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Title page

Type of manuscript:
Research paper

Termite symbiont bacteria as biological agents against anthrachnose of bird's eye chilli caused by *Colletotrichum* sp.

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ACKNOWLEDGMENTS
The authors would like to thank Department of Agricultural Production and Laboratory of Plant Protection, Politeknik Negeri Jember for their support in research and equipment.

FUNDING
This research received no external funding.

AUTHORS' CONTRIBUTIONS

GFD and ADW conceived and planned the experiment. VHPS, ADM, and ADW carried out the isolation and screening of antagonistic bacteria, including the in vitro dual culture assay. ZAW, RAR, and GFD performed molecular work and analysis. DGC and RDN performed data analysis and interpreted the plant damage and weather data. GFD, ADW, and RDN contributed to writing, review, editing, and visualization. All authors contributed to manuscript preparation, read, and approved the final version.

COMPETING INTEREST
The authors declare no conflict of interest.

Main text

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ABSTRACT

Symbiotic bacteria associated with termites are a largely unexplored resource with potential for use as biological control agents. This study investigates their effectiveness in managing anthracnose disease caused by *Colletotrichum* sp. in bird's eye chili, a disease known to impact crop productivity and yield. Despite its prevalence, farmers often rely on chemical pesticides, which pose environmental risks. The research was carried out over four months at the Plant Protection Laboratory, Bioscience Laboratory, and Innovation Garden of Politeknik Negeri Jember. The experimental procedures included bacterial isolation, preliminary antagonistic screening, hypersensitivity testing, in vitro assays against Colletotrichum sp., in vivo efficacy testing for disease suppression, and molecular identification. Four bacterial isolates—IR1A4, IR1A6, IR1A8, and IR4D3—demonstrated inhibitory effects against the pathogen. In vitro inhibition rates ranged from 22.52% to 80.47%, while in vivo suppression ranged from 7.89% to 18.42%. The IR1A6 isolate, closely related to *Bacillus amyloliquefaciens*, was the most effective in reducing pathogen growth. These findings highlight the potential of termite-derived bacteria as novel sources of biocontrol agents capable of suppressing fungal pathogens across diverse ecosystems.

Keywords: Antagonistic bacteria, beneficial microbe, *Colletotrichum* sp, biological control, termite

INTRODUCTION

Bird's eye chili (*Capsicum frutescens* L.) is a strategic horticultural commodity in Indonesia due to its significant economic and nutritional value. However, national statistics indicate unstable productivity, which is largely attributed to biotic constraints. One of the most serious problems is anthracnose disease, primarily caused by *Colletotrichum* spp. (Nurjasmi & Suryani, 2020). The pathogen can infect plants from the seedling to fruit-maturation stages, and its epidemiology is strongly influenced by environmental conditions; for example, high humidity during the rainy season accelerates disease development (de Silva et al., 2019; Soesanto, 2024). Symptoms typically begin as small black spots on fruit surfaces, then expand into dark, sunken lesions and soft rot, which may lead to premature fruit drop or necrosis of apical shoots. Post-harvest, the disease causes rapid deterioration of fruits, resulting in yield losses ranging from 50% up to complete crop failure (Andriyani et al., 2020; Widodo & Hidayat, 2018).

In field practice, farmers still rely heavily on chemical fungicides, such as those containing difenoconazole, to manage anthracnose (Hamidson et al., 2019). Although such chemicals can be effective, excessive use has environmental and health drawbacks, may disrupt beneficial soil organisms, and can promote resistance in pathogen populations (Aditiya, 2021; Dinata, 2023; Dinata et al., 2023). Therefore, the development of alternative, environmentally friendly control strategies is imperative. Biological agents (*Bacillus subtilis, Pseudomonas fluorescens, Paenibacillus polymyxa, Trichoderma* spp.) have shown promise in plant disease management as biostimulants, bioprotectants, or biofertilisers (Egamberdieva et al., 2017; Muhibuddin et al., 2021; Ramdan et al., 2021; Soumare et al., 2020).

Termites, as social insects, harbor diverse symbiotic microorganisms in their digestive tract that play crucial roles in lignocellulose degradation and nutrient cycling (Tampubolon, 2021). Recent studies have revealed that termite-associated bacteria, including *Bacillus, Pseudomonas, Lysinibacillus fusiformis,* and *Paenibacillus alvei* exhibit antagonistic properties against major plant pathogens such as *Ganoderma boninense, Rigidoporus microporus*, and *Phytophthora capsici* (Fitriana et al., 2022; Blanton et al., 2023). These findings suggest that termite symbionts possess untapped potential as biological control agents.

Thus, in this study, we investigate the potential of symbiotic bacteria derived from termite guts as biological control agents against *Colletotrichum* sp., the causal agent of anthracnose in bird's-eye chili. Our results are expected to contribute to the development of sustainable disease management strategies in tropical horticultural systems, reducing dependence on synthetic fungicides and supporting resilient agricultural production. To the best of our knowledge, this study represents the first report on the use of termite symbiont bacteria for controlling chili anthracnose.

MATERIALS AND METHODS

Research Site. This research was conducted in the Plant Protection Laboratory, Bioscience Laboratory, and Innovation Garden at Politeknik Negeri Jember from May to September 2024.

Isolated Bacteria. Based on the findings reported by Widura et al., (2024) and Sakti et al., (2024) a total of 33 bacterial isolates were initially collected, comprising isolates from a coffee plantation area and one nest obtained from the mushroom storage facility of Politeknik Negeri Jember.

Antagonist Test. To screen for antagonistic bacteria, the isolates were tested against the pathogenic *Colletotrichum* sp. using a modified co-culture method (Dinata et al., 2021; Herliyana et al., 2013). The pathogen isolates were previously purified from the Laboratorium Pengamatan Hama Penyakit Tanaman Pangan dan Hortikultura Jember using Potato Dextrose Agar media (Himedia). *Colletotrichum* sp. was then inoculated onto the medium and placed at the center of Petri dishes. A 0.5 cm filter paper, soaked in the bacterial suspension, was placed 3 cm away from the pathogen inoculation site.

$$\mathrm{R} = \frac{R1-R2}{R1}\ X100\%.$$

R = inhibitory test (100%),
R1 = Pathogen radius on control;
R2 = Pathogen radius with bacteria.

Phenotypic Properties Test. Four phenotypic property tests were used to characterize the obtained bacteria, namely the Gram reaction test, oxidation and fermentation (OF) test, potato soft rot test, and hypersensitive reaction test on tobacco leaves. The Gram reaction test was carried out using the KOH method by mixing a loopful of bacterial culture with 3% KOH solution on a glass slide; the formation of a viscous thread indicated a Gram-negative reaction, while no thread formation indicated Gram-positive bacteria (Schaad et al., 2001; Siswadi et al., 2023). The Oxidative-Fermentative (OF) test was conducted using OF medium to determine the metabolic characteristics of the bacteria in utilizing carbohydrates under oxidative and fermentative conditions. Bacterial cultures were inoculated into two tubes of OF medium; one tube was overlaid with sterile mineral oil to create anaerobic conditions, while the other was left without oil. A color change from green to yellow indicated acid production (Schaad et al., 2001). The potato soft rot test was performed by inoculating bacterial isolates onto sterile potato slices and incubating them at room temperature for 24–48 hours. The development of soft, watery, and macerated tissue indicated a positive result for pectinolytic activity (Schaad et al., 2001). The hypersensitivity reaction was performed by Fanani et al. (2015). The assay was carried out by suspending the bacterial isolates in 1 mL of sterile distilled water, which was then injected into the lower surface of young tobacco leaves. Observations were made for seven days or until necrotic symptoms appeared.

Assay for Inhibition Effectiveness of Symbion Bacteria on *Colletotrichum* Mycelial Growth. An in vitro antagonistic assay was conducted using the dual culture method (Suryanti et al., 2015; Dinata et al., 2026). Bacterial isolates were suspended in a glass tube containing 1 mL of distilled water and 0.5 cm diameter filter paper. The filter paper was then removed and drained on sterile tissue for 2–3 hours. The test was carried out by pairing pieces of *Colletotrichum* sp. colonies (0.5 cm in diameter) with filter paper containing bacteria at a distance of 3 cm in a Petri dish containing NA medium.

The percentage of inhibition was calculated using the formula (Dwiastuti et al., 2016):

$$\text{PIRG} = \frac{R1-R2}{R1} X100\%.$$

PIRG = Percentage Inhibition of Radial Growth
R1 = pathogen diameter (control),
R2 = pathogen diameter with bacteria.

The Effectiveness of Symbiont Bacteria in Suppressing Anthracnose on Chili. An in vivo assay was conducted on chili fruit by preparing a suspension of *Colletotrichum* sp. culture with a density of 10^6 spores/ mL. A bacterial suspension with a density of 10^7 cells/ mL was then prepared. Healthy fruits of uniform size were selected. The chili fruits were surface-sterilized with 1% NaOCl and rinsed with distilled water, then dipped in the bacterial suspension. The fruits were wounded using a sterile needle, and a suspension of pathogenic fungi was inoculated at the wound site. Observations were made on the incubation period and disease incidence on chili fruit (Puspitasari et al., 2014). The percentage of disease incidence was calculated using the following formula (Ginting, 2013):

$$DI = \frac{n}{N} x\, 100\%$$

DI= Disease incidence (%);
n = Number of symptomatic fruits;
N = Total number of fruits.

Experimental Design. The antifungal activity test was conducted using a Completely Randomized Design (CRD) with six treatments and four replications. Treatments included control, isolates IR1A4, IR1A6, IR1A8, IR4D3, and fungicides (difenoconazole 0.5 mL/L).

Molecular Identification of Termite Symbiont Bacteria.
DNA Extraction. Genomic DNA of the selected bacterial isolate was extracted using the Quick-DNA MagBead Plus Kit following the manufacturer's instructions. The quality and quantity of extracted DNA were assessed prior to amplification.

Polymerase Chain Reaction Amplification of 16S rRNA Gene.
The extracted DNA was amplified using MyTaq HS Red Mix (2×) (Bioline, BIO-25048) with bacterial genomic DNA as the template to amplify the 16S rRNA gene. PCR amplification was performed using universal bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The PCR reaction was carried out in a total volume of 25 µL consisting of ddH_2O up to 25 µL, 12.5 µL of MyTaq HS Red Mix (2×), 1 µL of 27F primer (10 µM), 1 µL of 1492R primer (10 µM), and 1 µL of DNA template (5–100 ng). The PCR conditions consisted of an initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 52°C for 15 s, and extension at 72°C for 15 s. The reaction was then held at 4°C. The expected amplicon size was approximately 1,500 bp. The PCR products were electrophoresed on 1% agarose gel in 1× TAE buffer. Electrophoresis was conducted at 100 V for 30 min. The results were visualized using a DigiDoc UV transilluminator (Bio-Rad, USA).

Sequencing and Sequence Analysis. PCR products showing clear bands were subjected to sequencing using the Sanger DNA sequencing method with capillary electrophoresis. The obtained sequences were edited and assembled using BioEdit and Chromas. Sequence similarity analysis was performed using the BLAST program available at the National Center for Biotechnology Information (NCBI) database (Camacho et al., 2009).
Phylogenetic analysis was conducted using MEGA (Molecular Evolutionary Genetics Analysis) version X, and a phylogenetic tree was constructed using the Neighbor-Joining method with 1,000 bootstrap replications (Kumar et al., 2018).

Data Analysis. Data on inhibition rate and percentage of disease incidence were analyzed using Analysis of Variance (ANOVA), followed by Duncan's test at $P < 0.05$. All statistical analyses were performed using SPSS version 21.

RESULTS AND DISCUSSION

Termite Symbiont Bacteria Isolates. A total of 33 bacterial isolates were initially obtained in this study. These isolates exhibited diverse colony morphologies and pigmentation, including transparent, milky, thick, greenish, and yellowish variants (Ashraf et al., 2024). Each morphologically distinct colony was subsequently purified to obtain a single isolate. Following the purification process, 31 bacterial isolates were successfully maintained and further evaluated for their antagonistic activity against *Colletotrichum* sp., tthe causal agent of anthracnose disease.

Preliminary Screening of Antagonism Bacteria. All 31 bacterial isolates demonstrated the ability to inhibit the growth of the pathogen *Colletotrichum* sp. Among them, 19 isolates exhibited strong antagonistic activity, with inhibition percentages exceeding 50%, indicating their considerable potential as biological control agents (Table 1). In contrast, the remaining 12 isolates showed inhibition levels below 50%, suggesting comparatively lower effectiveness, although they were still capable of suppressing pathogen growth to some extent. The observed variation in antagonistic activity is likely attributed to differences in metabolite production, enzymatic activity, bioactive compound synthesis, and compatibility with the growth medium (Fitriana et al., 2022).

Among the 31 bacterial isolates screened for antagonistic activity against *Colletotrichum* sp., five isolates—IR1A4, IR1A5, IR1A6, IR1A8, and IR4D3—demonstrated the highest inhibition rates, ranging from 72.09% to 83.72%. The variation in inhibition capacity among these isolates is presumed to result from differences in their metabolic potential, enzyme secretion, and secondary metabolite biosynthesis pathways (Adra et al., 2023).

The superior inhibitory performance of these isolates may be attributed to multiple antagonistic mechanisms, including the production of hydrolytic enzymes (e.g., chitinase, β-1,3-glucanase, protease), synthesis of antifungal compounds, competition for nutrients and ecological niches, and induction of systemic resistance in the host plant. These mechanisms are well documented in antagonistic genera such as *Bacillus, Paenibacillus*, and *Pseudomonas* (Compant et al., 2005; Pal & Gardener, 2006; Köhl et al., 2019). *Bacillus* species are known to produce cyclic lipopeptides such as surfactin, iturin, and fengycin, which can disrupt fungal cell membranes and inhibit spore germination (Ongena & Jacques, 2008; Cawoy et al., 2015) Similarly, *Pseudomonas* species species synthesize antimicrobial compounds including 2,4-diacetylphloroglucinol (DAPG), phenazines, and pyoluteorin that interfere with fungal respiration and membrane integrity (Sarma et al., 2015). In addition, siderophore-mediated competition for iron and the secretion of volatile organic compounds (VOCs) have also been reported as key suppression mechanisms against *Colletotrichum* spp. (Kim et al., 2022; Gu et al., 2020).

The high antagonistic activity observed in isolates IR1A4–IR4D3 suggests that they may employ a combination of enzymatic degradation, antibiotic production, and nutrient competition to inhibit Colletotrichum sp. effectively. These findings are consistent with previous studies showing that bacterial isolates exhibiting inhibition levels above 70% generally possess multiple biocontrol traits (Köhl et al., 2019; Fitriana et al., 2022). As noted by Soesanto et al. (2013a), every biological agent has distinct modes of action and levels of efficacy. Further biochemical and molecular characterization is necessary to elucidate the specific metabolites and genes involved in the antagonistic mechanisms of these isolates. Such understanding will contribute to the development of effective and environmentally friendly biocontrol strategies for managing anthracnose disease in chili cultivation. These findings support the further selection and development of high-performing isolates as environmentally friendly biological control agents.

Phenotypic Properties Test Out of the five bacterial isolates investigated, only the IR1A5 strain caused a necrotic reaction in the hypersensitivity test involving infiltration of bacterial suspension into tobacco leaves. The appearance of necrosis indicates a hypersensitive response of plant tissue to the IR1A5 isolate, suggesting that the plant recognizes this bacterium as a pathogen. This response identifies IR1A5 as having pathogenic potential, making it unsuitable for development as a biological control agent. According to Marsaoli et al. (2019), pathogenic bacteria typically trigger visible necrosis on tobacco leaves, whereas non-pathogenic strains do not elicit such symptoms. The hypersensitivity test is therefore essential for distinguishing between pathogenic and non-pathogenic microbes (Putri et al., 2024). The results of this test provide a critical foundation for ensuring the safety and effectiveness of bacterial isolates, especially for applications in biological control or crop productivity enhancement. Isolate IR1A5 exhibited necrotic symptoms in the hypersensitive reaction test; therefore, it was not subjected to further analyses. The remaining four isolates, namely IR1A4, IR1A6, IR1A8, and IR4D3, were identified as Gram-positive bacteria. Among them, isolate IR1A6 showed fermentative metabolism based on the OF test. All four isolates were non-pectinolytic, as indicated by negative results in the soft rot test.

In vitro Assay of Symbiont Bacterial Isolates Against *Colletotrichum* sp. The in vitro assay demonstrated that termite symbiont bacteria exhibited notable inhibitory effects against the growth of *Colletotrichum* sp. (Figure 1). The termite symbiont bacterial isolates exhibited inhibition efficiencies on the seventh day ranging from 22.52% to 80.47% (Table 2). Among them, isolate IR1A6 consistently showed the highest inhibiting mycelial growth of the pathogen, followed closely by IR1A4, whose performance was comparable to that of a chemical fungicide. The strong antagonistic activity of IR1A6 is believed to result from its rapid growth rate, which creates intense competition with Colletotrichum sp. for essential nutrients and space. These findings align with Soesanto et al. (2013b), who suggested that nutrient competition is a primary mechanism by which many biological control agents suppress pathogen development.

On the fourth day after inoculation, isolate IR1A6 proved to be the most effective in suppressing mycelial growth of *Colletotrichum* sp. The control group, which received no treatment, had the largest average mycelial width at 4.33 cm. In contrast, the fungicide treatment achieved the highest level of suppression, resulting in the smallest mycelial width of 1.30 cm. The IR1A6 isolate also performed strongly, with an average width of 1.88 cm on the fourth day, nearly matching the effectiveness of the fungicide. In subsequent days, IR1A6 continued to demonstrate strong inhibitory effects, recording the smallest average mycelial width of 1.49 cm by the seventh day, surpassing the fungicide, which had an average width of 1.79 cm. The IR1A4 isolate also showed promising results, with a width of 2.83 cm on the seventh day, although it was less effective than IR1A6 (Table 2).

On the seventh day of observation, isolate IR1A6 recorded the highest inhibition rate at 80.47%, surpassing the fungicide, which showed an inhibition rate of 76.53% (Table 2). The IR1A4 isolate also demonstrated strong inhibitory activity with an effectiveness of 62.90%, significantly higher than the control group, which showed no inhibition. In comparison, isolates IR1A8 and IR4D3 achieved inhibition rates of 22.85% and 22.52%, respectively. While lower than those of IR1A4 and IR1A6, these values were still notably better than the control.

Suppression of Symbiont Bacterial Against Anthrachnose Disease Caused by *Colletotrichum* sp. The inhibition assay evaluating the effect of termite- associated bacteria on the growth of pathogenic fungi was conducted for eight days. The effectiveness of inhibition was evaluated by observing anthracnose symptoms on chili fruits (Table 3). Symptoms typically begin as small black spots that gradually enlarge, darken, and cause fruit rot (Figure 2). White spores often appear around affected areas, followed by rotting and wilting of the fruit stalks. This is consistent with Anitasari (2016), who reported that these spots tend to grow, merge, and turn blackish, and in severe cases, the disease can lead to leaf desiccation and death. Moreover, small, slightly sunken black spots often appear on nearly mature fruits. As infection advances, fruits may shrivel, dry out, rot, and eventually drop. Besides affecting fruit, the pathogen can also infect branches and twigs (Sibarani, 2008).

The results revealed that all tested termite symbiont bacterial isolates significantly suppressed pathogen growth, with inhibition rates ranging from 7.89% to 21.05%, all outperforming the untreated control. In the absence of bacterial or fungicidal treatment, control fruits exhibited pronounced anthracnose symptoms, including enlarging lesions that progressed to fruit rot. These findings underscore a higher level of disease severity in the control group, reinforcing the conclusion of Souza et al. (2019) that *Colletotrichum* sp. propagates through wounds on chili fruits.

The ability of termite symbiont bacteria to inhibit pathogen growth is thought to be linked to the bioactive compounds they produce, which interfere with conidial germination and limit pathogen access to vital nutrients. These include lipopeptides (e.g., surfactin, iturin, fengycin), polyketides, siderophores, volatile organic compounds (VOCs), and hydrolytic enzymes such as chitinase and β-1,3-glucanase (Ongena & Jacques, 2008). The termite gut microbiome has evolved sophisticated degradation capabilities through functional synergy between gut symbionts and host enzymes, creating a collaborative digestive system in which stored plant material is broken down by a complementary suite of cellulases, xylanases, and esterases (Moreira et al., 2021). Similar mechanisms have been reported in *Bacillus*, *Pseudomonas*, and *Paenibacillus* spp., which produce compounds with strong antifungal activity against *Colletotrichum* spp. (Fitriana et al., 2022). Therefore, the high inhibition rates observed in this study may be linked to the combined effects of enzymatic degradation and secondary metabolite production, suggesting that termite-derived bacteria possess significant potential as biocontrol agents for sustainable anthracnose management. As noted by Elfina et al. (2024), these bacteria can suppress spore formation and reduce hyphal colonization in fruit tissues by releasing secondary metabolites or hydrolytic enzymes that block germination of *Colletotrichum* sp. spores.

Molecular Identification Results. The termite symbiont bacterial isolate IR1A6, which showed the highest antagonistic activity against *Colletotrichum* sp., was selected for molecular identification. The identification process began with DNA extraction using the Quick-DNA Magbead Plus Kit. The extracted DNA was subsequently amplified using the 2× MyTaq HS Red Mix kit. The resulting PCR products were then used for DNA sequencing (Rahayu & Nugroho, 2015). The results of PCR amplification were analyzed through electrophoresis. For isolate IR1A6, a clear band was observed at approximately 1700 bp (Figure 3), indicating successful amplification of the target gene fragment.

The sequencing data were edited using Geneious software to select high-quality DNA segments. The selected sequences were analyzed using BLAST on the NCBI website. BLAST analysis showed that isolate IR1A6 was closely related to Bacillus amyloliquefaciens (accession number PQ432901) with 100% query coverage, an E-value of 0.0, and 99.93% identity (Figure 4). *Bacillus amyloliquefaciens* is a plant growth-promoting bacterium widely studied as an environmentally friendly biological agent to support plant growth and increase productivity (Chen et al., 2007; Qiao et al., 2014). These bacteria stimulate growth and increase plant tolerance to both biotic stresses (pathogens) and abiotic stresses such as drought or salinity (Dimopoulou et al., 2021; Gamez et al., 2019; Kazerooni et al., 2021).

Studies have shown that *Bacillus amyloliquefaciens* can enhance growth parameters such as shoot weight, plant height, and root length, increase tomato yield by 47.93%, and reduce bacterial wilt disease by up to 55% (Do et al., 2024; Samaras et al., 2018). Additionally, these bacteria produce antifungal metabolites such as surfactin, iturin, fengycin, and indole acetic acid (IAA), which contribute to plant growth and biomass improvement (Shahid et al., 2021). According to Luo et al. (2022), *B. amyloliquefaciens* acts through various mechanisms, including increasing soil nutrient availability, regulating microbial populations, producing hormones and volatile compounds, inducing systemic resistance, and enhancing plant tolerance to abiotic stresses.

CONCLUSION

This study identified four isolates that showed the ability to inhibit the pathogen *Colletotrichum* sp. The isolates were IR1A4, IR1A6, IR1A8, and IR1D3. The ability of symbiont bacteria to suppress *Colletotrichum* sp. varied from 22.52% to 80.47% in vitro and from 7.89% to 18.42% in vivo. The IR1A6 isolate, which is closely related to *Bacillus amyloliquefaciens* (accession number PQ432901), was the most effective bacterial isolate in suppressing *Colletotrichum* sp. and was able to outperform the fungicide treatment.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Agricultural Production and the Laboratory of Plant Protection Politeknik Negeri Jember, for their support in research and equipment.

FUNDING

This research received no external funding.

AUTHORS' CONTRIBUTIONS

GFD and ADW conceived and planned the experiment. VHPS, ADM, and ADW carried out the isolation and screening of antagonistic bacteria, including the in vitro dual culture assay. ZAW, RAR, and GFD performed molecular work and analysis. DGC and RDN performed data analysis and interpreted the plant damage and weather data. GFD, ADW, and RDN contributed to writing, review, editing, and visualization. All authors contributed to manuscript preparation, read, and approved the final version.

COMPETING INTEREST

The authors declare no conflict of interest.

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Table 1. Antagonistic activity of termite symbiotic bacteria against *Colletotrichum* sp.

Isolate code	Inhibition activity (%)	Isolate code	Inhibition activity (%)	Isolate code	Inhibition activity (%)	Isolate code	Inhibition activity (%)
IR1A4	83.72	IR4D6	65.11	IR1A7	51.16	IR4D2	41.42
IR1A5	74.41	IR3C4	62.79	IR1A9	51.16	IR1A2	37.20
IR1A8	74.41	IR4D4	62.79	IR2B1	48.83	IR1A1	34.88
IR1A6	72.09	IR3C7	58.13	IR3C8	48.83	IR3C1	34.88
IR4D3	74.42	IR2B5	55.81	IR2B3	48.83	IR3C9	0
IR4D5	67.44	IR2B6	55.81	IR2B7	44.18	IR1A3	0
IR4D7	67.44	IR2B4	53.48	IR3C3	44.18		
IR3C2	65.11	IR3C5	51.16	IR3C6	41.86		
IR4D1	65.11	IR3C10	51.16	IR2B2	41.86		

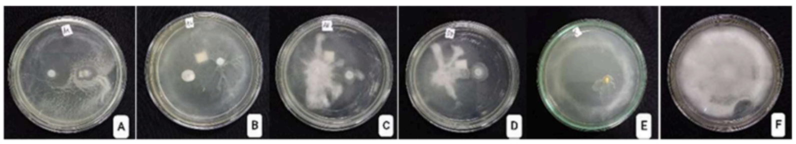

Figure 1. Antagonistic assay of bacterial isolates against *Colletotrichum* sp. on day 7. A. Isolate IR1A4; B. Isolate IR1A6; C. Isolate IR1A8; D. Isolates IR4D3; E. Fungicide treatment; F. Control.

Table 2. Mean width of *Colletotrichum* sp. mycelium growth against in the presence of bacteria isolates

Treatment	Mean mycelial line (cm)				Inhibition activity (%)
	4 DAI ($\bar{x}$ ± SD)	5 DAI ($\bar{x}$ ± SD)	6 DAI ($\bar{x}$ ± SD)	7 DAI ($\bar{x}$ ± SD)	
Control	4.33 ± 0.76 c	5.98 ± 0.33 b	6.90 ± 0.66 b	7.62 ± 0.26 c	-
IR1A4	2.60 ± 1.10 b	2.69 ± 1.37 a	2.74 ± 1.48 a	2.83 ± 1.54 a	62.90
IR1A6	1.88 ± 0.57 ab	1.83 ± 0.63 a	1.79 ± 0.59 a	1.49 ± 0.44 a	80.47
IR1A8	4.30 ± 0.47 c	5.26 ± 0.46 b	5.71 ± 0.48 b	5.88 ± 0.39 b	22.85
IR4D3	4.10 ± 0.55 c	5.29 ± 0.56 b	5.79 ± 0.47 b	5.90 ± 0.28 b	22.52
Fungicide	1.30 ± 1.27 a	1.69 ± 1.74 a	1.69 ± 1.66 a	1.79 ± 1.61 a	76.53

Numbers with the same letter within the same column are not significantly different, as determined by

Table 3. Average percentage of disease incidence on chili pepper fruits

Treatment	Average percentage of disease incidence on fruit (%)				Inhibition activity (%)
	5 DAI ($\bar{x}$ ± SD)	6 DAI ($\bar{x}$ ± SD)	7 DAI ($\bar{x}$ ± SD)	8 DAI ($\bar{x}$ ± SD)	
Control	80.00 ± 0.00 c	90.00 ± 0.00 d	92.50 ± 5.00 b	95.00 ± 5.77 b	-
IR1A4	52.50 ± 26.30 ab	62.5 ± 18.93 ab	70.00 ± 14.14 a	77.50 ± 9.57 a	18.42
IR1A6	40.00 ± 8.16 a	45.00 ± 12.91 a	70.00 ± 14.14 a	77.50 ± 9.57 a	18.42
IR1A8	40.00 ± 8.16 a	45.00 ± 5.77 a	67.50 ± 15.00 a	75.00 ± 12.91 a	21.05
IR4D3	67.50 ± 15.00 bc	82.50 ± 5.00 cd	87.50 ± 5.00 b	87.50 ± 5.00 ab	7.89
Fungicide	57.50 ± 22.17 abc	65.00 ± 17.32 bc	82.50 ± 5.00 ab	82.50 ± 5.00 ab	13.16

Numbers followed by the same letter within the same column are not significantly different based on Duncan's Multiple Range Test at the 5% significance level. DAI = days after inoculation.

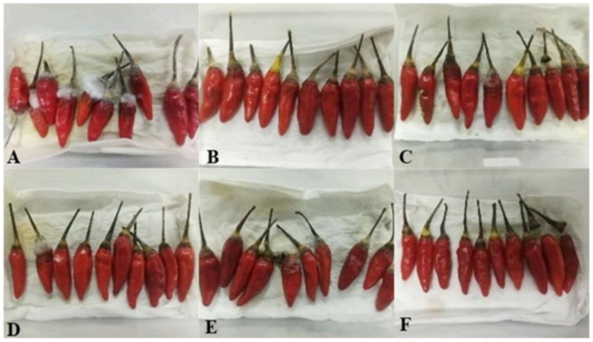

Figure 2. Symptoms of anthracnose on chili fruits at 8 days after inoculation. (A) Isolate IR1A8; (B) Isolate IR1A4; (C) Isolate IR1A6; (D) Isolate IR4D3; (E) Fungicide treatment; (F) Control

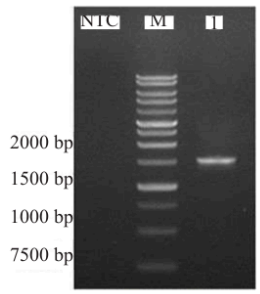

Figure 3. PCR product electrophoresis results.

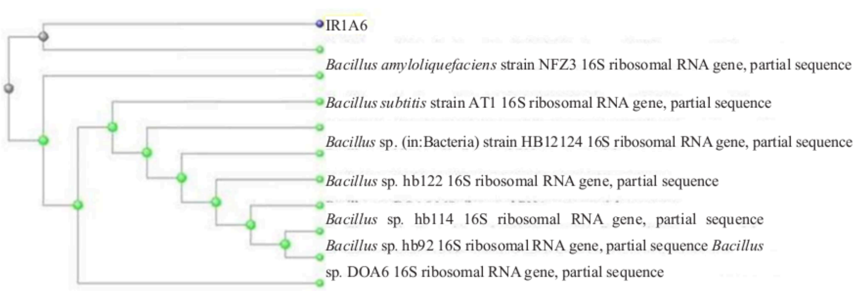

Figure 4. Phylogenetic tree of the IR1A6 isolate using the S-anger DNA Sequencing method with NCBI application.

Termite symbiont bacteria as biological agents against anthrachnose of bird's eye chilli caused by Colletotrichum sp.

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