J. Microbiol. Biotechnol. (2007), **17**(10), 1727–1732

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

A Green Fluorescent Protein-based Whole-Cell Bioreporter for the Detection of Phenylacetic Acid

KIM, JUHYUN[1], CHE OK JEON[2], AND WOOJUN PARK[1,2]*

[1]*Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Korea*
[2]*Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea*

Received: June 4, 2007 Accepted: August 6, 2007

Abstract Phenylacetic acid (PAA) is produced by many bacteria as an antifungal agent and also appears to be an environmentally toxic chemical. The object of this study was to detect PAA using *Pseudomonas putida* harboring a reporter plasmid that has a PAA-inducible promoter fused to a green fluorescent protein (GFP) gene. *Pseudomonas putida* KT2440 was used to construct a green fluorescent protein-based reporter fusion using the *paaA* promoter region to detect the presence of PAA. The reporter strain exhibited a high level of *gfp* expression in minimal medium containing PAA; however, the level of GFP expression diminished when glucose was added to the medium, whereas other carbon sources, such as succinate and pyruvate, showed no catabolic repression. Interestingly, overexpression of a *paaF* gene encoding PAA-CoA ligase minimized catabolic repression. The reporter strain could also successfully detect PAA produced by other PAA-producing bacteria. This GFP-based bioreporter provides a useful tool for detecting bacteria producing PAA.

Keywords: Biosensor, GFP, biodegradation, catabolic repression, transcriptional regulation

Phenylacetic acid (PAA) is an important organic chemical material that is widely used in the field of medicine, as a pesticide, and as an aromatizer [32]. PAA is a metabolic intermediate resulting from biodegradation of diverse compounds such as styrene and tropic acid [19, 26]. PAA can also be used as a bacterial carbon source and an antibiotic agent. For example, *Streptomyces humidus* and *Burkholderia* sp. have been shown to produce PAA as an antifungal agent [9, 20]. Production of PAA in *Bacillus licheniformis* also results in antimicrobial activity [14]. PAA is a precursor material of penicillin G [13, 22].

*Corresponding author
Phone: 82-2-3290-3067; Fax: 82-2-953-0737;
E-mail: wpark@korea.ac.kr

Many microorganisms possess aerobic PAA metabolism through a set of enzymatic reactions known as the PAA pathway [10, 19]. In *Pseudomonas putida* U, the PAA catabolic gene clusters are composed of 17 genes that are predicted to have five contiguous operons. These genes encode 17 proteins grouped in the following six putative functional units: a transport system (*paaL* and *paaM*), phenylacetic acid activating enzyme (*paaF*), ring-hydroxylation complex (*paaG*, *paaH*, *paaI*, *paaJ*, and *paaK*), ring-opening enzyme (*paaN*), β-oxidation-like system (*paaA*, *paaB*, *paaC*, and *paaE*), and two putative regulatory genes (*paaX* and *paaY*) [19, 25]. The functions of the *paaD* and *paaP* genes are unknown [2]. This pathway constitutes a common part of the complex functional unit (catabolon) integrated by several routes that catalyze the transformation of structurally related molecules into a common intermediate, phenylacetyl-CoA [25]. It has been reported that PAA-CoA, not PAA, is the true inducer of the PAA catabolic pathway [6, 7]. It is known that PaaX is a repressor protein that binds to the *paaA* promoter region. Binding of PaaX to its cognate DNA binding sites is abolished when the inducer, PAA-CoA, interacts with PaaX protein [6, 7]. Disruption of the *paaX* gene resulted in no regulation of PAA metabolic gene expression [25].

P. putida KT2440 is a metabolically versatile, saprophytic soil bacterium that has been extensively studied [10, 11, 16]. *P. putida* KT2440 is thought to degrade PAA *via* the PAA pathway, as previously reported [2, 10, 19]. The goal of this work was to detect PAA in the environment using *P. putida* KT2440. *P. putida* KT2440 was chosen because this strain is more resistant to environmental stress than the conventional *E. coli* reporter strain [10].

The *paa* gene clusters from *P. putida* KT2440 are organized similarly to the PAA degradation system in *P. putida* U [10, 19]. Northern blot analysis was conducted to confirm transcription of the first genes (*paaA*, *paaG*, and *paaL*) in each operon (Fig. 1A). Total RNA was isolated from 3 ml of exponentially growing cells using an RNeasy

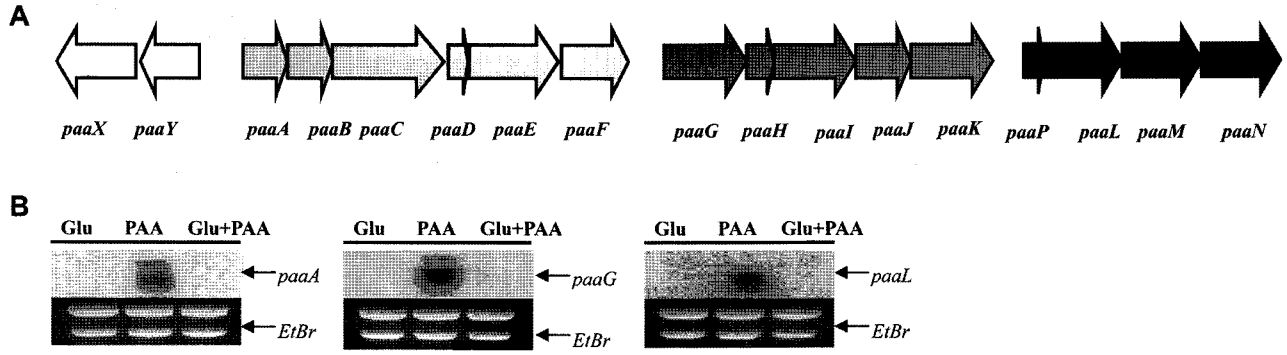

Fig. 1. A. Diagram showing regulatory and catabolic operons of the PAA degradation system in *P. putida* KT2440. **B**. Northern blot analysis of the transcriptional level of the first genes (*paaA*, *paaG*, and *paaL*) in each operon under minimal M9 medium containing either glucose (2.0%) or PAA (5.0 mM).
Total RNA of *P. putida* KT2440 was extracted during the early exponential growth phase (OD_{600}~0.3). Glu; glucose.

kit (Qiagen) following the manufacturer's instructions. RNA concentration was determined by measuring absorbance at 260 nm, and 5 μg of total RNA per sample was used. The fractionated RNA was transferred to a nylon membrane (Schleicher & Schuell) using a Turboblotter (Schleicher & Schuell) and the amount of *paaA*, *paaG*, and *paaL* mRNA determined by hybridizing the membrane with a ^{32}P-labeled probe specific for each gene. Because the *paaP* gene has a short ORF, the *paaL* gene was used to construct a probe to measure the transcription level instead of using the *paaP* gene itself. The level of their transcription was greatly increased when *P. putida* KT2440 was cultured in minimal medium containing PAA as the sole carbon source (Fig. 1B). However, no mRNA transcripts were observed when cells were grown in glucose-amended media (Fig. 1B). Overnight cultures grown in minimal media containing glucose were diluted (~1/100) in fresh minimal media containing glucose and PAA. PAA addition to glucose-amended media showed no increase of their mRNA transcripts (Fig. 1B), suggesting that the promoters examined here are subject to carbon catabolic repression in the presence of glucose. We confirmed that the first genes (*paaA*, *paaG*, and *paaL*) in each operon are highly inducible in the presence of PAA, although catabolic repression by glucose exists in PAA metabolism of *P. putida* KT2440.

We used a whole-cell bioreporter to detect environmental PAA. To construct a reporter plasmid, pRKP$_{paaA}$-*gfp*, a broad-host-range promoter probe vector, pRK415gfp [17, 31], was used. Bacterial bioreporters are microorganisms engineered to detect target chemicals compounds through inducible expression of reporter proteins [3, 24]. We used a green fluorescent protein (GFP)-fusion bioreporter instead of other bioreporters [23] because the GFP has several advantages: (1) It does not require any substrate other than oxygen to provide a detectable response, (2) it could express in most bacterial cells, and (3) it is extremely stable in bacteria [3]. Plasmid isolation, gel electrophoresis, transformation, and polymerase chain reaction (PCR) were performed using standard procedures [1]. Briefly, a 2,108-bp fragment from the *paaA* promoter containing the *paaX* and *paaY* regions (Fig. 1A) was amplified using primers paa-F (5'-CGCGGATCCCGGGCC GTTGCAGGGTAATGAGC-3') and paa-R (5'-CGCGAATTCCAAGG TGGCG GTGAA TGTGAG-3'). The amplicon was cloned into the EcoRI/BamHI cloning site of the pRK415gfp vector, generating pRKP$_{paaA}$-*gfp*. The constructed plasmid was then introduced into *E. coli* S17-1 by electroporation. Then, the pRKP$_{paaA}$-*gfp* plasmid was introduced by biparental conjugation into *P. putida* KT2440-R, thus creating *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*). Conjugation was performed by filter mating using *E. coli* S17-1 (pRKP$_{paaA}$-*gfp*) and *P. putida* KT2440-R as donor and recipient, respectively [26]. To modulate its transcription, the regulatory proteins were included in the construction of the reporter plasmid, although their molecular mechanisms have not been completely revealed [6]. The resulting reporter plasmid was introduced into either the soil model bacterium *P. putida* KT2440 or *E. coli*. To measure GFP expression, we did microscopic analysis and quantification of GFP fluorescence as previously described [16].

Bacterial cells harboring the reporter plasmid were cultured in minimal medium containing PAA (5 mM). *E. coli* cells showed no induction of *gfp* expression in any conditions (data not shown), although *E. coli* also possesses genes required to metabolize PAA [5]. It may be due to the difference in either the promoter region or RNA polymerase systems [18, 21]. It seems that the *paaA* promoter from *P. putida* is not inducible in *E. coli*. Whereas *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) produced high levels of *gfp* expression in minimal medium containing PAA (Fig. 2D), this strain produced no GFP when PAA was absent (Fig. 2B).

The levels of GFP indicated that GFP expression in the presence of media containing 5 mM PAA is 20-fold higher than that of the control (glucose, 5 mM; Fig. 2E). Additionally, the reporter strain showed a quantitative response to different concentrations of PAA (Fig. 2E). The GFP level in the

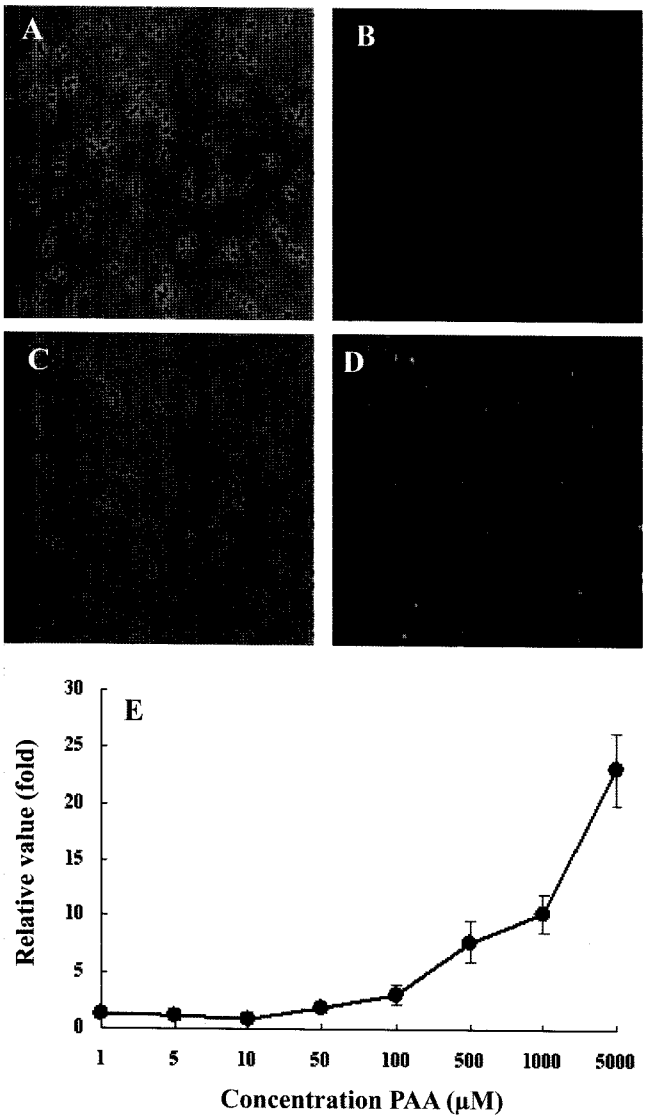

Fig. 2. Phase contrast micrograph of *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) cells grown on either glucose minimal medium (**A**) or PAA amended media (**C**). Fluorescence microscopic analysis of *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) responding to the absence (**B**) or presence (**D**) of PAA. Cells were cultured for 13 h in each medium. **E.** Quantification of GFP expression in the reporter strain at different PAA concentrations.
Cells were challenged for 13 h in minimal M9 medium containing pyruvate (10 mM) with different concentrations of PAA.

reporter strain could be quantified when cells were grown in the presence of greater than 50 μM PAA (Fig. 2E). Furthermore, the GFP level did not respond to the presence of other compounds, such as benzoic acid, toluene, glycerol, and styrene (Table 1). Pyridine (0.5 g/l) and salicylate (5 mM) inhibited the growth of the reporter cells, thus preventing GFP measurement (Table 1). The constructed reporter strain produced a high amount of GFP in a concentration-dependent manner with respect to PAA. Measurement of GFP induction using other carbon sources was carried out

Table 1. *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) response to different compounds.

Compound	GFP Fold induction[a]
Pyridine (0.5 g/l)	ND
Benzoic acid (5 mM)	0.88±0.17
Toluene (5 mM)	0.57±0.14
Salicylate (5 mM)	ND
Glycerol (1%)	1.35±0.07
Styrene (5 mM)	0.65±0.17
PAA (5 mM)	5.34±0.02

ND; no detection.
[a]The induction was measured by relative increase of GFP level after 11 h exposure to a particular compound, with respect to glucose-grown basal levels.

to understand catabolic repression. The reporter strain was freshly grown in minimal medium supplemented with different carbon sources until an OD_{600}~0.3 was observed, and then exposed to PAA (5 mM) for 3 h. Three ml of the culture was then harvested to measure the GFP level. As indicated by Northern blot analysis (Fig. 1B), PAA addition resulted in no increase of GFP level in reporter strains grown in LB- or glucose-amended media (Fig. 3). However, cells grown in the presence of either succinate or pyruvate with PAA showed increased GFP production (Fig. 3). Therefore, it seems that succinate and pyruvate did not impose a high level of catabolic repression on expression of the PAA degradative operon. It is most likely due to glucose-mediated catabolic repression in PAA catabolism. In contrast to the glucose effect, other carbon sources such as succinate and pyruvate did not lead to catabolic repression in our experiment. It has been shown previously that neither PAA-CoA ligase activity nor PAA uptake was detected in *P. putida* U grown in the presence of glucose (5 mM) [25]. Glucose-catabolic repression has been reported for some pathways responsible for the assimilation of sugars, amino acids, hydrocarbons, and aromatic compounds [4, 8]. In *E. coli*, the mechanisms of PAA catabolic repression have been extensively studied [6]; however, the molecular mechanisms related to catabolic repression in *Pseudomonas* strains are still unknown. In *P. putida*, the *crc* gene is known to be one of the regulators involved in catabolic repression of some metabolic pathways. The *crc* gene product, therefore, may play an important role in metabolism of 4-hydroxyphenyl pyruvate, benzoate, and 4-OH-benzoate in *P. putida* [8]. However, the pathway for PAA does not seem to be regulated by the Crc protein [8]. We will conduct future studies to find the integral gene related to catabolic repression of the PAA pathway. We hypothesized that glucose-dependent catabolic repression of the PAA system could be alleviated by the presence of the inducer, PAA-CoA. If PAA is partly diffused into cells by passive diffusion, we would expect to have more PAA-CoA when the *paaF* gene encoding PAA-CoA ligase is

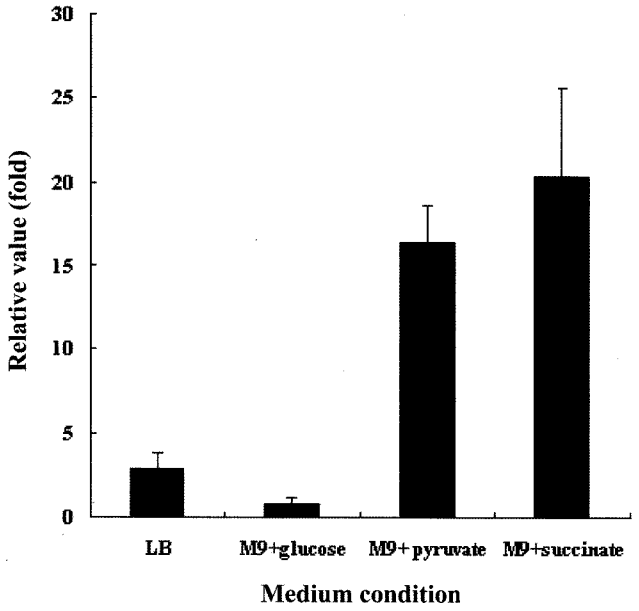

Fig. 3. Quantification of GFP expression in *P. putida* (pRKP$_{paaA}$-*gfp*) in response to different carbon sources.
Cells were grown in LB or M9 minimal media with glucose (10 mM), pyruvate (10 mM), and succinate (10 mM). PAA (5 mM) was added to exponentially grown cells in each medium. After PAA treatment, cells were incubated for 3 h.

overexpressed. By inserting a 1,499 bp fragment covering the *paaF* gene, which was amplified using primers paaF-F (5'-CGCGGATCCCCCGGCAGA ACGCAGGCA AAA A-3') and paaF-R (5'-CGCCTCGAG AGACCCTGCGCT-TGTTACC-3') into the cloning site of pBBR1MCS2 [15], the PaaF-overproducing plasmid was constructed. The resulting pBBR*paaF* gene was introduced into *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) to create *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*). Two reporter strains, *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) and *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*), were grown in fresh glucose-amended minimal medium until an OD_{600}~0.3 was attained. The culture was then incubated for 3 h in PAA-amended media and samples were harvested to measure the relative GFP expression level. As shown in Figs. 4A–4D, reduction of glucose-catabolic repression was observed in *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*), indicating more GFP fluorescence in the *paaF*-overexpressed strain. Three-fold more induction was measured in the *paaF*-overexpressed strain than in the *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) strain, even in the presence of glucose (Fig. 4E). It has been reported that a PAA passive diffusion system does not exist in *P. putida U* [7, 29]; however, based on the data obtained here, it seems likely that *P. putida* KT2440 has a PAA passive diffusion system. *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*) expression of GFP in the presence of glucose suggested that some PAA enters cells by passive diffusion at a very low rate, and is then converted to PAA-CoA, which is

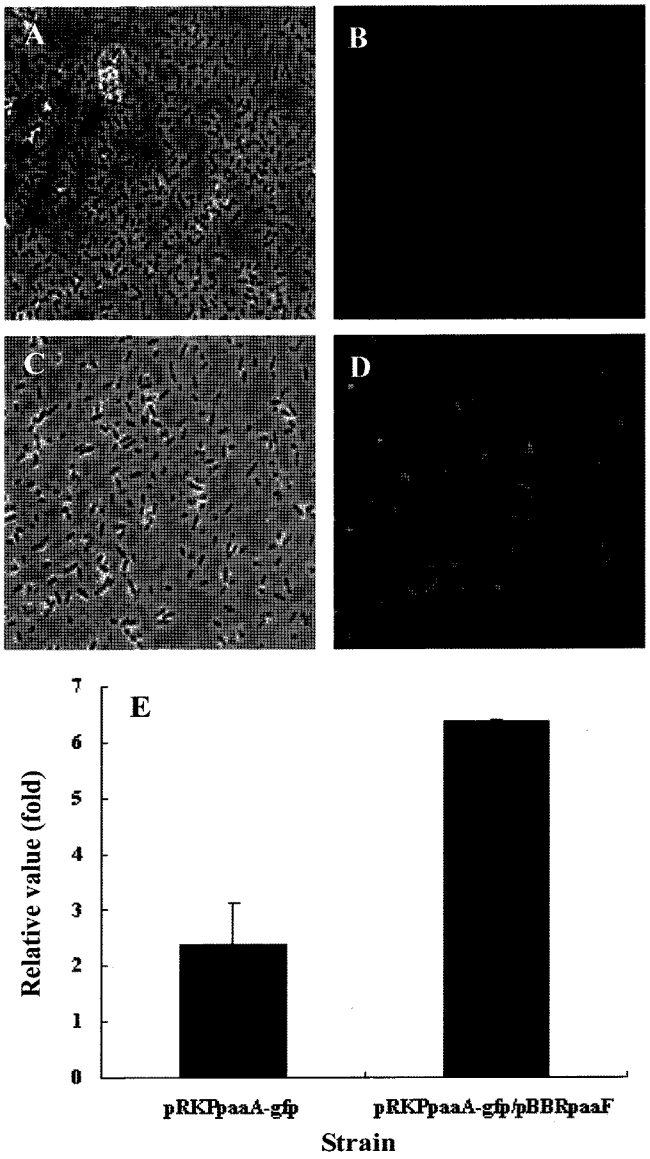

Fig. 4. Microscopic analysis and GFP quantification of *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) and *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*).
Both strains were pregrown on glucose-amended minimal medium. PAA was added to exponentially grown cells (OD_{600}~0.3). After the treatment of PAA (5 mM), cells were incubated for 3 h. **A**. Phase contrast micrograph of *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*) cells. **B**. Fluorescence micrograph of the same field of cells shown in panel **A**. **C**. Phase contrast micrograph of *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*) cells. **D**. Fluorescence micrograph of same field of cells shown in panel **C**. **E**. Quantification of GFP expression in each strain.

facilitated by the overexpressed *paaF* enzyme. Thus, PAA-CoA induces PAA operon promoters. PAA-CoA is known to be a true inducer in PAA catabolic systems [6, 7]. After cells were exposed to PAA for three hours, it is believed that glucose levels were not exhausted because cells were in a mid-exponential growth pattern in glucose-only-amended control media (data not shown). The relationship between

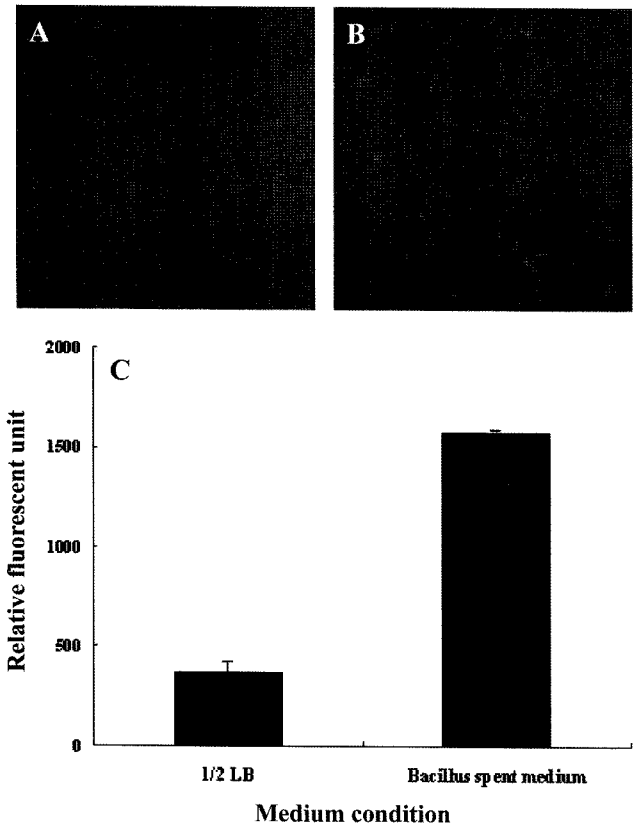

Fig. 5. Microscopic analysis of *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*) responding to spent medium of PAA-producing bacteria.

Cells were cultured for 13 h in each spent medium. Fluorescence micrograph of *P. putida* KT2440 (pRKP$_{paaA}$-*gfp*/pBBR*paaF*) cells grown on diluted LB medium (half of LB medium plus half of distilled water) (**A**) or spent medium (**B**) that was used to culture PAA-producing *Bacillus subtilis* cells. **C**. Quantification of GFP expression in each condition.

glucose consumption and growth phase has been reported [29]. We used this reporter strain to detect PAA produced by known PAA-producing bacteria such as *Bacillus subtilis* [12, 14]. The *paaF*-overexpressing reporter strain was cultured in spent LB medium after five days of PAA-producing bacterial growth. GFP fluorescence was detected only in cells cultured in the spent media (Fig. 5B). The level of GFP induction was four-fold compared with the control level in diluted LB medium. It could be possible to detect PAA-producing bacteria using the reporter strain we constructed instead of extensive GC-MS analysis, which is currently required for PAA detection. Many PAA-producing bacteria produce PAA up to more than 300 μM concentration [9, 14]. As shown in Fig. 2E, there was no GFP measurement in PAA concentration from 1 to 10 μM. However, in microscopic analysis, a low GFP induction was detected at 5 μM PAA (data now shown). Hwang *et al.* [9] showed that PAA inhibited the growth of fungi at about 70 to 350 μM PAA concentration. The PAA concentration of *B. cereus* HY-3 in nutrient broth was about 350 μM and *B. subtilis* HY-16 produced PAA up to 500 μM concentration [12]. It suggested that this reporter strain could be sufficient for detecting PAA-producing bacteria. Overproducing of the PAA transport system could increase the sensitivity of this bioreporter. Collectively, the GFP-based assay described here provides a useful method for detecting environmental PAA. This method will help find many organisms that produce PAA as their defense system in the environment.

Acknowledgments

This work was supported by an NCRC (National Core Research Center) grant (R15-2003-012-02002-0), and a grant (R0503442) from the BioGreen21 Program, Rural Development Administration, Republic of Korea to W. P. This work was also supported by the Brain Korea 21 project in 2007.

References

1. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Sruthl. 1999. *Current Protocols in Molecular Biology.* John Wiley & Sons, Inc., New York, N.Y., U.S.A.
2. Bartolomé-Martín, D., E. Martínez-García, V. Mascaraque, J. Rubio, J. Perera, and S. Alonso. 2004. Characterization of a second functional gene cluster for the catabolism of phenylacetic acid in *Pseudomonas* sp. strain Y2. *Gene* **341:** 167–179.
3. Burlage, R. S. 1997. Emerging technologies: Bioreporters, biosensors and microprobes, pp. 115–123. *In* Guy R. Kundsen, Michael J. McInerney, Linda D. Stetzenbach, and Michael V. Walter (eds.), *Manual of Environmental Microbiology.* ASM Press, Washington, D.C.
4. Collier, D. N., P. W. Hager, and P. V. Phibbs Jr. 1996. Catabolite repression control in *Pseudomonads. Res. Microbiol.* **147:** 551–561.
5. Ferrández, A., B. Miñambres, B. García, E. R. Olivera, J. M. Luengo, J. L. García, and E. Díaz. 1998. Catabolism of phenylacetic acid in *Escherichia coli.* Characterization of a new aerobic hybrid pathway. *J. Biol. Chem.* **273:** 25974–25986.
6. Ferrández, A., J. L. García, and E. Díaz. 2000. Transcriptional regulation of the divergent *paa* catabolic operons for phenylacetic acid degradation in *Escherichia coli. J. Biol. Chem.* **275:** 12214–12222.
7. García, B., E. R. Olivera, B. Miñambres, D. Carnicero, C. Muñiz, G. Naharro, and J. M. Luengo. 2000. Phenylacetyl-coenzyme A is the true inducer of the phenylacetic acid catabolism pathway in *Pseudomonas putida* U. *Appl. Environ. Microbiol.* **66:** 4575–4578.
8. García, M., F. L. Juan, B. Ana, P. A. Juan, L. M. José, and R. Fernando. 2004. The *Pseudomonas putida* crc global regulator controls the expression of genes from several chromosomal catabolic pathways for aromatic compounds. *J. Bacteriol.* **186:** 1337–1344.

9. Hwang, B. K., S. W. Lim, B. S. Kim, J. Y. Lee, and S. S. Moon. 2001. Isolation and *in vivo* and *in vitro* antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. *Appl. Environ. Microbiol.* **67:** 3739–3745.
10. Jiménez, J. I., B. Miñambres, J. L. García, and E. Díaz. 2002. Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. *Environ. Microbiol.* **4:** 824–841.
11. Kang, Y.-S., Y. Kim, C. Jeon, and W. Park. 2006. Characterization of naphthalene-degrading *Pseudomonas* species isolated from pollutant-contaminated sites: Oxidative stress during their growth on naphthalene. *J. Microbiol. Biotechnol.* **16:** 1819–1825.
12. Kawazu, K., H. Zhang, H. Yamashita, and H. Kanzaki. 1996. Relationship between the athogenicity of the pine wood nematode, *Bursaphelenchus xylophilus*, and phenylacetic acid production. *Biosci. Biotechnol. Biochem.* **60:** 1413–1415.
13. Kim, H. S., T. S. Kang, J. S. Hyun, and H. S. Kang. 2004. Regulation of penicillin G acylase gene expression in *Escherichia coli* by repressor PaaX and the cAMP-cAMP receptor protein complex. *J. Biol. Chem.* **279:** 33253–33262.
14. Kim, Y., J.-Y. Cho, J.-H. Kuk, J.-H. Moon, J.-I. Cho, Y.-C. Kim, and K.-H. Park. 2004. Identification and antimicrobial activity of phenylacetic acid produced by *Bacillus licheniformis* isolated from fermented soybean, *chungkook-jang*. *Curr. Microbiol.* **48:** 312–317.
15. Kovach, M. E., P. H. Elzer, D. S. Hill, G. T. Robertson, M. A. Farris, R. M. Roop Jr., and K. M. Peterson. 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166:** 175–176.
16. Lee, K., E. K. Ryu, K. S. Choi, M. C. Cho, J. J. Jeong, E. N. Choi, S. Lee, D.-Y. Yoon, I. Hwang, and C.-K. Kim. 2006. Identification and expression of the *cym*, *cmt*, and *tod* catabolic genes from *Pseudomonas putida* KL47: Expression of the regulatory *todST* genes as a factor for catabolic adaptation. *J. Microbiol.* **44:** 192–199.
17. Lee, Y., E. Ahn, S. Park, E. L. Madsen, C. Jeon, and W. Park. 2006. Construction of a reporter strain *Pseudomonas putida* for the detection of oxidative stress caused by environmental pollutants. *J. Microbiol. Biotechnol.* **16:** 386–390.
18. Lohrke, S. M., S. Nechaev, H. Yang, K. Severinov, and S. J. Jin. 1999. Transcriptional activation of *Agrobacterium tumefaciens* virulence gene promoters in *Escherichia coli* requires the *A. tumefaciens rpoA* gene, encoding the alpha subunit of RNA polymerase. *J. Bacteriol.* **181:** 4533–4539.
19. Luengo, J. M., J. L. García, and E. R. Olivera. 2001. The phenylacetyl CoA catabolon: A complex catabolic unit with broad biotechnological application. *Mol. Microbiol.* **39:** 1434–1442.
20. Mao, S., S.-J. Lee, H. Hwangbo, Y.-W. Kim, K.-H. Park, G.-Y. Cha, R.-D. Park, and K.-Y. Kim. 2005. Isolation and characterization of antifungal substances from *Burkholderia* sp. culture broth. *Curr. Microbiol.* **53:** 358–364.
21. Mencía, M., M. Monsalv, F. Rojo, and M. Salas. 1998. Substitution of the C-terminal domain of the *Escherichia coli* RNA polymerase α subunit by that from *Bacillus subtilis* makes the enzyme responsive to a *Bacillus subtilis* transcriptional activator. *J. Mol. Biol.* **275:** 177–190.
22. Miñambres, B., H. Martínez-Blanco, E. R. Olivera, B. García, B. Díez, J. L. Barredo, M. A. Moreno, C. Schleissner, F. Salto, and J. M. Luengo. 1996. Molecular cloning and expression in different microbes of the DNA encoding *Pseudomonas putida* U phenylacetyl-CoA ligase. *J. Biol. Chem.* **271:** 33531–33538.
23. Mitchell, R. J., J.-M. Ahn, and M. Gu. 2005. Comparison of *Photorhabdus luminescens* and *Vibrio fisheri lux* fusion to study gene expression patterns. *J. Microbiol. Biotechnol.* **15:** 48–54.
24. Oh, E.-T., J.-S. So, B.-H. Kim, J.-S. Kim, and S.-C. Koh. 2004. Green fluorescent protein as a marker for monitoring a pentachlorophenol degrader *Sphingomonas chlorophenolica* ATCC39723. *J. Microbiol.* **42:** 243–247.
25. Olivera, E. R., B. Miñambres, B. García, C. Muñiz, M. A. Moreno, A. Ferrández, E. Díaz, J. L. García, and J. M. Luengo. 1998. Molecular characterization of the phenylacetic acid catabolic pathway in *Pseudomonas putida* U: The phenylacetyl-CoA catabolon. *Proc. Natl. Acad. Sci. USA* **95:** 6419–6424.
26. Park, M., J. Bae, J. Han, E. Lee, S.-G. Lee, and S. Park. 2006. Characterization of styrene catabolic genes of *Pseudomonas putida* SN1 and construction of a recombinant *Escherichia coli* containing styrene monooxygenase gene for the production of (S)-styrene oxide. *J. Microbiol. Biotechnol.* **16:** 1032–1040.
27. Park, S.-H., D.-H. Lee, K.-H. OH, K. Lee, and C.-K. Kim. 2002. Detection of aromatic pollutants by bacterial biosensors bearing gene fusions constructed with the *dnaK* promoter of *Pseudomonas* sp. DJ-12. *J. Microbiol. Biotechnol.* **12:** 417–422.
28. Peso-Santos, T., D. Bartolomé-Martín, C. Fernández, S. Alonso, J. L. García, E. Díaz, V. Shingler, and J. Perera. 2006. Coregulation by phenylacetyl-coenzyme A-responsive *paaX* integrates control of the upper and lower pathways for catabolism of styrene by *Pseudomonas* sp. strain Y2. *J. Bacteriol.* **188:** 4812–4821.
29. Schleissner, C., E. R. Olivera, M. Fernandez-Valverde, and J. M. Leuengo. 1994. Aerobic catabolism of phenylacetic acid in *Pseudomonas putida* U: Biochemical characterization of a specific phenylacetic acid transport system and formal demonstration that phenylacetyl-coenzyme A is a catabolic intermediate. *J. Bacteriol.* **176:** 7667–7676.
30. Simon, R., U. Priefer, and A. Pufler. 1983. A broad host-range mobilization system in Gram-negative bacteria. *Biotechnology (NY)* **1:** 784–791.
31. Yin, S., M. Fuanthong, W. P. Laratta, and J. P. Shapleigh. 2003. Use of a green fluorescent protein-based reporter fusion for detection of nitric oxide produced by denitrifiers. *Appl. Environ. Microbiol.* **69:** 3938–3944.
32. Zumin, Q., H. Yunbing, Z. Dianmo, and L. Fufang. 2005. Study on the synthesis of phenylacetic acid by carbonylation of benzyl chloride under normal pressure. *J. Nat. Gas Chem.* **14:** 40–44.