How Do Endophytic Bacteria From Mango Affect the Growth of Fungus Botrytis cinerea and the Use of 16S rDNA Sequence To Identify Bacteria?

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The objective of this research was to test the effect of endophytic bacteria on growth of fungus, Botrytis cinerea. Branches from mango were cut into 5-6 centimeter pieces, surface sterilized and ground in saline solution using sterile mortars and pestles. One hundred microliters of plant suspension were plated on Tryptic Soy Agar medium. Colonies were observed after 48 hours of incubation. There were two types of colonies observed, both round in shape, and either creamy bright yellow or white in color. Total eight bacterial isolates, four yellow colonies and four white colonies, were grown in TS broth for 24 hours, then inoculated on TSA plates. B. cinerea agar discs were placed in the middle of the plates containing bacteria and on blank TSA as control. At 9 days after incubation, the diameter of fungal colonies were measured. For control, the average of fungal diameters was 18.67 mm. Limited fungal growth was observed in all bacteria tested. In comparison, B. cinerea growth was slower than C. gloeosporioides. The molecular identification of bacteria was continued using four bacterial isolates from previous research. The 16S rDNA fragments were successfully amplified, purified, and cloned into pGemT vector. Transformation was done using E. coli JM109 as host cells. White colonies indicating the inserted vectors as well as blue colonies indicating unsuccessful insertion were observed. Six white colonies from each of four isolates were subsequently grown and plasmid DNA was isolated. The polymerase chain reaction using Sp6/T7 primers was used to confirm the presence of the 16S rDNA inserts in all 24 isolated plasmids/clones. Two clones from each isolate were sent to a company for sequencing.

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