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*Research Article*

# A Novel Aspartic Protease with HIV-1 Reverse Transcriptase Inhibitory Activity from Fresh Fruiting Bodies of the Wild Mushroom *Xylaria hypoxylon*

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A novel aspartic protease with HIV-1 RT inhibitory activity was isolated and characterized from fruiting bodies of the wild mushroom *Xylaria hypoxylon*. The purification protocol comprised distilled water homogenization and extraction step, three ion exchange chromatographic steps (on DEAE-cellulose, Q-Sepharose, and CM-cellulose in succession), and final purification was by FPLC on Superdex 75. The protease was adsorbed on all the three ion exchangers. It was a monomeric protein with a molecular mass of 43 kDa as estimated by SDS-PAGE and FPLC. Its N-terminal amino acid sequence was HYTELLSQVV, which exhibited no sequence homology to other proteases reported. The activity of the protease was adversely affected by Pepstatin A, indicating that it is an aspartic protease. The protease activity was maximal or nearly so in the pH range 6–8 and in the temperature range 35–60°C. The purified enzyme exhibited HIV-1 RT inhibitory activity with an $IC_{50}$ value of 8.3 $\mu$M, but was devoid of antifungal, ribonuclease, and hemagglutinating activities.

## 1. Introduction

Mushrooms produce spectacular diversity of biologically active biomolecules encompassing laccase, lectins, nucleases, proteases, antifungal proteins, ribosome inactivating proteins, and polysaccharides, polysaccharide—peptides and polysaccharide—protein complexes [1]. Many of these proteinaceous and nonproteinceous molecules manifest potentially worthwhile activities including antitumor, HIV-1 reverse transcriptase inhibitory, and immunomodulatory activities [1, 2]. A variety of mushroom constituents are known to have health promoting activities and therapeutic potential. Some of them are under clinical investigations [3, 4]. Many mushrooms have a good taste and are highly nutritious. Thus mushrooms are popular in the diet of many people all over the world.

Proteases catalyze degradation with high (e.g., trypsine) or low (e.g., subtilisin) specificity. [5, 6]. They can be produced in sizeable quantities by employing microbial approaches. The requirements of brewing, dairy, meat, leather, detergent, and photographic industries make proteases useful and commercial [7]. The sale of industrial enzymes, a significant percentage of which is used for detergents, is a business involving millions of dollars [8]. Proteases