Biocontrol Potential of *Bacillus subtilis* strain 20W33 Against *Colletotrichum* sp. Causing Anthracnose in Avocado

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Abstract

Anthracnose, caused by *Colletotrichum* sp., is a major disease of avocado occurring in both field and postharvest stages. The pathogen was isolated from avocado as a sead in Mae Hai Market, Hang Dong, Chiang Mai, Thailand. Morphological characteristics and pathogenicity tests confirmed its role as the causal agent. Molecular identification based on ITS region analysis using primers ITS1 and ITS4 revealed a 5.8S rRNA fragment of approximately 580 bp, classifying the isolate within the genus *Colletotrichum*, although species-level identification was inconclusive. The antagonistic activity of *Bacillus subtilis* strain 20W33 was assessed using the dual culture method, showing a maximum inhibition of 51.59 ± 1.90%, which indicates a very low efficiency inhibition. Only cell culture inhibited fungal growth in agar well diffusion assays. Reducing sugar was very low (6.17 × 10^{-2} ± 0.0158 g/mL), indicating minimal chitinase production. The results suggest that further investigation is necessary before use *B. subtilis* 20W33 as a biological control agent against anthracnose in the future.

Introduction

Avocado (*Persea americana*) is currently affected by anthracnose cause by *Colletotrichum* sp. during both pre- and postharvest stages, characterized by brown to black necrotic lesions on leaves and fruits, resulting in significant economic losses. Although chemical fungicides are effective, their overuse leads to resistance and harmful residues. Biological control has therefore emerged as a safer alternative. *Bacillus subtilis* has been reported to inhibit *Colletotrichum* through the production of antifungal enzymes and metabolites. This study aimed to identify the causal pathogen and evaluate the potential of *Bacillus subtilis* strain 20W33 to inhibit *Colletotrichum* sp. under laboratory conditions.



Objectives

1. To evaluate the inhibitory efficacy of *Bacillus subtilis* strain 20W33 against the mycelial growth of the causal agent of anthracnose disease in avocado.
2. To characterize the fungal pathogen causing anthracnose disease in avocado.

Methodology

Study of morphological charateristic.

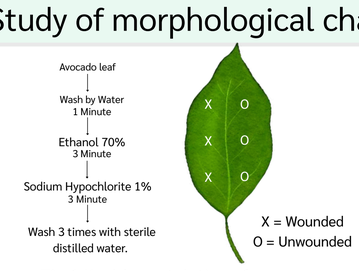


Fig 1 Koch's postulation of *Colletotrichum* sp. in Avocado leaf

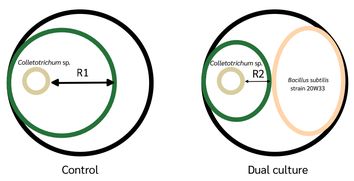


Fig 2 the dual culture technique to test the efficacy of *Bacillus subtilis* strain 20W33 aginst *colletotrichum* sp.

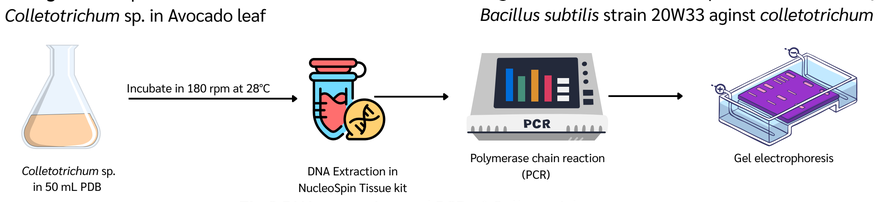


Fig 3 DNA extraction and PCR of *Colletotrichum* sp.

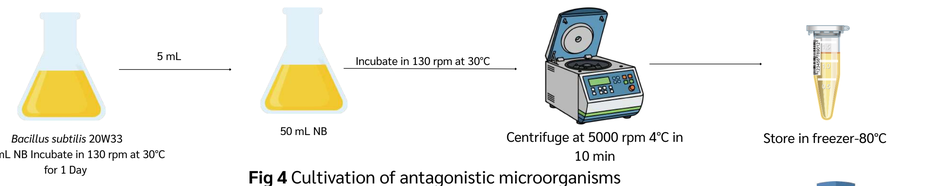


Fig 4 Cultivation of antagonistic microorganisms

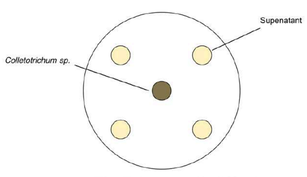


Fig 5 Agar well diffusion

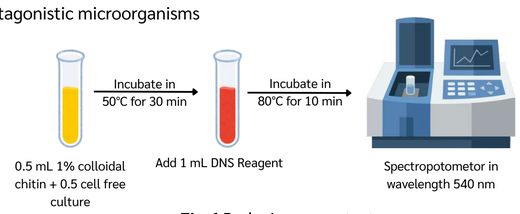


Fig 6 Reducing sugar test

Results & Discussion

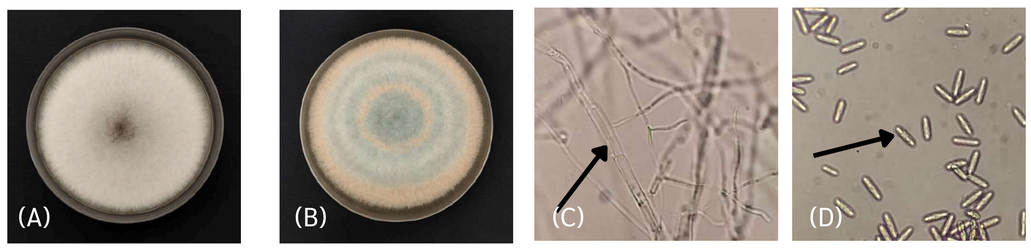


Fig 7 A) Morphological characteristic of *Collectotrichum* sp. (A) at 7 day (B) at 14 day C) hyphae D) conidia



Fig 8 Koch's postulation of *Colletotrichum* sp. in Avocado leaf in 14 day

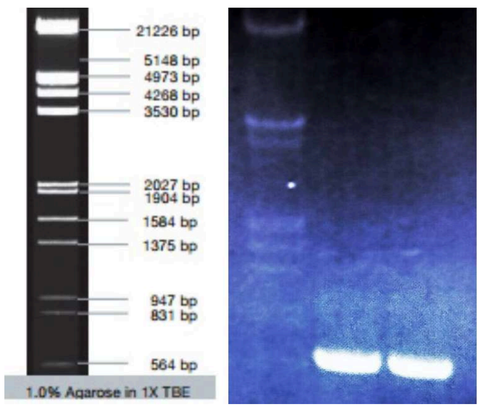


Fig 9 PCR amplification of the ITS region of *Colletotrichum* sp. yielded a fragment of approximately 580 bp.

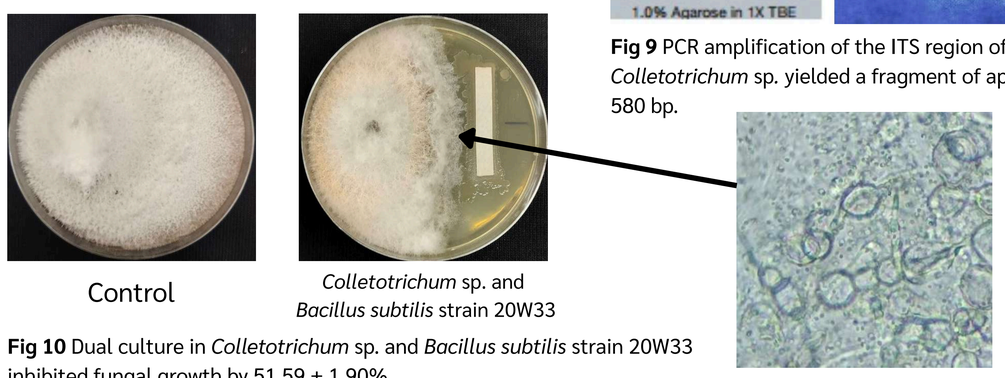


Fig 10 Dual culture in *Colletotrichum* sp. and *Bacillus subtilis* strain 20W33 inhibited fungal growth by 51.59 ± 1.90%.

Fig 11 Swollen hyphae

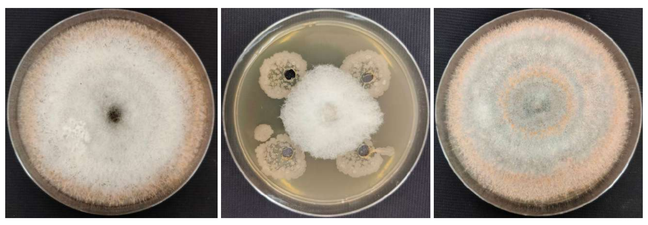


Fig 12 Agar well diffusion of *Collectotrichum* sp. with cell culture and cell free culture

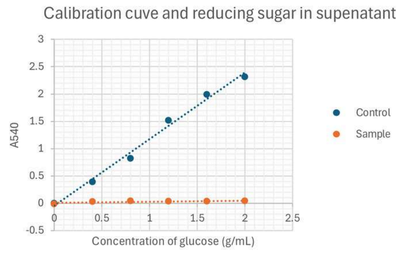


Fig 13 reducing sugar test was low (6.17 × 10^{-2} ± 0.0158 g/mL)

Conclusion

- The pathogenic fungus caused anthracnose and showed a 580-bp ITS region, suggesting it belongs to *Colletotrichum* sp., though species identification was inconclusive.
- *Bacillus subtilis* strain 20W33 inhibited fungal growth by 51.59 ± 1.90% which indicates a low efficiency inhibition.
- Only cell culture inhibited the fungus; cell free culture showed no effect.
- Reducing sugar was very low (6.17 × 10^{-2} ± 0.0158 g/mL), indicating minimal chitinase production.
- Therefore, *B. subtilis* 20W33 has potential as a biological control agent against anthracnose.

Acknowledgements

- Division of Biochemistry and biochemical innovation, Department of Chemistry, Faculty of Science,Chiang Mai University.
- Chiang Mai Agricultural Technology Promotion Center (Palnt Protection), DOAE.

Reference: Muhammad *et al*. (2016). Isolation of a potential antifungal *Bacillus subtilis* 37-JM07 strain from straw and its biocontrol efficacy to combat green mold disease of commercial mushroom, *Pleurotus ostreatus*, 12(3), 91-103.Heo *et al.* (2024). Concentration Characterization and evaluation of *Bacillus subtilis* GYUN-2311 as a biocontrol agentagainst *Colletotrichum* spp. on appleand hot pepper in KoreaFront. Microbiol. 14(1322641). K. S Kimaru *et al*. (2020). Effects of anthracnose disease on avocado production in kenya. cogent food & agriculture, 6(1799531), 1 - 14.