

Purification and characterization of a novel antifungal class I chitinase from the latex of *Ficus benjamina* L.

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Introduction: *Ficus benjamina* is a lactiferous tree from Southeast Asia, although distributed worldwide. It is resistant to several diseases and its latex is rich in defense proteins. **Objective:** To purify a chitinase from *F. benjamina* latex and test it against phytopathogenic fungi. **Material and Methods:** The latex was harvested in cold 50 mM Tris-HCl buffer, pH 8.5, containing 1 mM iodoacetamide. To remove rubber, samples were dialyzed against distilled water at 4 °C and centrifuged at 12000 x g, 20 min, 4 °C. The latex proteins were fractionated with ammonium sulfate and the 30-60% fraction subjected to affinity chromatography on chitin column. The purified chitinase (FbLx-Chi-1) was analyzed to establish optimal pH and temperature; heat, pH, and salinity stability; and the effects of metallic ions and DTT on the enzyme activity. The antifungal activity of FbLx-Chi-1 was assessed against *Colletotrichum gloeosporioides*, *Fusarium pallidoroseum*, and *F. oxysporum*. **Results and Discussion:** The protein peak eluted with 10 mM acetic acid from the chitin column loaded with the 30-60% fraction showed a unique band of 30 KDa (FbLx-Chi-1) in SDS-PAGE, which is similar to the MW of other plant chitinases, and has a N-terminal sequence similar to other class I chitinases. FbLx-Chi-1 showed maximum activity at 60 °C, pH 5.5, and was stable up to 60 °C and at a pH range of 2-10. NaCl, up to 250 mM, did not interfere with the chitinolytic activity. Mg²⁺ and Ca²⁺ enhanced, but Al³⁺ and Mn²⁺ inhibited the enzyme. DTT inhibited 100% activity, indicating that FbLx-Chi-1 contains disulfide bond(s). Noteworthy, FbLx-Chi-1 inhibited the hyphal growth of all tested fungi after 24 h contact, probably by damaging the fungus cellular wall as observed by scanning electron microscopy. **Conclusions:** FbLx-Chi-1 is a new high stable chitinase purified from latex that displays antifungal activity against fungi of agronomical importance.

Keywords: *Ficus benjamina*, chitinase, plant Defense

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