

Studies on Intergeneric Protoplast Fusion and Nuclear Transfer between *Ganoderma lucidum* and *Coriolus versicolor*

Seol Hee Park, Eung Chil Choi and Byong Kak Kim

Department of Microbial Chemistry, College of Pharmacy

Seoul National University, Seoul 151-742, Korea

(Received July 9, 1991)

Abstract □ Stable intergeneric hybrids involving allodiploid were obtained through protoplast fusion and nuclear transfer between the auxotrophic mutants of two basidiomycetes, *Ganoderma lucidum* and *Coriolus versicolor*.

keywords □ Basidiomycetes, *Ganoderma lucidum*, *Coriolus versicolor*, Protoplast fusion and nuclear transfer, Stable intergeneric hybrid

In the past decade, several reports on intraspecific and interspecific protoplast fusion in filamentous fungi have been published.

On the other hand, very few papers have been reported on intergeneric protoplast fusion, especially in basidiomycetes, though intergeneric and even more distant fusion in yeasts have been reported. The main problem in using protoplast fusion as a breeding tool so far is the difficulty in obtaining a stable hybrid since the nuclear fusion rarely occurs, especially in more distant fusions. Direct transfer of isolated nuclei may render it possible to hybridize taxonomically more distant species and may also clearly alleviate the need for introduction of conventional selective markers in fusion experiments.

Two fungi, *Ganoderma lucidum* and *Coriolus versicolor* being distantly related higher fungi, have been chosen because they have unique, biologically active components against various human diseases and grow fast and moreover, their mycelial morphologies are clearly different. These are useful characteristics as far as the experiments and the analysis of the results are concerned. The purpose of this study is to find a possibility of intergeneric protoplast fusion and nuclear transfer between the two basidiomycetes, to obtain stable hybrids distinctly different from parental lines by this process, and to partially characterize these nonparental progenies.

Protoplast fusion and nuclear transfer were made

by modified methods of Peberdy using polyethylene glycol (M.W. 6,000) in CaCl₂-glycine solution. The intergeneric fusion frequencies between the mycelial protoplasts of the auxotrophic mutants of *G. lucidum* and *C. versicolor* ranged from 0.03% to 0.56% (Table I). Viable hybrids were obtained by the transfer of the nuclei isolated from the protoplast into the donor protoplast. In segregation studies, the fusants obtained through intergeneric protoplast fusion and nuclear transfer segregated spontaneously on a complete medium into various strains including parental strains, nonparental auxotrophs and nonparental prototrophic hybrids (Table II). Those hybrids were different from their parents in their growth rate and mycelial morphology. It was evident that prototrophic hybrids of aneuploid or diploid could be constructed after spontaneous segregation from the fusants produced through the protoplast fusion on a minimal medium. Comparison of the intergeneric hybrids between *G. lucidum* and *C. versicolor* that were produced through protoplast fusion and nuclear transfer was made by analyzing the isozymes of esterase and peroxidase. The isozyme patterns of both fungi were distinct. The comparison of the parental fungi and their hybrids indicated that in the intergeneric hybrids an interaction occurred between the two genomes. Different quantities of the isozymes from the parental fungi were observed in the hybrids. Furthermore some new isozyme bands were also observed. The hybrids

Table I. Intergeneric fusion between various auxotrophic mutants of *C. versicolor* and *G. lucidum*

Cross (Genotype)		Symbol	Fusion frequency (%)
Protoplast	Protoplast		
CE 17 (arg)×LP 20 (cyt)		CE17-LP20-F	0.10
CE 17 (arg)×LP 120 (leu)		CE17-LP102-F	0.56
CE 6 (glu)×LP 20 (cyt)		CE6-LP20-F	0.03
CE 6 (glu)×LP 120 (arg)		CE6-LP20-F	0.44
Protoplast	Nuclei		
CE 17 (arg)×LP 20 (cyt)		CE17-LP20n-F	-
CE 17 (arg)×LP 120 (leu)		CE17-LP102n-F	-
CE 6 (glu)×LP 20 (cyt)		CE6-LP20n-F	-
CE 6 (glu)×LP 120 (leu)		CE6-LP102n-F	-

that showed characteristic new isozyme bands and that had different mycelial morphology from their parents, were proved to be diploids by DNA quantitation. One of those diploids, CE17-LP20-F6 was induced to segregate by benomyl (100 µg/ml). Most of the progenies of this diploid were prototrophic and showed similar novel bands of esterase isozymes. These isozyme patterns and genetic traits of the segregants of this diploid suggest that crossing over in many regions of the two genomes made this diploid a stable homozygotic state and hence made a stable novel strain that was different from their parents.

LITERATURE CITED

1. Bok, J. W., Park, S. H., Choi, E. C., Kim, B. K. and Yoo, Y. B.: Studies on protoplast formation and regeneration of *Coriolus versicolor*. *Kor. J. Mycol.* **18**, 115-126 (1990).
2. Davis, B. J.: Discontinuous electrophoresis-II. *Ann. N. Y. Acad. Sci.* **121**, 404 (1964).
3. Ferenczy, L.: Fungal protoplast fusion: basic and applied aspects. In *Cell Fusion: Gene transfer and transformation*, p. 154-160, ed. Beer, Jr. R. F. and Bassett, E. G., Raven Press, N. Y.

Table II. Spontaneous segregation of intergeneric fusants obtained from protoplast fusion between *G. lucidum* and *C. versicolor*

Cross		No. of colonies tested	Phenotype of heterokaryon			
A	B		Parentals		Non-parentals	
			A	B	proto	auxo
CE-17×LP-20 (arg) (cyt)		76	4	1	69	2
CE-17×LP-120 (arg) (leu)		94	12	6	68	8

(1984).

4. Ferenczy, L. and Pesti, M.: Transfer of isolated nuclei into protoplast of *Saccharomyces cerevisiae*. *Curr. Microbiol.* **7**, 157-160 (1982).
5. Park, W. M., Lee, Y. S., Kim, S. H. and Park, Y. H.: Characterization of isolates of *Ganoderma lucidum* by electrophoretic patterns of enzymes. *Kor. J. Mycol.* **14**, 93-99 (1986).
6. Park, S. H., Choi, E. C. and Kim, B. K.: Studies on protoplast and nuclear fusion of *Ganoderma* species. *Proc. Internat. Sym. on New Drug Development from Natural Products*, May 2-3, p. 172-207 Seoul, Korea (1989).
7. Peberdy, J. F.: Fungal protoplast: isolation, reversion and fusion. *Ann. Rev. Microbiol.* **33**, 21-39 (1989).
8. Tamaki, H.: Genetic analysis of intergeneric hybrids obtained by protoplast fusion in yeast. *Curr. Genet.* **10**, 491-494 (1986).
9. Yang, Q. Y. and Jong, S. C.: Medical mushrooms in China. In: *Mushroom Science XII (Part I)*, Proceedings of the Twelfth International Congress on the Science and Cultivation of Edible Fungi, ed. Grabbe, G. and Hilber, Q. Braunschweig Press, Braunschweig. (1989).
10. You, C. H., Yoo, Y. B., Byun, M. O. and Park, Y. H.: Studies on the transfer of isolated nuclei from *Pleurotus sapidus* into *Pleurotus ostreatus*. *Kor. J. Mycol.* **16**, 210-213 (1988).

Notice to Authors for

ARCHIVES OF PHARMACAL RESEARCH

- (1) This scientific journal publishes research reports on pharmacal and allied sciences.
- (2) Submission of manuscripts to the *ARCHIVES* is allowed only to the society members, or it shall be approved by the Editorial Committee.
- (3) The manuscripts that have not been published previously and are not being submitted elsewhere will be considered for publication. All manuscripts except those invited, are subject to review both by the editors and qualified outside referees, and are classified into the following five categories:
 - A) Original Articles: reports of original research in the field of pharmacal sciences will be published in this section.
 - B) Notes: original research reports of a limited scope will be grouped under this heading.
 - C) Communications: short communications that report new findings of great importance will be given prompt publication after review. The full articles of them can be published later.
 - D) Reviews: summaries covering recent advances and critically evaluating published articles will be published as reviews, and are solicited by invitation. Unsolicited reviews may be accepted from authors after submission of preliminary detail concerning the manuscript.
 - E) Guest Lectures: the manuscripts of guest lectures invited by the Society can be published under this heading.
- (4) The manuscripts shall be written only in English. Scientific names shall be adopted from the respective international codes of nomenclature.
- (5) The title should be as condensed as is consistent with clarity and should be followed by the author and institution names and address.
- (6) The manuscripts should be written concisely and in the order of abstract, keywords, introductory paragraph (without center heading), experimental methods, results, discussion, conclusion, acknowledgements, and literature cited.

The names of journals or books (in the literature cited) and scientific names and other Latin names shall be written in italics, and the volume numbers in bold letters. These differentiations shall be indicated in the manuscripts by underlining a direct line for the bold letters and a wavy line for the italics.
- (7) Figures shall be submitted as copy directly suitable for engraving. All illustrations shall be carefully drawn in India ink or black ink on white tracing paper, preferably twice the size of the finished block (maximum width six cm or twelve cm).

Each figure of photograph, which is to bear a legend, shall accompany a separate sheet of legends for the figures. The locations of the figures with Arabic numerals shall be indicated in the manuscript. Tables with their titles in the upper part shall be numbered with Roman numerals (*e.g.*, Table I, II, III, . . .)
- (8) C.G.S. units shall be used for weights and measures and Arabic numerals shall be used. Standard abbreviations in *Chemical Abstracts* shall be used throughout the experimental section.