NOTE

Mutation Spectrum of Manganese (II) Peroxidase Gene in the *Pleurotus* ostreatus Mutants Induced by Gamma Radiation

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The mutational spectra in the manganese (II) peroxidase gene (mnp) of the *Pleurotus ostreatus* mutants induced by gamma radiation (Co^{60}) give evidence to prove the effect of gamma radiation on the gene. mnp of each mutant was cloned, sequenced and analyzed. Among the 1941 base pairs of the sequenced region of the mnp genes of 4 mutants (PO-5, -6, -15 and -16), nine mutational hotspots on which the same base was mutated simultaneously were found, additionally 6 mutations were also found at different positions in the mnp gene. These mutation-spectra were predominantly A:T \rightarrow G:C transitions (50.1%). By the analysis of putative amino acid sequences, PO-5 and PO-16 mutants have 3 and 1 mutated residues, respectively. Since the mutational spectra reported herein are specific to the mnp gene, we propose that the mutational hotspots for the gamma radiation could be in the gene(s) within cells.

Key words: gamma radiation, manganese (II) peroxidase gene, mutational spectrum, Pleurotus ostreatus

Irradiation by gamma ray may cause some mutations to the genes of cells through the DNA repair mechanisms within cells (Thacker, 1999). Lafleur and colleagues studied the mutational spectrum of the lacZ gene on the M13mp 10 DNA or plasmid after irradiation with gamma ray. They suggested that the mutational spectrum depended on irradiated conditions and DNA repair mechanisms of host cells (Reitsma-Wijker et al., 2000; Kuipers et al., 2000a; Kunipers et al., 2000b) and that the repair mechanisms of the base excision repair, SOS repair and nucleotide excision repair are the causes of the mutational spectra in the target genes (Wijker and Lafleur, 1998; Kuipers et al., 2000a; Braun et al., 1997). In fact, because the extra chromosomal DNA as plasmid could exist due to the functionally useful gene, for example the resistant gene to antibiotics, the information from the cell-free irradiated system could be too simple to explain the effect of the gamma ray irradiation on the genes of cells.

Many factors could affect the mutational spectra of genomic and episomic DNA in a cellular environment after gamma ray irradiation. After the 250 Gy of gamma ray irradation, the C/G basepair substitutions were the main type of gamma ray induced mutations in *E. coli* and

the spontaneous mutational hot spots at position 620-632 in the *lacI* gene reduced (Wijker et al., 1996). In *lacI* transgenic mice, the mutation frequency of the lacI gene was increased by gamma radiation (Winegar et al., 1994; Hoyes et al., 1998). In hamster ovarian cells (CHO), the gamma radiation induced aprt- or hprt-deficient mutants with transversions or deletions, respectively (Miles and Meuth, 1989; Thacker, 1986). In white-rot fungus, Phanerochaete chrysosporium, the phenoloxidase negative mutants were induced by gamma radiation (Liwicki et al., 1985). Zolan and colleague (1988) isolated the radiationsensitive meiotic mutants of Coprinus cinereus. The genetic diversity increased in the gamma radiationinduced mutants resulted from RAPD analysis (Lee et al., 2000). Therefore, it is necessary to get some more information about the mutation spectra of several genes from the gamma radiation induced individual mutants.

Manganese (II) peroxidase (MnP) of *Pleurotus ostreatus* (oyster mushroom) is one of the main enzymes that degrade lignin which is the most difficult fraction of lignocellulosic materials to degrade (Asada *et al.*, 1995). The enhanced mutants of ligninolytic ability induced by gamma ray irradiation were isolated from *P. ostreatus* PO-1 and characterized, previously (Lee *et al.*, 2000). The present study has been carried out to investigate the mutation spectrum of *mnp* genes of *P. ostreatus* PO-1 mutants induced by gamma ray irradiation. Mycelia of *P. ostreatus*

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PO-1 (Korea, KCTC 16812) and its mutants (PO-5. -6. -7, -14, -15 and -16) induced by gamma ray irradiation previously were cultured in potato dextrose broth (PDB) media according to the previous study (Lee *et al.*, 2000).

The mutants of PO-5, -6, -7 were induced with 1 kGy of gamma radiation (Co-60), and PO-14 was induced with 2 kGy. The PO-15 and PO-16 mutants were derived from PO-14 after re-irradiation with 1 kGy of gamma radiation. Genomic DNAs were extracted according to Graham (1994) and the polymerase chain reactions (PCRs) were carried out with AccuPower PCR PreMixTM (Bioneer Co., Korea) in 50 µl of reaction solution containing 20 ng of genomic DNA and 5 pmol of each primer. Specific primers for the mnp gene of P. ostreatus were constructed with the sequence of 5'-CCC TAC ATC GCA ATG ACC TT-3' (MNP-A2, forward) and 5'-ACT TTG CTT ACG CAG GTG GG-3' (MNP-B2, reverse), which were retrieved from the Genebank database (accession number POU21878) (Asada et al., 1995). All PCR reactions were subjected to the initial denaturation at 94°C for 5 min. These were then processed through 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, and extension at 72°C for 1 min. These cycles were followed by a single cycle of 5 min at 72°C using Gene Amp PCR System 2400 (Perkin Elmer). Aliquots of the amplified DNA were separated by electrophoresis on 0.8% agarose gel at 10 V/cm. The PCR products were cloned into pGEM T-vector (pGEM T-easy Vector System, Promega). The total RNAs of PO-1 mutants were isolated with an RNeasy kit (Quiagen) and were used to amplify cDNA of the mnp gene using the above primers. The amplified cDNAs of the mnp gene were also cloned into the pGEM-T vector. The cloned genes were sequenced by automatic sequencer (LI-COR IR2 System). The sequences were analyzed with BCM Search Launcher (http://dot.imgen.bcm.tmc.edu::9331). The mnp gene of P. ostreatus PO-1 (KCTC 16812) amplified by PCR with MNP-A2 (contain the ATG start codon) and MNP-B2 (contains the TAA terminal codon) primers consisted of 1941 base pairs, but P. ostreatus IFO30160 had 1936 base pairs (Asada et al., 1995). The intron 5 and 7 of mnp of PO-1 are one and four bases longer, respectively, than those of IFO30160. However, both mnp genes contained 15 introns and shared the identical exon-intron boundaries (Fig. 1). The nucleotide sequence of the PO-1 mnp gene differed 6.59% (128 bases/1941 bases) from mnp of IFO30160. Among the 361 amino-acid residues of predicted encoding MnP protein of PO-1, four residues (Ala-15, Val-20, His-21 and Arg-253) differed from IFO30160 (Val-15, Ala-20, Gln-21 and Lys-253, respectively) (Fig. 2).

Using MNP-A2 and MNP-B2 primers, 1941 base pairs of the *mnp* genes of 4 mutants (PO-5, -6, -15 and -16) induced by gamma radiation were amplified. The restriction maps and sequences of randomly selected clones of PCR products of each mutant did not differ from each

other (data not shown). Among the 1941 base pairs of the mnp gene, nine hot spots on which the same base was mutated simultaneously among the mutants were found (Table 1). Additional mutations were found at different positions in the mnp genes of PO-5, -15 and -16 mutants as 4, 2, and 1 base, respectively. The types of these mutations consisted of transversions (25%) as $C \rightarrow G$ (2/4), $C \rightarrow A$ (1/4) and $T \rightarrow G$ (1/4), and transitions (75%) as $G \rightarrow A$ (2/12), $A \rightarrow G$ (3/12), $C \rightarrow T$ (2/12) and $T \rightarrow C$ (5/12) (Table 2). Therefore, transversions occurred only at pyrimidine bases, but all types of transitions occurred in the mnp locus. However, tandem double base substitutions and base insertions/deletions did not occur. The genomic mnp gene of PO-14 was not cloned and its sequence was not compared to those of PO-15 and -16, derived from PO-14 after 1 kGy reirradiation. If the mnp gene of PO-14 was also mutated at the nine hot spots similar to PO-5 and PO-6, it means that the mutated bases of PO-15 and PO-16 did not result from the reirradiation of 1 kGy. In this case, the specific mutated bases found in PO-15 and -16 were really induced by the reirradiation (Table 1) and back mutations at least in the nine hot spots did not occur. All of the gamma radiation induced mutations in the mnp gene were base substitutions (Table 2), a similar result which was also obtained by other studies (Miles and Meuth, 1989; Wijker et al., 1996; 1998; Wijker and Lafleur, 1998). The majority of all types of base substitutions occurred on G and C bases in other studies (Sargentini and Smith, 1994; Wijker et al., 1996), whereas our result showed a clear difference. In the mnp gene, the A and T bases were 56.5% of all base substitutions. The predominance of mutations at C bases could partly be explained by the fact that the lacI gene had more C sites than A sites (Wijker et al., 1996). However, it dose not explain the mnp gene. Although the contents of C and G bases of mnp (50.76%) were slightly higher than those of A and T bases, A and T bases were more mutated in the mnp gene. Since the change of mutation spectrum is a result from the repair of the damaged base or base pair by the DNA repair system of the cell, the precision and/or the difference of repair systems could reflect the mutation spectrum. Von Sonntag (1987) suggested that the damaged bases induced by gamma ray radiation exhibited no preference for a specific base (or base pairs). But the mutation spectrum in the present results could reflect a preference to a specific base on DNA sequence of certain genes depending on the repair mechanisms of the cell.

The PO-1 strain of *P. ostreatus* (isolated in Korea) has a different sequence in the *mnp* gene from other strain, IFO30160 (*P. ostreatus* IFO30160 Hiratake; Asada *et al.*, 1995; Fig. 1). These strains may evolve individually into other strains through the genetic barrier by regional separation. Therefore, they would have a different spectrum by spontaneous mutation (Fig. 1). The mutational hot spots on which the same base at the same position of all

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studied mutants were found to be as 56.25% of all base substitutions (Table 1). In the 9 hot spots of the *mnp* gene, 5 spots were equal to the naturally mutated sites and among them 4 sites were mutated by gamma radiation to

the same bases as those of IFO30160 strain. It means that 3.1% of natural mutation sites (128 bases) could be regarded as hot spots in the *mnp* genes of PO-1 and IFO30160 strain (Fig. 1, Table 1). This phenomenon has

P0U21878 P0-1	CCCTACATOGCAATO	5 16 30 B ACCTITISCTTICSCTT B ACCTITISCTTICSCTT	TOTOCOCTOGTCCTT	GTCTTCGCTGTGACT	GTCCAAGTCGCTCAA	76 90 Ggtgagccaccgcgt Ggtgagccaccgcgt	90 90
P0U21878 P0-1	cccctcctactgatt	5 106 120 agcctaacacgacgt agcctaacacgacgt	gcttcagCCGTATCT	TTGCCTCAGAAACGC	GCAACTTGTGCTGGT	166 180 GGACAAGTTACCGCC GGACAAGTTACCGCC	180 180
P0U21878 P0-1	AACGCGGCCTGTTGT	196 210 GTTCTGTTCCCGCTC GTTCTCTTCCCGCTC	ATGGAAGACCTGCAG	226 240 AAGAACCTGTTCGAC AAGAACCTGTTCGAC	GACGGCGCATGCGGC	256 270 GAAGATgtacgeteg GAAGATgtacgetea *	270 270
P0U21878 P0-1	ctccatccactacga	5 286 300 i ctcttacttattgct i ctcttactaattgct	atctatgccagGCCC		TGACCTTCCACGACG		360 360
POU21878 PO-1	CTTCCAGGGGgtagg	tottogcccattitg tottoggccattitg	tatggcgccctgtt tattgcgtccctgtt	406 420 attgatttgtcgagg actgattcgtcgagg	acagTGTTATGGGAG	GCGCCGATGGCTCTG	450 450
P0U21878 P0-1	TCATCACATTCTCCC	ATACTGAGGTCAACT ATACTGAGGTCAACT	TCCCAGCCAACCTCG TCCCAGCCAACCTTG *	GAATCGACGAGATCG * *	TCGAGGCTGAGAAAC TCGAGGCCGAGAAAC	CGTTCCTTGCAAGGC	540 540
P0U21878 P0-1	ACAACATCTCCGCAC ACAACATCTCCGCAC	556 570 i GCGATTTgtacgtta i GCGATTTgtacgcta	ttcactggcctattc ttcacgagcctacgc	tagactgaggattga *	cactcactactitgc cactcaccactctcc * * *	gcagGGTTCACTTCG gcagGGTTCACTTCG	630 630
P0U21878 P0-1	CCGGCACCCTCGCCG CCGGCACCCTCGCCG	TTACCAACTGTCCTG TTACCAACTGCCCTG *	GTGCTCCCCGAATCC *	CGTTCTTCTTAGGTC CGTTCTTCTTAGGAC	GCCCTCCTGCCAAGG GTCCCCCTGCCAAAG	COGCATCACCCATTG COGCGTCACCCATAG	720 720
P0U21878 P0-1	GATTGGTTCCGGAAC GATTGGTTCCGGAAC	736 750 CATTCGgtcagcagc CATTCGgtaagcagc	tgttttgggaattt tatttttgtaatttt	gccgcagactgttaa gccgcagcctgttaa	cgaccttgaatagAT cgaccttgaatagAT	ACCATTACAGATATC ACCATCACAGATATC *	809 810
P0U21878 P0-1	CTGGCCCGAATGGAT CTGGCCCGAATGGAT	826 840 GACGCTGGATTCGTC GACGCTGGATTTGTC	TCTGTCGAGGTTGTC TCCGTCGAGGTTGTC *	TGGCTTCTTTCCGCg TGGCTCCTTTCCGCg	tacgataaacaccat tacgcttgacaccat * **	ttgctccagaactca ttgctccagaaccta	899 900
P0U21878 P0-1	tggetgaegtagegt tggetgaegtagegt	916 930 acagCCACTCTGTCG acagTCACTCTGTTG	CTGCAGCTGACCATG CTGCAGCTGACCATG	TTGACGAAACTgtaa TTGACGAAACTgtaa	gcattacccaaagat gcattaccctaaaat	tacgacgttcg tacgatgttctfttg	985 990
P0U21878 P0-1	acgetgacttaactt aagetgacttaactt	1006 1020 tttgtgaagATTCCT tttgtgaagATTCCT	GGgtaagctttgcat GGgtgagttttgcat * *	acaaatttattttc attaatttccctttc	tettgtggettacae tettgtggettacae	gcactcagAACGCCG acactcagAACGCCA *	1075 1080
P0U21878 P0-1	TTCGACTCAACGCCA TTCGACTCAACGCCA	1096 1110 AACCTTTTCGATTCA AACCTTTTCGATTCA	CAAATCFTCATCGAG CAAATCFTCATCGAG	ACGCAACTCCGTGGA ACGCAACTCCGCGGC * *	ATTTCCTTCCCAGGQ ATTTCCTTCCCAGGQ	taggoggttctccta taatcaattttccca	1165 1170
P0U21878 P0-1	atgcccaatccacgt atactcaatccatat	1186 1200 acatattgctcactt ccatattgctcactc	gttgaatagCACTGG gttgaatagCACTGG	TGGGAATCACGGCGA TGGGAACCACGGCGA *	AGTACAATCCCCACT AGTACAATCCCCACT	CAAGGGTGAAATGAG CAGGGGTGAAATGAG	1255 1260
P0U21878 P0-1	ACTCCAGTCAGATCA ACTCCAGTCAGATCA	1276 1290 CTTGgttagtcttta CTTGgttagtctttc	cactgctctcttgca cactgctattttgca * *	cogagaagcgatatc ccgagtagtgatatc * * *	tgacatgacgtgccc tgacatgacatgcat	accgatttgacag∏ accgatttgacag∏	1345 1350
P0U21878 P0-1	CGCTCGAGgtacttg	aaactaatcattcat aaactaatcatttat *	aataacictcccca gatgatatttcccca + + +++ +	ttaaaacatccaaat ttaaaacatccaaat	gtatcagACGATAGG gtatcagACGATAGG	ACATCCTGCGAATGG ACATCCTGCGAATGG	1435 1440
P0U21878 P0 1	CAGTCCATGACTAgt	aagtatcatgctcta aagtatcatgttcta *	ecgettecetttete tegettecetttete *	ctcctaagctcattg ctcctgagctcacta	tcaacgataattagA tcaacgataattagA	TGATCAACAGAAGAT CGATCAACAGAAGAT *	1525 1530
P0U21878 P0-*	CCAAGACCGCTTCTC *	CGACACACTGTTCAA CGACACGCTGTTCAA	GATGTOGATGCTTGG GATGTOGATGCTTGG	ACAGAACCAGGACGC (TCAGAACCAGGACGC (*	CATGATCGATTGCTC (CATGATTGATTGCTC (CGATGTCATCCCTGT CGATGTCATCCCCGT	1615 1620
P0U21878 P0 1	CCCCGCTGCCCTTGT	AACCAAACCCCATCT AACCAAACCCCATCT	CCCCGCCGGGAAGAG CCCCGCCGGGGAAGAG	TAAGACCGACGTTGA / CAAGACCGACGTTGA / *	ACAAGCCgtgcgtcg : ACAAGCCgtacgtcg :	tgcaatgtatcaggg tgcaatgtatcaggg	1705 1710
P0U21878 P0-1	attacgcaacgtcgc	tgaattgitticcic tgaattgitticcic	cctagTGTGCCACCG cctagTGTGCCACCG	GCGCCTTCCCAGCCC 1	CGGTGCTGACCCTG (gtgagtaaacaggcg gtgagtaaacaggcg	1795 1800
P0U21878 P0~1	acgtagacaccagaa	agcotottaccoatt (gattttictttagGC (gattcttctttagGC (CCAGTCACCTCTGTT (CTCGTGTgteagtg	tcaacctatgtctct	1885 1890
P0U21878 P0 1		1906 1920 ccaacgaacacccta (CCCACCTGCGTAAG (CAAAGT 1936			

Fig. 1. Multiple DNA sequence alignment of the *mnp* genes of *Pleurotus ostreatus* IFO30160 (POU21878) and PO-1. Introns are indicated by low-ercase type. The different bases are shown by asterisks. The PCR primers are underlined.

not yet been described in other studies. All of the hot spots of the *mnp* genes of PO-1 mutants determined in this study were not mutated to original or other bases by re-irradiation (1 kGy) of gamma radiation (PO-15 and PO-16; Table 1). In the *lac1* gene model system in *E. coli*, after gamma-ray irradiation, the hot spots have not been

found although some hot spots occurred spontaneously (Wijker et al., 1996, 1998).

Among the nine hot-spots of the *mnp* gene, three occurring in the exon did not bring about the change of amino acids. The specifically mutated bases in PO-5 and PO-16 were involved in the change of 3 and 1 residues, respec-

Table 1. Sequence analysis of the mnp genes in gamma radiation-induced mutants of Pleurotus ostreatus PO-1

Position	Exon/Intron	PO-1		Mutant			Amino acid	Amino acid	Target sequence
rosition	EXOII/ IIIIIOII		PO-5	PO-6	PO-15	PO-16	changes	number	5' to 3'
89	I1	G*	a	a	a	a			acege g teeet
233	E2	T	C				I → D	5.0	GAACC T GTTCG
459	E4	T				C	L→P 56		TCACA T TCTCC
716	E5	C	G	G	G	G	F→L	98	TCACC C ATAGG
766	15	g	a	a	a	a	$p \rightarrow p$	164	atttt g ccgca
773	15	c	g	g	g	g			cgcag c ctgtt
798	E6	C	T	T	T	T	$T \rightarrow T$	174	gATAC C ATCAC
880	16	c	a				1 /1	174	cttga c accat
1169	19	c	t**	t	t	t			tttee e aatae
1184	19	a	g**	g	g	g			tccat a tccat
1192	19	t			g				catat t getca
1371	I11	a			g				aaact a atcat
1530	E13	T	C				$I \rightarrow T$	204	GAAGA T CCAAG
1597	E13	T	C**	C	C	С] →]	284 306	ATGAT T GATTG
1752	E14	Α	G				$T \rightarrow A$	339	GTGCC A CCGGC
1881	I15	T	c**	c	c	c	ı 'A	339	tcaac t tatgt

^{*}A lowercase is a base in intron and a capital letter is a base in exon. Blank is the same base of PO-1.

^{**}Mutated to equal base of *P. ostreatus* IFO30160 strain.

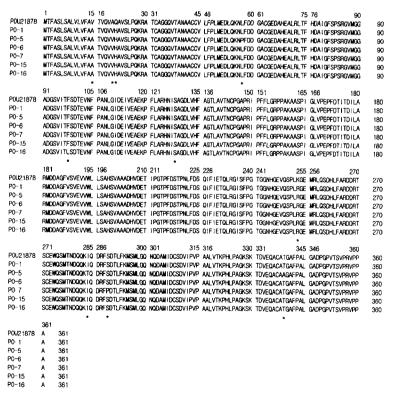


Fig. 2. Alignment of predicted amino-acid sequences of manganese (II) peroxidase (MnP) of the radiation-induced mutants of *Pleurotus ostreatus* PO-1. POU21878 is the amino acid sequence of MnP of *P. ostreatus* IFO30160. The different residues among seven enzymes are shown by asterisks.

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Table 2. Summary of the types of mutations on the <i>mnp</i> gene of <i>P</i>	leu-
rotus ostreatus mutants induced by gamma radiation	

Mutation type	mnp (1941 bp)				
Transitions					
$A \rightarrow G$	3/16 (18.8%)				
$G \rightarrow A$	2/16 (12.5%)				
$T \rightarrow C$	5/16 (31.3%)				
$C \rightarrow L$	2/16 (12.5%)				
Transversions					
$C \rightarrow A$	1/16 (6.3%)				
$C \rightarrow C$	2/16 (12.5%)				
$T \longrightarrow C$	1/16 (6.3%)				

tively, in MnP proteins. However, in PO-6 and PO-15, there were not any changes in the putative protein sequences (Table 1, Fig. 2). From PO-7 mutant, we did not clone the genomic DNA of the mnp gene but cloned the cDNA of the mnp gene from total RNA. The cDNA of PO-7 mnp gene has been mutated in two sites and the putative amino acid sequence of the PO-7 was different from PO-1 (Fig. 2). The mnp genes of PO-5, PO-7 and PO-16 mutants of PO-1 may produce different proteins (Fig. 2). In this study, the properties of the mutated proteins have not been determined, but these products of the mnp genes of mutants could be useful for degrading the recalcitrant lignin of biowastes because they showed an enhanced ligninolytic activity (Lee et al., 2000). The MnP of PO-7 differs in two amino acids (at 128 and 289; Fig. 2) from that of PO-1 and it has not been investigated whether these two different amino acids could play an important role in lignin degradation.

The frame shift mutations did not occur in PO-1 mutants. In the gamma radiation induced mutation spectrum described here, the deletion was not accounted for in the *mnp* gene (Table 1). In mammalian cell lines, the ionizing radiation induced mutations with large deletions as the main type (Thacker, 1986; Miles and Meuth, 1989; Nelson et al., 1994; Giver et al., 1995; Thacker, 1999), but in E. coli, it is not the main type of mutation, that is, the sizes of the deletions were from one base to several hundred bases (Wijker et al., 1996). This difference in the amount of induced deletions could be derived from the differences of target genes and/or differences in the repair system of double strand breaks in prokaryotic and eukaryotic cells. Because the mutants used in this study were isolated with several criteria such as growth rate in lignin medium and formation of fruiting body, it seems that the mutants with severely deleted mutations could be excluded.

In this study, the mutation spectrum of the *mnp* gene was investigated in the gamma radiation induced mutants of *P. ostreatus* PO-1. Previously, these mutants were independently isolated from the survivals of the mycelial fragments after gamma ray irradiation at the dose range of 1-2 kGy and confirmed the enhanced

ability of ligninolysis and the diversity of genetic similarity by RAPD analysis (Lee *et al.*, 2000). It seemed that the DNA sequences of the *mnp* genes of the mutants could include the common mutated positions as hot spots and the specific positions in individual mutants. These results could be useful for evaluating the tolerant limitation of change in the functional genes, at least the *mnp* gene, in this eukaryotic organism and modifying the molecular structure of proteins through changing the structural genes by gamma radiation.

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