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# SCREENING OF MICROBIAL BIOPESTICIDES AGAINST POST-HARVEST FUNGAL PATHOGEN *COLLETOTRICHUM* SPP: A PRELIMINARY STUDY

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**Abstract:** The most common fungal pathogens are found in the *Colletotrichum* genus, which causes economically significant anthracnose or blight disease in various tropical and subtropical fruits and vegetables. Despite that, the excessive application of chemical pesticides in agriculture has caused many problems, such as poor soil fertility, pollution, and risk to human health due to the toxic accumulation of pesticides in the soil. This study was carried out to isolate potential bacterial colonies from five different soil sources around Universiti Malaysia Terengganu (UMT) and to screen the isolated bacteria for antagonistic effects against *Colletotrichum* species. In total, 50 bacterial strains were isolated and subjected to selective screening to distinguish bacterial strains capable of inhibiting *Colletotrichum* sp. Based on the selective screening, nine bacterial strains with different morphology were selected and tested further in quantitative screening by calculating their Percentage of Inhibition of Radial Growth (PIRG). The results showed that S5-H strain demonstrated the highest inhibitory effect at (75.67 ± 2.86)%, followed by S5-I strain (68.89 ± 5.93)%, S5-D strain (66.89 ± 6.15)%, S3-C strain (60.33 ± 3.19)%, S5-F strain (57.89 ± 4.66)%, S5-G strain (53 ± 5.79)%, S2-I strain (41.33 ± 7.26)%, S4-B strain (40 ± 7.07)% and S3-D strain (38.33 ± 8.90)%. Based on the results, bacterial isolate S5-H with yellow, round, convex, and entire colony from mangrove muddy sediment was shown to have the potential to be utilized as biopesticides against *Colletotrichum* sp. Nevertheless, this study requires further work in the future, focusing on species identification by 16S rRNA phylogeny and the mechanism of action exhibited by the S5-H strain.

Keywords: Biopesticides, *Colletotrichum* sp., antagonistic effect, growth inhibition, blight disease.

## Introduction

The most common fungal pathogens are found in the *Colletotrichum* genus, which causes anthracnose or blight in various tropical and subtropical fruits and vegetables. According to Siddiqui and Ali (2014), nearly every crop cultivated worldwide is vulnerable to one or more fungal attacks from *Colletotrichum* spp. Besides that, diseases found in economically important crops such as cereal, legumes, vegetables, and tree fruits are often associated with the *Colletotrichum* genus. For example, 35 isolates representing species of *Colletotrichum* were obtained from chilies in Korea showing anthracnose disease symptoms (Oo *et al*., 2017), and 52 isolates of *Collectotrichum* spp. were collected from 24 strawberry farms in Taiwan (Chung *et al*., 2020). According to Poonpolgul and Kumphai (2007), anthracnose is a disease that causes severe economic losses, affecting between 10% and 80% of agricultural output in developing countries. Studies on the *Colletotrichum* genus have found that many phytopathogenic *Colletotrichum* spp. live a hemibiotrophic lifestyle (Gan *et al*., 2013) and are extremely varied, with distinct subgroups within a single species complex having varying host ranges and levels of virulence, ranging from destructive pathogens to potential endophytes (Bhagya *et al*., 2011). The fungi produce enzymes which enable the fungus to invade and harm their host. For instance, the fungus produces polygalacturonase and pectatelyase enzymes to degrade plant cell walls. The fungus

is also found to cause rotting on infected plants and fruits by emitting black spore masses (Siddiqui & Ali, 2014) and producing cellulase enzymes, which catalyze the breakdown of the host cell (Peeran *et al*., 2014).

Over the years, chemical pesticides have been used to manage harvests effectively against pests and diseases. Despite that, excessive usage of chemicals in agriculture has caused many problems, such as poor soil fertility, pollution, and risk to human health due to the toxic accumulation of chemicals in the soil (Toan *et al*., 2019). According to Petit *et al*. (2012), it is inevitable that fungicides will be applied to control pests on crop plants. *Propiconazole*, *Tricyclazole*, *Carbendazim*, *Metalaxyl*, and *Difenoconazole* are among the fungicides commonly used to treat the affected plants. These chemicals are categorized as Endocrine-Discupting Chemicals (EDCs). However, it may harm crop physiology, particularly photosynthesis (Petit *et al*., 2012). Other than that, resistance may arise as the pests can develop a mechanism to circumvent the mode of action by the chemicals, likely due to mutations in the target site of fungicides, which can prevent the fungicides from effectively binding their intended target (Cortaga *et al*., 2023). *Colletotrichum* species exhibit other resistance mechanisms, such as the ability to produce efflux pumps that actively pump out fungicides from the cell to reduce intracellular concentration of the antifungal agent (Liu *et al*., 2021). When pests are not properly controlled, they can destroy crops and reduce yields, resulting in economic damage and food crisis. Food security can be seriously jeopardized, especially in areas primarily relying on agriculture for survival (Saina *et al*., 2013). Moreover, farmers likely will apply more pesticides in greater quantities and from a wider range of classes of pesticides (Ntow *et al*., 2006). The excessive use of these chemicals can harm non-target organisms, causing ecological imbalances and biodiversity loss, and can lead to human health issues ranging from acute poisoning to chronic conditions and even more severe long-term consequences (Kumar *et al.,* 2013; Jaishankar *et al*., 2014).

Recently, numerous bacterial strains have been recognized as biocontrol agents that are considered a promising strategy against *Colletotrichum* species. *Bacillus* genus generates several bioactive molecules that have biocontrol properties against a variety of plant pathogens. For example, *Bacillus velezensis,* an antagonistic strain obtained from the soil rhizosphere of Korean ginseng farm, had shown biocontrol action against apple bitter rot by suppressing the mycelial development of the fungicide-resistant fungus (Kim *et al*., 2021). Similarly, *Bacillus amyloliquefaciens* was shown to have potential as a biocontrol agent of *Colletotrichum gloeosporioides* as the mycelial structure of *C. gloeosporioides* was damaged and distorted due to the repeated attack of *B. amyloliquefaciens* (Wang *et al*., 2020). Furthermore, *Bacillus subtilis* demonstrated growth inhibition efficacy against *Colletotrichum acutatum,* with colony reduction ranging from 64.7% to 67.5%. The mycelium growth diminished significantly when the fungal strains were grown on the agar surface with *B. subtilis,* and an increase in swelling hyphae was found along with the growth inhibition (Arroyave-Toro *et al.*, 2017).

Screening of more biocontrol agents against pests is essential due to the need for reduced reliance on chemical pesticides and toward sustainable bioremediation efforts. Therefore, this preliminary study is very important to isolate more bacteria from the soil with good potential as biopesticides by isolating potential bacterial colonies from five different soil sources from Universiti Malaysia Terengganu (UMT) and screening the isolated bacteria strain for antagonistic effect against *Colletotrichum* sp.

## Material and Methods

### *Sample Collection*

Samples were taken from five different soil sources from UMT. The five sources were from compost from Gheart Base (5°24.566'N, 103°5.251'E), muddy sediment from the lake near UMT Jaya Holding Sdn Bhd (5°24.608'N, 103°5.264'E), mangrove soil from mangrove

near hatchery (5°24.900'N, 103°5.090'E), soil sediment from a dumpster near Faculty of Ocean Engineering Technology and Informatics (FTKKI, 5°24.679'N, 103°5.298'E) and mangrove soil from mangrove near Biowalk (5°24.575'N, 103°5.503'E). The top 10 cm soil samples (4 g) were collected using a clean plastic spoon to avoid cross-contamination and transferred into a 15 mL Falcon tube. All soil samples were stored at 4°C until use.

### *Maintenance and Culture of Fungal Strain*

*Colletotrichum* sp. strain was obtained from the Laboratory for Pest, Disease and Microbial Biotechnology (LAPDiM), UMT. Glycerol stock of *Colletotrichum* sp. was streaked on Potato Dextrose Agar (PDA) and incubated at 30°C for seven days before being sub-cultured in PDA for further use in this study.

### *Isolation of Bacterial Strains*

Serial dilution of soil samples was performed by mixing 0.1 g of the soil in 0.9 mL of sterile distilled water. The mixture was serially diluted up to $10^{-6}$ (Nasran *et al.,* 2020). Next, Luria-Bertani (LB) agar was prepared by mixing 5 g of yeast extract, 10 g of tryptone, 10 g of sodium chloride, and 16 g of agar in 1 L of distilled water. LB media was autoclaved at 120°C for 20 minutes. Then, 0.1 mL of dilution was transferred and spread on the surface of the LB agar plate before being incubated for 24 hours at 30°C. Ten bacterial colonies were selected based on growth performance and subcultured multiple times using the streaking method until pure colonies were obtained.

### *Selective Screening of Bacterial Strain Capable of Inhibiting Colletotrichum* sp.

Initially, *Colletotrichum* sp. was cultured on PDA and incubated at 30°C for 72 hours. The isolated bacteria strains were inoculated around the fungal pathogen at a distance of 3.0 cm using the streaking method. The treatment plate consists of *Colletotrichum* sp. and bacteria strain, while the control plate only contains *Colletotrichum* sp. and distilled water. Both plates were incubated at 28°C for five days.

### *Quantitative Screening of Bacterial Strain Capable of Inhibiting Colletotrichum* sp.

The bacteria strains that showed a positive response where the bacteria were able to grow against the fungal pathogen and inhibit the fungal growth above 50% in selective screening were selected for quantitative screening. The screening method for quantitative screening was conducted in the same manner as selective screening in section 2.4. Three replicates of quantitative screening for the selected bacteria strain were carried out to record the average inhibition of radial growth.

### *Determination of Antagonistic Effect of Isolated Bacterial against Colletotrichum sp.*

The data for the Percentage of Inhibition of Radial Growth (PIRG) were recorded after the five days of the incubation period by measuring the growth radius of *Colletotrichum* sp. using the following formula (1):

$$\text{PIRG (\%)} = (R1 - R2) / R1 \times 100, \quad (1)$$

where R1 denotes the radial growth of fungal strain in the control plate, and R2 indicates the radial growth of fungal strain in the treatment plate.

## Result and Discussion

In this study, a total of 50 pure bacterial strains were isolated from five different soil sources. Two factors that influenced the selective screening of bacteria strains capable of inhibiting *Colletotrichum* sp. are the ability of the isolated bacteria to grow against the fungal pathogen and the PIRG (%). Based on the results, nine bacteria strains were selected as positive samples due to their strong ability to grow against the fungal pathogen, and the PIRG (%) was above 50%. The nine selected bacteria are S2-I from lake muddy sediment, S3-C and S3-D from dumpster soil sediment, S4-B from mangrove muddy sediment at biowalk, and S5-D, S5-F, S5-G, S5-H, S5-I from mangrove muddy sediment beside hatchery (Table 1). On the other hand, 41 bacteria strains were considered negative samples due to their weak ability to grow against the fungal pathogen, and the PIRG (%) was 50% and below (Table 2).

Table 1: The selective screening of bacterial strain capable of inhibiting *Colletotrichum* sp. that exhibits a positive response after five days of incubation at 30°C

| Isolate | Fungal growth, in Diameter (cm) | | | Inhibitory effect (%) |
|---|---|---|---|---|
| | **Day 0** | **Day 3** | **Day 8** | |
| S2-I | 0.5 | 0.7 | 1 | 75 |
| S3-C | 0.5 | 0.8 | 1 | 75 |
| S3-D | 0.5 | 0.8 | 1 | 75 |
| S4-B | 0.5 | 0.9 | 2 | 50 |
| S5-D | 0.5 | 0.7 | 1 | 75 |
| S5-F | 0.5 | 0.8 | 1.1 | 72.5 |
| S5-G | 0.5 | 0.9 | 1 | 75 |
| S5-H | 0.5 | 0.9 | 1 | 75 |
| S5-I | 0.5 | 0.7 | 1 | 75 |
| Control plate | 0.5 | 0.9 | 4 | 0 |

Table 2: The representative of negative samples from selective screening that exhibits no inhibitory effect after five days of incubation at 30°C

| Isolate | Fungal Growth, in Diameter (cm) | | | Inhibitory effect (%) |
|---|---|---|---|---|
| | **Day 0** | **Day 3** | **Day 8** | |
| S1-C | 0.5 | 0.8 | 2.5 | 37.5 |
| S1-D | 0.5 | 0.8 | 2.5 | 37.5 |
| S1-E | 0.5 | 0.8 | 2.7 | 32.5 |
| S1-F | 0.5 | 0.8 | 2.7 | 32.5 |
| S2-E | 0.5 | 0.9 | 3 | 25 |
| S2-F | 0.5 | 0.9 | 3 | 25 |
| S4-E | 0.5 | 0.9 | 2.4 | 40 |
| S4-F | 0.5 | 0.9 | 2.4 | 40 |
| S4-G | 0.5 | 0.9 | 2.7 | 32.5 |
| S4-H | 0.5 | 0.9 | 2.7 | 32.5 |

Based on the selective screening results (Table 1), S2-I, S3-C, S3-D, S4-B, S5-D, S5-F, S5-G, S5-H, and S5-I were the top nine isolated bacteria that have PIRG (%) ranging from 55-75% and have high ability to grow against the fungal pathogen. The colony morphology of all nine isolates was varied. The colony morphology of S2-I was white, round, and flat. In contrast, S3-C has a white, mucus-like texture and round colony, while S3-D strain has irregular, opaque, smooth surface colonies. S4-B has an opaque, brittle, flat, and round colony. The colony morphology of S5-D and S5-H is yellow, but S5-D has punctiform and round colonies, while S5-H has round, convex, and entire colonies. Moreover, the S5-F strain has a white, viscid, and round colony. Meanwhile, S5-G and S5-I have translucent and irregular colonies, but their texture is different, where S5-G has a viscid texture colony, and S5-I has a mucus-like colony (Table 3).

Table 3: Observed colony morphology of the selected isolated bacteria on the Luria-Bertani (LB) plate

| Isolate | Shape | Colony Color | Margin | Elavation |
|---|---|---|---|---|
| S2-I | Circular | White | Undulate | Flat |
| S3-C | Circular | White | Undulate | Raised |
| S3-D | irregular | Creamy white | Undulate | Flat |
| S4-B | Circular | Creamy white | Filiform | Flat |
| S5-D | Circular | Yellow | Filiform | Raised |
| S5-F | Circular | White | Filiform | Raised |
| S5-G | Irregular | translucent | Entire | Raised |
| S5-H | Circular | Yellow | Filiform | Raised |
| S5-I | Irregular | translucent | Filiform | Raised |

Several studies have shown that if an isolate's PIRG (%) is above 50%, it has a high potential to be a biofungicide with antifungal activity against fungal pathogens. For instance, the soil bacterium *Streptomyces* sp. 20C1 has a 96% inhibition rate, while *Saccharomonospora* spp. 21A2 (Ting *et al*., 2010) and *Paenibacillus polymyxa* Kp10 (Nasran *et al*., 2020) had 78% and 66.52% inhibition rates, respectively. All were isolated from the soil. Furthermore, *Bacillus subtilis* ATCC 55614 and *Bacillus velezensis* TS3B-45, which were isolated from mango leaves, recorded PIRG values of 77% and 80%, respectively (Reyes-Estebanez *et al*., 2020).

Quantitative screening results demonstrated that the highest inhibition effect by S5-H strain at (75.67 ± 2.86)%, followed by S5-I strain at (68.89 ± 5.93)%, S5-D strain at (66.89 ± 6.15)%, S3-C strain at (60.33 ± 3.19)%, S5-F strain at (57.89 ± .66)%, S5-G strain at (53 ± 5.79)%, S2-I strain at (41.33 ± 7.26)%, S4-B strain at (40 ± 7.07)% and S3-D strain at (38.33 ± 8.90)% (Table 4). As expected, the control treatment, in which only distilled water was inoculated, recorded no inhibition effect.

Table 4: Antagonistic effect of selected bacteria isolates on *Colletotrichum sp.* after five days of incubation at 30ºC

| Isolate | Sample Origin | Inhibitory effect (%) |
|---|---|---|
| S2-I | lake muddy sediment | 41.33±7.26 |
| S3-C | dumpster soil sediment | 60.33±3.19 |
| S3-D | dumpster soil sediment | 38.33±8.90 |
| S4-B | mangrove muddy sediment (Biowalk) | 40±7.07 |
| S5-D | mangrove muddy sediment (Hatchery) | 66.89±6.15 |
| S5-F | mangrove muddy sediment (Hatchery) | 57.89±4.66 |
| S5-G | mangrove muddy sediment (Hatchery) | 53±5.79 |
| S5-H | mangrove muddy sediment (Hatchery) | 75.67±2.86 |
| S5-1 | mangrove muddy sediment (Hatchery) | 68.89±5.93 |
| Control | - | 0±0.00 |

Based on Table 4, S5-H, isolated from mangrove sediment at Mangrove Forest near Hatchery in UMT, has the highest potential as biopesticides against *Colletotrichum* sp. among the others due to the highest inhibition percentage of fungal radial growth at 75.67%. Morphology identification of the S5-H strain was a yellow colony with a slimy texture, convex elevation, a shiny, smooth surface, and a circular-shaped colony. The morphology of bacteria isolated from mangrove sediments that is circular, yellow, and convex shape is typically from the genus *Sphingomonas* and *Mycobacterium* (Zhou *et al*., 2008; Guo *et al.,* 2010). Therefore, S5-H might belong to the genera *Sphingomonas* or *Mycobacterium* since there are similarities in their colony morphology. However, species identification cannot be solely based on morphology characterization. It requires biochemical tests and 16S rRNA DNA sequencing to identify the species.

There are many other bacteria species that are known as microbial biocontrol agents against *Colletotrichum* sp. According to Nasran *et al.* (2020), *Paenibacillus polymyxa* has been proven to inhibit *Colletotrichum* species through a dual culture test. Moreover, *Staphylococcus sciuri* (Alijani *et al.,* 2019), *Serratia* sp. (Granada *et al*., 2020), *Bacillus subtilis* (Reyes-Estebanez *et al.,* 2020), *Pseudomonas aeruginosa* (Sandani *et al*., 2019) and *Strenotrophomonas rhizophila* (Reyes-Perez *et al.,* 2019) are considered effective microbial biocontrol agents against *Colletotrichum* species in a variety of post-harvest fruits. Some studies have associated the antifungal properties of biofungicide antagonists against *Colletotrichum* species to the actions of cell wall-degrading enzymes such as chitinase and -1-3-glucanase produced by *Streptomyces* species to restrict the growth of the fungus (Mukherjee & Sen, 2006; Choudhary *et al.,* 2014) and production of extracellular metabolites, such as hydrolytic enzyme that suppress pathogenic plant fungi (Prapagdee *et al*., 2008). Furthermore, according to Alfaro-vargas *et al*. (2022), scanning electron microscopy (SEM) images of *Trichoderma asperellum* as a biofungicide of *Colletotrichum* sp. showed that it generates damage to the hyphae morphology and spores of the fungi by producing peptaibols. Separately, the genus *Bacillus* is reported to produce lipopeptides, which can inhibit fungi by stimulating the host's defense mechanisms as well as acting as an antibiosis agent (Choudhary & Johri, 2009). Hence, this evidence can support that the S5-H strain may have one or more antifungal mechanisms that caused the inhibition of radial growth of *Colletotrichum* sp. and will be addressed in the next communication.

## Conclusion

In conclusion, the present study highlighted nine bacteria isolates that successfully grew against *Colletotrichum* sp., and one bacterial isolate recorded the highest inhibitory effect on the mycelial growth of the fungal pathogen at (75.67 ± 2.86)%. Bacterial isolate S5-H from mangrove muddy sediment was shown to inhibit *Colletotrichum* sp. through a screening test that could be utilized as biopesticides. Nevertheless, this study requires further work focusing on species identification using 16S rRNA phylogeny assessment on the S5-H strain and elucidating the mechanism of inhibition by the S5-H strain.

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## Conflict of Interest

The authors declare that they have no conflicts of interest that might influence the outcome of the work reported.

**References**

Akinpelu, D. A., Abioye, E. O., Aiyegoro, O. A., Akinpelu, O. F., & Okoh, A. I. (2015). Evaluation of Antibacterial and Antifungal Properties of Alchornea laxiflora (Benth.) Pax. & Hoffman. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 684839. https://doi.org/10.1155/2015/684839

Cother, N. J., & Priest, M. J. (2009). A simple and effective method for the elimination of bacteria from fungal cultures. *Australasian Plant Pathology*, *38*(2), 132–134. https://doi.org/10.1071/AP08096

Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., & Sarniguet, A. (2011). Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews*, *75*(4), 583–609. https://doi.org/10.1128/MMBR.00020-11

Gomez-Gil, B., Fajer-Avila, E., & García-Vargas, F. (2007). Vibrios of the spotted rose snapper Lutjanus guttatus Steindachner, 1869 from northwestern Mexico. *Journal of Applied Microbiology*, *102*(6), 1518–1526. https://doi.org/10.1111/J.1365-2672.2006.03199.X

Ismail, N. A., Kasmuri, N., Hamzah, N., Jaafar, J., Mojiri, A., & Kindaichi, T. (2023). Influence of pH and concentration on the growth of bacteria - fungus and benzo[a] pyrene degradation. *Environmental Technology & Innovation*, *29*, 102995. https://doi.org/10.1016/J.ETI.2022.102995

Ko, S. S., Kunimoto, R. K., & Ko, W. H. (2001). A simple technique for purifying fungal cultures contaminated with bacteria and mites. *Journal of Phytopathology*, *149*(9), 509–510. https://doi.org/10.1046/J.1439-0434.2001.00662.X

Mensah-Attipoe, J., Toyinbo, O., Mensah-Attipoe, J., & Toyinbo, O. (2019). Fungal growth and aerosolization from various conditions and materials. In *Fungal infection*. Intech Open. https://doi.org/10.5772/INTECHOPEN.81565

Nottingham, A. T., Hicks, L. C., Ccahuana, A. J. Q., Salinas, N., Bååth, E., & Meir, P. (2018). Nutrient limitations to bacterial and fungal growth during cellulose decomposition in tropical forest soils. *Biology and Fertility of Soils*, *54*(2), 219–228. https://doi.org/10.1007/s00374-017-1247-4

Popowska, M., Luis Balcazar, J., Giraud, E., Baron, S., Granier, S. A., Larvor, E., Jouy, E., Cineux, M., Wilhelm, A., Gassilloud, B., Le Bouquin, S., Kempf, I., & Chauvin, C. (2017). Aeromonas diversity and antimicrobial susceptibility in freshwater-an attempt to set generic epidemiological cut-off values. *Frontiers in Microbiology*, *8*, 503. https://doi.org/10.3389/fmicb.2017.00503

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H. W., Perrone, G., Seifert, K. A., Susca, A., Tanney, J. B., Varga, J., Kocsubé, S., Szigeti, G., Yaguchi, T., & Frisvad, J. C. (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. Studies in Mycology, *78*(1), 141. https://doi.org/10.1016/J.SIMYCO.2014.07.004

Serwecinska, L. (2020). Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. *Water*, *12*(3313). https://doi.org/10.3390/w12123313

Shi, X.-X., Qiu, H.-P., Wangid, J.-Y., Zhang, Z., Wang, Y.-L., & Sun, G.-C. (2019). A handy method to remove bacterial contamination from fungal cultures. *PLOS ONE*, *14*(11). https://doi.org/10.1371/journal.pone.0224635

Zulkifli, N. A., & Zakaria, L. (2017). Morphological and molecular diversity of aspergillus from corn grain used as livestock feed. *Journal of Biosciences*, *24*(1), 26–34. https://doi.org/10.1016/J.HJB.2017.05.002