

Bio-Control of *Alternaria alternata* during Banana Storage by Purified AFP Using Isoelectric Focusing Technique

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Abstract

Interestingly, antifungal protein AFP was purified from *Aspergillus giganteus* supernatants with modified isoelectric focusing procedure after adaptation of the secretion conditions. Subsequently, the antifungal activity as well as the mode of action against *Alternaria alternata* was tested *in vitro*. Moreover, different concentrations of AFP were applied to banana fruits for 15 days at 20°C *in vivo*. Obtained results illustrated that *A. giganteus* was able to secrete about 39.78 ± 2.39 mg AFP·l⁻¹ Olson medium. The employed ammonium sulfate (AS 75%) precipitation procedure followed by dialysis steps yielded about 16 - 22 mg AFP·l⁻¹ culture supernatant with general mean of 18.67 ± 1.98 mg·l⁻¹. The lost amount of AFP during purification using AS and 3KDa cut-off dialysis membrane is about 50% thus, purification procedure must be further improved. Indeed, concluded results from MIC and hyphal extension inhibition test noticed that AFP was efficiently affected by either growth or hyphae form of *A. alternata in vitro*. The MIC of AFP against *A. alternata* was 2 µg·ml⁻¹. However, short, thick and highly septated hyphae with damaged constricted apical regions extruding from condensed mycelium aggregates in treated hyphae compared to the untreated culture was remarkably shown. The mode of action of *in vitro* experiment manifested that AFP was effective to act the fungal cell and permeabilize the cell membrane of *A. alternata*. Furthermore, the *in vivo* experiment showed that AFP could reduce post-harvest decay on banana caused by *A. alternata*. AFP at concentration of 15 and 25 µg·ml⁻¹ exhibit *Alternaria* decayed reduction by 45.45% and 77.27%, respectively. While no *Alternaria* decayed area was observed when 50 µg·ml⁻¹ was applied during the storage time. Quantification of DNA by species-specific PCR could exude a positive correlation between the DNA amount and decayed area. In conclusion, AFP can be efficiently used as a bio-preservative agent during storage and handling of banana fruits, and considered as an excellent biological alternative to combat secondary growth of filamentous fungi.

Keywords

Antifungal Protein (AFP), *Alternaria alternata*, Isoelectric Focusing, Antifungal Activity,