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LETTER

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Enhanced Bacterial Biosensor for Fast and Sensitive Detection of Oxidatively DNA Damaging Agents

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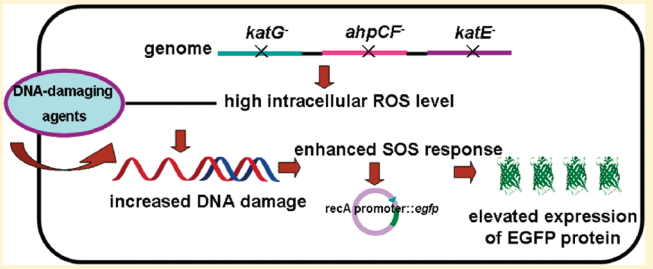
Supporting Information

ABSTRACT: A number of known and potential chemicals may cause substantial damage to genomic DNA, further inducing mutagenesis and carcinogenesis. To screen potentially genotoxic compounds from a multitude of chemicals, fast and senstive bioanalytical technologies are desirable. By taking advantage of the DNA damage-dependent SOS response (a regulatory signal initiated by damage to DNA or the physiological consequences of such damage in prokaryotes) in reactive oxygen species (ROS)-sensitive bacterium and the enhanced green fluorescent protein reporter, we constructed a composite bacterial biosensor for detection of DNA damage agents. The sensitivity of the bacterium to ROS induced DNA damage is 10−20-times enhanced by the knockout of one alkyl hydroperoxide reductase gene and two catalase genes. This biosensor can be used for fast and sensitive detection of DNA damaging agents among which some cannot be detected by previous bacterial biosensors, demonstrating the potential and promising applications for evaluation of DNA damage and for screening of DNA damaging agents in large scale.



Reactive oxygen species (ROS) may induce a series of oxidation related adverse changes at molecular levels, such as DNA damage,[1] protein oxidation,[2] and lipid peroxidation.[3] In particular, damage to DNA by ROS may cause heritable and permanent changes in the genome. It is known that a number of exogenous and endogenous factors may elevate the intracellular ROS level, including chemical exposure,[4] ionizing and UV irradiation,[5] genomic defects,[6,7] and metabolic disorders.[8,9] Interestingly, some organisms (e.g., competitive organisms) may utilize ROS to protect themselves.[10,11] In addition to the known DNA damaging agents, there are more potential and unknown DNA damaging agents needed to be identified from a multitude of chemicals. It is highly desirable to develop analytical technologies for fast and sensitive screening of DNA damaging agents.

A number of biosensors have been designed for detection of oxidative stress to which organisms are exposed *in vivo* and *in vitro*.[12−15] Because of the existing antioxidant defense mechanisms, in general, cell based biosensors are not very sensitive to ROS. Attempts have been made to selectively knock out specific antioxidant genes to enhance the sensitivity of the cells to ROS.[16,17] However, the cellular responses being utilized were not specific to ROS. Here we propose to combine the knockout of antioxidant genes and DNA damage inducible SOS (a regulatory signal initiated by damage to DNA or the physiological consequences of such damage in prokaryotes) reporter to develop a novel type of bacterial biosensors for sensitive detection of ROS inducing chemicals and assessment of DNA damaging effects.

A bacterial biosensor (*E. coli* L5 strain) was first constructed from an *E. coli* LC106 strain by transfection with a modified plasmid pET-16b, bearing an enhanced green fluorescence protein (EGFP) reporter gene under the control of *recA* promoter (pETPrecAegfp5, see Table S1 in the Supporting Information). One alkyl hydroperoxide reductase gene (*ahp*) and two catalase genes (*katG* and *katE*) were knocked out in the *E. coli* LC106 strain.[6] In *E. coli*, alkyl hydroperoxide reductase scavenges endogenous H_2O_2 primarily at the micromolar levels, and the two catalases, products of *katE* gene and *katG* gene, can remove H_2O_2 at the millimolar levels. The knockout of the three genes in LC106 cells may allow the rapid accumulation of the intracellular H_2O_2 when the cells are exposed to an oxidatively DNA damaging agent. H_2O_2 is relatively inert and cannot directly induce oxidative DNA damage. However, the accumulated H_2O_2 can be broken down to produce a highly reactive hydroxyl radical probably through intracellular transition metal mediated Fenton reactions[7] and therefore cause substantial DNA damaging and induce SOS reponse for activation of the DNA repair pathway. This is the biochemical basis for us to develop an improved bacterial biosensor for detection of oxidative DNA damage in this work.

As depicted in Scheme 1, substantial DNA damage will occur in *E. coli* L5 cells upon exposure to a DNA damaging agent. Then the key *recA* gene will be induced to initiate the SOS response that would allow the cells to repair and/or bypass the generated DNA damage. Meanwhile, EGFP protein as a reporter under the

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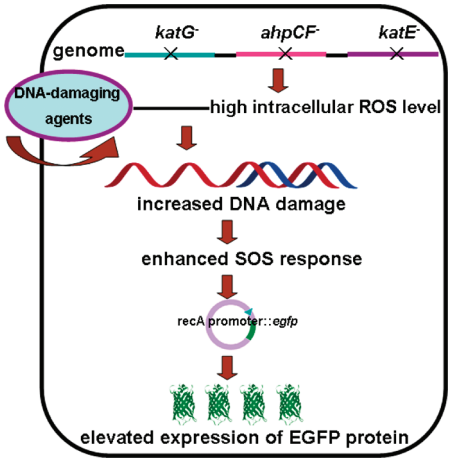
Scheme 1. Enhanced Fluorescence Response of *E. coli* L5 Cells (*katG*$^-$ *katE* $^-$*ahp*$^-$) upon Exposure of DNA Damaging Agents



control of the *recA* promoter will also be expressed at a high level, signaling the presence of DNA damaging agent(s). The expressed EGFP protein from the reporter gene displays 35 times the enhanced fluorescence signal over the wild type GFP due to the double mutation of Phe64Leu and Ser65Thr.[18]

EXPERIMENTAL SECTION

Materials and Chemicals. The used *E. coli* strains and plasmids were listed in Table S1 in the Supporting Information. L5 strain was constructed from the LC106 strain (*katG*$^-$ *ahp*$^-$ *katE*$^-$) carrying the vector pETPrecAegfp5, with the *egfp* gene under the control of the *recA* promoter. The B5 strain was constructed from a protein expression BL21(DE3) strain carrying the same vector pETPrecAegfp5 as L5 strain. H_2O_2 was purchased from Beijing Chemical Works, China. Acrolein (CP purity) was purchased from Aladdin-Reagent, Shanghai, China. Ampicillin was purchased from Inalco. Other reagents were purchased from Sigma-Aldrich.

Bacterial Growth and Chemical Induction. First, L5 cells were inoculated at 37 °C without shaking in fresh anaerobic GCTMA medium (0.2% glucose, 0.2% casein acidic hydrolysate, 0.2 mM tryptophan, 5 μg mL^{-1} thiamine, M9 salts,[19] and 60 μg mL^{-1} ampicillin) to log phase ($OD_{600} \sim 0.15$). Then L5 cells were induced for 2 h shaken at 130 r min^{-1} at 37 °C aerobically as follows: 20 μL of chemicals in aqueous solution with a varied concentration were added to aliquots of L5 cells with a final volume of 200 μL in 600 μL centrifuge tubes. Untreated L5 cells were added with 20 μL of sterile water as a control. B5 cells grew in GCTMA medium at 37 °C to log phase (OD_{600} = 0.2−0.3) at a speed of 130 r min^{-1}, and the induction of B5 cells was conducted in the same way as that for the L5 cells. The BL21(DE3) strain and LC106 cells were grown in aerobic and anaerobic GCTM medium (same as GCTMA but no ampicillin), respectively, to the log phase, and 20 μL of sterile water were added as the blank for the induction process. The two blank strains were used for subtraction of intracellular autofluorescence.

Fluorescence and Optical Density Detection. The *E. coli* cells were excited at 480 nm, and the fluorescence was scanned from 500 to 600 nm in the 96-well plate (Corning) with the spectral scanning multimode reader (Varioskan Flash, Thermo).

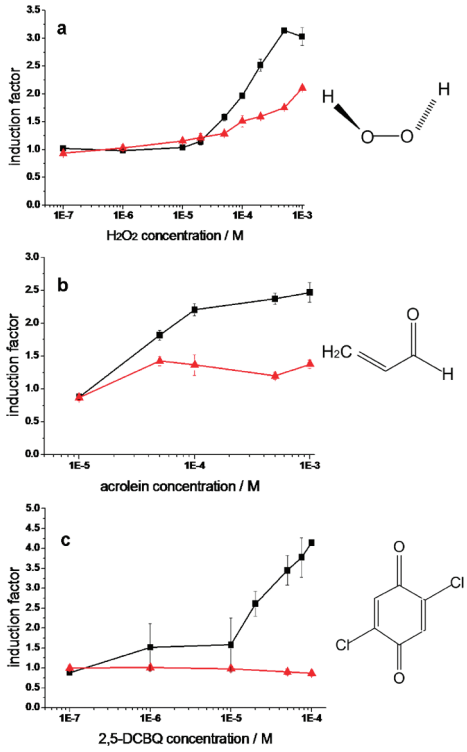


Figure 1. The dose-dependent fluorescence response of L5 and B5 strains to DNA damaging agents. L5 and B5 cells grew to log phase anaerobically and aerobically, respectively. The two strains were subsequently treated aerobically by H_2O_2 (a), acrolein (b), and 2,5-DCBQ (c). The response of the L5 strain was indicated as black squares and the B5 strain as red triangles. The induction factor is estimated as the ratio of the SFU value of treated cells to the untreated cells. The SFU value indicates the ratio of the fluorescence intensity to the optical density at 600 nm of relative cells (n = 3).

The cell density (OD_{600}) was determined simultaneously using the same device.

Data Analysis. The SFU (specific fluorescence unit) value indicates the value of the detected fluorescence intensity divided by the optical density of *E. coli* cells at 600 nm (OD_{600}) at the same time point. The absolute SFU value indicates the SFU value of the treated sample minus that of the blank strain without the pETPrecAegfp5 vector. The induction factor of the treated sample was calculated by the absolute SFU value at 509 nm of the treated sample divided by that of the untreated sample. The low detection limit is defined as the dose at which the induction factor reaches a value as twice that of the background.[20]

RESULTS AND DISCUSSION

First, we examined the fluorescence response of L5 cells to a representative DNA damaging agent, H_2O_2. As shown in Figure 1a, when L5 cells were exposed to H_2O_2 for 2 h, the induction factor of treated samples showed a significant dose-dependent response. It is evident that H_2O_2 even at the concentration as low as 50 μM can cause substantial DNA damage and trigger an SOS response in L5 cells. In contrast, B5 cells, which are contructed from another *E. coli* strain BL21-(DE3) transfected with the same plasmid pETPrecAegfp5 and carry the three antioxidant genes, showed a lower induction response. As previously proposed, a substance can be identified

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to be genotoxic if at any of its concentrations induction factor reaches a value of 2 or more.[20−22] The induction factor of L5 cells treated by 100 μM H_2O_2 was over the value of 2; in contrast, the B5 cells double its fluorescence intensity by a treatment of 1 mM H_2O_2. H_2O_2 can display its ROS induced genotoxicity in both strains, however, by contrasting the response of the two strains, we can conclude that the sensitivity of the L5 strain is at least 10 times higher than that of the B5 strain, suggesting that the knockout of the three antioxidant genes can significantly enhance the sensitivity of the strain to oxidative stress. Compared to previously reported bacterial biosensors that can detect H_2O_2 only at the millimolar level,[21,22] the constructed L5 strain also displays 10 times higher sensitivity. By the knockout of two SOD genes in *E. coli*, which can scavenge the superoxide anion radical (one important ROS), the cellular sensitivity to H_2O_2 cannot be significantly improved (Supporting Information, Figure S1). These results suggest that the knockout of the *ahp* gene and the two catalase genes are more beneficial to the detection of ROS, consistent with previous work.[6,7,23]

Interestingly, two chemicals acrolein and 2,5-dichlorobenzoquinone (2,5-DCBQ), which cannot or barely be identified by the B5 strain as genotoxic compounds, can be sensitively detected by our L5 strain.

Acrolein has three unsaturated and electrophilic groups (two ethylenic bonds and one aldehyde group) and can be found in cellular lipid peroxidation breakdown products and in tobacco smoke (about 60 μg acrolein per cigarette[24]) and heated cooking oils (5−250 mg acrolein per kg of oil[25]). It is known that electrophilic acrolein can directly react with genomic DNA and form stable DNA adducts and induce inter- and intrastrand cross-links and protein−DNA cross-links.[26−29] However, it is evident that acrolein cannot induce significant SOS response in the B5 strain even at the concentration as high as 1 mM (Figure 1b), suggesting that the SOS response-dependent B5 strain cannot be used to detect some chemicals even with known DNA-damaging ability. In contrast, the L5 strain is more sensitive to the exposure of acrolein (Figure 1b). In this case, acrolein can be detected at the concentration as low as 50 μM, suggesting the sensitivity of the L5 strain is enhanced over that of the B5 strain at least 20 times. It is yet controversial whether acrolein can induce ROS caused DNA damage.[24−29] Here we cannot yet predict whether the enhanced sensitivity is associated with the elevated level of ROS in L5 cells by the exposure to acrolein.

2,5-DCBQ is one of the reactive metabolites of a ubiquitous environmental pollutant, pentachlorophenol (PCP). PCP has been classified as a 2B group carcinogen by IARC and listed as an environmental priority pollutant by EPA.[30] As a germicidal agent, PCP is widely used in industry and agriculture though it has been restricted or banned in many countries. PCP can cause liver cancer, hemendothelioma, and pheochromocytoma in mice[31] and lymphoma and leukemia in humans[32,33] with long-term exposure. However, few bacterial assays have shown the mutagenesis of PCP.[34,35] It is known that 2,5-DCBQ can form a covalent BQ−dG adduct *in vitro*.[36] It has also been proved that the oxidative DNA damage caused by 2,5-DCBQ will increase when H_2O_2 or transition metal was added, demonstrating the oxidative potential of 2,5-DCBQ to DNA.[36,37] Like the response to acrolein, the L5 strain displayed a significant dose-dependent response to 2,5-DCBQ and detectable response even at the concentration as low as 10 μM. In contrast, the B5 strain had no response to 2,5-DCBQ over the investigated dosage (0−100 μM). It is obvious with the knockout of the three antioxidant genes, the L5 strain became more sensitive and could be used to detect more DNA-damaging agents.

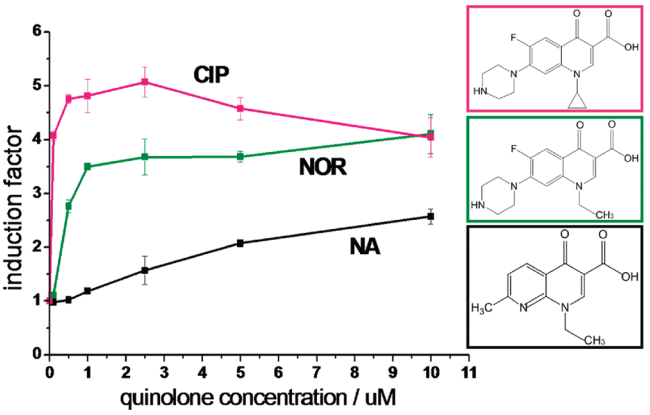

Figure 2. Dose-dependent fluorescence response of L5 strain to antibiotic quinolones. L5 cells grew to log phase anaerobically. Then the strain cells were induced aerobically by ciprofloxacin (CIP), norfloxacin (NOR), and nalidixic acid (NA). The induction concentrations of quinolones are 0, 0.1, 0.5, 1, 2.5, 5, and 10 μM. The chemical structures CIP, NOR, and NA shown in the boxes have the same colors of box edgings with the lines in the graph (n = 3).

To further investigate the potential applications of the constructed bacterial biosensor (L5 strain), three chemicals (ciprofloxacin, norfloxacin, and nalidixic acid) belonged to same family of quinolone antibiotics but displayed different DNA damaging potency were tested. The quinolones have been widely used in clinical treatment and may be associated with an increasing risk of tendinitis and tendon rupture in all ages.[38] These quinolones exert their bacteriostatic action by trapping the topoisomerases−DNA complexes and induction of chromosome fragmentation.[39−41] It was observed that the L5 strain displayed a significant dose-dependent fluorescence response for all three quinolone antibiotics over the tested dosage (0−10 μM). The induction factor of the L5 strain to ciprofloxacin exceeded a value of 4 at a concentration of 0.1 μM, showing the possible detection of nanomolar concentrations of DNA damaging agents by the L5 strain. Norfloxacin also demonstrated strong induction response since the concentration of 0.5 μM. Nalidixic acid needed a higher concentration to trigger the SOS response. The DNA damage potency of the three quinolone antibiotics increases in the order of nalidixic acid, norfloxacin, and ciprofloxacin, and evidently ciprofloxacin displayed the strongest DNA damaging potency (Figure 2). The measured genotoxic potential of the tested quinolones in this study are consistent with their chromosome fragmentation potency and ROS-induction ability as shown by previous study,[41] further demonstrating the reliability of the constructed biosensor.

In this work, we have designed a bacterial biosensor for sensitive detection of DNA damaging agents. By the knockout of antioxidant genes, the designed bacterial biosensor can be utilized to detect more DNA damaging chemicals. As demonstrated here, acrolein and 2,5-DCBQ can be detected by the antioxidant gene-knocked out L5 strain but not by the B5 strain, in which the three antioxidant genes have not been knocked out yet. Although the detection of fluorescent EGFP protein in bacterial cells can be more sensitive by separating EGFP protein from intracellular autofluorescent components using capillary electrophoresis-laser induced fluorescence,[42] direct measurements

of DNA damaging chemicals by bacterial biosensors may provide an alternative choice for high throughput analysis. It is known that oxidative damages caused by ROS are closely associated with the aging process and various diseases such as dementia, diabetes, and cancer. Meanwhile, oxidative DNA damage can affect DNA replication, DNA repair, and gene expression and may be mutagenic to cells and further carcinogenic to organisms. Therefore, the designation of biosensor with enhanced sensitivity to oxidative stresses has significance in the study of oxidative DNA damage in cells and may stimulate the development of optimum measures to prevent oxidative DNA damage from endogenous and exogenous chemicals. The biosensor will be a potentially useful tool for detection of environmental carcinogens in hospital waste, industrial wastewater, agricultural polluted soil, nuclear waste, and so on.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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