Title : Biocontrol Potential of *Bacillus subtilis 20W33* Against *Colletotrichum sp.* Associated with Anthracnose Disease in Avocado

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ABSTRACT

Anthracnose is a widespread disease affecting many plant species, including avocado. The disease manifests on both leaves and fruits, occurring in the field as well as during postharvest storage. Anthracnose is caused by fungi in the genus *Colletotrichum sp.* In this study, the pathogen was isolated from avocado fruits obtained from Mae Hia Market and Hang Dong District in Chiang Mai , Thailand. Then the fungus was cultured on potato dextrose agar (PDA), the fungal mycelia initially appeared white and fluffy, as the colony matured, it developed to a white-to-orange coloration. Microscopic observation revealed that the conidia were oval in shape. Pathogenicity tests were conducted by re-inoculating the fungus onto healthy avocado leaves using both wounded and unwounded methods. After 10–15 days, brown necrotic lesions that expanded into circular spots with dark discoloration were observed on the wounded leaves. The fungal isolate was further identified using molecular techniques by analyzing the internal transcribed spacer (ITS) region of ribosomal DNA with ITS1 and ITS4 primers, followed by sequence similarity analysis.The antagonistic activity of *Bacillus subtilis* strain 20W33 against the fungal pathogen was evaluated using the dual culture method, which demonstrated an inhibition rate of 28.04, 22.10, 39.09 % in 5 days. In addition, the effect of secondary metabolites produced by *Bacillus subtilis* 20W33 on fungal growth was assessed using the agar well diffusion method. The optimal incubation period for secondary metabolite production was determined by measuring chitinase enzyme activity. Chitinase activity was evaluated by quantifying reducing sugars released from chitin hydrolysis using the 3,5-dinitrosalicylic acid (DNS) method. It was found that at all bacterial growth stages, chitin degradation occurred, with an average

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reducing sugar content of 0.061711 mg. Therefore, further studies are required to determine the optimal timing for applying the antagonistic microorganism to effectively control the plant pathogen.

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