SL305 Phylogenetic Analysis of Tricholoma matsutake on the Basis of rRNA Genes

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Tricholoma matsutake(S. Ito et Imai) Singer, its Korean name Song-Yi, is an Tricholomataceae, belongs to fungus which ectomycorrhizal T. matsutake genomic DNAs digested with Hymenomycetes, and Basidiomycota. various restriction enzymes were hybridized with the probe DNA which was derived from Saccharomyces cerevisiae 25S rRNA gene. The length of rDNA repeat was estimated to be about 11.5kb from Southern hybridization. Three DNA fragments were obtained by colony hybridization from the genomic library of T. matsutake. Total length of the 3 cloned fragments was estimated to be 7.4 kb in length. 6 portions of the cloned DNA fragments were used for Northern hybridization as probe DNAs to know whether the cloned DNA habor all four rRNA genes. It was shown that all four rRNA genes were present in the cloned DNAs and were organized as IGS2 - 18S - ITS1 - 5.8S - ITS2 - 25S - IGS1 -5S rRNA - IGS2. The presence and order of above result were confirmed by the DNA sequence determination. The resultant 7,390 bp of DNA sequence was analysed with Genetyx program and BLAST programs via Internet. Some features of particular importance were found: 1) the ITS2 was extraordinarily large as compared to the coding regions, 2) the IGS2 has long tracts of 'T', which is considered to take part in transcription termination, 3) TATA-like sequence was located 102bp upstream of 5S rRNA gene, 4) direct repeat sequences were found immedietely upstream of 5' end of 18S rRNA gene. ITS shows relatively high similarity to those of some species of Basidiomycetes, implying that the regions are to some extent evolutionary conserved. Of the all four rRNA genes, 5S rRNA gene shows the highest %G+C content (61.02%), indicating that 5S rRNA molecule is more stable than other rRNA molecules against evolutionary divergence. Secondary structure of 5S rRNA is also highly conserved in the features of eukaryotic structure. 5.8S rRNA of T. matsutake interacts with 5' end of 25S and this structure is approximately rRNA sequence, identical representative model of eukaryotes. 18S rRNA sequence of T. matsutake shows both highly conserved regions and variable region, which was also found in 25S rRNA sequence. Phylogenetic analysis was carried out with PHYLIP package.