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Enhancement of Pendimethalin Degradation Activity in *Bacillus* sp. MS202 using Gamma Radiation

Young-Keun Lee*, Hwa-Hyoung Chang, Ho-Jin Lee,
Heesoon Park, Kyung Hee Lee and Min-Ho Joe

Radiation Application Research Division, ARTIJ, Korea Atomic Energy Research Institute, Jeongeup 580-185, Korea

Abstract - To induce the enhanced mutants of dinitroaniline herbicide pendimethalin degrading bacterium, *Bacillus* sp. MS202 was irradiated with gamma radiation at the dose of LD_{99} (3.35 kGy). Three enhanced mutants (MS202m7, MS202m14, MS202m18) were isolated from the candidates by the generation-isolation method. Clear zone formation and the GC analysis confirmed that the degrading activity of each enhanced mutant (MS202m7, MS202m14, MS202m18), the formation of pendimethalin metabolite, increased by 11%, 45%, and 32% than a wild type, respectively. It suggested that these mutants induced by gamma radiation could be useful for the application of pesticide degradation.

Key words : pendimethalin degradation, *Bacillus* sp., gamma radiation, enhanced mutant

INTRODUCTION

Pendimethalin is a dinitroaniline herbicide inhibiting the plant cell division after germination or following emergence from the soil (Tomlin 2003). US Environmental Protection Agency has classified pendimethalin as persistent bioaccumulative toxics (US EPA 1999). Microorganisms in soil degrade some of pesticide residues (Engelhardt *et al.* 1973; Chen and Mulchandani 1998). It has been known that the degradation of pendimethalin has two different pathways of aerobic and anaerobic conditions (Singh and Kulshrestha 1991; Gita *et al.* 2000). Recently, we isolated *Bacillus* sp. MS202 that differed from other bacteria in the way of pendimethalin degradation. It initially involved in the reduction of $-NO_2$ group into $-NH_2$ in an aerobic condition (Lee *et al.* 2004). Ionizing radiation, such as gamma radiation, has a clear mutagenic potential inducing many different types of DNA damages (Tubiana *et al.* 1990). Mutations of genes induced by gamma radiation are able to alternate phenotypes of bacteria (Friedberg 2003). Therefore, mutant induction by gamma radiation will be used to investigate the functionally related genes in bacteria (Lee *et al.* 2003). In this study, we reported the induction of the degrading activity-enhanced mutants by gamma radiation from the newly isolated pendimethalin degrading *Bacillus* sp. MS202.

* Corresponding author: Young-Keun Lee, Tel. 063-570-3300, Fax. 063-570-3309, E-mail. yklee@kaeri.re.kr

MATERIALS AND METHODS

Bacillus sp. MS202 isolated from the soil of Masan City, Gyeongnam province, Korea, was used. The pendimethalin degrading activity on agar medium containing pendimethalin (1,000 mg L^{-1}) was observed by clear zone formation around their colonies. Pendimethalin metabolites were analyzed using the TLC and GC (Agilent 6890, USA, HP-1 Capillary Column, FID Detector) analysis. The enhanced

mutants of degrading activity were isolated by the generation-isolation method as detailed below. Radiation sensitivity of *Bacillus* sp. MS202 to gamma radiation (^{60}Co, capacity: 75,000 Ci, dose rate: 920 Gy hr^{-1}, AECL) was determined. To induce the enhanced mutants, *Bacillus* sp. MS202 was irradiated with gamma radiation at the dose of LD_{99}, and the size of clear zones of survived colonies on pendimethalin agar was determined. Three candidates (first generation, F1) of the enhanced mutants were isolated from colonies which formed clear zone lager than that of wild type. Each of F1 colonies was inoculated on pendimethalin agar and 4 colonies were randomly isolated as second generation (F2). The pendimethalin metabolites of F2 colonies were analyzed by the GC analysis. From the GC results, one colony shown the highest productivity of metabolite was selected and spread on pedimethalin agar for the isolation of third generation (F3) colonies. The metabolites of F3 colonies were also analyzed by GC and then one colony was also selected. From the late colony, 4 colonies were also isolated as fourth generation (F4) and compared the results of the GC analysis to isolate the most enhanced mutant among them. In each generation experiment, one colony of the wild type *Bacillus* sp. MS202 was randomly selected and used to determine the degrading activity.

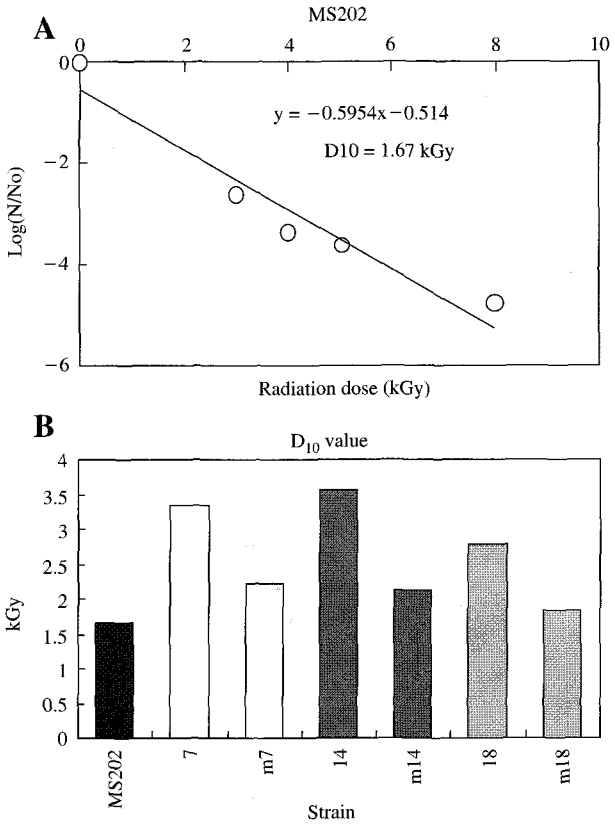

Fig. 1. Radiation sensitivity of *Bacillus* sp. MS202 (A) and the mutants induced by gamma radiation (B). F1 generation: 7, 14, 18; F4 generation: m7, m14, m18.

RESULTS AND DISCUSSION

Bacillus sp. MS202 degraded pendimethalin and produced single metabolite (called M1) by the TLC and GC analysis. Rf values of pendimethalin and M1 were 0.70 and 0.37, respectively, at the TLC analysis with hexan : diethyl ether (3 : 1) as a mobile phase. The retention times of pendimethalin and M1 in the GC analysis were 18.5 min and 16.5 min, respectively. Molecular weights of M1 and pendimethalin previously determined by the GC-MS analysis were 251 and 280, respectively, and the difference of molecular weight between M1 and pendimethalin was produced by the reduction of $-NO_2$ group of pendimethalin into $-NH_2$. Thus, M1 was suggested as 6-amino-2-nitro-*N*(1-ethylpropyl)-3, 4-xylidine or 2-amino-6-nitro-*N*(1-ethylpropyl)-3, 4-xylidine (Lee *et al.* 2004). Pendimethalin can be degraded in soil by microorganisms such as bacteria (*B. megaterium*, *Pseudomonas* sp., *Pyricularia* sp., *Rizobium* sp., *T. viride*) (Kole *et al.* 1994) and fungi (*F. oxysporum*, *P. variotii*, *R. bataticola*) (Singh and Kulshrestha 1991). It has been known that the degradation of pendimethalin has two different pathways of aerobic and anaerobic conditions (Singh and Kulshrestha 1991; Gita *et al.* 2000). In an aerobic condition, dealkylation of pendimethalin occurred initially and partial reduction followed, but *vice versa* in an anaerobic condition. However, *Bacillus* sp. MS202 differed from the other bacteria in the way of pendimethalin degradation. It initially involved in the reduction of $-NO_2$ group into $-NH_2$ in an aerobic condition. This result suggests that the way of pendimethalin degradation could be dependant not only on culture conditions, but also on strains of bacteria (Lee *et al.* 2004).

To isolate the degrading activity-enhancing mutants by gamma radiation, we constructed the generation-isolation method (described in Materials and Methods). D_{10} value of *Bacillus* sp. MS202 to gamma radiation was 1.67 kGy (Fig. 1A). The pendimethalin degrading *Bacillus* sp. MS202 was

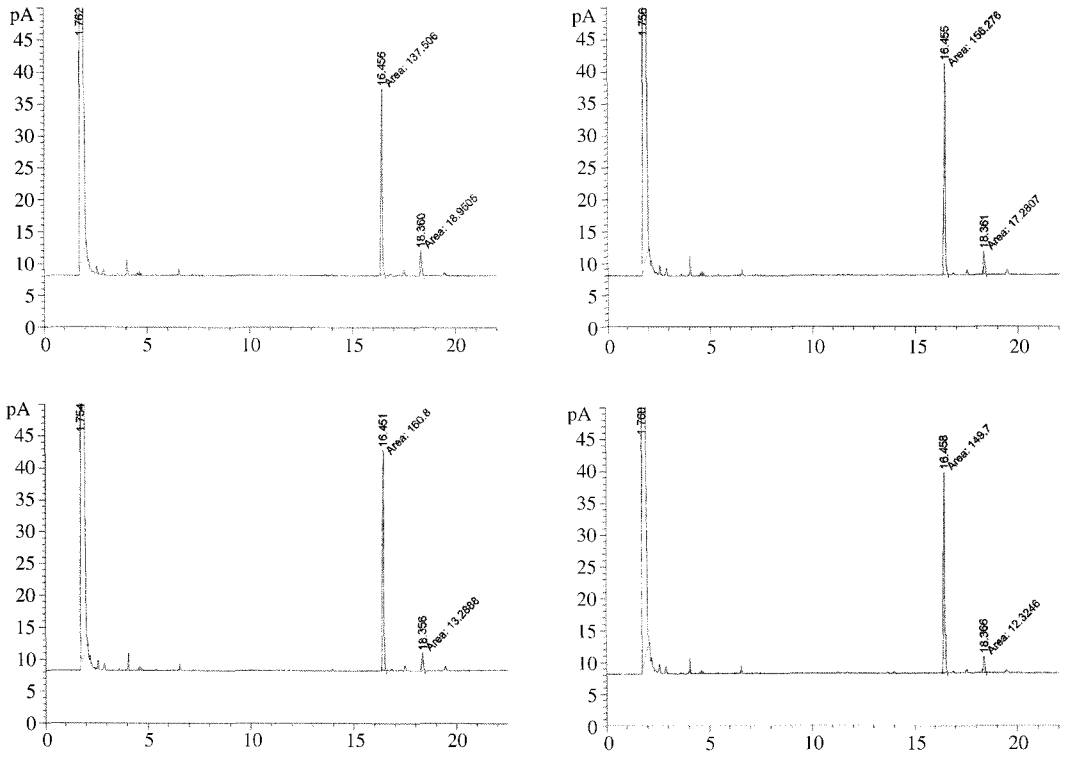

Fig. 2. GC analysis of the pendimethalin (18.5 min) and its metabolite, M1 (16.5 min) produced by *Bacillus* sp. MS202 and its F1 mutants induced by gamma radiation. Clockwise rotation: *Bacillus* sp. MS202, MS202-7, MS202-14, and MS202-18.

irradiated with gamma radiation at the dose of LD_{99} (3.35 kGy) and the F1 candidates for the enhanced mutants were isolated as MS202-7, MS202-14 and MS202-18 by the comparison of clear zone size and M1 productivity (Fig. 2). The degrading activity of F1 (MS202-7, MS202-14 and MS202-18) increased by 24%, 67% and 66% than that of the wild type. We finally selected three enhanced mutants (MS202m7, MS202m14 and MS202m18) from three F1 candidates, respectively, by the generation-isolation method. The GC analysis confirmed that the degrading activity of each enhanced mutant (MS202m7, MS202m14, MS202m18), the formation of pendimethalin metabolite, increased by 11.0%, 45.1%, and 31.6% than the control, respectively (Table 1, Fig. 3). During four generations, the best enhanced mutant at each generation was selected as forefather to obtain daughter colonies. Although the pendimethalin degrading activity of forefather was higher than that of wild strain, degrading activities of the daughters were varied diversely (Table 1). This pattern of degrading activity was found in all of the tested generations. In the control, the standard deviation of the degrading activities from the 4 colonies of the wild type *Bacillus* sp. MS202

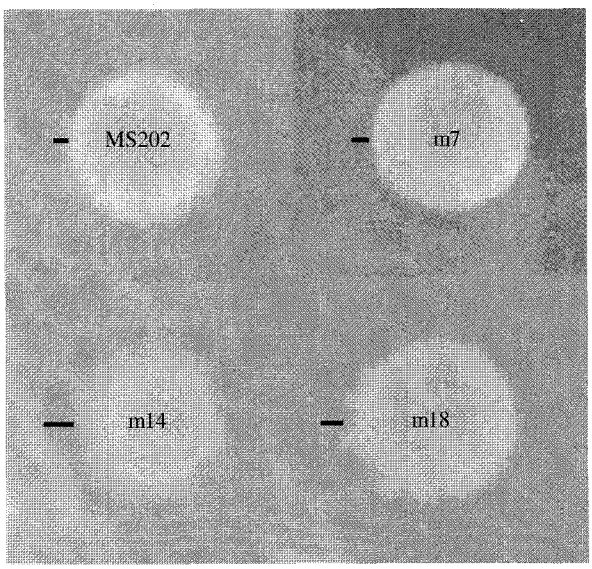

Fig. 3. Pendimethalin degrading activity of *Bacillus* sp. MS202 and its enhanced mutants induced by gamma radiation. MS202: *Bacillus* sp. MS202, numbers indicate mutants. Bar indicates the clear zone of pendimethalin degradation.

was only 4.03. The standard deviation of degrading activity of the previous generation of the mutants reduced in the

Table 1. Pendimethalin degrading activity of gamma radiation induced mutants of *Bacillus* sp. MS202

Generation		Degrading activity of mutants to that of the control (%)				
		A	B	C	D*	Average±SD
MS202-7	F2	88	97	133	137	114±25
	F3	82	86	118	120	102±21
	F4	65	82	91	111	87±19
MS202-14	F2	51	83	163	178	119±61
	F3	143	192	327	778	360±289
	F4	93	98	114	145	113±24
MS202-18	F2	131	167	186	291	189±74
	F3	71	109	109	110	100±19
	F4	110	117	128	132	120±13

*D column indicated the isolated colony for the next generation

next generation. This result suggested that the diversity of pendimethalin degrading activity in the enhanced mutants induced by gamma radiation could decrease in descendant at least during four generations. Although we did not exactly prove the causes of the stabilization in the degradation activity of descendant strain, it could be related to the simultaneously upregulated expressions of the repair and resistance related genes in mutants induced by gamma radiation (Lee *et al.* 2004). To estimate indirectly the upregulated expression of resistance genes, the radiation sensitivity was determined in the enhanced mutants (Fig. 1B). Three F1 mutants (MS2020-7, -14 and -18) showed 3.35, 3.57 and 2.78 kGy of D_{10}, respectively, but it decreased to 2.21, 2.12 and 1.84 kGy in the F4 enhanced mutant of MS202m7, m14 and m18, respectively higher than that of *Bacillus* sp. MS202. This result suggested that the enhanced mutant could become more resistant to the gamma radiation than the control and tend to stabilize the radiation resistance into the basal level through generations.

The enhanced mutants of the pendimethalin-degrading bacterium (*Bacillus* sp. MS202) could be induced by gamma radiation. And *Bacillus* sp. MS202 was different from the other bacteria in the way of pendimethalin degradation. That is, it initially involved in the reduction of $-NO_2$ group into $-NH_2$ in an aerobic condition. Methodologically, the pendimethalin degrading-enhanced mutants induced by gamma radiation could be isolated with easy by the generation-isolation method. It seems to be possible for these enhanced mutants of *Bacillus* sp. MS202 induced by gamma radiation to be utilized for the remediation of pendimethalin contamination.

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