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Original Article

Application of thermal stability difference to remove flammutoxin in fungal immunomodulatory protein, FIP-fve, extract from *Flammulina velutipes*

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ABSTRACT

Fungal immunomodulatory protein (FIP-fve) is a potential functional food ingredient. However, undesirable component flammutoxin (FTX) would occur in the extracted fraction of FIP-fve. In this paper, an application of heating processing instead of the intensive separation process was employed in fractionation of FIP-fve, meanwhile, exclusion of FTX was reached. Contents of FIP-fve and FTX were monitored by HPLC-UV-ESI-MS. Both FIP-fve and FTX had higher thermal stability in a lower concentration solution. Cold water could effectively extract FIP-fve and FTX from fresh mushroom without acetic acid and disulfide-bond breaking agent β-mercaptoethanol commonly used in biochemical studies. Heating cold water extract contained 580 μg/mL FIP-fve and 452 μg/mL FTX at 60 °C for 5 min could effectively exclude FTX and remain 75% of FIP-fve. Adding 0.1 M trehalose or 20% ethanol did not significantly alter the stability of both proteins. The method developed is an applicable procedure for preparing FIP-fve solution free of FTX.

Abbreviations: FIP-fve, fungal immunomodulatory proteins from *Flammulina velutipes*; FTX, flammutoxin; FDS, putative uncharacterized protein; UHA, Unheated ammonium sulfate fraction; HA, heat treatment and ammonium sulfate fractionation; CW, cold water extract in water; CWT, cold water extract in 0.1 M trehalose; CWE, cold water extract in 20% ethanol..

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1. Introduction

Flammulina velutipes (referred to as the golden needle mushroom) is an economical, popular edible mushroom. Various components of *F. velutipes*, such as polysaccharides and glycoproteins, are found to be bioactive. Among these functional proteins, FIP-fve, a novel bioactive polypeptide isolated from the fruiting bodies of *F. velutipes* [1], has been classified as a member of fungal immunomodulatory proteins (FIPs). FIPs such as LZ-8, FIP-vvo, and FIP-gts have been isolated and purified from *Ganoderma lucidum* (Lingzhi), *Volvariella volvacea*, and *Ganoderma tsugae*, respectively [2–4]. FIPs have been reported to assist the production of interleukin (IL)-2, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) in peripheral blood lymphocytes [1,3]. The oral administration of 10 mg/kg FIP-fve has been reported to significantly inhibit the size of tumors and increase the life span of BNL 1MEA.7R.1 (BNL) hepatoma-bearing mice. Other studies have reported that compared to FIP-gts, FIP-fve exhibits higher resistance to the digestion of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) [5]. Chu et al. [6] demonstrate that oral FIP-fve has an anti-inflammatory effect on house dust mite-induced airway inflammations. In addition, these results suggest that FIP-fve possesses a strong potential to be used as an alternative therapy in food or pharmaceutical products being commercially developed for allergic airway diseases such as asthma. Based on previous studies, FIP-fve is stable and demonstrates significant potential for commercial development into functional food or even pharmaceutical products.

Lin et al. [7] have isolated a cardiotoxic and cytolytic 22-kDa protein from the basidiocarps of *F. velutipes* and designated it as flammutoxin (FTX). FTX has been reported to cause the lysis of mammalian erythrocytes. Later, Bernheimer and Oppenheim [8] have purified a 32-kDa hemolytic protein from *F. velutipes* and referred to it as FTX assuming that the FTX isolated from the study of Lin et al. [7] was derived from their 32-kDa FTX via partial proteolysis. Furthermore, they have studied the susceptibility of erythrocytes of different mammals to FTX and have reported that hemolytic activity is inhibited by sucrose. Tomita et al. have isolated FTX as a single hemolysin of 31 kDa from *F. velutipes*, determined by the 28N terminal residues, and studied the molecular basis of the cytolytic action of this protein [9].

Classically, hemolysins have been defined as exotoxins capable of causing the lysis of red blood cells and nucleated cells. Hemolysins are pore-forming toxins, which interact with specific ligands on the surface of various target cells [10].

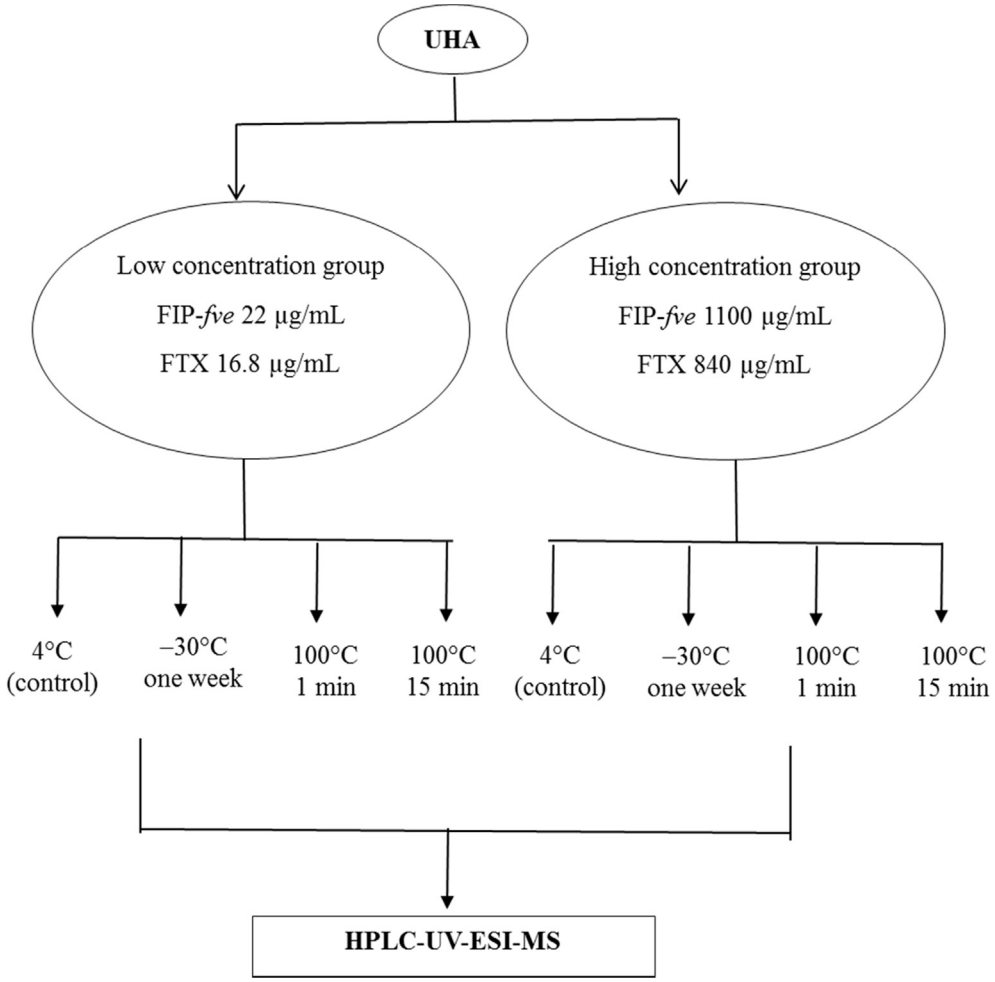

Fig. 1 – Scheme for thermal stability test of FIP-fve and FTX proteins in *Flammulina velutipes* extract UHA.