seven fungal isolates (J21, JJ22, 50K6, KPK21, KPK22, PP21, and PP22) exhibited antagonistic activity, with percentages of inhibition >50% under in vitro conditions (Figure 2 and 3). Among these, isolates PP21 and KPK22 demonstrated the highest antagonistic activities. Molecular identification

targeting the partial of 28S rRNA in Large Subunit rRNA region confirmed that both isolates are two species in the genus *Trichoderma*, which is widely recognized as an effective biocontrol agent against *Fusarium* wilt disease in banana. Analysis based on the LSU region sequences cannot distinguish a species sufficiently as described in Table 2. Therefore, the designation of isolates PP21 and KPK22 remains at the genus level of *Trichoderma*. Correa-Delgado et al. (2024) indicates that *Trichoderma* isolates obtained from indigenous habitat possess strong inhibitory effects against *Foc* subtropical race 4, mediated through both competitive interactions and the production of antifungal compounds.

Inhibitory activity, by quantification of the percentage of *Foc* colony diameter suppression, was highest in isolate KPK21, reaching 83.7%. The activity of the isolate was not significantly different from isolates PP22 and PP21, which exhibited inhibition values of 78.99% and 80.39%, respectively. Percentages of inhibition were lower slightly in isolates KPK22 and J21, at 61.84% and 71.18%, respectively. The lowest percentages of inhibition were in isolates JJ22 and 50K6, with values of 57.29% and 54.72%, respectively.



**Figure-2.** Percentages of inhibition of *Trichoderma* spp. isolates against *Foc* colony growth nine days after in vitro dual assay. Means that do not share a letter are significantly different based on Tukey's test at  $p=0.01$ .



**Figure-3.** Growth inhibition of *Trichoderma* spp. isolates (PP21, PP22, KPK21, KPK22, J21, J22 and 50K6) against *Foc* isolates on in vitro dual test.

### Compatibility test within the microbes

The compatibility test results that all bacterial isolates were compatible with *Trichoderma* sp. KPK22. However, only two bacterial isolates, *Burkholderia* sp. S10KTR316 and S10KTR325, exhibited compatibility with both *Trichoderma* isolates, PP21 and KPK22 (Table 3). Based on these findings, and

considering their high antagonistic activity, the two *Burkholderia* isolates were selected for further co-culture experiments with the antagonistic fungal isolates to evaluate potential synergistic effects in controlling *Foc* isolate in vitro. Li et al. (2024a) and Li et al. (2024b) reported that *T. harzianum* strains are compatible with *B. vietnamiensis* B418 in co-culture modes.

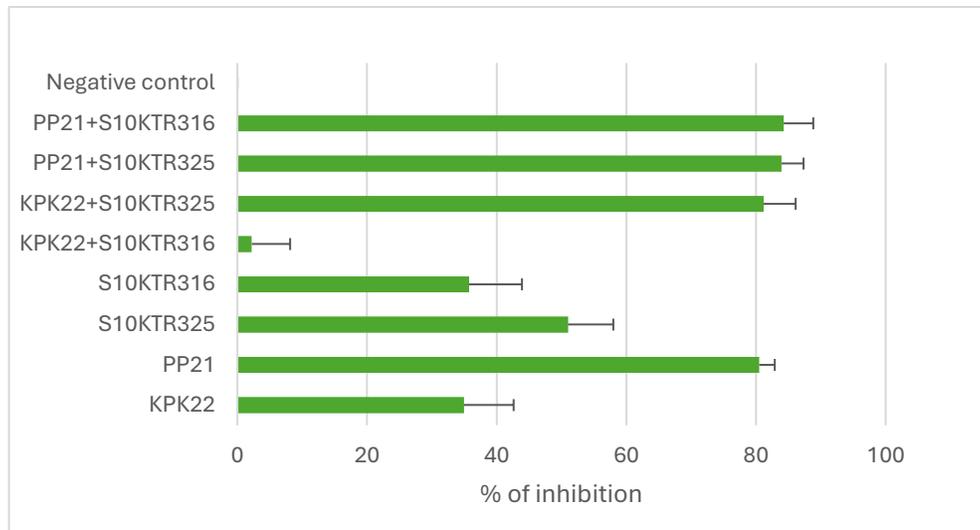
**Table-3.** Compatibility reaction of selected bacterial and fungal isolates.

Isolates	Compatible response	
	<i>Trichoderma</i> sp. KPK22	<i>Trichoderma</i> sp. PP21
<i>Burkholderia</i> sp. S10KTR316	+	+
<i>Burkholderia</i> sp. S10KTRR325	+	+
<i>Bacillus</i> sp. PUKTBS542	+	-
<i>Bacillus</i> sp. PUKTBS528	+	-
<i>Bacillus</i> sp. PUKTBS3	+	-

### Antagonistic assay of cell-free supernatants of single and co cultured *Trichoderma* spp. and *Burkholderia* spp.

Based on the results shown in Figures 4 and 5, *Burkholderia* sp. S10KTR16 and S10KTR25, as well as *Trichoderma* sp. PP21 and KPK22 demonstrated strong inhibitory activity against *Foc* and was therefore chosen for further investigation. The cell-free supernatants at a concentration of 35% from co-cultured and single cultured *Burkholderia* sp.

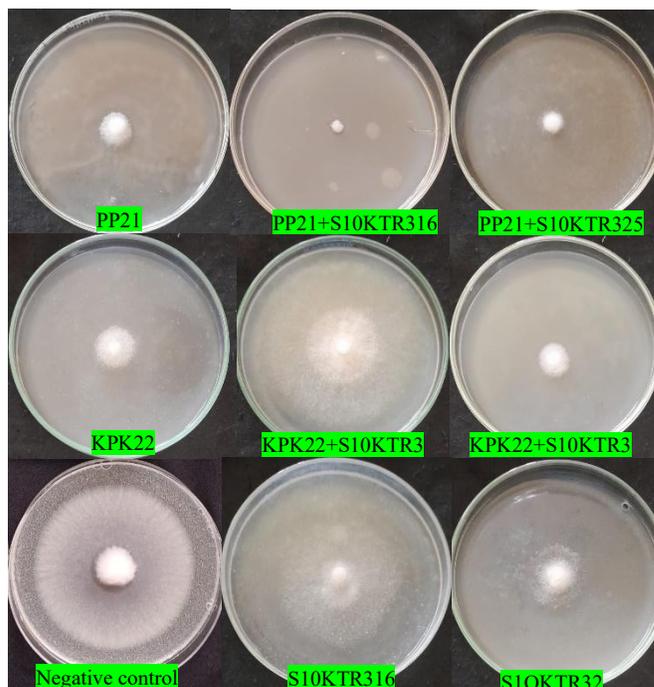
S10KTR325 and both *Trichoderma* sp. PP21 and KPK22 obstructed the growth of *Foc*. The inhibition activity increased in the co-cultured cell-free supernatants compared with the single-culture system, except for the co-culture of *Burkholderia* sp. S10KTR316 and *Trichoderma* sp. KPK22, with a percentage of inhibition of only 2.2%. The maximum percentages of inhibition were achieved by the cell-free supernatants either from single-cultured or co-cultured *Trichoderma* sp. PP21 with *Burkholderia* sp. S10KTR316 and S10KTR325.



**Figure-4.** Percentages of inhibition of cell-free supernatants from *Burkholderia* sp. (S10KTR325 and S10KTR316) and *Trichoderma* sp. (PP21 and KPK22) against *Foc*. Each data unit represents the mean  $\pm$  SD of nine replicates. The same superscript letters denote a value which is not significantly different at the test level of 5 % based on Tukey's test at  $p=0.01$ .

The activity of the cell-free supernatants derived from the co-culture of *Trichoderma* sp. KPK22 and *Burkholderia* sp. S10KTR325, exhibited inhibition at a rate starting from 81.17%. Interestingly, the single culture of *Trichoderma* sp. PP21 was equally effective

in inhibiting the growth of *Foc* as the co-culture treatment. The results of the study indicate that the inhibitory efficacy of KPK22, *Burkholderia* sp. S10KTR325, and S10KTR316 was significantly lower than that of PP21.



**Figure-5.** Inhibition of *Foc* growth on media containing cell-free supernatant from *Burkholderia* sp. (S10KTR325 and S10KTR316) and *Trichoderma* sp. (PP21 and KPK22) six days after treatment.

The results of this study reveal that cell-free supernatants from microbial cultures, both single and co-cultures of *Burkholderia* sp. and *Trichoderma* sp., significantly suppressed *Foc* colony growth. Co-cultures of *Burkholderia* sp. and *Trichoderma* sp. produced higher inhibition levels compared to single culture except combination of KPK22 and S10KTR316. The combination of *Burkholderia* sp. S10KTR316 and *Trichoderma* sp. KPK22 showed the lowest percentage of inhibition, with only 2.2% suppression. The high inhibition levels of *Foc* colony caused by co-culture of the three combination *Burkholderia* sp. and *Trichoderma* sp. indicates the formation of synergistic interactions between secondary metabolites produced by both microbes. Fan et al. (2025) found that co-culture *T. harzianum* and *Pseudomonas fluorescens* induced new volatile organic compound which is more effective to control *F. oxysporum*. Li et al. (2024a) also found that co culture *T. harzianum* T11-W and *B. vietnamiensis* B148 induced biocontrol activity of volatile organic produced by microorganism. Interestingly, in this study, the combination of *Burkholderia* sp. S10KTR316 and *Trichoderma* sp. KPK22 lower percentage of *Foc* inhibition, suggesting that both microbes induce metabolite antagonism or physiological incompatibility between the two isolates, which could hinder the production of bioactive compounds.

Both the single-cultures and co-cultures comprising *Trichoderma* sp. PP21 with *Burkholderia* strains S10KTR316 and S10KTR325 showed the highest percentages of *Foc* colony inhibition. The result reveals that *Trichoderma* sp. PP21, consistent with previous studies, has strong biocontrol activity in controlling plant diseases pathogen (Andrade-Hoyos et al., 2020; Halifu et al., 2020). The co-culture of *Trichoderma* sp. KPK22 and *Burkholderia* sp. S10KTR316 also showed a high percentage of inhibition, achieving up to 81.17%. Although not the most optimal combination, the synergy between these two microbes indicates their antimicrobial potential. The results of the study further reveal that the single-cultured supernatant of *Trichoderma* sp. PP21 produced antifungal compounds as effective as that of the co-culture of *Burkholderia* sp. S10KTR325 and S10KTR316. This result was confirmed and well-documented that secondary metabolites produced by *Trichoderma* spp., such as viridin and trichodermin, have strong antimicrobial activity against a wide range of plant pathogens. As in this study that *Trichoderma*

still exhibit the bioactive compounds which maintain high antifungal activity, supporting the application of *Trichoderma* as a bio-fungicide in sustainable agricultural systems. Khan et al. (2020) and Zhang et al. (2021) further reported that secondary metabolites from *Trichoderma*, including viridin, trichodermin, and peptaibols, possess significant biocontrol potential and can be effectively applied as extracts or supernatants to inhibit the growth of pathogenic fungi. Single applications of *Trichoderma* sp. PP21 also offer practical benefits in biocontrol product formulation, as it reduces production complexity and minimizes the risk of microbial antagonism. DNA amplification of the LSU region showed that the isolate *Trichoderma* sp. PP21 has a close phylogenetic relationship with *T. lentiformae*. The species is combinations of *T. harzianum* species complex (Chavveri et al., 2017). *T. lentiformae* species produced superior antagonism compounds against *R. solani*, *P. capsici* and *Fusarium* sp. (Rodríguez-Martínez et al., 2025). Accordingly, *Trichoderma* sp. PP21 is a potential candidate for further product development as a primary agent in the biological control of plant pathogens. To accurately confirm species identity, further analysis using genetic markers from additional genomic regions is recommended.

The single culture of *Trichoderma* sp. PP21 demonstrated superior potential as a biocontrol agent however in this study combination of *Burkholderia* sp. S10KTR325 and S10KTR316 with *Trichoderma* sp. PP21 provides an insight into the specificity of synergism between microbes. The inhibitory activity of supernatants from *Burkholderia* sp. S10KTR325 and S10KTR316 significantly increased when combined with the supernatant of *Trichoderma* sp. PP21. Several reports support the findings of this study such as co-culture between *Trichoderma* and bacteria particularly with the genera *Pseudomonas* and *Bacillus*, demonstrate synergistic effects in pathogen suppression and plant growth promotion across diverse horticultural crops (Dugassa et al., 2021; Prigigallo et al., 2023). According to Li et al. (2024a) the co-cultivation of *T. harzianum* and *B. vietnamiensis* significantly increased antagonistic potential through the production of VOCs. However, when the supernatants of both bacterial isolates were combined with *Trichoderma* sp. KPK22, the co-culture with S10KTR325 showed enhanced inhibitory activity, whereas the co-culture with S10KTR316 exhibited a drastic reduction in activity.

**Table-4.** GC–MS profile of bioactive compounds produced by single and co-culture of *Trichoderma* spp and *Burkholderia* spp.

Compound code	% of bio-active compound of single and co-culture <i>Trichoderma</i> spp. and <i>Burkholderia</i> spp							
	KPK22	PP21	S10KT R316	S10KT R325	KPK22+ S10KTR 316	KPK22+ S10KTR 325	PP21+ S10KTR 316	PP21+ S10KTR 325
1	3.71							
2								2.45
3			6.62		12.06		3.39	7.24
4	6.04							
5		18.83	18.64					13.23
6	0.30							
7		6.32	7.94		12.49			5.51
8		2.06						
9	20.86	0.20	5.15	19.25	12.49	25.40	29.80	8.56
10	0.37							0.32
11							1.72	0.32
13	0.94							
16						1.82		
17	1.66		23.30	2.36	1.46		1.73	1.41
18	1.57	1.22						
19			2.68	2.05	1.70			
20	24.32	16.91	23.30	24.22	23.57	33.55	20.57	19.54
21	2.64							
22								1.05
23								0.83
24	0.44							
25		1.1	3.40		1.69			
26	1.28		1.0	2.01			1.73	2.17
27						2.76		
28						3.52		
29		1.09	2.14		1.46			
30								1.99
31	3.12							
32								0.21
33	2.6					4.4		
34								2.83
35			4.96	3.93	2.62	4.49		2.25
36	5,06							
37						2.44		
38	1.08			1.20				1.40

1=Propylene glycol, 2=N-acetyl isoxazolidine, 3=Butanoic acid, 5=4, 2,3-butanediol, 6= Butanoic acid, 3 methyl-, 7= 2-Isopropoxyethylamine, 8= Butanoic acid, 2 methyl, 9=1-amino-3-methoxypropan-1-ol, 10=2-Piperidinone, 11= Benzeneacetic acid, 12= Silane, ethenyldiethylmethyl-, 13=Pentane,1-(ethenyloxy)-, 14=1,4-diazabicyclo[4.3.0]nonan-2,5-dione, 3-methyl, 15=6-methyl-cyclohex-2-en-1-ol, 16= Cyclo(L-prolyl-L-L-Valine), 17= Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-, 18=2-butenal, dimethylhydrazone, 19=5-isopropylidene-3, 20= Hexadecanoic acid, 21= cyclohex-2-enone,3-(N',N'-dimethylhydrazino)-, 22=2,5-cyclohexadien-1-one,3,5-dihydroxy-4,4-dimethyl-, 23= Pyrrolo[1,2-a][yrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-, 24=2-(methyl amino)cyclohexan-1-ol, 25=2-(E)-heptenoic acid, (4S)-4-[(R)-alanyl]amino]-6-methyl-, 26= Propanamide,N-(1-cyclohexyl)ethyl, 27= Cyclobutylcarboxamine, 28= Propane-2-(ethenyloxy)-, 29= Phenethylamine, 30=4-hydroxy-6-methyl-3-nitro-2-pyridone, 31=2,5-piperazinedione, 32=1-Oxaspiro[4.4]nonan-4-one, 33=1-isopropylidiaziridine, 34=7-hydroxycoumarin.

**Note:** Bio-compounds were selected with quality exceeding 40% and demonstrated significant antifungal activity, based on the PASS Online system.

The GC-MS analysis revealed that the identified bio-compounds from all treatments with quality above 40% and relatively high-level antifungal activity (PASS Online) are between six and seventeen compounds (Table 4). In general, 2-piperidinone, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) were found in all treatments, both in single microbial cultures and co-cultures. Prasad et al. (2021) also reported that *B. seminalis* produced Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) and the compounds exhibit significant antimicrobial effects against *F. oxysporum*, *Aspergillus niger*, *Microsporium gypseum*, and *Trichophyton mentagrophytes*. While, Pérez-Cordero et al. (2024) stated that cultures of *B. cepacea* supplemented with molasses produced two compounds, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), which exhibited strong inhibitory activity (77%) against *C. gloeosporioides* isolates. The *Trichoderma* isolates has also been reported to produce these compounds in co-culture with other microbes. Ajijah et al. (2023) further reported that the diketopiperazine (DKP) compounds from co-culture of *T. koningiopsis* and *Pseudomonas protegens* resulted in synergistic interactions, producing high antifungal activity against *F. cerealis*, the causal agent of fusarium head blight in wheat. In addition to these two compounds, *Trichoderma* also produces the secondary metabolite 2-piperidinone (Sudha et al., 2021). Although involving different species within the same genus, Li et al. (2024a) demonstrated that *B. vietnamense* co-cultured with *T. harzianum* generated a greater diversity and relative abundance of metabolites

compared to monoculture. Furthermore, these two microbes efficiently induced antifungal secondary metabolites, and their synergistic activity enhanced biocontrol effectiveness in strawberry plants (Li et al., 2024b). In line with these findings, the types of metabolites produced by the co-culture of *Trichoderma* sp. PP21 and *Burkholderia* sp. S10KTR325 were more diverse compared to other treatments. This was further supported by the results showing that the co-culture of *Trichoderma* sp. PP21 with *Burkholderia* sp. S10KTR325 exhibited the highest inhibition against the development of *Foc* colonies compared to single microbial isolates and other co-culture treatments. Furthermore, these microbes can be integrated into biocontrol consortia, especially as compatibility tests indicated no antagonistic interactions between *Burkholderia* sp. and *Trichoderma* spp., supporting their potential for coexistence. Conversely, the co-culture of strains *Trichoderma* sp. KPK22 and *Burkholderia* sp. S10KTR316 exhibited a significant reduction in antagonistic activity. This may be attributed to possible interactions in which the metabolites produced exert inhibitory effects. In addition, this co-culture generated fewer types of antifungal metabolites (Table 4). Although a definitive conclusion cannot yet be drawn, it is suspected that these factors contribute to the reduced antagonistic potential observed in this co-culture.

## Conclusion

The combination of supernatants from *Trichoderma* sp. PP21 and *Burkholderia* sp. S10KTR316 or S10KTR325, as well as the co-culture between *Trichoderma* sp. KPK22 and *Burkholderia* sp. S10KTR316, demonstrated efficiency more than 80%,

yielding the highest percentages of inhibition against the pathogen under study, except for the combination of *Burkholderia* sp. S10KTR316 and *Trichoderma* sp. KPK22, which exhibited the lowest suppression efficacy at 2.2%. The single supernatant of *Trichoderma* sp. PP21 showed >80% effectiveness, comparable to that of co-culture treatments. All treatments, both in single microbial cultures and co-cultures consistently producing antifungal secondary metabolites. These results suggest that other than co culture approach, in this case single-culture also represents a promising resource for bio fungicide development.

### Acknowledgement

The authors are thankful to the Head of Research Organization who has approved this proposal and facilitated it so that the research runs smoothly and Hort 2018/178 which supplied the soil source from the experiment for use in this research.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Source of Funding:** The Research Organization for Agriculture and food, Indonesian National Research and Innovation Agency Grant No. 7/III.11/HK/2025.

### Biosafety Declaration

The *Burkholderia* isolates obtained from the plant rhizosphere have undergone at least basic biosafety analyses and did not show optimal growth at 37 °C. In addition, pathogenicity tests using model organisms did not reveal any negative effects. Based on these results, the isolates have a good biosafety profile and can be considered safe for biocontrol applications.

### Contribution of Authors

Riska: Conceived the idea, planned, conducted and supervised all research experiments and managed funds for the research work.

Idris H, Jumjunidang, Nurmansyah, Budiyanti T, Warman R, Cahyaningrum H, Tarigan R, Mansyah E, Afrizon & Atman: Provided technical assistance, guidance and research facilities for the execution of this research and reviewed and edited the final draft.

All authors read and approved the final draft of the manuscript.

### References

- Ajijah N, Fiodor A, Dziurzynski M, Stasiuk R, Pawlowska J, Dziewit L and Pranaw K, 2023. Biocontrol potential of *Pseudomonas protegens* ML15 against *Botrytis cinerea* causing gray mold on postharvest tomato (*Solanum lycopersicum* var. *cerasiforme*). *Front. Plant Sci.* 14:1288408. <https://doi.org/10.3389/fpls.2023.1288408>.
- Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ, 1990. Basic local alignment search tool. *J. Mol Biol.* 215(3):403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Andrade-Hoyos P, Silva-Rojas HV and Romero-Arenas O, 2020. Endophytic *Trichoderma* species isolated from *Persea americana* and *Cinnamomum verum* roots reduce symptoms caused by *Phytophthora cinnamomi* in avocado. *Plants.* 9:1 17. <https://doi.org/10.3390/plants9091220>.
- Azeem H, Iftikhar Y and Raza M, 2024. Biological control of plant pathogens by using antagonistic bacteria: A review. *Pak. J. Phytopathol.* 36(01):45–52. <https://doi.org/10.33897/pjp.2024.361.07>
- Bouanaka H, Bellil I, Harrat W, Boussaha S, Benbelkacem A and Khelifi D, 2021. On the biocontrol by *Trichoderma afroharzianum* against *Fusarium culmorum* responsible of fusarium head blight and crown rot of wheat in Algeria. *Egypt. J. Biol. Pest Control.* 31:68. <https://doi.org/10.1186/s41938-021-00416-3>.
- BPS (Badan Pusat Statistik), 2024. Statistik Hortikultura 2023. Dalam Direktorat Statistik Tanaman Pangan, Hortikultura (Penyunting Sulistina, T. H. Marpaung). Volume 5. Jakarta: BPS-Statistic Indonesia.
- Burnett J, 1976. *Fundamentals of Mycology* (2nd Edition). Edward Arnold Publishers, London
- Chaverri P, Branco-Rocha F, Jaklitsch W, Gazis R, Degenkolb T, and Samuels GJ, 2015. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycol.* 107(3): 558–590
- Correa-Delgado R, Brito-López P, Cardoza Silva RE, Jaizme Vega MC, Laich F and Gutiérrez S, 2024. Biocontrol potential of a native *Trichoderma* collection against *Fusarium*

- oxysporum* f. sp. *cubense* subtropical race 4. Agric. 14(11): 2016. <https://doi.org/10.3390/agriculture14112016>
- Dugassa A, Alemu T and Woldehawariat Y, 2021. *In-vitro* compatibility assay of indigenous *Trichoderma* and *Pseudomonas* species and their antagonistic activities against black root rot disease (*Fusarium solani*) of faba bean (*Vicia faba* L.). BMC Microbiol. 21:1–11. <https://doi.org/10.1186/s12866-021-02181-7>
- Elshafie HS and Camele I, 2021. An Overview of Metabolic Activity, Beneficial and Pathogenic Aspects of *Burkholderia* Spp. Metabolites. 11(5):321. <https://doi.org/10.3390/metabo11050321>
- Elshafie HS, Camele I and Grasso S, 2017. Antifungal activity of *Burkholderia gladioli* pv. *agaricicola* against some phytopathogenic fungi. Chem. Biodivers. 14(5): e1700126. <https://doi.org/10.1002/cbdv.201700126>
- Fan H, Wang Q, Bai J, Chen Y, Yang C, Hrynsphan D, Savitskaya T, Wang Z and Chen J, 2025. Review on the metabolic synergistic mechanisms in fungal-bacterial co-culture systems for VOCs biodegradation: from a microscopic perspective. Rev. Env. Sci. Bio. Technol. 24: 7
- FAOSTAT, 2023. Rome: FAO. <http://www.fao.org/faostat/en/#data/QC> Accessed 16/10/2025
- Halifu S, Deng X, Song X, Song R and Liang X, 2020. Inhibitory mechanism of *Trichoderma virens* ZT05 on *Rhizoctonia solani*. Plants. 9:912. <https://doi.org/10.3390/plants9070912>
- Heo J, Kim J, Lee S and Lee CW, 2022. Biological control activity of *Burkholderia contaminans* AY001 against *Fusarium* wilt and bacterial spot diseases of tomato. Biology. 11(4):619. <https://doi.org/10.3390/biology11040619>
- Istikorini Y and Budiman T, 2023. Uji potensi mikroba rizosfer sebagai pengendali hayati penyebab penyakit tanaman. Jurnal Silviculture Tropika, [PDF file]. Retrieved from <https://journal.ipb.ac.id/index.php/jsilvik/article/download/52709/27082>
- Karuppiah V, Sun J, Li T, Vallikkannu M and Chen J, 2019. Co-cultivation of *Trichoderma asperellum* GDFS1009 and *Bacillus amyloliquefaciens* 1841 causes differential gene expression and improvement in the wheat growth and biocontrol activity. Front. Microbiol. 10: 1068. <https://doi.org/10.3389/fmicb.2019.01068>
- Kema G-H, Drenth A, Dita M, Jansen K, Vellema S and Stoorvogel J-J, 2021. *Fusarium* wilt of banana, a recurring threat to global banana production. Front. Plant Sci. 11:628888.
- Khan N, Martínez-Hidalgo P, Ice TA, Maymon M, Humm EA, Nejat N, Sanders ER, Kaplan D and Hirsch AM, 2018. Antifungal activity of *Bacillus* species against *Fusarium* and analysis of the potential mechanisms used in biocontrol. Front. Microbiol. 9:2363.
- Khan RAA, Najeeb S, Hussain S, Xie B and Li Y, 2020. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms. 8(6): 817. <https://doi.org/10.3390/microorganisms8060817>
- Li T, Tang J, Karuppiah V, Li Y, Xu N and Chen J, 2020. Co-culture of *Trichoderma atroviride* SG3403 and *Bacillus subtilis* 22 improves the production of antifungal secondary metabolites. Biol. Control 140: 104122. <https://doi.org/10.1016/j.biocontrol.2019.104122>
- Li W, Fu Y, Jiang Y, Hu J, Wei Y, Li H, Li J, Yang H and Wu Y, 2024b. Synergistic biocontrol and growth promotion in strawberries by co-cultured *Trichoderma harzianum* TW21990 and *Burkholderia vietnamiensis* B418. J. Fungi. 10(8):551.
- Li W, Wang X, Jiang Y, Cui S, Hu J, Wei Y, Li J, Li H, Yang H and Wu Y, 2024a. Volatile organic compounds produced by co-culture of *Burkholderia vietnamiensis* B418 with *Trichoderma harzianum* T11-W exhibits improved antagonistic activities against fungal phytopathogens. Inter. J. Mol. Sci. 25(20): 11097.33–752.
- Lim J, Lee H, Kim Y and Kim J, 2023. Antifungal activity of *Burkholderia gladioli* against *Fusarium* species through production of secondary metabolites. Microbiol. Spectr. 11(2): e04805-22. <https://doi.org/10.1128/spectrum.04805-22>
- Llorens E and Agustí-Brisach C, 2022. Biocontrol of Plant Diseases by Means of Antagonist Microorganisms, Biostimulants and Induced Resistance as Alternatives to Chemicals. Plants. 11(24): 3521. <https://doi.org/10.3390/plants11243521>

- Ntushelo K, Ledwaba LK, Rauwane ME, Adebo OA and Njobeh PB, 2019. The Mode of Action of *Bacillus* Species against *Fusarium graminearum*, Tools for Investigation, and Future Prospects. *Toxins*.11(10):606. <https://doi.org/10.3390/toxins11100606>
- Pérez-Cordero A, Chamarro-Anaya L, Barboza-García A, Baldiris-Avila R and Montes Robledo A, 2024. Antifungal Activity of Secondary Metabolites Produced by *Burkholderia cepacia* Against *Colletotrichum gloeosporioides*. *Curr. Res. Env. App. Mycol. (J. Fungal Biol.)*, 14(1): 281–288.
- Prasad J-K, Pandey P, Anand R and Raghuwanshi R, 2021. Drought exposed *Burkholderia seminalis* JRBHU6 exhibits antimicrobial potential through pyrazine-1, 4-dione derivatives targeting multiple bacterial and fungal proteins. *Front Microbiol.* 12:633036.
- Prigigallo MI, Staropoli A, Vinale F and Bubici G, 2023. Interactions between plantbeneficial microorganisms in a consortium: *Streptomyces microflavus* and *Trichoderma harzianum*. *Microb. Biotechnol.* 00:1–21. <https://doi.org/10.1111/1751-7915.14311>.
- Rodríguez-Martínez E-S, Rios-Velasco C, Sepúlveda-Ahumada D-R, Buenrostro-Figueroa J-J, Correia K-C, Guigón-López C and Alvarado-González M, 2025. *Trichoderma* Species from semiarid regions and their antagonism against the microorganisms that cause pepper wilt. *J. Fungi* 11(3):174.
- Shao MW, Chen HJ, Huang AQ, Zheng L, Li CJ, Qin D, Sun YH, Lin Z, Fu G, Chen YH and Li YJ, 2025. Modulation of rhizosphere microbiota by *Bacillus subtilis* R31 enhances long-term suppression of banana *Fusarium* wilt. *iMetaOmics.* 2(2): p. e70006.
- Stracquadanio C, Quiles JM, Meca G and Cacciola SO, 2020. Antifungal activity of bioactive metabolites produced by *Trichoderma asperellum* and *Trichoderma atroviride* in liquid medium. *J. Fungi.* 6:263. <https://doi.org/10.3390/jof6040263>.
- Sudha A, Rajesh M, Senthilkumar M, Vijayakumar M, and Sumathi E, 2021. Volatile organic compounds of some antagonists against *Lasiodiplodia theobromae*, a pathogen of coconut. *Int. J. Agric. Biol.* 26(6):731-740
- Tyagi A, Tamang TL, Kashtoh H, Mir RA, Mir ZA, Manzoor S, Manzar N, Gani G, Vishwakarma SK, Almalki MA and Ali S, 2024. A review on biocontrol agents as sustainable approach for crop disease management: Applications, production, and future perspectives. *Horticulturae.* 10(8):805. <https://doi.org/10.3390/horticulturae10080805>
- Wang L, Jia S, Du Y, Cao H, Zhang K, Xing J and Dong J, 2025. Biocontrol Potential of *Bacillus subtilis* A3 Against Corn Stalk Rot and Its Impact on Root-Associated Microbial Communities. *Agronomy* 15(3):706.
- Zakqy N, Wuryandari Y and Purnawati A, 2024. Potential of The Biological Agent *Bacillus* spp in Inhibiting *Fusarium* Wilt Disease and its Effects on The Growth and Production of Cayenne Pepper (*Capsicum frutescens* L.). *J. Pembelajaran dan Biol. Nukleus.* 10(1): 27–32. <https://jurnal.ulb.ac.id/index.php/nukleus/article/view/5366>
- Zhang J-L, Tang W-L, Huang Q-R, Li Y-Z, Wei M-L, Jiang L-L, Liu C, Yu X, Zhu H-W, Chen G-Z and Zhang X-X, 2021. *Trichoderma*: A treasure house of structurally diverse secondary metabolites with medicinal importance. *Front. Microbiol.* 12:723828. <https://doi.org/10.3389/fmicb.2021.723828>