PRELIMINARY PRODUCTION OF CHITINASES OF NATIVE STRAINS OF *Isaria fumosorosea* Wize IN A SOLID CULTURE MEDIA

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Introduction. *Isaria fumosorosea* Wize has been successfully used as a biocontrol agent of whiteflies and other insect pests (1). Like most entomopathogenic fungi, *I. fumosorosea*, infects its host by breaching the cuticle (2). Various metabolites allow the pathogen to physically penetrate the host as well as inhibit its regulatory system, these include proteases, chitinases, and lipases (3). These enzymes allow the fungus to breach the insect cuticle and disperse through the hemocoel. Different studies suggest proteases and chitinases as major determinants of fungal virulence in the complex and multifactorial phenomenon insect host/pathogen relationship (4).

Due to the importance of knowing the ability of native strains to produce enzymes involved in the processes of penetration to the target insect cuticle and thus determine its potential as a possible biological control of pests of agricultural importance, the objective of this study was to evaluate the production of chitinases native strains of *I. fumosorosea* on solid culture medium.

Methods. All fungi were grown on potato dextrose agar for 14 days at 25 ± 2 º C. After were prepared suspensions of 1 x 10^7 conidia ml-^{1} for inoculate petri dishes that contained chitinases medium ($NH_4H_2PO_4$, KCl, $MgSO_4$ .7H2O, $CaCl_2$, bacteriological agar and 1% colloidal chitin) in which was placed a sterile filter paper circle in the center, then the suspension was added and incubated at 25 ± 2 º C. The activity measurement was performed at 120 h of incubation. For the interpretation of the enzymatic reactions we used the criterion that relates the speed of growth and enzyme activity by the activity index which is equal to: IA (activity index) = total diameter colony + halo/ diameter of colony (5). The results were subjected to ANOVA using the IBM ® SPSS v.19 Inc., to compare the production of chitinases from fungi tested. The experiments were performed in triplicate and repeated at least twice.

Results. The production of chitinases among the strains tested showed no significant differences (p> 0.05) at 120 h of incubation (Figure 1). Although the production of enzymes involved in the penetration of the cuticle of the insect has been considered an important pathogenicity factor (6) can be observed between different isolates variations associated with virulence including the production of chitinases, due to the tolerance and specificity of the host and as a result of the genetic variability of each of these isolates (7).

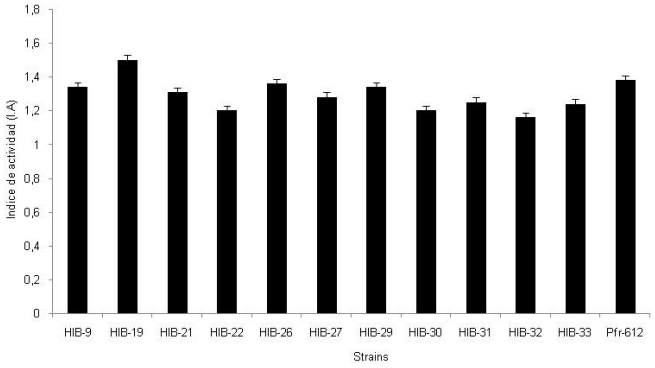

Figure 1. Production of chitinases among different strains of *I. fumosorosea* at 120 h of incubation under laboratory conditions, 25 ± 2 ºC. Lines in the bars indicate the standard error.

Conclusions. The strains tested showed chitinolytic enzyme production by the formation of translucent halos on agar plates with 1% colloidal chitin, however no significant differences in enzyme production between strains tested. This method is useful for analize a broad spectrum of strains for determine their preliminary chitinase activity.

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