Mating System and Genetic Variations of *Tricholoma crassum* (Berk.) Sacc. in Some Area of Thailand by Isozyme Electrophoresis and PCR-RFLP Method

Malinee Tantiyaporn & Aree Ritthiboon

Department of Applied Biology, Faculty of Science, King Mongkut's Institute of Technology, Ladkrabang, Bangkok 10520, Thailand, email: ktmaline@kmitl.ac.th

Tricholoma crassum (Tricholomataceae, Agaricales) is a large, good edible species. It has nutritional quality, having 10.02 gm carbohydrate, 0.287 gm fats, 18.58 gm protein, 2.71 mg calcium, and 3.35 mg iron in every 100 gm of fresh weight. It is found growing abundantly throughout Thailand.

The mating system of *T. crassum* was studied. The monokaryon isolates were selected from ten sporocarp collections in five provinces in Thailand—Ubonratchathani, Sakonnakorn, Mahasarakham, Srisaket, and Nakornratcha-sima. Suitable growth conditions for the mycelial cultivation were PDYA medium pH 7 and 25 °C incubation. Twelve fast-growing monokaryotic strains were selected for mating system studies. The mating system was determined by pairing the monokaryotic mycelia of each collection in all pairwise combinations. The presence of clamp connections after mating indicated sexual compatibility. The ratio of compatible crosses to all combination crosses were 1:4, which indicated tetrapolar heterothallism. In addition, multiple alleles among the monokaryons for each sporocarp of the five provinces were examined on the basis of their mating interactions, and the results were two factors (A and B), and each possessed 16 different alleles.

One hundred and thirty-eight monokarytic isolates of *T. crassum* from the above five provinces were cultured on growth media. The suitable conditions for the mycelial growth were PDYB medium at 25 °C for 21 days. All samples were analyzed for isozyme variability on polyacrylamide gel electrophoresis with 11 enzyme systems: isocitrate dehydrogenase, leucine aminopeptidase, acid phosphatase, phosphogluconate dehydrogenase, alkaline phosphatase, alcohol dehydrogenase, glucose–6–phosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, laccase, and esterase. Eight of the systems showed polymorphism. Cluster analysis based on isozyme variability using the NTSYSpc 2.00 and UPGMA methods revealed two clusters at a similarity coefficient of 0.67. The first cluster consisted of monokaryotic isolates from Nakornratchasima, Mahasalakham, and eight samples of Ubonratchathani. The second cluster consisted of the isolates from Sakonnakhorn, Srisaket, and 30 samples of Ubonratchathani.

The genetic variations of nine additional samples of *T. crassum* from four additional provinces—Roiyed, Burerum, Patumthani, and Nakhon Pathom—were studied by the technique of PCR-RFLP. Two pairs of primers, ITS1- ITS4 and O1-LR12R, were used respectively for PCR amplification on ITS (internal transcribed spacer) and IGS (intergenic spacer) regions of the nuclear ribosomal gene, followed by digestion with *Hind*III, *Dde*I, *Hae*III, *Eco*RI, and *Hinf*I. Data analyses of the PCR-RFLP products based on the similarity index and the UPGMA method in the WinBoot program revealed three clusters that were related to their geographic origins, except the samples from Burirum, which showed genetic variation from the same areas at a similarity coefficient of 0.8 and were grouped into the third cluster.