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学 位 論 文 要 旨

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題目: Molecular biological study about the physiology and ecology of ectomycorrhizal fungus *Tricholoma matsutake*

（ 外生菌根菌マツタケの生理･生態に関する分子生物学的研究 ）

Tricholoma matsutake is one of the most economically important mushroom in Japan. In order to develop artificial cultivation the fruiting body, ecology, physiology and genetics of this mushroom were studied.

Firstly, to analyze the relationship between ecological difference (difference of host tree) and species of mushroom, nucleotide sequences of mitochondrial small subunit ribosomal DNA (mt SSU rDNA) V4 variable domain and those of nuclear rDNA internal transcribed spacer (rDNA-ITS) region of *T. matsutake* and related species were compared. Sequence analysis of samples collected from different forest environments and geographic regions throughout Asia revealed that host specificity and geographic distribution had no effect on the species of *T. matsutake*. In addition, the results revealed that although *T. matsutake*, *T. fulvocastaneum* and *T. bakamatsutake* formed a grouped within the same clade, *T. magnivelare* was very closely related to *T. matsutake*.

Secondly, to understand physiological characteristics such as starch utilization, the gene encoding the glycoside hydrolase family 15 glucoamylase (*TmGlu1*) in the ectomycorrhizal fungus *T. matsutake* was cloned and characterized. After the culture of *T. matsutake* mycelia in media containing different forms of starch as a carbon source, increased extracellular glucoamylase activity in the culture medium and a correspondingly higher transcriptional level of *TmGlu1* in mycelia were detected, particularly in amylose-supplemented medium, when compared with those in the glucose medium. These results suggest that starch, especially amylose, affects the transcription of *TmGlu1* and downstream glucoamylase activity, which is directly related to starch utilization. Similar results were obtained when compound forms of starch were used to culture mycelia. Meanwhile, glucoamylase genes from saprophytic

and ectomycorrhizal fungi formed a single clade. The observed inducibility of *TmGlu1* and lack of distinct phylogenetic differences among glucoamylase genes of saprophytic and ectomycorrhizal fungi suggest that glucoamylase may relate to some common functions in these two types of fungi.

Finally, to establish the genetics of *T. matsutake* for breeding, we sequenced and characterized the genes for homeodomain1 (HD1) protein gene (*Tmhox1*), the flanking mitochondrial intermediate peptidase (MIP) gene (*Tmmip*) and upstream region of *Tmhox1* of an ectomycorrhizal basidiomycete, *T. matsutake*. Gene location of *A* mating type locus in *T. matsutake* was unique. Because, homeodomain 2 (HD2) protein gene was absent in the upstream region of *Tmhox1*. From the results of Southern hybridization HD1 gene was used as probe, it was suggested that presence of HD2 gene in the *T. matsutake* genome far from HD1 gene and *T. matsutake* strain use in this study might be homokaryon. Phylogenetic analysis using amino acid sequence of HD1 protein among basidiomycetous mushrooms showed that evolution of HD1 protein has no relationships with that of physiological, ecological and morphological characteristics of basidiomycetous mushrooms. Thus, we suggest that no phylogenetic relationships between morphological species and ability of clamp cell formation and ability of formation of mycorrhiza were started to evolve independently in an early stage of the evolutionary history. if morphological changing is the main routes of evolution, ability of mycorrhiza formation, clamp cell formation, and starch utilization seem to be equipped or lost in the unrelated to the evolutional lineage.

Form these studies, three important possibility for artificial cultivation were found; i) *T. matsutake* can essentially grow in symbiosis with both conifer and broad leaf tree; ii) *T. matsutake* can utilize effectively relatively short chainof starch; ii) lifecycle of *T. matsutake* might be homothallic. These findings obtained the new strategy for *T. matsutake* artificial cultivation.