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Incompatibility Factors and Genetic Analysis of *Pleurotus sajor-caju*

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여름느타리버섯의 交配系 및 交配菌株의 遺傳 分析

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Abstract: The mating system of monokaryotic isolates in *Pleurotus sajor-caju* was controlled by two incompatibility factors A and B of tetrapola mating system. The mycelia of dikaryotic isolates grew faster than those of their component monokaryons, but no correlation between dikaryotic and their component monokaryotic isolates was found. The primodia formed well on the potato dextrose agar (PDA) media not only in the dikaryotic isolates but also in the monokaryons under irradiated conditions. The dikaryotic isolates produced normal sporophores; however, the monokaryotic isolates produced abnormal sporophores when they were cultivated with sawdust substrates. Some dikaryotic isolates derived by mating between monokaryotic isolates showed high yields of sporophores more than those of parental strains. Both the dikaryotic mycelial growth rate and primodia formation number on the PDA plate showed significant correlation with its sporophore products on sawdust substrates.

Keywords: *Pleurotus sajor-caju*, Genetics, Incompatibility analysis.

Pleurotus sajor-caju is one of most attractive species for mushroom growers since it has been introduced for the first time in 1984 as a suitable species for the summer cultivation in Korea (Go *et al.*, 1984). It grew well on rice straw substrates and produced high yields of sporocarps.

Furthermore, the mushroom has some advantages in which it is an unique species which grows and produces sporophores during the summer season where temperature exceeds than 20°C. Cultivation of mushroom is possible throughout the year. Since *P. sajor-caju* has been introduced as an alternative species with *P. ostreatus*.

Therefore the cultivation area of the mushroom will be continuously expanded in the near future. It will also be necessary to improve the mushroom strains to enhance their productivity.

Incompatibility factors of *P. ostreatus* reported already were tetrapolar mating system where the heterothallism or self incompatibility is genetically controlled by two incompatibility factors A and B (Anderson, 1968; Eger, 1974; Go *et al.*, 1980). The A factor seems to be controlled by three linked genes. The number of combination factors derived from a fruit body will often be more than four (Terakawa, 1960). Eugenio and Anderson (1968) estimated

in the number of repeated alleles in a finite sample used, that there are approximately 63 A factors and minimum of 190 B factors in the populated species. This means that each unique combination of A and B factors represents 63× 190=11, 970 mating types.

In mycelial growth, the dikaryotic mycelial growth rate of *P. sapidus* exceeded that of their component monokaryons and hence the mono- and the dikaryotic growth rate showed high correlations. No correlation, however, was found between growth rate of the dikaryons on nutrient agar and sporocarp production on an aspen sawdust oat substrates (Wang *et al.*, 1972). Also no correlation was found between growth rates of derived dikaryons and of monokaryons in *P. flabellatus* (Chandrashekar *et al.*, 1981).

On the other hand, very little information on the genetics of *P. sajor-caju* is available except for only short communication of sexuality of the mushroom by Roxen and Jong (1977). The species of *P. sajor-caju* was recently introduced as a new edible mushroom although the mushroom has been widely cultivated in the Southeast Asia. Jandaik made his first trial to artificial cultivate in the world in 1974. At that time, some cultivation experiments (Bano *et al.*, 1982; Go *et al.*, 1984; Jandaik *et al.*, 1974) and nutrient ingredient analysis of the sporocarps were performed (Jandaik *et al.*, 1975).

This experiment was performed to find out the incompatibility factors of *P. sajor-caju* and to obtain basic data for mycelial growth and sporocarp production for improving the strains.

Materials and Methods

Organism

Pleurotus sajor-caju used in this experiment was kindly provided by Dr. Lee who brought it from India and stocked at the Institute of Agricultural Science as stock number ASI 2070. The organism was cultured on the potato dextrose agar (PDA) media in a tube at 25°C about for 7 days and preserved at 4°C in the refrigerator.

Single Spore Isolation and its Dikaryotization

The basidial spores were collected on sterile Petri dishes aseptically from a mature sporocarp of the mushroom. The spores were suspended into sterile water and made dilution into about 10^5 per ml. The suspension was inoculated on the water agar media and spread uniformly with a curved glass rod. The inoculated Petri dishes were incubated at 25°C about for 4 days until the sporous hyphae was visible with a micromanipulator with 50 fold magnification. The single spore colony was transferred into a tube containing PDA media. After propagating of the colony on media, the mycelium was examined on clamp connection of the mycelium with a microscope with 600 fold magnification to confirm either the monokaryon or dikaryon. Twelve monokaryons were isolated from one sporocarp. The monokaryotic isolates were mated by inoculating in 3 cm distance between two colonies on the PDA plate. Dikaryotization was made in all combinations with the monokaryons.

Mycelial Growth and Sporophores Production

The dikaryotic isolates were inoculated on the PDA plate in a Petri dish and incubated at 25°C for 7 days and the mycelial growth was measured. The completely grown mycelia were kept under irradiated conditions for 14 days to induce primodia which resulted in agglutination of mycelia on the agar plate. The dikaryotic isolates were cultivated with sawdust substrates mixed with 20 %(v/v) rice bran in a 800 ml broad mouth bottle made of polypropylene (P.P.) to produce their sporophores. The sporocarp was weighted when the sporocarps grew for 7 days after pinheading in fresh conditions.

Table I. The results of mating between pairs of the 12 monokaryonic isolates from a sporophore of *P. sajor-caju*.

Monokaryotic isolates		I			II						III		IV
		M-1	M-2	M-14	M-4	M-6	M-7	M-11	M-17	M-20	M-9	M-10	M-3
I	M-1	−	−	−	+	+	+	+	+	+	−	−	−
	M-2	−	−	−	+	+	+	+	+	+	−	−	−
	M-14	−	−	−	+	+	+	+	+	+	(+)	−	−
II	M-4	+	+	+	−	−	−	−	−	−	−	−	−
	M-6	+	+	+	−	−	−	−	−	−	−	−	−
	M-7	+	+	+	−	−	−	−	−	−	−	−	−
	M-11	+	+	+	−	−	−	−	−	−	−	−	−
	M-17	+	+	+	−	−	−	−	−	−	−	−	−
	M-20	+	+	+	−	−	−	−	−	−	−	−	−
III	M-9	−	−	(+)	−	−	−	−	−	−	−	−	+
	M-10	−	−	−	−	−	−	−	−	−	−	−	+
IV	M-3	−	−	−	−	−	−	−	−	−	+	+	−

+ : Clamp formation − : No clamp (+) : Clamp formation only on the barrage

Results and Discussion

The results of mating between monokaryotic isolates in *P. sajor-caju* were shown in Table I. Monokaryotic isolates of M-1, M-2 and M-14 formed the clamp connections when they were mated with any one of M-4, M-6, M-7, M-11 and M-17, and M-10 was dikaryotized only with M-3, respectively. The clamp connections were uniformly distributed on the mycelia of both sides of inoculum. Monokaryotic isolates of M-9 and M-14, however, produced clamp connections only on the barrage in the central part region of both isolates. Based on the mating results the monokaryotic isolates were divided into four group I, II, III and IV as shown in Table I. Group I could be mated with group II, and group III could be mated with group IV, respectively. This means that the mating system of monokaryotic isolates of *P. sajor-caju* was controlled by two incompatibility factors A and B of tetrapolar heterothallism. In the case of M-9 and M-14, clamp connections borne only on the region of barrage at the junction of both isolates. This means that the two isolates showed common B reaction (Chang *et al.*, 1969; Kemp, 1980; Raper, 1978). The common B (A≠ B=), heterokaryon was hard to distinguish from the normal dikaryon with mycelial morphology but common B isolates produced only clamp connections on the junction line (Chandrashekar, 1982). At this point of view, the group I was classified as factor A_1 B_1, group II, A_2 B_2, group III, A_2 B_1 and group IV, A_1 B_2, respectively. The results have corresponded with those of *P. ostreatus* (Andenson *et al.*, 1973; Eger, 1974; Go *et al.*, 1981) *P. sapidus* (Eugenio *et al.*, 1968), *P. flabellatus* (Chandrashekar, 1981), however, they are different from that of *P. spodoleucus* which has bipolar (Nagai, 1959) even in the same genus.

The mycelial growth rates of derived dikaryons (DD) and their component monokaryons (CM) were given in Table II. The dikaryotic isolates grew faster compared with that of their

Table II. Mycelial growth rate of derived dikaryons and their component monokaryons of *P. sajor-caju* on the PDA at 25°C for 7 days.

D. D.		C. M.	
Isolates	Mycelial growth (mm/7 days)	Isolate	Mycelial growth (mm/7 days)
D-1	42.7	M-14	34.2
		M-11	33.3
D-2	28.1	M-1	29.2
		M-4	29.7
D-3	42.5	M-7	32.2
		M-2	25.8
D-4	41.9	M-11	33.3
		M-2	25.9
D-5	38.3	M-1	29.2
		M-11	33.3
D-6	38.0	M-20	23.1
		M-7	32.2
D-7	38.6	M-4	29.7
		M-10	33.6
D-8	31.7	M-14	34.2
		M-9	27.5
D-9	33.4	M-6	30.7
		M-14	34.2
Average	37.2		

D.D. : Drived dikaryons
C.M. : Component monokaryons

Table III. Primodia number of derived dikaryons and their component monokaryons of *P. sajor-caju* on the PDA media on 14th day after inoculating under irradiated conditions.

D.D.		C.M.	
Isolates	Number of primodia	Isolates	Number of primodia
D-1	112	M-14	3
		M-11	0
D-1	0	M-1	96
		M-4	0
D-3	114	M-7	37
		M-2	3
D-4	50	M-11	0
		M-2	3
D-5	4	M-1	96
		M-11	0
D-6	8	M-20	0
		M-7	37
D-7	36	M-4	0
		M-10	29
D-8	175	M-14	3
		M-9	2
D-9	136	M-6	4
		M-14	3
Average	71		

D.D.: Derived dikaryons
C.M.: Component monokaryons

component monokaryons, although D-2, D-8 and D-9 isolates were similar to those of their component monokaryons. These results correspond with those of Wang *et al.* (1972) in *P. sapidus* and Chandrashekar *et al.* (1981) in *P. flabellatus.* Among the dikaryotic isolates, D-1, D-3 and D-4 showed best mycelial growth. These isolates grew more than 40 mm for 7 days, compared with about 30 mm of their component monokaryons in the same periods. This result indicates that the growth ability of mushroom can be enhanced by combining of the monokaryons.

No correlation between mycelial growth rate of dikaryons and that of their component monokaryons was found as shown in Table V. In *P. sapidus,* dikaryotic mycelial growth rate exceeded those of their component monokaryons, and mono- and dikaryotic growth rate showed a high correlation (Wang *et al.*, 1972). However, in case of *P. flabellatus,* no correlation was found between growth rate of derived dikaryons and those of their component monokaryons (Chandrashekar *et al.*, 1981). In our results, *P. sajor-caju* was different from that of *P. sapidus* but corresponed with that of *P. flabellatus.* This means that correlations between mono-and dikaryotic mycelial growth rates were various depending upon the species.

The species, *P. sajor-caju* formed primodia

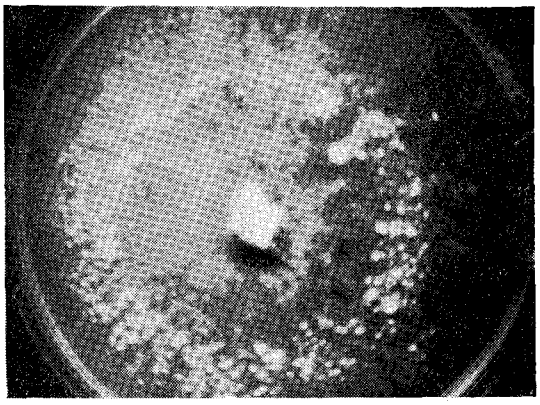

Fig. 1. The primodia of the monokaryotic isolate in *P. sajor-caju* on the PDA media.

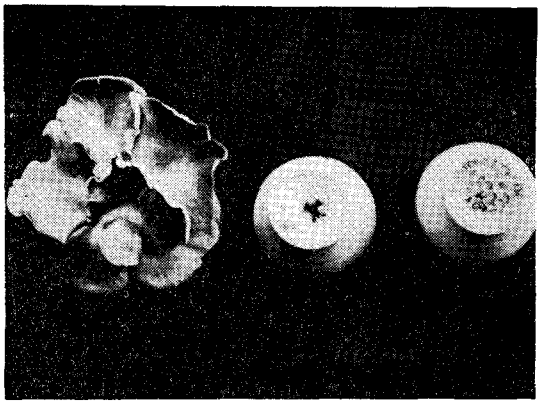

Fig. 2. Fruit body morphology of the dikaryon (left one) and the monokaryon (two bottle of the right) in *P. sajor-caju*.

well on the PDA plate not only in the dikaryons but also in the monokaryons except M-4, M-11 and M-20 isolates. These primodia were formed by agglutination of the mycelia like tiny cotton ball as shown in Fig. 1. The primodia formed from 7 days after they were under irradiated conditions. The property of primodia forming on the agar plate is of great significant among *Pleurotus* species. The number of primodia of the mono- and dikaryons is shown in Table III. The dikaryons had more primodia than those of monokaryons except D-2, D-5 and D-6 isolates. The number of primodia between mono- and dikaryons was not correlated (Table V).

Table IV. Yields of fresh mushrooms, cap diameter and stem length of the derived dikaryotic isolates of *P. sajor-caju*.

Isolates	Yield (g/800cc bottle)	Cap diameter (mm)	Stem length (mm)
D-1	60	58	42
D-2	—**	—	—
D-3	58	58	38
D-4	67	55	44
D-5	71	60	42
D-6	65	59	40
D-7	70	67	48
D-8	—	—	—
D-9	42	76	3
O.P.S.*	67	76	50

* O.P.S.: Original parents strain
**—; Contaminated by weed molds

The sporophores occurred on the sawdust substrates in a 800 ml P.P. bottle not only in the dikaryotic isolates but also in the monokaryons. As shown in the Figure 2, the dikaryotic isolate had normal sporophores fully grown and produced basidial spores. However, the monokaryotic isolates were abnormal and they couldn't be grown fully. The cap was not opened, being only about 5~8 mm in diameter where the stem was about 5~10 mm in length, respectively. After a 4-day period the sporophores failed and died. Esser (1978) reported that some monokaryotic species of 12 genera of *Agaricales* and 6 genera of *Polyporus* produced sporophores which be called monokaryotic fruiting. According to him, the ability of producing sporophores by monokaryotic mycelia originated from a single uninucleate basidial spore which had a fruit body or fruit-like structure formed. In the monokaryotic mycelium, there are at least two genes that control the potential of fruiting as in the case of dikaryotic mycelia.

The primodia of the dikaryotic isolates formed well on the sawdust substrates 3 to 5 days after

Table V. Correlations between the derived dikaryons and their component monokaryon in *P. sajor-caju*.

Characteristics	Correlation coefficient(r)
Mycelial growth rate of the monokaryons and that of the derived dikaryons	0.05
Number of primodia of the monokaryons and that of the derived dikaryons	0.41
Mycelial growth rate of the derived dikaryons and the yields of fresh sporophores	0.87*
Number of primodia formation of the dikaryons and the yields of fresh sporophores	0.71*
Yields of fresh sporophores and cap diameters	0.95**
Yields of fresh sporophores and stem length	0.47*

*Significant at 0.05 **Significant at 0.01

inducing to fruit. The fruit bodies appeared singly and developed up to 7~10 cm in diameter of the cap for 7 days after pinheading. The sporophore yields of the dikaryotic isolates were shown on Table IV. The dikaryotic isolates of D-4, D-5 and D-7 showed higher yields of sporophores than those of their parental strains. Among them, D-5 isolate produced sporophores 6 % more than that of its orinigial strain. This result shows the possibility of selecting high yielding strains through the cultivation trials. Meanwhile, D-2 and D-8 isolates were so severely infected by *Trichoderma* spp. that the isolates didn't produce their sporophores.

The mycelial growth rate and primodia number of the dikaryotic isolates on the PDA media showed high correlations with their sporophores products on sawdust substrates (Table V). This result suggested that it is an easy way to improve high yielding strain in this species because it is possible to select the desired strain among isolates in the laboratory before cultivating it with sawdust substrates. Significant correlations between total sporophore yields, its cap diameter and stem length were also found, respectively.

적 요

여름느타리버섯 *Pleurotus sajor-caju*은 2個의 不和合性 因子를 가진 4極性 交配系를 가지고 있으며, 2核菌絲體는 單核菌絲體보다 菌絲生長이 빨랐으나 相互相關關係는 없었다. 여름느타리버섯은 2核菌絲體 뿐만아니라 單核菌絲體도 agar培地 上에서 原基形成이 잘되는 特徵이 있으며 2核菌絲體에서 그 形成數가 많은 傾向이었다. 單核菌絲體의 子實體는 그 形態가 奇型이었으며 成熟하지 못하고 死滅되었으나 2核菌絲體의 子實體는 正常으로 生長하였다. 交配菌株 中 D-5 菌株는 母菌株 보다 子實體 收量이 높았다. Agar培地 上에서 2核菌絲體의 菌絲生長과 原基形成 數는 톱밥 瓶栽培로 얻은 子實體 收量과 高度의 相關이 있었으며, 子實體 收量은 傘의 直徑과 줄기의 길이와도 高度의 相關을 보였다.

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