Protoplast Fusion between *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm. and *P. citrinopileatus* Singer

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Protoplast fusion has been used as a method to create mushroom hybrids, especially when using conventional methods cannot achieve this result. *Pleurotus ostreatus* and *P. citrinopileatus,* along with many other species of the genus *Pleurotus,* are widely industrially cultivated in many parts of the world, including Thailand.

Protoplasts fusion between *P. ostreatus* and *P. citrinopileatus* in this experiment was carried out by growing 4-day-old monokaryotic mycelia derived from a single spore isolate of each species on malt extract broth. The mycelia were agitated at 100 rpm for 2 hours with 9 mg membrane-filtration sterilized Lysing Enzyme (Sigma L-1412) in 1 mL osmotic stabilizer (0.6 M $MgSO_4(7H_2O)$ in 0.05 M sodium maleate buffer, pH 5). The freshly prepared protoplasts were then mixed and incubated in 40% PEG (polyethylene glycol 6,000)/0.05 M $CaCl_2(2H_2O)$ for 20 minutes at room temperature. All protoplasts were regenerated on regeneration medium containing 30 g agar, 20 g malt extract, 20 g glucose, 1 g peptone, and 1000 mL 0.6 M sucrose for 7–12 days. There were 487 regenerated colonies detected, but only three of them were selected as fusants by posessing clamp connections on their mycelia. The fusants (Fu1, Fu2, and Fu3) were proved to be "hybrids" of *P. ostreatus* and *P. citrinopileatus* as the experiment progressed.

The mycelia of the fusants were significantly faster in growth and larger in size than the parental strains, which were relevant to the theories that fusants, which are dikaryotic ($n + n$), grow faster (Toyomatsu and Mori, 1987) and have larger hyphae (Abe et al., 1982) than their monokaryotic (n) parental strains. The experiments were performed by subculturing the mycelia of each fusant and of the two parents on MEA plates and incubating for 9 days at room temperature followed by determining the diameters of each colony for at least 10 replications as the mycelial growth and measuring the diameters of the mycelia microscopically using a calibrated eyepiece micrometer for 100 replications as the hyphal size.

The fusants showed esterase and malate dehydrogenase isozyme bands common to those of their parents when esterase, malate dehydrogenase, and alcohol dehydrogenase were studied as the method modified from that of Pasteur et al. (1988).

The fruiting bodies of Fu1, which was the only fusant successful in fruiting on sawdust plastic bags, showed recombined characteristics of the parents. Its fruiting bodies were yellow in color, which was similar to *P. citrinopileatus,* but its spore print was creamish color, which was similar to *P. ostreatus. P. ostreatus* has a creamish color both in its fruiting bodies and spore prints, while *P. citrinopileatus* has golden yellowish color in its fruiting bodies but is pinkish grey in spore sprints.