Characteristics of Artificial Hybrids between *Lentinula edodes* and *Coriolus versicolor*

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Genetic recombination is a more powerful method for developing superior strains than selection or mutation. Protoplast fusion has proven to be a suitable tool for overcoming the natural barriers of incompatibility and establishing heterokaryosis in many fungi (Croft and Dales, 1979; Peberdy, 1989). Intergeneric and even inter-order protoplast fusions have been successfully performed in basidiomycetes (Bok *et al.*, 1994; Liang and Chang, 1989; Park *et al.*, 1991).

Lentinula edodes and *Coriolus versicolor* showing anatomical incompatibility were grown on complete media (Kim *et al.*, 1996). Protoplast fusion and nuclear transfer were successfully utilized in producing hybrids between *L. edodes* and *C. versicolor.*

The optimum protoplast yield was obtained by the treatment with a combination of Novozyme 234 (10 mg/ml, Novo Industry, Denmark) and cellulase Onozuka (10 mg/ml, Yakult, Japan). The optimum conditions for protoplast isolation and regeneration were described in Table I. For the protoplast fusion, auxotrophs with specific markers were used (Table II). Polyethylene glycol (M.W. 4000) in 10 mM $CaCl_2$-glycine solution (pH 8.0) induced protoplast fusion and nuclear transfer. The inter-order fusion frequency between the protoplasts from LE(eb)26 and CV17 was 7.4×10^{-6} (Table III). Viable hybrids were obtained by the transfer of the nuclei isolated from the protoplast of LE207 into the protoplast of CV17 (Table IV). The ratio of hybrid formation was higher than those of the protoplast fusion.

Those hybrids were different from their parents in the growth rate, mycelial morphology, pigment production, and clamp connection (Tables III and IV). The auxotrophs of *L. edodes* were characterized by fast growing white aerial mycelia and the auxotroph of *C. versicolor,* CV17, was characterized by fast growing pigmented mycelia and advancing zone. The growth rate of the hybrids was variable and the aerial mycelia ranged from sparse to dense. Some of the hybrids produced pigment without advancing zone and others made advancing zone without pigment production. Some of the CV17-like or non-parental nuclear transfer products revealed clamp connection, whereas parental CV17 lack clamp connection. The presence of clamp connection in hybrids implies formation of heterokaryons.

The nucleus number of the protoplasts was determined by using Giemsa or 4,6-diamidino-2-phenylindole (DAPI) as a specific dye for staining nucleus. DNA contents of the parents and the hybrids were determined with Hoescht 33258 (Table V). The hybrids differed in their DNA contents, whereas nucleus number per cell was consistent. Compared with the parents, some of the hybrids contained more than three times the amount of DNA. This result demonstrates that the nuclei of LE207 transferred into the protoplast of CV17. However, there were hybrids that possessed less DNA than CV17. It represents not all of nuclei were integrated into the hybrid nucleus.

The isozyme patterns of peroxidase, esterase, superoxide dismutase and acid phosphatase were examined. The isozymes of both parents were distinct and

Table I. Optimal conditions for the production and regeneration of protoplasts of *L. edodes* and *C. versicolor*

Conditions	*L. edodes*	*C. versicolor*
Agar concentration	0%	2.0%
Mycelial age	6-8 days old	2.5 days old
Isolation stabilizer	0.6 M mannitol	0.6 M sucrose
Reaction time	3.5-4 hours	2-6 hours
Temperature	30°C	30°C
pH	4.0	nd*
Regeneration stabilizer	0.6 M sucrose	0.6 M sucrose

nd*: not determined

Table II. List of auxotrophs used in fusion of *L. edodes* with *C. versicolor*

Strain	Auxotroph symbol	Phenotype	Regeneration frequency	Back mutation frequency
L. edodes	LE207	Ser	5.54×10^{-3}	8.46×10^{-4}
	LE(eb)26	Ile, Arg, Thy	1.84×10^{-3}	4.81×10^{-4}
C. versicolor	CV17	Arg	6.47×10^{-3}	1.68×10^{-6}

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Table III. Characterization of the protoplast fusants between LE(eb)26 and CV17

Strain	Morphology[a]	Backcross to parents[b]		Aerial mycelia[c]	Mycelial growth[d]	Pigment[e]	Advancing zone[f]
LE(eb)26	Le			++++	F	–	–
CV17	C			+	F	+	+
LE(eb)26-CV17-F		Le	C				
3	Le	+	–	++++	M	–	–
5	Le	+	–	++++	M	–	–
6	Le	+	–	++++	M	–	–
11	C	–	+	++	M	+	+
13	C	–	+	++	M	+	+
14	C	–	+	++	M	+	+
16	C	nd*		++	M	+	+
18	C	–	+	++	S	+	–
19	C	–	+	++	S	+	+
21	C	–	+	+++	S	+	+
22	C	–	+	+++	S	+	+
24	C	–	+	++	S	+	+
25	C	–	+	+	M	–	+
26	C	–	+	++	M	+	+
12	N	–	+	++	M	+	+
15	N	–	+	++	M	+	+
23	N	–	+	+	S	+	+
30	N	nd		++	S	+	+

[a]Le: LE(eb)26, C: CV17, N: non-parental type, [b]+: compatible – : noncompatible, [c]++++: indicate best yields, [d]F: fast growth, M: moderate growth, S: slow growth, [e]brown pigment production, [f]+: zonate, – : not zonate, nd*: not determined

the isozyme patterns of the hybrids were different from those of their parents. The parental isozyme bands as well as the new ones were observed in the hybrids (data not shown).

Table IV. Characterization of nuclear transfer products between LE207 and CV17

Strain	Morphology[a]	Backcross to parents[b]		Clamp connection[c]	Aerial mycelia[d]	Mycelial growth[e]	Pigment[f]	Zone[g]
LE207	L			+	++++	F	–	–
CV17	C			–	+	F	+	+
LE207-CV17-F		C	L					
10	L	–	+	+	++++	F	–	–
11	L	–	+	+	++++	F	–	–
44	L	–	+	+	++++	F	–	–
46	L	–	+	+	++++	F	–	–
59	L	–	+	+	++++	F	–	–
72	L	–	+	+	++++	F	–	–
4	C	+	–	–	+++	F	+	+
6	C	+	–	+	++	nd	+	–
9	C	+	–	–	++	F	+	+
17	C	+	–	nd*	nd	S	+	+
17-1	C	+	–	+	++	F	+	–
23	C	+	–	+	+++	F	+	–
28	C	+	–	nd	+	S	+	–
63	C	+	–	+	++	F	+	–
86	C	+	–	+	++	M	–	nd
8	N	+	–	–	++	S	+	nd
19	N	+	–	–	+	F	+	–
27	N	+	–	+	+	F	+	–
73	N	–	+	+	++++	S	+	–
89	N	+	–	nd	+++	M	+	+

[a]L: LE207, C: CV17, N: non-parental type, [b]+: compatible – : noncompatible, [c]+: presence of clamp connection – : absence of clamp connection, [d] ++++: indicate best yields [e]F: fast growth, M: moderate growth, S: slow growth, [f]brown pigment production, [g]+: zonate, – : not zonate, nd*: not determined

Table V. DNA contents of parents and hybrids between LE 207 and CV17

Strain	DNA (μg) /10^6 cell	Nucleus No./cell	DNA (pg)/ nucleus
LE207	5.72	1.12	5.11
CV17	3.02	1.25	2.42
LE207-CV17-F4	2.25	1.19	1.89
LE207-CV17-F17-1	2.31	1.29	1.79
LE207-CV17-F37	21.42	1.21	17.70
LE207-CV17-F46	16.72	1.27	13.17
LE207-CV17-F51	9.59	1.29	7.43
LE207-CV17-F63	23.30	1.30	17.92
LE207-CV17-F89	2.33	1.27	2.33

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