

【ノート】

Purification and characterization of intracellular serine protease produced in mycelia during fruit-body growth stage of *Hypsizygus marmoreus*

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[Abstract]

The purification and characterization of a neutral protease (PE-4) produced in mycelia during fruit-body formation in *Hypsizygus marmoreus* was carried out in the present study. The enzyme was purified to homogeneity using ammonium fractionation, ion exchange chromatography on DEAE-Toyopearl, hydrophobic exchange chromatography on Butyl-Toyopearl, and gel chromatography on Superdex 200. Protease activity was enhanced by Co²⁺, in particular, and was strongly inhibited by phenylmethanesulfonyl fluoride (PMSF), aprotinin and Pefabloc SC, which are known serine protease inhibitors. Phosphoramidon did not inhibit the protease. The N-terminal amino acid sequence was identical with the serine protease from the fruit-body of *H. marmoreus*, hmsp. The same serine protease was present in both vegetative mycelia and the fruit-body in the growth stage. Protease activity activated in mycelia at fruit-body formation was inhibited by PMSF, EDTA, and phosphoramidon but not completely. These results also suggest that several neutral proteases besides PE-4 were present during kinkaki, which is removal of both the spawn and the uppermost layer of the medium, in the mature fruit-body in the mycelia.

Key words: Edible mushroom, Fruit-body, *Hypsizygus marmoreus*, Inhibitor, Serine protease

[摘要]

ブナシメジ (*Hypsizygus marmoreus*) 栽培において、菌かき後に培養基内で活性が上昇する中性プロテアーゼは、本菌の子実体形成に深く関わることが考えられる。子実体収穫後の培養基をプロテアーゼ源として、菌糸体内由来の中性プロテアーゼ (PE-4) の酵素精製と諸性質の検討を行った。PE-4 は、セリンプロテアーゼ阻害剤である PMSF, Aprotinin と Pefabloc®SC によって完全に活性を失ったが、Phosphoramidon に感受性を示さなかった。阻害剤の影響および N 末端アミノ酸配列の結果から、PE-4 はセリンプロテアーゼであると結論した。金属プロテアーゼ阻害剤である Phosphoramidon は、本菌の子実体形成を著しく阻害するため、PE-4 は子実体形成に深く関わらないことが示唆された。菌かき後から子実体成熟期における菌糸体より粗酵素を抽出し、中性プロテアーゼ活性に対する Phosphoramidon, PMSF と EDTA の阻害度を測定したところ、PMSF による完全な阻害は受けなかった。子実体形成においては、PE-4 以外に活性化されているプロテアーゼの重要性が示唆された。