

Studies on Protoplast Fusion of Basidiomycetes

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To investigate the possibility of interorder protoplast fusion and nuclear transfer between two basidiomycetes, *Lentinus edodes* (Berk.) Sing. and *Coriolus versicolor* (L.: Fr.) Quel. showing anastomotal incompatibility, protoplast fusions and nuclear transfers were attempted by modified Peberdy methods. For isolation of auxotrophic mutants with markers from the mycelia of *L. edodes* on minimal medium, UV irradiation and ethidium bromide (EtBr) enrichment after UV irradiation were used. The minimum inhibitory concentrations (MICs) of several fungicides, antibiotics, and amino acid analogues, which are able to inhibit growth of *L. edodes*, were determined. In addition, five *p*-fluorophenylalanine-resistant mutants and eight benomyl-resistant mutants were selected by UV irradiation. Then those mutants were used in protoplast fusion and nuclear transfer. The protoplasts of the fungi were readily obtained from the 8-day-cultured mycelia in liquid medium of *L. edodes* and the 4-day-cultured mycelia on 1.5% agar media of *C. versicolor* by treatment with a combination of Novozym 234 (10–15 mg/ml) and cellulase Onzuka R-10 (10 mg/ml) for 3.5–6 hr at 30°C. As an osmotic stabilizer for protoplast formation, 0.6 M mannitol was the best for *L. edodes* and 0.6 M sucrose was the best for *C. versicolor*; and for regeneration of the protoplasts 0.6 M sucrose was the best for the two mushrooms. The regeneration frequency of the protoplasts from the mycelia of *L. edodes* was 0.18–

0.55% and that of *C. versicolor* was 0.65%. Back mutation frequency of those auxotrophs used in fusion was 10^{-3} – 10^{-6} . Polyethylene glycol (M.W. 4000) in 10 mM CaCl_2 –glycine solution (pH 8.0) induced protoplast fusion and nuclear transfer. The interorder fusion frequency between the mycelial protoplasts of the mutants of *L. edodes* and those of *C. versicolor* was 7.4×10^{-6} . Viable hybrids were obtained by the transfer of the nuclei isolated from the protoplasts into the donor protoplasts. The rate of hybrid formation was higher than that of the protoplast fusion. Those hybrids were different from their parents in growth rate and mycelial morphology. In segregation studies, the fusants obtained through interorder protoplast fusion and nuclear transfer segregated on complete medium containing benomyl. A comparison of the interorder hybrids between *L. edodes* and of *C. versicolor* that were produced through protoplast fusion and nuclear transfer was made by using isozyme analysis of peroxidase, esterase, acid phosphatase, and superoxide dismutase. In most cases the isozyme patterns of both mushrooms were distinct. A comparison of the parental mushrooms and their hybrids showed that in the interorder hybrids an interaction occurred between the two genomes. For examination of the ultrastructure of the protoplasts and their fusants, the protoplasts and hybrids were embedded and polymerized in Epon and observed using scanning and transmission electron microscopy.