The Journal of Microbiology, March 2003, p.52-57
Vol. 41, No. 1

NOTE

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

Hwa-Hyoung Chang[1], Young-Keun Lee[2]*, Jae-Sung Kim[2], Ki-Sung Lee[1], and Kyu Seong Cho[2]

[1]Research Center for Bio-Medicinal Resources, PaiChai University, Daejeon, 302-735, Korea
[2]Radiation Application Division, Korea Atomic Energy Research Institute, Yusung P.O. Box 105, Daejeon, 305-600, Korea

(Received Nov 4, 2002 / Accepted Dec 10, 2002)

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 →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 radiation-sensitive meiotic mutants of *Coprinus cinereus*. The genetic diversity increased in the gamma radiation-induced 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*

* To whom correspondence should be addressed.
(Tel) 82-42-868-8056 (Fax) 82-42-862-6980
(E-mail) yklee@kaeri.re.kr

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 PreMix™ (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→G (2/4), C→A (1/4) and T→G (1/4), and transitions (75%) as G→A (2/12), A→G (3/12), C→T (2/12) and T→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

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

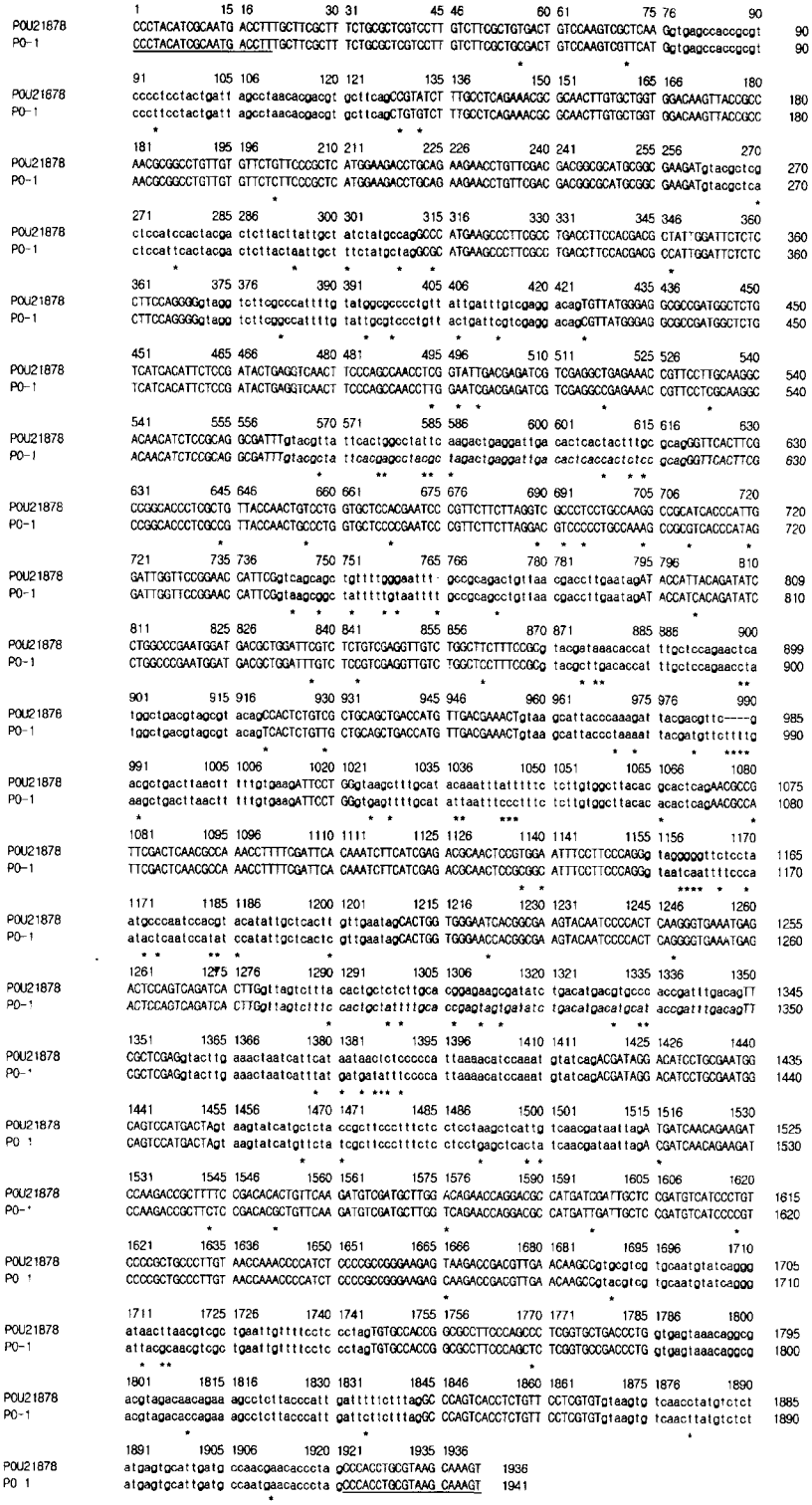

Fig. 1. Multiple DNA sequence alignment of the *mnp* genes of *Pleurotus ostreatus* IFO30160 (POU21878) and PO-1. Introns are indicated by lowercase type. The different bases are shown by asterisks. The PCR primers are underlined.

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 *lacI* 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 changes	Amino acid number	Target sequence 5' to 3'
			PO-5	PO-6	PO-15	PO-16			
89	I1	G*	a	a	a	a			accgc g tccct
233	E2	T	C						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	I5	g	a	a	a	a	P→P	164	atttt g ccgca
773	I5	c	g	g	g	g			cgcag c ctgtt
798	E6	C	T	T	T	T			gATAC C ATCAC
880	I6	c	a				T→T	174	cttga c accat
1169	I9	c	t**	t	t	t			tttcc c aatac
1184	I9	a	g**	g	g	g			tccat a tccat
1192	I9	t			g				catat t gctca
1371	I11	a			g				aaact a atcat
1530	E13	T	C						GAAGA T CCAAG
1597	E13	T	C**	C	C	C	I→T	284	ATGAT T GATTG
1752	E14	A	G				I→I	306	GTGCC A CCGGC
1881	I15	T	c**	c	c	c	T→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.

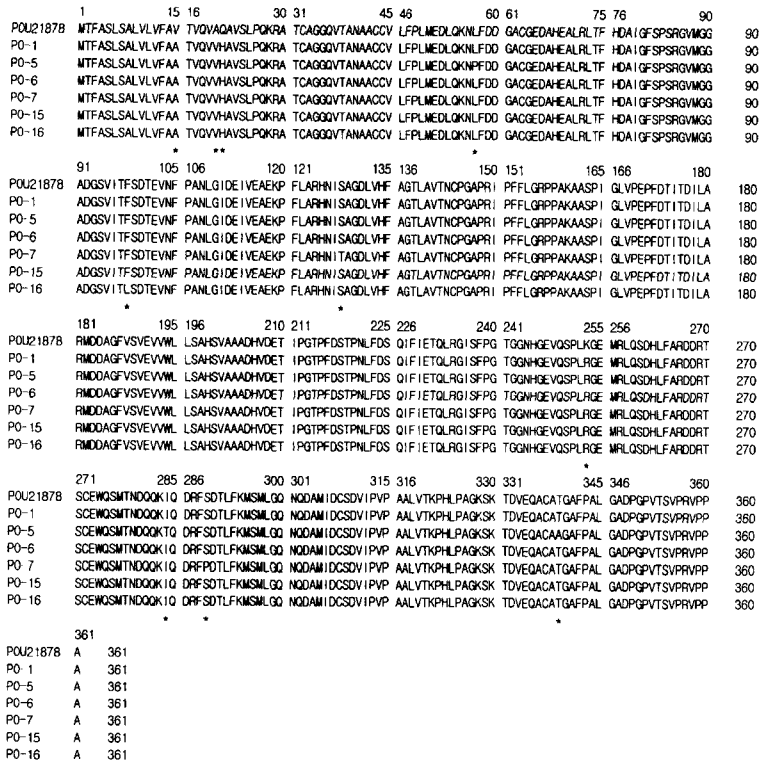

Fig. 2. Alignment of predicted amino-acid sequences of manganese (II) peroxidase (MnP) of the radiation-induced mutants of *Pleurotus ostreatus* PO-1. POU21878 is the amino acid sequence of MnP of *P. ostreatus* IFO30160. The different residues among seven enzymes are shown by asterisks.

Table 2. Summary of the types of mutations on the *mnp* gene of *Pleurotus ostreatus* mutants induced by gamma radiation

Mutation type	mnp (1941 bp)
Transitions	
A→G	3/16 (18.8%)
G→A	2/16 (12.5%)
T→C	5/16 (31.3%)
C→T	2/16 (12.5%)
Transversions	
C→A	1/16 (6.3%)
C→G	2/16 (12.5%)
T→G	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.

Acknowledgments

This study has been carried out under the Nuclear R&D Program by the Ministry of Science and Technology (MOST), Korea.

References

Asada, Y., A. Watanabe, T. Irie, T. Nakayama, and M. Kuwahara. 1995. Structures of genomic and complementary DNAs coding for *Pleurotus ostreatus* manganese (II) peroxidase. *Biochim. Biophys. Acta* 1251, 205-209.

Braun, J.E.F., A.H. Wanamarta, E.J. Westmijze, N.M. Wientjes, C.A. Wijker, M.V.M. Lafleur, and J. Retel. 1997. Influence of nucleotide excision repair of *Escherichia coli* on radiation-induced mutagenesis of double-stranded M13 DNA. *Mutat. Res.* 384, 45-53.

Giver, C.R., S.L. Nelson, M.Y. Cha, P. Pongsaensook, and A.J. Grosovsky. 1995. Mutational spectrum of X-ray-induced TK-human cell mutants. *Carcinogenesis* 16, 267-275.

Graham, G.C. 1994. A simplified method for the preparation of fungal genomic DNA for PCR and RAPD analysis. *Biotechniques* 16, 49-50.

Hoyes, K.P., P.J. Wadeson, H.L. Sharma, J.H. Hendry, and L.D. Morris. 1998. Mutation studies in *lacI* transgenic mice after exposure to radiation or cyclophosphamide. *Mutagenesis* 13, 607-612.

Kuipers, G.K., B.J. Slotman, H.A. Poldervaart, M.J. van Vilsteren, C.A. Reitsma-Wijker, and M.V.M. Lafleur. 2000a. The influence of combined Fpg- and MutY-deficiency on the spontaneous and γ-radiation-induced mutation spectrum in the *lacZα* gene of M13mp10. *Mutat. Res.* 461, 189-195.

Kuipers, G.K., B.J. Slotman, H.A. Poldervaart, M.J. van Vilsteren, C.A. Reitsma-Wijker, and M.V.M. Lafleur. 2000b. The role of nucleotide excision repair of *Escherichia coli* in repair of spontaneous and gamma-radiation-induced DNA damage in the *lacZα* gene. *Mutat. Res.* 460, 117-125.

Lee, Y.-K., H.-H. Chang, J.-S. Kim, J.K. Kim, and K.-S. Lee. 2000. Lignocellulolytic mutants of *Pleurotus ostreatus* induced by gamma ray radiation and their genetic similarities. *Rad. Phys. Chem.* 57, 145-150.

Liwicki, R., A. Paterson, M.J. MacDonald, and P. Broda. 1985. Phenotypic classes of phenoloxidase-negative mutants of the lignin-degrading fungus *Phanerochaete chrysosporium*. *J. Bacteriol.* 162, 641-644.

Miles, C. and M. Meuth. 1989. DNA sequence determination of

gamma-radiation-induced mutations of the hamster *aprt* locus. *Mutat. Res.* 227, 97-102.

Nelson, S.L., C.R. Giver, and A.J. Grosovsky. 1994. Spectrum of X-ray-induced mutations in the human *hprt* gene. *Carcinogenesis* 15, 495-502.

Reitsma-Wijker, C.A., B.J. Slotman, and M.V.M. Lafleur. 2000. Mutagenic effect by penylalanine during γ-irradiation of plasmid DNA in aqueous solution under oxic conditions. *Mutat. Res.* 454, 71-76.

Sargentini, N.J. and K.C. Smith. 1994. DNA sequence analysis of spontaneous and gamma-radiation (anoxic)-induced *lacI*^{d} mutations in *Escherichia coli umuC*122::Tn*5*: differential requirement for *umuC* at G:C vs. A:T sites and for the production of transversions vs. transitions. *Mutat. Res.* 311, 175-189.

Thacker, J. 1986. The nature of mutants induced by ionizing radiation in cultured hamster cells. III. Molecular characterization of HPRT-deficient mutations induced by gamma-rays or alpha-particles showing that the majority have deletions of all or part of the *hprt* gene. *Mutat. Res.* 160, 267-275.

Thacker, J. 1999. Repair of ionizing radiation damage in mammalian cells. Alternative pathways and their fidelity. *C. R. Acad. Sci. III* 322, 103-108.

Von Sonntag, C. 1987. The chemical basis of radiation biology. Taylor & Francis, New York.

Wijker, C.A. and M.V.M. Lafleur. 1998. Influence of the UV-activated SOS response on the gamma-radiation-induced mutation spectrum in the *lacI* gene. *Mutat. Res.* 408, 195-201.

Wijker, C.A., M.V.M. Lafleur, M.J. van Vilsteren, H. Steeg, G.R. Mohn, and J. Retel. 1996. Gamma-radiation-induced mutation spectrum in the episomal *lacI* gene of *Escherichia coli* under oxic conditions. *Mutat. Res.* 349, 229.-239.

Wijker, C.A., N.M. Wientjes, and M.V.M. Lafleur. 1998. Mutation spectrum in the *lacI* gene, induced by gamma-radiation in aqueous solution under oxic conditions. *Mutat. Res.* 403, 137-147.

Winegar, R.A., L.H. Lutze, J.D. Hamer, K.G. O'Loughlin, and J.C. Mirsalis. 1994. Radiation-induced point mutations, deletions and micronuclei in *lacI* transgenic mice. *Mutat. Res.* 307, 479-487.

Zolan, M.E., C.J. Tremel, and P.J. Pukkila. 1988. Production and characterization of radiation-sensitive meiotic mutants of *Coprinus cinereus*. *Genetics* 120, 379-387.