An Effective Strategy for a Whole-Cell Biosensor Based on Putative Effector Interaction Site of the Regulatory DmpR Protein

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Abstract

Introduction and Rationale: The detection of bioavailable phenol is a very important issue in environmental and human hazard assessment. Despite modest developments recently, there is a stern need for development of novel biosensors with high sensitivity for priority phenol pollutants. DmpR (Dimethyl phenol regulatory protein), an NtrC-like regulatory protein for the phenol degradation of Pseudomonas sp. strain CF600, represents an attractive biosensor regimen. Thus, we sought to design a novel biosensor by modifying the phenol detection capacity of DmpR by using mutagenic PCR.

Methods: Binding sites of ‘A’ domain of DmpR were predicted by LIGSITE, and molecular docking was performed by using GOLD to identify the regions where phenol may interact with DmpR. Total five point mutations, one single at position 42 (Phe-to-Leu), two double at 140 (Asp-to-Glu) and 143 (Gln-to-Leu), and two double at L113M (Leu-to-Met) and D116A (Asp-to-Ala) were created in DmpR by site-directed mutagenesis to construct the reporter plasmids pRLuc42R, pRLuc140p143R, and pRLuc113p116R, respectively. Luciferase assays were performed to measure the activity of luc gene in the presence of phenol and derivatives, while RT-PCR was used to check the expression of luc gene in the presence of phenol.

Results: Only pRLuc42R and pRLuc113p116R showed positive responses to phenolic effectors. The lowest detectable concentration of phenol was 0.5 μM (0.047 mg/L), 0.1 μM for 2, 4-dimethylphenol and 2-nitrophenol, 10 μM for 2, 4, 6-trichlorophenol and 2-chlorophenol, 100 μM for 2, 4-dichlorophenol, 0.01 μM for 4-nitrophenol, and 1 μM for o-cresol. These concentrations were measured by modified luciferase assay within 3 hrs compared to 6–7 hrs in previous studies. Importantly, increased expression of luciferase gene of pRLuc42R was observed by RT-PCR.

Conclusions: The present study offers an effective strategy to design a quick and sensitive biosensor for phenol by constructing recombinant bacteria having DmpR gene.

Introduction

Phenol and its organic compounds are the most important environmental pollutants at the global level that have been released into the environment in substantial quantities by natural events and industrial activities. Phenol is toxic, carcinogenic, mutagenic, and teratogenic even in low concentration and is mentioned in the list of priority pollutants of U.S. Environmental Protection Agency [1].

The detection of bioavailable phenol is a very important issue in environmental and human hazard assessment studies. Currently, chromatography coupled to the mass spectrometer detection is used for the analysis of phenol [2]. However, these methods are not used extensively because of being cost intensive and the chemicals used in these processes may harm the surrounding environment [3]. Biosensors can provide rapid measurement without labor-intensive and time-consuming sample preparation and apparently allow for a promising way to assess the biologically available phenol in the environment. Previously, different biosensors for phenol have been described such as the amperometric [4,5], enzymatic [6], and optical biosensor [7].

Microbes offer attractive targets for the construction of biosensors for monitoring the status of the environment. Whole-cell microbial sensors have become one of the latest approaches of molecular tools in environmental monitoring [8]. Microorganisms, for their low cost, lifespan, and range of suitable pH and temperatures, have been widely used as the biosensing recognition elements in the construction of biosensors [9]. Currently available whole cell biosensors have many advantages as they can provide an inexpensive and simple way of determining contaminants. As they are living organisms, they give a more accurate response on the toxicity of different compounds. Some stress-induced biosensors report the mutagenic effects of samples with great sensitivity. Biosensors are unexcelled in gene expression and physiological study of bacteria in complex environments. One of the greatest
limitations of whole-cell biosensor development is the availability of strong promoters that respond only to relevant stimuli. To surmount this obstacle, more knowledge on gene regulatory networks in bacteria is needed [10]. Numerous whole cell biosensors for phenol have been developed in which different reporter and regulator genes with various lowest detection ranges of up to 291.4 mg/L (highest) [11] and 0.082 mg/L (lowest) [12] were reported. However, there is always a need for less cumbersome and sensitive approaches to monitor phenol levels. A different approach to construct a microbial biosensor is to connect a strictly regulated promoter sequence to a sensitive reporter gene was reported previously [13]. The ability of the bacteria to survive in a contaminated environment is usually based on a genetically encoded resistance system, the expression of which is regulated very precisely.

DmpR (Di methyl phenol regulatory protein), the product of the *Pseudomonas* strain CF600 DmpR gene [14,15], mediates the expression from Pdmp, the promoter of the Dmp operon, is activated when DmpR detects the presence of an inducing phenol [15]. Previous studies of the sensory 'A' domain may provide some insights regarding interaction between phenol and Dmp protein [16]. The natural interaction of DmpR with a subset of phenols suggested that the modification of its sensor domain might result in protein which can detect a broader range of phenolic derivatives [17]. Till date, there is no crystal structure for the DmpR protein. However, a threading model for N-terminal 'A' domain of DmpR has been reported [18,19].

The aim of this study was to develop a new whole-cell luminescence-based bacterial sensor for highly selective and sensitive detection of bioavailable phenol in the environment. We accomplished this by choosing the DmpR gene with Po/Pr operator/promoter gene from *Pseudomonas* sp. CF600 [14,15] as a receptor for phenol as an analyte and luc genes from pGL3 vector (Promega, USA) as a reporter. Pr promoter of DmpR was identified as a σ70 dependent promoter that is regulated by σ34 dependent Po promoter [20]. Previous mutational studies [15,21,22] were based on the random analysis in DmpR gene. In this study, mutant residues were predicted by docking tools instead of being chosen randomly. Mutant forms of DmpR with altered effector specificity were made by a dock-based site directed mutagenesis method. Three mutant plasmids pRLuc42R, pRLuc140p143R and pRLuc113p116R were constructed by creating mutations in the DmpR gene. The pRLuc42R contains one single mutation F42L (Phenylalanine at 42→Leucine), whereas the pRLuc140p143R and pRLuc113p116R possess two point mutations each (a) D140E (Aspartic acid at 140→Glutamic acid), and (b) Q143L (Glutamine at 143→Leucine); (a) L113M (Leucine at 113→Methionine) and (b) D116A (Aspartic acid at 116→Alanine). Mutant with the single mutation F42L (Phe at 42→Leu) was particularly interesting since it showed an increased response to the phenol inducer. 'A' domain of the DmpR is responsible for sensing the phenol and its derivative. Mutations were induced in this region by computational prediction and validated by mutagenic PCR in the wet lab.

### Experimental Procedures

#### 2.1. Bacterial Strains and Culture Conditions

*Escherichia coli* strain DH5α (hsdR, recA, thi-1, relA1, gyrA96) [23] was used for the maintenance of plasmids. *Pseudomonas* sp. CF600 [24] was obtained from Dr. Victoria Shingler (Umea, Sweden) and grown in Nutrient broth at 37°C. The *E. coli* DH5α harbouring the plasmids was cultured at 37°C in Luria Bertani media (1% tryptone, 0.5% yeast extract and 0.5% NaCl) containing 100 mg/ml ampicillin. The list of used strains and vectors is given in Table 1.

#### 2.2. Construction of pRLuc42R Biosensor

Whole cell biosensor (pRLuc42R) sensing principle is shown in Figure 1.

#### 2.2.1. Plasmid isolation, cloning and PCR

Genomic DNA of *Pseudomonas* sp. CF600 was prepared by using a Genomic DNA isolation kit (RBC, Taiwan). Plasmids were isolated with a Qiagen spin column kit (CA, USA). Cloning, ligation, and transformation were carried out by using standard techniques [25]. PCR was performed by using forward primer 5'-CGATCGATGCTCAGCTCGAGGC-CAGGTTAGGCGTAGGACGCA-3' and a reverse primer, 5'-GCTAGCATGGTATAAAGTTGGACACCGATGTTAGATAGAAGAA-3', with XhoI and HindIII sites at the ends. PCR conditions were as follows: 95°C for 5 min followed by 33 cycles of 95°C for 45 s, 55°C for 45 s, and 72°C for 1 min, followed by 72°C for 7 min. *Taq* DNA polymerase and deoxynucleoside triphosphates were from New England Biolabs (USA).
2.2.2. Site-directed mutagenesis and cloning. Mutagenesis was performed by using the mutagenic PCR [26]. The primers used for site-directed mutagenesis are as follows, (a) for F42L (to generate pRLuc42R) forward primer: TGTTGCTGCAGCTTTCAGCGATGG and reverse primer: GCCATCGCTGAAGCTGCAGCAA (b) for D140E and Q143L (to generate pRLuc140p143R) forward Primer: AC- 

GAACTGGGGCTGATGCA and reverse primer: TGCAT-CAGCCCCAGTTCCGT (c) for L113M and D116A (to generate pRLuc113p116R) forward Primer: ACCGAGATGGA-TATC 

GCCAAGGAA and reverse primer: TTCCTTGGCGA-

TATC CATCTCGGT (Altered codons are underlined). Mutagenic PCR product containing mutated DmpR (1692 bp) and the promoters, Pr and Po fragments (total 2377 bp) were gel-isolated and digested with XhoI and HindIII (New England Biolabs, USA), and introduced upstream of the firefly Luciferase (luc) in the pGL3 basic expression vector to generate approximately 7 kb of pRLuc42R plasmid (Figure 2). The same method was used to generate pRLuc, pRLuc140p143R and pRLuc113p116R. Plasmids were transformed into E. coli DH5α by the CaCl₂ transformation method. The nucleotide sequences of the pRLuc42R were determined by DNA sequencing from the TCGA (The Centre for Genomic Application, New Delhi, India).

2.3. Proposed Binding Sites

LIGSITE algorithm [27] was used to predict the active site of ‘A’ DmpR model for phenol and phenolic derivatives. The binding pocket which were predicted by LIGSITE, were given an input to GOLD software for docking analysis.

2.4. Treatment of Phenolic Compounds and Activity Assays for Luciferase

Various phenolic compounds (Sigma and Aldrich Chemical Co., MO, USA) were dissolved in ethanol and added to media at final concentrations ranging from 10 nM to 1 mM. The E. coli DH5α cells harboring the plasmid were grown overnight at 37°C in 4 ml of LB medium containing 100 mg/ml ampicillin. Cells were sub-cultured and grown to the log phase of 0.2 in OD at 600 nm [28] followed by supplementation with phenolic compounds at various concentrations. After 1 h, 50 μl of the cells were withdrawn, and kept frozen at −70°C with the addition of 5 μl of 1 M KH₂PO₄ and 20 mM EDTA (pH 7.8). Cells were lysed by incubating at room temperature for 10 min with the addition of 150 μl of lysis solution [1.25 mg/ml lysozyme, 2.5 mg/ml BSA, 1X CCLR] (Promega, WI, USA). Supernatants were obtained by centrifugation. For the luciferase activity, 20 μl of supernatant was mixed with 20 μl of firefly luciferin solution (Promega, WI, USA). The bioluminescence was measured for 20 s by luminometer Berthold Detection System (Germany).

2.5. Calculations

Induction of the pRLuc42R sensor by phenol and phenolic derivatives was expressed as normalised luminescence (NL) calculated as follows [9]:-

\[ \text{Induction (NL)} = \frac{\text{Luminescence signal in the presence of phenol}}{\text{Luminescence signal in the absence of phenol}} \]

Figure 1. Schematic illustration of sensing principle of whole cell biosensor (pRLuc42R) to detect phenol at glance.

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Where $S_{LS}$ is the luminescence of the sensor in the dilution of the phenolic compound, $S_{LB}$ is the background luminescence of the sensor bacteria, and $CF$ is the correction factor.

$$NL = \frac{S_{LS}}{S_{LB}} \cdot CF,$$

Where $L_B$ is the background luminescence of the control bacteria, and $L_S$ is the luminescence of the control bacteria in phenol/phenolic compound. The detection limit for the sensor bacteria for different phenols was defined as a concentration of the compound which induced the sensor twice above the background level, i.e. $NL \geq 2$. Linearity curve for phenol induction is presented in Figure 3.

2.6. Chemical Analysis of Phenolic Compounds

Chemical analysis of phenolic compounds was performed according to the standard method recommended by American Public Health Association [29]. Synthetic water was filtered through a 0.2 µm membrane (Sartorius, Germany) and then serially diluted in 1 ml volume. Subsequently, 25 ml of 0.5 N NH4OH, 10 ml of 2% 4-aminoantipyrine (Sigma, MO, USA), and 10 ml of 8% K3Fe(CN)6 (Sigma, MO, USA) were added in order and mixed thoroughly. OD at 500 nm was measured.

2.7. RNA Isolation and Real Time PCR

Total RNA was isolated by RNeasy Mini Kit (Qiagen, USA) from pRLuc42R, pRLuc, pRLuc140p143R, pRLuc113p116R and pGL3-promoter vector (Promega, USA) containing E. coli cells treated with or without phenol. Total RNA was converted into cDNA by cDNA synthesis kit (Roche, USA). The RT-PCR (Model 7500, Applied Biosystems, CA, USA) was performed in a MicroAmp Optical 96 well reaction plate (Applied Biosystems,
The primers for luc and DmpR gene were designed by using Primer Express 1.0 (Applied Biosystems, CA, USA). The forward and reverse primers for luc gene were 5’-TGGAGAGCACTGCATAAG-3’ and 5’-CGTTTCATAGCTTCTGC-CAA-3’, respectively, while the forward and reverse primers for the DmpR gene were 5’-AAATCCGAGCTCCGATTTC-3’ and 5’-CCACCGGATTGACCATTTC-3’. The specificity of primers was examined under the standard PCR conditions prior to quantitation by real-time RT-PCR. The primers have been synthesized by TCGA (New Delhi, India). Each RT-PCR reaction contained the following: 1× SYBR Green Master Mix (containing assay buffer, dNTPs, and Polymerase enzyme), primer pair (1 μM), and cDNA, in 25 μl of reaction volume. Real time RT-PCR was carried out for 30 min at 60°C followed by initial 2 min incubation at 95°C to prevent carryover reactions. The reaction was terminated by dissociation step by heating at 95°C for 15 s, 60°C for 1 min and finally 95°C for 15 s. The PCR amplification was then performed for 40 cycles with each cycle at 95°C for 15 s and 60°C for 1 min. All reactions were carried out in triplicate using the ABI 7500 Real Time PCR. The threshold cycle, Ct, values were averaged from the values obtained from each reaction, and the mRNA expression was calculated as a relative level of expression.

2.8 Statistical Analysis

The data represents mean±SD of three independent experiments. The data was analyzed by one-way ANOVA followed by Newman-Keuls Multiple Comparison Test. The p value of <0.05 represents statistical significance in different groups.

Results and Discussion

Among the most abundant environmental pollutants, the aromatic compounds (such as phenol or phenolic compounds) are of major concern because of their persistence and toxicity. The environmental problems caused by such coherent pollutants have increased the demand for the development of sensitive pollutant and toxicity detection methods. The fusion of reporter genes to promoters that are induced when cells are exposed to chemicals is one promising approach that has been used to formulate biosensor for such application [30].

3.1. Construction of Whole Cell Biosensor pRLuc42R

For confirmation of DmpR protein binding to phenol, E. coli whole cell luminescence biosensor (pRLuc42R) was constructed. The mutated sequence (mutation was suggested by docking and computational study of 2377 bp [includes DmpR, Pr and Po]) were inserted upstream of luc in pGL3 basic expression vector to generate pRLuc42R as a final construct to form whole cell biosensor for phenol. Reporter enzymes such as chloramphenicol acetyltransferase, firefly luciferase, and β-galactosidase from E. coli are frequently used for studies of gene regulation, gene activity, and expression in eukaryotic cells [31]. Firefly luciferase, in particular, has been extensively applied in molecular and cell biology studies because of its negligible background, less incubation time, high sensitivity, and the relative simplicity of the assay [32,33]. A previous study [34] has shown that firefly luciferase can also be used for analysis in prokaryotic cells. Therefore, firefly luciferase was used as a reporter in this study.

3.2. Active Site Prediction

In our previous study [35], we performed the docking procedure for phenol and N-DmpR model with GOLD by using both scoring functions (GOLD Score and Chemscore). The GOLD Fitness score and Chemscore for the selected phenolic compounds are shown (Table 2). In selected phenolic derivatives, phenol showed highest GOLD Fitness score of 33.42 whereas the lowest Chemscore was 19.98.

Table 2. The GOLD Fitness score, Chemscore for the selected phenolic derivatives (Ligand) are shown.

| Ligand            | Goldscore | Chemscore |
|-------------------|-----------|-----------|
| Phenol            | 33.42     | 19.98     |
| 2,4-dimethylphenol| 31.4      | 21.89     |
| 2-chlorophenolphenol | 29.54    | 22.85     |
| 4-nitrophosphol| 32.46     | 21.29     |

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Figure 3. Linearity curve is presented as phenol concentration v/s NL value of luminescence. Firefly luciferase activities expressed in E. coli cells with pRLuc42R grown at different concentration of phenol. The pRLuc42R strain showed highly sensitive response at a low concentration of 0.5 μM. R² = 0.9913. NL - Normalised luminescence, μM - Micro molar concentration.

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3.3. Responses of pRLuc42R to Various Phenolic Compounds

The cells with pRLuc42R (Figure 5) showed very promising results with various phenolic compounds (p < 0.001 compared with pRLuc, pRLuc113p116R, or pRLuc140p143R). Priority pollutant phenols were chosen for the testing including phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, Pentachlorophenol, 4-chloro-3-ethylphenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2-methyl-4, 6-dinitrophenol, o-cresol, 4-chloro-3-ethylphenol, and xylene. Xylene was used as a negative control.

The effective compounds were phenol, 2-chlorophenol, 2,4-dichlorophenol, 4-chloro-3-ethylphenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2-methyl-4, 6-dinitrophenol, o-cresol, 4-chloro-3-ethylphenol, and xylene. Xylene was used as a negative control.

The effective compounds were phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, and cresol; and the minimum concentrations (in μM) for detection of these effective compounds were 10, 100, 10, 0.1, 0.1, 0.01, and 1, respectively. These are the minimum detection limits so far for these compounds [12,15,17,31] (Table 3). The sensor is more sensitive to 4-nitrophenol (0.01 μM), 2-nitrophenol (0.1 μM) and 2,4-dimethylphenol (0.1 μM) than phenol (0.5 μM). This is an advantage since these phenolic derivatives, especially the 2,4-dimethylphenol was considered as the compound responsible for carcinogenic influence while nitro phenols can exert mutagenic influence [40].

On the other hand, 2, 4-dinitrophenol, 2-methyl-4, 6-dinitrophenol, pentachlorophenol, 4-chloro-3-ethylphenol, and Xylene were not detected by the system. The detection range and maximum responses of luciferase activity varied according to the compounds tested. The strong bioluminescence responses were caused by phenol, 2,4-dimethylphenol, and 2-chlorophenol. It has been known that phenol and methyphenol compounds had strong effects on the regulatory proteins involved in phenol degradation. The effective concentration of phenolic compounds for the regulatory proteins is also one of the major concerns. However,
only a single concentration of effectors, usually around 2–3 mM was used in the measurement for DmpR in previous reports [12, 15, 18, and 34]. In the present studies, phenolic compounds in a range from 0.01 mM to 1 mM were examined. These results suggested that the residue L42 (Leucine at 42), M113 (Methionine at 113) and A116 (Alanine at 116) might be involved in inducer binding while the inducer binding might not be occurring in case of E140 (Glutamic acid at 140) and L143 (Leucine at 143). It is noteworthy that our system shows very low concentration ranges of phenolic compounds for detection. This aspect of the bacterial biosensors described here could be useful in detecting phenolic compounds at lower concentrations.

3.4. Luciferase Assay and Real Time PCR

E. coli cells harbouring pRLuc42R plasmid were grown with the addition of various concentrations of phenol, and their luciferase activities were measured. Luciferase shows stronger signals in almost all ranges of phenol and phenolic derivative concentration. The pattern of responses began to appear around 10 nM and continued to increase up to 1 mM. These results indicate that the DmpR/phenol complex binds to Po operator to activate luciferase genes most effectively, owing to the fact that the cloned DmpR is an activator gene implicated in the metabolism of phenol [14]. In addition, it was shown that the insect luciferase is stably expressed when they are grown to the log phase of 0.2 (instead of 0.3 or 0.4) in OD at 600 (p < 0.001) (Figure 6). As a result, the time consumption for the assay was decreased by 2–3 hours, as compared to 6–7 h in previous studies [12,34] which made it a relatively faster luciferase assay for phenol detection. The total time taken to complete the assay includes the incubation time for reaching the desired OD and induction with phenol. The quantitation of the luminescence is possible with a liquid scintillation counter, a luminometer, or even X-ray film.

Real time PCR was performed to confirm the responses to phenol with pRLuc42R, pRLuc and pRLuc140p143R. The threshold cycle (Ct) values for expression of luc gene in pRLuc42R, pRLuc, pRLuc140p143R, and pGL3 were 8.02±0.21, 10.80±0.63, 14.63±0.78, 9.24±0.27

Table 3. Comparative table for the existing bacterial biosensors for phenol.

| S.No. | Description | pRLuc42R Phenol Biosensor (This study) | Shingler et al. (1994) | Wise and kuske (2000) | Park et al. (2003) | Leedjarv et al. (2006) |
|-------|-------------|----------------------------------------|------------------------|----------------------|----------------------|------------------------|
| 1.    | Regulatory gene for Phenol-reporter gene | Mutated DmpR+luc | DmpR wt & DmpR-Xyl | DmpR-LucZ | CapR+Luc | DmpR+lux |
| 2.    | Wild Type/Mutant | Both | Both | Wild type | Wild type |
| 3.    | Mutants (Positions) | Docking based at 42nd position of A domain | Random | Random | NA | NA |

Pollutants

| Priority pollutant Phenols(USEPA) | Detection Limit | Detection Limit | Detection Limit | Detection Limit | Detection Limit |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| I Phenol                        | 500 nM          | 3.2 μM          | 2.5 μM          | 10 μM           | 0.092 mg/l      |
| II 2-chlorophenol               | 1 μM            | 2.5 μM by Mutant | 100 μM          |
| III 2, 4-dichlorophenol         | 10 μM           | 25 μM by Mutant |
| IV 2, 4, 6-trichlorophenol      | 0.1 μM          | Not Detected    |
| V Pentachlorophenol             | Not detected    | Not Detected    |
| VI 4-chloro-3-ethylphenol       | 100 μM          | 75 μM by Mutant |
| VII 2, 4-dimethylphenol         | 0.1 μM          | 75 μM by Mutant |
| VIII 2-nitrophenol              | 100 μM          | 75 μM by Mutant |
| IX 4-nitrophenol                | 10 μM           | 75 μM by Mutant |
| X 2, 4-dinitrophenol            | Not detected    | Not Detected    |
| XI 2-methyl-4,6-dinitrophenol   | Not detected    | Not Detected    |
| Other Phenolic derivatives      |                 |                 |                 |
| A Cresol & derivatives          | 10 μM           | 3.2 μM-2 mM     |
| Non Phenolic derivatives        |                 |                 |
| Xylene                          | Not detected    |                 |

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and 14.64±0.43, respectively. The ΔC_T was calculated with reference to the pGL3 promoter vector. As a result, real time PCR shows approximately 7 folds and 100 folds increased expression of luciferase gene of pRLuc42R (by 2-ΔΔCT Method), when compared to unmodified system pRLuc and pRLuc140p143R, respectively. Folds change has been compared with 1 mM phenol treated and untreated cells of all construct (Figure 7). In luciferase assay, decrease in the response occurring at high concentration of phenolic compounds appears to be due to the toxic effect of phenolic compounds on the cells. It has been suggested that these phenolic compounds may interact with specific components of the electron transport chain, thus inhibiting electron transport and oxidative phosphorylation [41,42]. Even though it cannot be excluded that cell viability may affect the total activities at high concentration of phenolic compounds, major increase in responses seem to occur in harmless or sub-lethal ranges. Taken together, the results indicate that the pRLuc42R protein has different properties in effecter specificity from other relevant regulators for catabolic pathways of phenolic compounds.

3.5. Assessment of Phenolic Compounds in Synthetic Water

It is shown above that E. coli cells with DmpR could be used as whole cell biosensor to monitor various pure phenolic compounds. The results show that this system can also detect phenolic compounds in synthetic water. The total amount of phenolic compounds in water measured by a chemical analysis exceeded the phenol amount obtained. The whole cell luminescent biosensor is similar to the chemical analysis method in a sense that they can detect the total phenolic compounds (data not shown). However, its results must be considered in a more complex way due to the differential effects of different phenolic derivatives. Moreover, the concentration of each phenolic compound in synthetic water might be achieved by further refined instrumental analysis.

The biosensor described here is very simple, economical and fast in measurement, possibly with sustaining sensitivity and
reproducibility. It might reflect various aspects of pollutants such as bioavailability and toxicity during the measurement [43,44]. So far the whole cell biosensors have offered unique alternative methods in identifying and quantifying phenol and its derivatives [12,15,18,34]. However, the present sensor does not detect some non-phenolic derivatives out of eleven priority pollutant phenols (http://www.epa.gov/) but this is capable in detection of seven some phenolic derivatives out of eleven priority pollutant phenols [12,15,18,34]. However, the present sensor does not detect methods in identifying and quantifying phenol and its derivatives far the whole cell biosensors have offered unique alternative as bioavailability and toxicity during the measurement [43,44]. So the DmpR regulatory gene. Single mutant (pRLuc42R) was the problems by tailoring microorganism through mutagenesis to transducer. As a sensing element, microorganism has not only structural model of its N-terminal domain. Environ Microbiol 4: 29–41. of aromatic effectors on the prokaryotic enhancer-binding protein XylR: a composition-based statistics and other refinements. Nucleic Acids Res 29: acides. Nucleic Acids Res 33: D266–268. member of the AAA + superfamily of proteins. Journal of Structural Biology: 371–372. Available at: http://hayweb.dsey.de/science/annual_reports/2006_report/part2/contrib/73/18327.pdf. Accessed 2012 July 27.

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Author Contributions

Conceived and designed the experiments: SG AK RK DM. Performed the experiments: SG MS. Analyzed the data: SG AK NS RK. Contributed reagents/materials/analysis tools: SG AK NS RK. Wrote the paper: SG.
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