Molecular and Biochemical Detection of Fusarium Oxysporum F.sp. Cubense as the Causal Pathogen of Fusarium Wilt Disease on Banana (*Musa* Spp.)

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ABSTRACT

The study was conducted to detect several isolates of *Fusarium oxysporum* f.sp. *cubense* (*Foc*) through molecular analysis and activity of their extracellular enzyme. Six isolates of *Foc* collected from some locations in Indonesia were artificially inoculated on "Ambon Kuning" cultivar of banana plant to assess their pathogencity levels in causing *Fusarium* wilt disease. For molecular analysis, the DNA of one-week culture isolates was extracted by three different extraction methods prior to PCR assay using specific primer for tropical race 4 (TR4) of *Foc*; whilst the activity of their extracellular enzyme was consecutively determined with reduction sugar, agar diffusion and SDS-PAGE assays. Statistical analysis revealed that all isolates insignificantly caused *Fusarium* wilt symptoms on tested banana with disease severity index (DSI) ranging from 3, which were exposed by Bnt1 and Lmp3 isolates, to 3.6 by Btu3 isolate. The maximum DNA concentration was obtained by CTAB extraction method (766.25 µg mL^{-1} in average), followed by SDS and Alkaline Lyses extraction methods with 553.75 and 211.25 µg mL^{-1} in average, respectively. PCR analysis showed that only Bnt2 and Kjg1 isolates were positively reacted to a pair of specific primer for TR4 of *Foc*, with DNA size of 1400 bp approximately. Both reduction sugar and agar diffusion assays demonstrated that Kjg1 isolate significantly produced more extracellular enzyme which was indicated by its maximum concentration (6.53 x 10^{-2} mg mL^{-1}) and its largest halo diameter on HCl-amended PDA (20 mm). Meanwhile, SDS-PAGE assay viewed diverse bands of tested fungi ranging from 20.6 (Btu3 isolate) to 80 kDa (Kjg1 isolate) which represented four different extracellular enzymes. The positive PCR results of Bnt2 and Kjg1 isolates molecularly highlighted the presence of TR4 of *Foc* infecting banana in Indonesia. This study also concluded that various activities of extracellular enzymes did not influence the virulence level of *Foc* isolates.