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A novel bioluminescent bacterial biosensor using the highly specific oxidative stress-inducible *pgi* gene

Javed H. Niazi[a], Byoung Chan Kim[b], Joo-Myung Ahn[a], Man Bock Gu[a,*]

[a] College of Life Sciences and Biotechnology, Korea University, 5-1 Anam-dong, Seongbuk-gu, Seoul 136-701, Republic of Korea
[b] Diagnositics Group, Institut Pasteur Korea, 9-1 Hawolgokdong, Seongbuk-gu, Seoul 136-791, Republic of Korea

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ABSTRACT

A new oxidative stress-responsive bacterial biosensor was constructed using the promoter of the *pgi* gene fused to the *luxCDABE* reporter. This strain (PGRFM) responded in a dose-dependent manner to methyl viologen (MV), a model redox chemical that results in oxidative stress. The responses of strain PGRFM to redox chemicals was strongly dependent on the available carbon source. For example, when the strain was grown under nutrient-limited conditions in the presence of glucose or gluconate it was capable of responding to low MV concentrations (0.6–19.3 ppm), whereas the same cells grown in LB (a nutrient rich media) only responded to higher concentrations (4.9–625 ppm). This allowed us to select PGRFM's growth conditions and extend the range of concentrations at which a stress-inducing chemical could be detected. Further, strain PGRFM responded to structural analogs of MV (*i.e.*, ethyl and benzyl viologen), demonstrating that this strain is responsive to the presence of superoxide radicals, regardless of the chemical by which they are generated. Strain PGRFM's response patterns to these analogs were distinct from each other, which determined their strength to induce oxidative stress. As well, a significant induction was seen when this strain was exposed to hydrogen peroxide, illustrating that strain PGRFM is responsive in the presence of both the superoxide ($O_2^{\bullet -}$) and hydroxyl ($OH^{\bullet}$) radicals.

1. Introduction

Xenobiotics that can induce damage via radicals are referred to as redox cycling compounds. Such compounds are reduced to form free radical species and the resulting free radicals subsequently donate their unpaired electron to O_2, thus generating superoxide anion radicals ($O_2^{\bullet -}$) (Goeptar et al., 1995). Some examples of xenobiotic compounds that undergo redox cycling include paraquat (methyl viologen: MV), diquat (Sabapathy, 1994), menadione (Rosen et al., 1989) and doxorubicin (Goeptar et al., 1993). Methyl viologen is known to induce toxicity by generating activated oxygen species, which are highly reactive and modify cellular macromolecules (Bus and Gibson, 1984). Likewise, doxorubicin is known to produce both $O_2^{\bullet -}$ and hydroxyl radicals ($OH^{\bullet}$) (Faure et al., 1996). A number of other chemicals that induce free radical damage have been reported. For example, carbon tetrachloride (CCl_4), in the presence of oxygen, is subsequently converted into a peroxyl radical (Goeptar et al., 1995; Recknagel et al., 1989), while ethanol exposure is associated with $O_2^{\bullet -}$ generation (Nordmann et al., 1992) and iron nitrilotriacetate (Fe-NTA) induces oxidative DNA damage and lipid peroxidation (Umemura et al., 1990).

It is well documented that $O_2^{\bullet -}$ participates in a broad spectrum of potentially toxic reactions, such as the peroxidation of polyunsaturated lipids, the depolymerization of hyaluronic acid, the inactivation of proteins and damage to the cellular DNA (Bus and Gibson, 1984; Hassan and Fridovich, 1980). However, $O_2^{\bullet -}$ itself is involved in the formation of $OH^{\bullet}$, which is a much more potent oxidant than $O_2^{\bullet -}$, via the reactions shown in the following equations (Bus and Gibson, 1984; Kappus, 1987).

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 \quad (1)$$

$$H_2O_2 + O_2^{\bullet -} + H^+ \rightarrow OH^{\bullet} + O_2 + H_2O \quad (2)$$

These reactions occur in the cells as a result of respiration and exposure to toxic chemicals and it has been suggested that most of the hydrogen peroxide generated in the cells arises from $O_2^{\bullet -}$ metabolism (Gonzalez-Flecha and Demple, 1997).

The oxidative toxicity caused by chemicals that generate $O_2^{\bullet -}$ or $OH^{\bullet}$ can be detected using specific promoter elements from the SoxRS or OxyRS regulon genes as the key component of the biosensor. For example, *Escherichia coli* biosensors constructed with promoters from the *zwf* or *fpr* genes, both of which belong to the SoxRS regulon, are specifically induced by $O_2^{\bullet -}$ (Niazi et al., 2007).

* Corresponding author. Tel.: +82 2 3290 3417; fax: +82 2 928 6050.
E-mail address: mbgu@korea.ac.kr (M.B. Gu).

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Whereas, *katG* belongs to the OxyRS regulon, the genes of which are specifically induced by H_2O_2 or $OH^\bullet$ (Belkin et al., 1996). Since $O_2^{\bullet-}$ can also lead to the generation of H_2O_2 or $OH^\bullet$, the toxicity experienced is much more complex and biosensors specific for $O_2^{\bullet-}$ may fail to respond to H_2O_2 or $OH^\bullet$ (Niazi et al., 2007). Similarly, $OH^\bullet$-specific biosensors do not respond to $O_2^{\bullet-}$ (Belkin et al., 1996; Niazi et al., 2007). Therefore, it is imperative that a biosensor that can simultaneous detect both $O_2^{\bullet-}$ and H_2O_2 or $OH^\bullet$ be developed and characterized. This can be accomplished using a bacterial biosensor that is constructed using a specific promoter element that is induced by both $O_2^{\bullet-}$ and H_2O_2 or $OH^\bullet$.

Bacterial biosensors make use of bacteria to sense and detect small amounts of a chemical, or to report on the physiological and biochemical changes in the cells caused by a sample. These bacterial biosensors have the ability to sense the stress signal through a process in which many enzymes are involved, such as those involved in the electron transport system and catabolic reactions. Bacteria possess a wide variety of stress responses, including for oxidative stress, nutrient starvation and membrane damage (Martinez-Antonio et al., 2003; Pagan and Mackey, 2000; Storz et al., 1990). Specific promoter elements are activated by a particular type of stimulant, such as chemicals that induce oxidative stress response. For example, the SoxRS and OxyRS regulon genes collectively respond to neutralize the stress at various levels (Storz and Imlay, 1999). These genes not only take part in responding to stresses but also have indigenous roles including metabolism. However, all the SoxRS regulon genes are collectively induced by oxidative stresses resulting from redox chemicals (Storz and Imlay, 1999) and this induction may also be influenced by changes in the growth conditions (Martinez-Antonio et al., 2003). As such, the promoter elements of such genes aid in the mitigation of oxidative damage through the production of specific proteins. Furthermore, these promoters can serve as potential candidates for the construction of biosensors.

Different reporter genes, such as *gfp* and *luxCDABE*, have been used in the development of bacterial biosensors (Hakkila et al., 2002; Mitchell and Gu, 2004b). The biosensors containing *luxCDABE* reporter genes are faster and have more sensitive detection capabilities compared to the fluorescent biosensors containing *gfp* reporter system, which is applicable only for long-term exposure (Li et al., 2008). In addition, normal GFP proteins are very stable and so cannot be used as a dynamic and sensitive reporter in bacteria.

Consequently, a number of bioluminescent strains have been developed and used in that screening of specific toxic chemicals. For example, strains have been constructed that detect H_2O_2 (Belkin et al., 1996), $O_2^{\bullet-}$ (Niazi et al., 2007), heat shock (Van Dyk et al., 1994, 1995), and DNA damage (Vollmer et al., 1997). These bacterial biosensors have promising applications within the biotechnology and environmental sciences fields (Kim et al., 2005; Lee et al., 2005; Mitchell and Gu, 2004a,b). To construct them, the promoter elements from genes induced during a particular type of toxicity are fused to *luxCDABE* in *E. coli* and the damage is then sensed through the translation of the signals into increased levels of bioluminescence (Min et al., 1999; Mitchell and Gu, 2004a; Van Dyk et al., 2001). However, these bacterial biosensors are sensitive to minor changes in their growth conditions and this is due to the actions of transcriptional regulatory genes, which are responsible for sensing stimuli and subsequently inducing and/or repressing genes (Martinez-Antonio et al., 2003). Therefore, growth conditions, such as carbon sources, starvation, and temperature, can seriously affect the functions of transcriptional units or genes and by limiting the growth conditions for a bacterial biosensor, researchers could extend or modify the biosensors ability to respond to a particular type of toxicity.

In this study, we report on a novel bacterial biosensor (PGRFM), containing the promoter element from the *pgi* gene, which responds to both $O_2^{\bullet-}$ and H_2O_2 or $OH^\bullet$. We also report on an extension of this biosensor's sensitivity to detect oxidative stress inducing chemicals by altering the growth conditions. The results indicate that the selection of growth conditions, *i.e.*, different carbon sources, had a significant effect on the range of concentrations that could be detected. Further, our results show that strain PGRFM was responsive to chemicals structurally similar to methyl viologen. The results of this study clearly demonstrate that it is possible to alter, and potentially expand, the sensitivity of bacterial biosensors, a finding that holds promise for instances where toxicants, such as pesticides, drugs and other xenobiotics, need to be monitored.

2. Materials and methods

2.1. DNA microarray analysis and real time RT-PCR

The method for microarray analysis using *E. coli* 6.0 K genome oligonucleotide microarray chips (Genomictree, Daejeon, Korea) and real time RT-PCR analysis with three primers and probe sets for *pgi* and 16S rDNA genes are described in the Supporting information Section I(a).

2.2. Construction of strain PGRFM

The promoter region in the upstream of the *pgi* gene was amplified by PCR using *E. coli* RFM443 (*rpsL*-(*StrR*), *galK2*, *lac*Δ74) genomic DNA and specially designed primers. The amplified product was fused to *luxCDABE* operon and a bioluminescent strain (PGRFM) was developed as described in the Supporting information Section I(b).

2.3. Experimental conditions

The responses of recombinant *E. coli* strain (PGRFM) against oxidative stress-inducing chemicals was monitored as previously described (Niazi et al., 2007) with slight modification when needed. Strain PGRFM was cultured overnight in Luria-Bertani (LB) medium containing ampicillin (50 μg/mL) and kanamycin (25 μg/mL). Unless otherwise stated, strain PGRFM was also grown in minimal medium (64 mM K_2HPO_4, 34 mM NaH_2PO_4, 20 mM $(NH_4)_2SO_4$, 10 mM $MgSO_4$, 10 μM $CaCl_2$ and 1 μM $FeSO_4$, pH 7.4) supplemented with one of the following 10 mM carbon and energy sources: glucose, gluconate, mannitol or mannose (Echave et al., 2003). Sub-culturing was performed using the same conditions and media. Experiments were performed by incubating the 96-well plate in a luminometer (MLX, Dynex Inc.). The minimum detectable RLU with the luminometer is 0.001 (see Supplementary information Section I(c)).

3. Results

3.1. Analysis of pgi response to methyl viologen by DNA microarray and real-time RT-PCR

DNA microarray analysis using *E. coli* cells treated with 13.75 ppm methyl viologen, a known redox chemical, found that the *pgi* gene was induced over 4-fold, along with the other SoxRS regulon genes, when compared to the untreated cells (Table 1). Induction of the *pgi* expression level by MV was further validated by RT-PCR, where the expression ratio 60 min after initiating

Table 1
Response ratios of the 10 most strongly induced *E. coli* genes after the treatment with 13.75 ppm methyl viologen (MV) for 1 h

Gene	Function	Expression ratio (exposed sample/control sample)
fpr	Ferredoxin-NADP reductase	126.4
soxS	Regulation of superoxide response regulon	53.00
nfo	Endonuclease IV	12.18
zwf	Glucose 6-phosphate dehydrogenase	6.25
fldA	Flavodoxin 1, energy production/transport	5.07
fumC	Fumarace C = fumarate hydratase class II	4.14
pgi	Phosphoglucose isomerase, located at the juncture of glycolytic and PP-pathways	4.08
sodA	Manganese superoxide dismutase	3.06
inaA	pH inducible, involved in stress response	3.16
pqiA	Paraquat inducible protein A	2.44

The results are from a DNA microarray analysis using the *E. coli* whole genome chip (Genomictree).

the exposure was 3.58 ± 0.39, a value that is consistent with the microarray analysis (Tables 1and S1). Many of the other genes belonging to the SoxRS regulon have already been studied and reported on (Table 1) (Kim et al., 2005; Martin and Rosner, 2002; Niazi et al., 2007), but little is known about the *pgi* gene. Therefore, we selected this gene and its promoter for further analysis.

3.2. Construction and characterization of strain PGRFM

A new bacterial biosensor (PGRFM) was constructed using the promoter element of the *pgi* gene by fusing it upstream of the *luxCDABE* operon that is present in plasmid pUCD615. The *pgi* promoter activity was then monitored through the bioluminescence when PGRFM was exposed to MV. The initial studies with MV showed that PGRFM responded in a dose-dependent manner to various concentrations of MV, where as the maximum induction was achieved 70 min after initiating the exposure. Induction of *pgi::luxCDABE* began to decline after this time. This loss in bioluminescence is likely due to the lower metabolic activity of the cells as they entered the stationary phase of growth or a limitation in oxygen brought on by high cell numbers (Meighen, 1991; Neilson et al., 1999). The bioluminescent response increased in a dose-dependent manner up to a MV concentration of 625 ppm in nutrient-rich medium (LB), although the responses were also seen from this strain up to a concentration of 5000 ppm, but the level of bioluminescent response did not increase with respect to higher MV levels beyond 625 ppm (Fig. 1). The saturated responses seen with these higher concentrations, *i.e.*, above 625 ppm MV, are likely due to the over toxicity of the sample at these concentrations. The minimum MV concentration to which PGRFM showed a 2-fold induction in LB-medium was 4.9 ppm, and this was seen about 60–90 min after initiating the exposure.

To further probe and characterize the *pgi* promoter activity, strain PGRFM was exposed to chemicals that also induce oxidative stress and are structurally similar to MV, such as ethyl viologen (EV) and benzyl viologen (BV). Strain PGRFM showed distinct responses to these structural analogs, which reflect the potential of both of these chemicals to induce oxidative stress (Fig. 2A and B). BV was highly toxic to the cells since the concentration giving the maximum response was 78 ppm. However, this concentration resulted in a relative bioluminescence (RBL) value of 199. Tests with higher concentrations resulted in a reduced response, very likely due to the over toxicity of the compound. In contrast, strain PGRFM showed higher responses as the EV concentration increased, up to 2500 ppm, indicating that EV was less toxic than BV. The responses of the cells to MV and EV followed a similar trend but the response level for EV was consistently about 25–50% of the response from MV at the same concentration. The variation in the responses to these structurally similar chemicals is very likely due to the differences in the alkyl groups that are attached to each, *i.e.*, $-CH_3$ and CH_2-CH_3 in MV and EV, respectively (Fig. 2B).

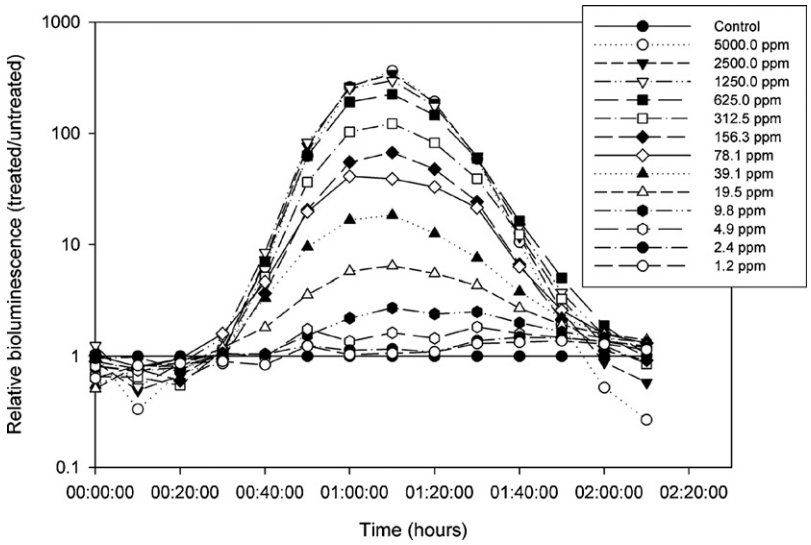

Fig. 1. Induction profiles of strain PGRFM (*pgi::luxCDABE*), grown in Luria-Bertani (LB) after being exposed to various concentrations of methyl viologen (MV). The relative bioluminescence values are defined as the bioluminescence (arbitrary light units) of the exposed sample divided by that of the untreated (control) sample.

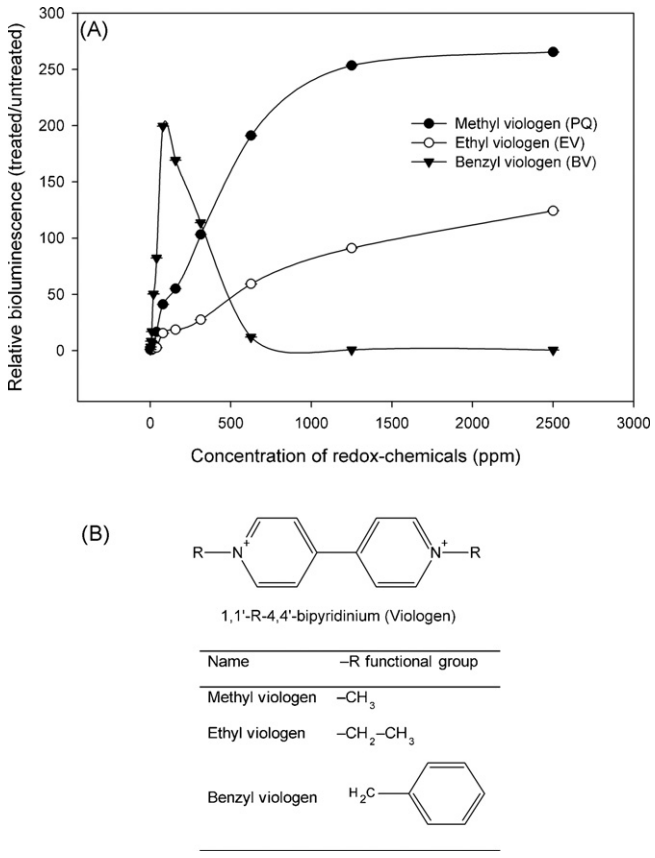

Fig. 2. Response of strain PGRFM to structural analogs of methyl viologen. (A) Relative bioluminescent responses (RBL) from strain PGRFM (*pgi::luxCDABE*) exposed to various concentrations of the viologens, including methyl viologen (MV), ethyl viologen (EV) and benzyl viologen (BV). Tests were performed in LB media. The values shown are the maximum RBL values seen over a 2 h exposure. (B) General structure of 4,4′-dipyridinium derivatives (viologens) that induce oxidative stress. The functional group –R, for the different viologens (MV, EV, or BV) is shown.

3.3. Response of strain PGRFM to MV under different growth conditions

Strain PGRFM was grown in minimal medium with only glucose, gluconate, mannitol or mannose as the carbon source. This allowed us to fine tune the responses of this strain to sense lower concentrations of MV, thereby enhancing the sensitivity of this strain. Initially the cells were treated with various concentration of MV and the bioluminescence was recorded. Strain PGRFM, when grown under nutrient-limited conditions, still showed significant responses to MV. However, the MV concentration to which the cells responded varied dependent on the carbon source. For example, strain PGRFM was highly sensitive to MV when it was grown on glucose or gluconate (Fig. 3). The minimum and maximum detectable MV concentration when grown on glucose were 0.6 ppm (RBL = 12.3) and 4.9 ppm (RBL = 15.8), respectively. Likewise, the minimum detectable concentration for the gluconate-grown cells was 1.22 ppm (RBL = 3.2) while the maximum was 19.5 ppm (RBL = 23.8). In contrast, when strain PGRFM was grown on mannitol or mannose, it showed only moderate responses but these were seen with higher MV concentrations (4.9–40 ppm) than those seen when the cells were grown in the glucose or gluconate media. However, the response strengths of strain PGRFM when under these nutrient-limited growth conditions was comparatively low when contrasted with the tests done with cells grown in the nutrient-rich medium (LB) (Figs. 1 and 3). However, the use of these different growth conditions permitted us to expand the range of MV concentrations that could be detected and lowered the minimum detectable level by 8-fold from 4.9 (in LB) to 0.6 ppm (in minimal-glucose media). Therefore, by fine-tuning the growth conditions for PGRFM, it was possible to detect this stress-inducing compound at lower concentrations, especially when the cells were grown on glucose and gluconate (Fig. 3).

3.4. Response of strain PGRFM to other stress-inducing chemicals

Strain PGRFM also responded in a dose-dependent manner when exposed to various concentrations of hydrogen peroxide (Fig. S1(A)), which also contributes to oxidative stress but via $OH^{\bullet}$ production. Likewise, only very mild responses were seen when this strain was exposed to 4-chlorophenol (4-CLP), mitomycin C (MMC) or N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), which cause membrane—(4-CLP) and DNA-damage (MMC and MNNG) (Fig. S1(B and C)). However, the maximum RBL values for these compounds were lower than 5 (Fig. S1(B and C)), *i.e.*, MMC (maximum RBL = 4.9 at 10 ppm), 4-CLP (maximum RBL = 3.7 at 50 ppm) and MNNG (maximum RBL = 2.4 at 9.3 ppm), suggesting that these responses likely result from an indirect oxidative stress response. Taken together, it is clear that PGRFM specifically responded to chemicals that caused oxidative stress by generating either reactive oxygen species, $O_2^{\bullet -}$ or H_2O_2 or $OH^{\bullet}$.

4. Discussion

The defense mechanisms by which cells sense and respond to stress utilizes the induction of a specific set of genes, such as those within the SoxRS and OxyRS regulon, which are induced by the presence of chemicals that induce $O_2^{\bullet -}$ and $OH^{\bullet}$, respectively (Compan and Touati, 1993; Storz and Imlay, 1999). Such genes are potential candidates for the development of stress-specific bacterial biosensors. Many bacterial biosensors have been reported on due to their promising applications in the fields of biotechnology and environmental sciences (Howbrook et al., 2001; Kim et al., 2005; Lee et al., 2005; Niazi et al., 2007). However, these recombinant bacterial sensors are subject to differences in their growth conditions. One previous study reported that changes in growth conditions could perturb the complex network

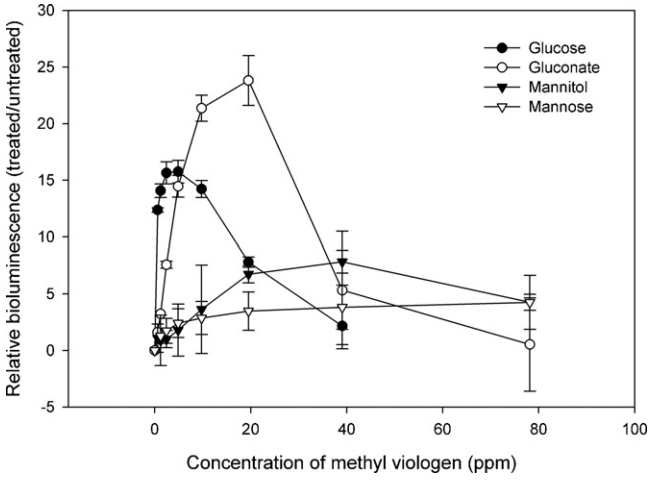

Fig. 3. Effect of different growth substrates on the responses of strain PGRFM to MV. Strain PGRFM was grown in minimal medium (MM) containing a single carbon source (glucose, gluconate, mannitol, or mannose) and exposed with various concentrations of MV. The values shown are the maximum RBL values seen over a 2 h exposure.

of metabolic interactions that govern the physiological state of the cell (Martinez-Antonio et al., 2003). Such perturbations may affect the ability of a bacterial biosensor to sense and respond to a stimulant. Therefore, in this study, we report on a novel bacterial biosensor (PGRFM) constructed using the promoter element from the *pgi* gene from *E. coli*, which was found to be highly sensitive to different growth substrates. This may be because of the involvement of *pgi* in a key metabolic reaction located at the juncture of the glycolytic and pentose-phosphate pathways (Canonaco et al., 2001).

This study demonstrated that this gene is strongly induced by $O_2^{\bullet -}$, such as those generated by MV, through DNA microarray and real time RT-PCR analyses (Tables 1and S1). These results are in agreement with previous studies that show that the *pgi* gene is an active member of the SoxRS regulon (Martin and Rosner, 2002; Pomposiello et al., 2001) and an important gene, which takes part in glucose metabolism (Canonaco et al., 2001). Our results showed that *pgi* was responsive not only to $O_2^{\bullet -}$ but also to H_2O_2 or $OH^{\bullet}$ (Fig. S1(A)), unlike other SoxRS genes. However, when the media composition was altered and a minimal media was used, this strain was able to detect lower concentrations of MV than when cultured in a rich medium (Figs. 1 and 3). Whereas the rich medium facilitated strain PGRFM's responses to higher concentrations of MV, up to 5000 ppm, this strain was also less sensitive since the minimum detectable concentration was 4.9 ppm of MV (Fig. 1). On the other hand, when the same cells were grown in a minimal media containing glucose or gluconate, their sensitivity was enhanced and they responded to concentrations as low as 0.6 or 1.22 ppm, respectively (Fig. 3).

We observed that cells grown in minimal medium with any one of the carbon sources tested had a slower growth rate (doubling time = 45–50 min) compared to those grown in LB-medium (doubling time = 20–25 min). To compare the results from each of the media, therefore, the same number of cells were used in each exposure (OD_{600} = 0.3), irrespective of the carbon source or growth medium. Despite the fact that the same number of cells were used, the responses to MV were distinct with respect to the media and MV concentrations, but the time required to reach the maximum induction remained unaltered (within 2 h). This allowed us to enhance the sensitivity of PGRFM and to extend the dynamic range of MV detection. It was reported in previous studies that different growth conditions results in altered metabolic and physiological changes (Canonaco et al., 2001; Martinez-Antonio et al., 2003). Thus, the differences in strain PGRFM's responses to MV in the presence of different growth substrates were likely due to such metabolic and physiological changes. Moreover, it may be possible to employ this phenomenon to optimize the responses from other biosensing cells. In addition, the dose-dependent trend with respect to time and concentration of MV and OD is discussed in the Supporting information, Section II(a).

As with MV, strain PGRFM responded strongly to EV and BV, which differ in the type of alkyl group attached (Fig. 2A and B). Since each of these compounds generate $O_2^{\bullet -}$, it is clear that strain PGRFM responds to superoxide stress. The differences in these responses, however, can be attributed to the alkyl groups, which interfere with the permeability of the viologens through the cytoplasmic membrane of the bacterium (Jones and Garland, 1977; Jones et al., 1976). PGRFM also mildly responded to different toxicants, such as MMC, MNNG, and 4-CLP (Fig. S1(B and D)). This is not entirely surprising, however, since it has been reported that DNA damaging chemicals, such as mytomycin C, also result in oxidative damage due to oxygen radicals being formed during redox cycling (Kappus, 1987; Lown, 1983). However, these responses were distinct from the responses to MV, EV and BV in terms of their response patterns and strengths (Fig. 2 and Figs. 2A and S1(B and D)).

Various bacterial biosensors have been constructed and used in detection of oxidative stresses are almost all are specific for either $O_2^{\bullet -}$ or H_2O_2 ($OH^{\bullet}$), but not both. For example, a *katG*-lux fusion strain was developed for the detection of oxidative stresses caused by H_2O_2 or $OH^{\bullet}$ (Belkin et al., 1996; Howbrook et al., 2001; Mitchell and Gu, 2004b) while $O_2^{\bullet -}$ radicals were detected using *soxS*, *inaA*, *zwf*, and *fpr*-lux fusions (Kim et al., 2005; Niazi et al., 2007). However, the detection of oxidative stresses from both reactive oxygen species using same type biosensor is scarce, except with one biosensor that contains a *sodA*-lux fusion and is responsive to paraquat (MV, $O_2^{\bullet -}$) and H_2O_2 ($OH^{\bullet}$) (Lee and Gu, 2003). Our results demonstrate that strain PGRFM is induced by both the superoxide radical and H_2O_2 in a dose-dependent manner.

Based on the DNA microarray analysis presented here and elsewhere in the literature (Martin and Rosner, 2002; Pomposiello et al., 2001), it is clear that the *pgi* gene is induced by oxidative stress. However, some major differences between the *pgi* gene and other oxidative stress-responsive genes were seen, including the finding that it responds to both the $O_2^{\bullet -}$ and $OH^{\bullet}$ and that it plays a key role in catabolic pathways (Canonaco et al., 2001). Only a few genes, such as *sodA*, *pqi*, and *fur* are known to be responsive to both types of oxidative stresses ($O_2^{\bullet -}$ and $OH^{\bullet}$) (Lee and Gu, 2003; Michan et al., 1999; Pomposiello et al., 2001; Storz and Imlay, 1999; Zheng et al., 2001). The fact that $O_2^{\bullet -}$ metabolism also leads to the generation of H_2O_2 and $OH^{\bullet}$ suggests that there is a need for a biosensor that can detect toxicities caused by both $O_2^{\bullet -}$ and H_2O_2 or $OH^{\bullet}$. Therefore, PGRFM is a potential candidate. Its response to oxidative toxicity was further enhanced to obtain a greater sensitivity and dynamic range by altering the growth conditions. The advantages of this methodology can also be expanded to other bacterial biosensors that are used to detect a variety of toxic chemicals. Finally, the portability of these biosensing biosensors in conjunction with CCDs or CMOS is discussed extensively in the Supporting information, Section II(b).

5. Conclusions

The *pgi* gene was selected for the development of bacterial biosensor because of two main factors: (a) it showed a significant induction in the DNA microarray and real time RT-PCR analyses when *E. coli* was exposed to MV and (b) its involvement in a key metabolic reaction. Therefore, a new oxidative stress-responsive recombinant bacterial biosensor (PGRFM) was constructed in which the expression of the *luxCDABE* reporter genes was controlled by the *pgi* promoter. This strain responded in a dose-dependent manner to the presence of MV, a model superoxide radical-generating compound. Furthermore, PGRFM also responded to structural analogs of MV, but with a different range of effective concentrations. During tests with MV, it was also demonstrated that the response of this strain to redox chemicals was strongly dependent upon the available carbon source. When strain PGRFM was grown in a minimal media containing glucose or gluconate, the range of MV concentrations that could be detected was shifted from 4.9 to 625 ppm (in LB media) to 0.6–19.3 ppm, an increase in the sensitivity by more than 8-fold. Likewise, the bioluminescence of strain PGRFM was also significantly induced by hydrogen peroxide. This result clearly shows the special nature of the *pgi* gene since the expression of only a few genes is induced by both the $O_2^{\bullet -}$ and $OH^{\bullet}$. In conclusion, this study reports on the detection of both $O_2^{\bullet -}$ and $OH^{\bullet}$ toxicity by same type biosensor and the characterization of the PGRFM biosensor under a variety of stressful conditions. Finally, this study illustrates a method to enhance the sensitivity of this biosensor, with the potential for

this enhancement to be employed with previously characterized bioluminescent biosensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.06.026.

References

Belkin, S., Smulski, D.R., Vollmer, A.C., Van Dyk, T.K., LaRossa, R.A., 1996. Appl. Environ. Microbiol. 62 (7), 2252–2256.
Bus, J.S., Gibson, J.E., 1984. Environ. Health Perspect. 55, 37–46.
Canonaco, F., Hess, T.A., Heri, S., Wang, T., Szyperski, T., Sauer, U., 2001. FEMS Microbiol. Lett. 204 (2), 247–252.
Compan, I., Touati, D., 1993. J. Bacteriol. 175 (6), 1687–1696.
Echave, P., Tamarit, J., Cabiscol, E., Ros, J., 2003. J. Biol. Chem. 278 (32), 30193–30198.
Faure, H., Coudray, C., Mousseau, M., Ducros, V., Douki, T., Bianchini, F., Cadet, J., Favier, A., 1996. Free Radic. Biol. Med. 20 (7), 979–983.
Goeptar, A.R., Scheerens, H., Vermeulen, N.P., 1995. Crit. Rev. Toxicol. 25 (1), 25–65.
Goeptar, A.R., Te Koppele, J.M., Lamme, E.K., Pique, J.M., Vermeulen, N.P., 1993. Mol. Pharmacol. 44 (6), 1267–1277.
Gonzalez-Flecha, B., Demple, B., 1997. J. Bacteriol. 179 (2), 382–388.
Hakkila, K., Maksimow, M., Karp, M., Virta, M., 2002. Anal. Biochem. 301 (2), 235–242.
Hassan, H.M., Fridovich, I., 1980. In: Jakoby, W.B. (Ed.), Enzymatic Basis of Detection. Academic Press, New York, pp. 311–322.
Howbrook, D.N., Lynch, J.M., Bainton, N.J., 2001. Enzyme Microb. Technol. 29 (8–9), 521–526.
Jones, R.W., Garland, P.B., 1977. Biochem. J. 164 (1), 199–211.
Jones, R.W., Gray, T.A., Garland, P.B., 1976. Biochem. Soc. Trans. 4 (4), 671–673.
Kappus, H., 1987. Arch. Toxicol. 60 (1–3), 144–149.
Kim, B.C., Youn, C.H., Ahn, J.M., Gu, M.B., 2005. Anal. Chem. 77 (24), 8020–8026.
Lee, H.J., Gu, M.B., 2003. Appl. Microbiol. Biotechnol. 60 (5), 577–580.
Lee, J.H., Mitchell, R.J., Kim, B.C., Cullen, D.C., Gu, M.B., 2005. Biosens. Bioelectron. 21 (3), 500–507.
Li, Y.F., Li, F.Y., Ho, C.L., Liao, V.H., 2008. Environ. Pollut. 152 (1), 123–129.
Lown, J.W., 1983. Mol. Cell. Biochem. 55 (1), 17–40.
Martin, R.G., Rosner, J.L., 2002. Mol. Microbiol. 44 (6), 1611–1624.
Martinez-Antonio, A., Salgado, H., Gama-Castro, S., Gutierrez-Rios, R.M., Jimenez-Jacinto, V., Collado-Vides, J., 2003. Biotechnol. Bioeng. 84 (7), 743–749.
Meighen, E.A., 1991. Microbiol. Rev. 55 (1), 123–142.
Michan, C., Manchado, M., Dorado, G., Pueyo, C., 1999. J. Bacteriol. 181 (9), 2759–2764.
Min, J., Kim, E.J., LaRossa, R.A., Gu, M.B., 1999. Mutat. Res. 442 (2), 61–68.
Mitchell, R.J., Gu, M.B., 2004a. Biosens. Bioelectron. 19 (9), 977–985.
Mitchell, R.J., Gu, M.B., 2004b. Appl. Microbiol. Biotechnol. 64 (1), 46–52.
Neilson, J.W., Pierce, S.A., Maier, R.M., 1999. Appl. Environ. Microbiol. 65 (8), 3473–3482.
Niazi, J.H., Kim, B.C., Gu, M.B., 2007. Appl. Microbiol. Biotechnol. 74 (6), 1276–1283.
Nordmann, R., Ribiere, C., Rouach, H., 1992. Free Radic. Biol. Med. 12 (3), 219–240.
Pagan, R., Mackey, B., 2000. Appl. Environ. Microbiol. 66 (7), 2829–2834.
Pomposiello, P.J., Bennik, M.H., Demple, B., 2001. J. Bacteriol. 183 (13), 3890–3902.
Recknagel, R.O., Glende Jr., E.A., Dolak, J.A., Waller, R.L., 1989. Pharmacol. Ther. 43 (1), 139–154.
Rosen, G.M., Hassett, D.J., Yankaskas, J.R., Cohen, M.S., 1989. Xenobiotica 19 (6), 635–643.
Sabapathy, N.N., 1994. Toxicology 91 (1), 93–98.
Storz, G., Imlay, J.A., 1999. Curr. Opin. Microbiol. 2 (2), 188–194.
Storz, G., Tartaglia, L.A., Farr, S.B., Ames, B.N., 1990. Trends Genet. 6 (11), 363–368.
Umemura, T., Sai, K., Takagi, A., Hasegawa, R., Kurokawa, Y., 1990. Cancer Lett. 54 (1–2), 95–100.
Van Dyk, T.K., DeRose, E.J., Gonye, G.E., 2001. J. Bacteriol. 183 (19), 5496–5505.
Van Dyk, T.K., Majarian, W.R., Konstantinov, K.B., Young, R.M., Dhurjati, P.S., LaRossa, R.A., 1994. Appl. Environ. Microbiol. 60 (5), 1414–1420.
Van Dyk, T.K., Smulski, D.R., Reed, T.R., Belkin, S., Vollmer, A.C., LaRossa, R.A., 1995. Appl. Environ. Microbiol. 61 (11), 4124–4127.
Vollmer, A.C., Belkin, S., Smulski, D.R., Van Dyk, T.K., LaRossa, R.A., 1997. Appl. Environ. Microbiol. 63 (7), 2566–2571.
Zheng, M., Wang, X., Templeton, L.J., Smulski, D.R., LaRossa, R.A., Storz, G., 2001. J. Bacteriol. 183 (15), 4562–4570.