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Radiation Sensitivity of Basidiospore and Mycelium in *Pleurotus ostreatus*

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Abstract

To assess the effects of gamma-ray (Co-60) on radiation sensitivity and genetic similarity of the basidiospore and mycelium in oyster mushroom, *Pleurotus ostreatus*, the D_{10} values and RAPD patterns were analysed. Three strains were isolated from basidiospores (PO-B1, -B2, and -B3 from 2 kGy irradiation group) and five strains from mycelia (PO-M1, -M2 from 1 kGy, PO-M3 from 2 kGy, and PO-M4 and -M5 from 2+1 kGy irradiation group). The D_{10} values of basidiospore and mycelium of *P. Pleurotus ostreatus* were 1,250 Gy and 500 Gy, respectively. The growth rates of the eight strains on the five media were various and the activities of extracellular chitinases of them were generally higher than those of the control. By the gamma-ray radiation, 22-25% of genetic similarities were changed in the basidiospore strains and 23-36% of them in the mycelium strains. From these results, it seems that the basidiospore could be more radio-resistant than the mycelium of *P. ostreatus* and that the genetic similarity of the mycelium of *P. ostreatus could* be changed easier than that of the basidiospore by the gamma-ray radiation.

Key Words : basidiospore, gamma-ray, mycelium, *Pleurotus ostreatus*, RAPD, sensitivity

1. Introduction

The productivity of lignocellulosic biowastes is greatly expanded along the technical improvement of agricultural industry all over the world. One of the useful methods for reusing biowastes is the cultivation of edible mushrooms [1]. Edible mushroom could convert lignocellulosic biowastes to fruiting body which have definite nutritive and medicinal values [2-4]. The bioconversion products of inedible biowastes by edible mushroom could be also useful as animal feed [5]. Among hemicellulose, cellulose, and lignin fractions of biowastes, the lignin is the most difficult fraction to be degraded by mushroom [6,7]. Thus, it is necessary to induce and/or isolate the improved strains of edible mushroom with more lignocellulolytic activity.

Recently, several studies have shown that gamma-ray radiation could change the genomic structure. In the diploid mutants of rice derived from gamma-ray irradiated tetraploids, the rDNA

intergenic spacers (IGSs) were rearranged [8]. The gamma-ray radiation induced the positive [9] or negative mutants [10,11] of specific genes. In mutants of *Trichoderma harzianum* induced by gamma-ray radiation [12], the change of genetic diversity have been analyzed by DNA fingerprinting of randomly amplified polymorphic DNA (RAPD). It seems that gamma-ray radiation could be valuable tool to induce useful strains of edible mushroom for recycling the biowastes. On the way to meet these aims, the effects of gamma-ray radiation on the radiation sensitivity and the genetic similarity of the basidiospore and the mycelium in edible oyster mushroom, *Pleurotus ostreatus*, were investigated.

2. Materials and Methods

2.1. Irradiation and Isolation of Survivals

Basidiospores and mycelia of *P. ostreatus* (isolated in Kang-Won province, Korea) were irradiated with gamma-ray (Co-60, 60,000Ci of capacity, Atomic Energy of Canada Ltd.) at the dose of 1 kGy to 20 kGy in the potato-dextrose broth (Difco, pH 5.2) at room temperature. Dose rate (300 Gy/hr) was determined by Fricke dosimetry [13]. The irradiated basidiospores and mycelia were spread on potato-dextrose agar (PDA) plate within 30 mins after irradiation and recovered at 25℃ for 2 days. Three strains were isolated from irradiated basidiospores (PO-B1, -B2, and -B3 from 2 kGy irradiation group), five strains from irradiated mycelia (PO-M1, -M2 from 1 kGy, PO-M3 from 2 kGy irradiation group, and PO-M4 and -M5 from 1 kGy irradiation group of PO-M3). The germination rate (100 X number of the germinated basidiospore/number of the spread basidiospore) and its survival rate (100 × number of the survival mycelium/number of the spread mycelium) of the basidiospore and the mycelium were examined. The dose response curves were obtained and the D_{10} values (D_{10} = -1/slope(b)) were calculated from the linear regression curve ; y = a + bx [14].

2.2. Growth Rates on the Five Solid Media

The growth rates of the eight strains and the control were measured on solid media such as complete- (PDA), minimal- (MM), lignin- (LIG), cellulose- (CMC), and glucoside-media (GLU) [15].

2.3. Activities of the Extracellular Chitinases

The activities of the extracellular exo- and endo-chitinase were measured by the methylumbellifery (MUF)-residue reaction [16] using MUF-N-acetyl-β-D-glucosaminide and MUF-N,N′-diacetyl chitobioside, respectively. After reaction, the fluorescence was detected by spectrofluorometer (Hoefer, TKO100; excitation: 365 nm, emission: 460 nm) [17]. The unit of enzyme activity was determined by the following formula ; Enzyme activity (unit) = Emission intensity × 1000/ST = Emission intensity X 10/mMhr (S: concentration of substrate, 25 μM, T: reaction time, 4 hr).

2.4. Randomly Amplified Polymorphic DNA (RAPD) Analysis

The genomic DNA was isolated according to Graham [18] and the polymerase chain reactions (PCRs) were carried out in 25 μl of reaction solution containing 20 ng of genomic DNA template and 5 pmol of random primer (Operon Technologies, Table 1) using AccuPower™ PreMix-Top (Bioneer, Korea). All PCR reactions were subjected to the initial denaturation at 94℃ for 5 min. They were then processed through 55 cycles consisting of denaturation at 94℃ for 30 sec, annealing at 38℃ for 30 sec, and extension

Table 1. Primers Used in RAPD Reaction

Primer	Source	G-C contents (%)	Sequences
#1	OPF-2	60	5′-GAGGATCCCT-3′
#2	OPF-3	60	5′-CCTGATCACC-3′
#3	OPF-5	60	5′-CCGAATTCCC-3′
#4	OPF-7	60	5′-CCGATATCCC-3′
#5	OPF-8	60	5′-GGGATATCGG-3′
#6	OPF-1	60	5′-ACGGATCCTG-3′
#7	OPF-6	60	5′-GGGAATTCGG-3′
#8	OPF-9	60	5′-CCAAGCTTCC-3′
#9	OPF-13	60	5′-GGCTGCAGAA-3′
#10	OPG-2	70	5′-GGCACTGAGG-3′
#11	OPG-3	70	5′-GAGCCCTCCA-3′
#12	OPG-5	60	5′-CTGAGACGGA-3′
#13	OPH-2	60	5′-TCGGACGTGA-3′
#14	OPH-3	60	5′-AGACGTCCAC-3′
#15	OPH-4	70	5′-GGAAGTCGCC-3′

at 72℃ for 1 min. These cycles were followed by a single cycle of 5 min at 72℃ using Gene Amp PCR System 2400 (Perkin Elmer). Aliquots of the amplified DNA were separated by electrophoresis on a 0.8% agarose gel at 10 V/cm. All of the bands were numbered and marked as T if a certain band appeared or A if not. Genetic similarity coefficients of the isolated strains were calculated with CLUSTAL-W software [19].

3. Results and Discussion

3.1. Radiation Sensitivity

The germination rate, survival rates, and radiation sensitivities of basidiospore and mycelium of *P. ostreatus* were determined (Fig. 1). The germination (Fig. 1A) and survival curve (Fig. 1C) of the basidiospore were sigmoid and the D_{10} values were calculated from the linear regression formulae (Fig. 1B and 1D) as 2,500 Gy

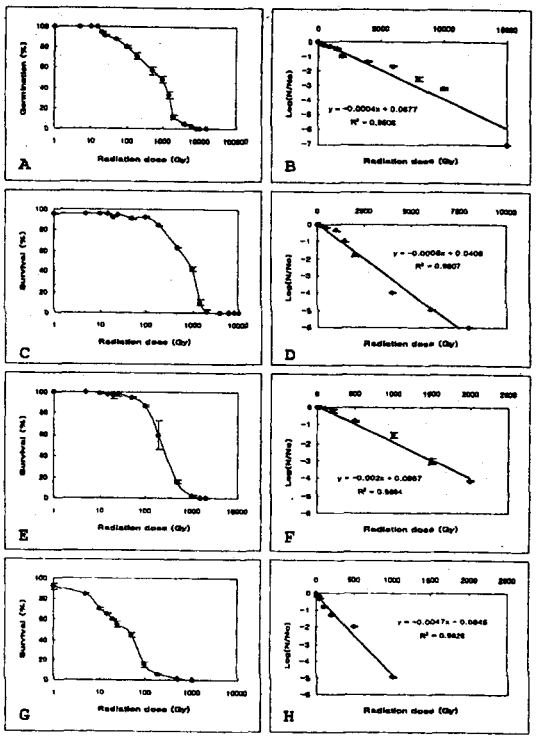

Fig. 1. Germination and Survival Rate of the Basidiospore and the Mycelium in *Pleurotus ostreatus* After Gamma-Ray Radiation. A, B ; Germination Curve of Basidiospore, C, D ; Survival Curve of Basidiospore,. E, F ; Survival Curve of Mycelium (control), G, H ; Survival Curve of PO-M3 Derived from 2 kGy Irradiation Group of the Control. Data Represents Mean ± Standard Error of Mean and Experiments Were Separately Duplicated.

and 1,250 Gy, respectively. The survival curve of mycelium after irradiation of gamma-ray was also sigmoid (Fig. 1E) and the D_{10} value was 500 Gy (Fig. 1F). In the strain PO-M3 derived from 2kGy irradiation group of mycelia, the radiation sensitivity was also determined (Fig. 1G) and the D_{10} value was 213 Gy (Fig. 1H). It is suggested that the radio-sensitive mutant could be induced by the gamma-ray radiation. As shown in Fig. 1,

Table 2. Growth Patterns and the Activities of Extracellular Chitinase of Irradiated Strains (*Pleurotus ostreatus*) by the Gamma-ray Radiation

Strain	Radiation dose (kGy)	Length of mycelium at 7-day (mm in diameter)					Activity of enzyme (unit/1mg of protein)	
		PDA	MM	LIG	CMC	GLU	Exo-chitinase	Endo-chitinase
Control	0	31	27	3	33	27	1269	1643
PO-B1	2	17	15	7	15	20	455	1004
PO-B2	2	30	40	3	40	26	7527	2195
PO-B3	2	25	25	3	11	25	1187	197
PO-M1	1	35	15	18	20	25	16489	11314
PO-M2	1	28	27	7	25	26	15701	15701
PO-M3	2	32	23	3	25	22	8310	6430
PO-M4	2+1	42	36	5	28	18	10950	7848
PO-M5	2+1	40	32	5	24	20	8785	12216

basidiospore was more resistant than the mycelium against the gamma-ray radiation. Aziz and colleagues [20] showed that the D_{10} values of the spores of filamentous fungi, *Aspergillus* species were ranged from 360 to 630 Gy and that the radio-resistance could be related to the lipid content of the cell walls. It seems that the basidiospore of *P. ostreatus* could be more radio-resistance than the spore of *Aspergillus* species because of the different characteristics of cell wall components in each spore.

3.2. Growth Patterns and Activities of Extracellular Chitinases

The growth rates on the five solid media and the activities of two chitinases of the eight strains and the control were measured and shown in Table 2. The growth rates of the basidiospore strains were more remarkably altered than those of the mycelial strains and the ratio constants to the control were 0.61-1.15. In LIG media the PO-M1 showed 6 times higher growth rate than the control. It seems that the gamma-ray radiation could change the growth rate on the tested media. The activities of the extracellular exo- and endo-chitinases of all eight strains were 1.3-12.0 times higher than those of the control except PO-B1 and -B3. From the changed activities of the two chitinases in *P. ostreatus*, it is suggested that the growth patterns of *P. ostreatus* could be altered variously because, in general, chitinase used to play a role in cell growth and differentiation [21]. It seems that the gamma-ray radiation could not alter the specific genes regularly but ramdomly from the results of the above characteristics.

3.3. Genetic Similarity

The RAPD patterns of the eight strains were analyzed using fifteen 10-base primers (Table 1) and shown in Fig. 2. Five primers (#1-#5) produced polymorphic DNA as 1-12 bands in the three strains (PO-B1, -B2 and -B3) and ten primers (#6-#15) produced as 3-8 bands in the other strains. The genetic similarities of three

Table 3. Genetic Similarity Coefficient Matrix Among Strains of *Pleurotus ostreatus* Irradiated by the Gamma-ray Radiation Based on RAPD Markers

Strain	Control	PO-B1	PO-B2	PO-B3	PO-M1	PO-M2	PO-M3	PO-M4	PO-M5
Control	1.00								
PO-B1	0.50	1.00							
PO-B2	0.51	0.51	1.00						
PO-B3	0.53	0.50	0.57	1.00					
PO-M1	0.93				1.00				
PO-M2	0.91				0.89	1.00			
PO-M3	0.73				0.71	0.73	1.00		
PO-M4	0.77				0.73	0.78	0.91	1.00	
PO-M5	0.64				0.64	0.64	0.82	0.84	1.00

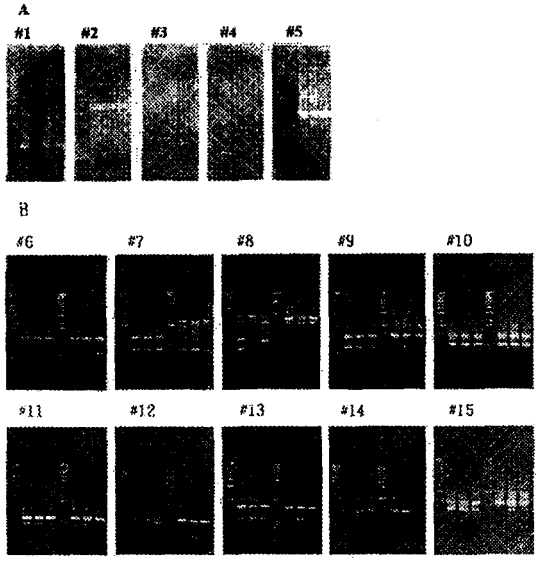

Fig. 2. RAPD Fingerprints of Eight Strains (*Pleurotus ostreatus*) Irradiated by Gamma-Ray Radiation. A) Basidiospores : PO -B1, -B2 and -B3 Derived from 2 kGy Irradiation Group. Lane 1 : 1kb Marker, Lane 2 : Control, Lane 3 : PO-B1, Lane 4 : PO-B2, Lane 5 : PO-B3. B) Mycelia : PO-M1 and PO-M2 (1 kGy); PO-M3 (2 kGy); PO-M4 and PO-M5 (2+1 kGy). Lane 1 : 1 kb Marker, Lane 2 : Control, Lane 3 : PO-M1, Lane 4 : PO-M2, Lane 5 : 1 kb Marker, Lane 6 : PO-M3, Lane 7 : PO-M4, Lane 8 : PO-M5. #1-#15 : Primers (See Table 1).

strains (PO-B1, PO-B2, and PO-B3) derived from irradiated basidiospores decreased to 50%, 51% and 53% of the control, respectively (Table 3). RAPD analysis revealed that there is approximately 75% genetic similarity among the basidiospores of *P. ostreatus* [22]. As a result, 22-25% of genetic similarity has been changed by the 2 kGy of gamma-ray radiation in the basidiospores. In the other strains derived from irradiated mycelia of *P. ostreatus*, the genetic similarities decreased to 64%-93% of the control (Table 3). Compared with the basidiospores, 23-36% of genetic similarity has been changed in the 2 kGy irradiation group. It seemed to be resulted from the differences of radiation sensitivities between them, that is, the basidiospore is more radio-resistant than the mycelium. However in the 1 kGy irradiation group, 7-9 % of the genetic similarlity has been changed. It suggested that the genetic similarity could be altered depending on the radiation dose of gamma-ray.

From the above results, it is suggested that growth patterns of *P. ostreatus* could be changed not specifically but randomly and diversely. And it seems that the genetic similarity of the mycelium could be altered at ease and dose dependently

compared with that of the basidiospore by the gamma-ray radiation.

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