（様式第３号）

学 位 論 文 要 旨

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題目：Regulation of the GH 15 family protein (glucoamylase) gene expression during mycelia growth and fruiting body development in the basidiomycetous *Pholiota microspora*
（ *Pholiota microspora* の菌糸体増殖および子実体発生における GH 15 タンパク質（グルコアミラーゼ）発現調節に関する研究）

Mushroom fruiting is a highly organized and accurate process, which requires the coordination between genetic, environmental, and physiological factors. Because the significant difference on morphology during the development of mushroom, with the development of molecular technology, amount of genes have been identified genes specially expressed at certain stage. In this study, by analysis the different life style, glucoamylase gene is believed play important function during fruiting process. Also, mushroom capable to digest cellulose shed a light on the importance of carbon source utilization, starch is the second largest carbon sources in nature, most of them stored in the form of grain seeds. Several kinds of starch have been used as carbon sources to investigate the induction mechanism on fruiting.

The glucoamylase gene (*PnGlu1*) from *Pholiota nameko* was amplified and characterized. The 1743bp coding region of PnGlu1encoded a polypeptide consisting of 581 amino acids with a signal peptide comprising 17 amino acids at the N-terminal. The deduced protein sequence has a two domain structure consisting of an N-terminal domain with the glycoside hydrolase family 15 **(**GH 15) signature and a C-terminal carbohydrate binding module (CBM) 20. Southern blot analysis revealed that only a single copy of *PnGlu1* was present in the haploid genome of *P. nameko*. To investigate the capability of starch utilization in *P. nameko*, *PnGlu1* expression level has been examined on minimal media containing different carbon source such as glucose, soluble starch, corn starch, wheat starch, and potato starch. Quantitative reverse transcription-PCR showed that corn and soluble starch strongly stimulated *PnGlu1* gene expression of *P. nameko*.

In order to reveal the regulation of glucoamylase gene from *Pholiotanameko*, the promoter region about 2734 bp sequences upstream of start codon of *PnGlu1* gene have been amplified and characterized. Two TATA box like sequence, consensus sequence of two mating factor alpha1 (MATα1) and three mating factor a1 (MATa1) binding sites, respectively,in yeast have been discovered. To investigate the relationships between regulation of *PnGlu1* and mating type system, *PnGlu1* gene expressionwas investigate in monokaryon, dikaryon and four transformants those were introduced homeoprotein genes. *PnGlu1* gene expression was induced in dikaryon, and *PnGlu1* gene expressionfour transformants are higher than that in monokaryon.In order to investigate the relationships between regulation of *PnGlu1*and fruiting body development, *P. nameko* was cultivated in sawdust media, quantitative reverse transcription-PCR was carried out. *PnGlu1* expression level, glucoamylase activity and

glucose contents were dramatically increase until fruiting. These results suggested that glucoamylase gene expression was closely related with dikaryotization and fruiting body development.

Soluble and corn starches strongly induced glucoamylase gene expression give a hint that mushroom capable to digest certain sources of starch may be due to the structure of linkage bonds between glucose and branch points. Also, the induction of carbon source on the fruiting process is a complex mechanism, in this research, the research result in this study is a preliminary attempt for understand the induction process, to understand the process completely, much work need to be done in the near future.

The glucoamyalse gene is highly expressed in dikaryon strain suggested that homeodomain protein regulated this gene expression, by analysis the promoter region of glucoamylase gene, a hypothesis have been proposed to demonstrated probably regulatory mechanism.