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Prediction and classification of the modes of genotoxic actions using bacterial biosensors specific for DNA damages

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ABSTRACT

We report on a novel approach to predict the mode of genotoxic action of chemicals using a series of DNA damage specific bioluminescent bacteria. For this, a group of seven different DNA damage sensing recombinant bioluminescent strains were employed. Each of these strains was tested against model DNA damaging agents, such as mitomycin C (MMC), 1-methyl-1-nitroso-*N*-methylguanidine (MNNG), nalidixic acid (Nal) and 4-nitroquinoline N-oxide (4-NQO). These biosensors were grouped based on their responses to a specific mode of genotoxic action, such as (a) DNA damage cascade response (biosensor with *nrdA*-, *dinI*- and *sbmC*-lux), (b) SOS response or DNA repair (strains carrying *recA*-, *recN*- and *sulA*-lux), and (c) DNA damage potentially by alkylation (biosensor with *alkA*-lux). The differential response patterns and its strength of these strains to various model genotoxicants allowed classifying the chemical's potential genotoxic mode. Therefore, it is possible to elucidate and classify the mode of genotoxic impacts of an unknown sample and that together they may be utilized in the pre-screening steps of new drugs, newly synthesized chemicals, food and environmental contaminants.

1. Introduction

Genotoxicity testing is performed for the detection of DNA damage, or to assay for the formation of a DNA adduct and chromosomal damage. It can also used as a preclinical safety assessment tool to screen newly synthesized drug candidates based on the detection of potential carcinogenicity and heritable mutations based on their responses to genotoxic actions (Witte et al., 2007).

In vitro genotoxicity testing usually involves at least two different endpoints at several levels of biological complexity (Kroes, 1995). Typical testing assays are performed using a living organism, either prokaryote or eukaryote (Kroes, 1995). One example of a eukaryote-based assay is the comet assay (single-cell gel electrophoresis) and is a simple method for measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells. Because of its sensitivity, versatility and accuracy, it has been applied in genotoxicity testing, human biomonitoring and molecular epidemiology and ecogenotoxicology studies, as well as in fundamental research in DNA damage and repair (Collins, 2004). However, eukaryote-based assays usually are expensive, time consuming, and often associated with complicated protocols (Rusling et al., 2007). Therefore, prokaryote-based assays are being used because of their ease of use, its speed and its inexpensiveness for toxicological prescreening (Yagi, 2007). The Ames (*Salmonella typhimurium*) test is an example of a widely used short-term bacterial assays for identifying materials that induce genetic damage leading to gene mutations (Deserres and Shelby, 1979; Lee and Lee, 2007).

Most prokaryotic genotoxicity assays do have some limitations. For example, (a) bacteria are potentially more resistant to many toxicants and (b) some genotoxic compounds become toxic only after they enter the body and are processed in the liver, and these are likely to be overlooked during the tests performed with the help of prokaryotic systems (Ron, 2007). In addition, (c) another shortcoming that is not only restricted to prokaryotic-system-based assays but also most other genotoxicity assays is that the test is simply a 'black-box'. The tests report whether any DNA damage occurred, but offer no information on the potential mode that lead to this damage (Kroes, 1995). However, because the former two limitations (a) and (b) are natural characteristics of prokaryotes, those limitations would not be overcome. The shortfalls of other prokaryote-based assays can be presumed to be resolved through the use of a group of specific recombinant bioluminescent bacteria each carrying specific DNA damage marker promoter as biosensors (Kim et al., 2005).

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To demonstrate this expectation, we used several promoters controlling genotoxic response genes that are highly induced when *E. coli* experiences DNA damage and constructed different bioluminescent bacterial strains that respond to different types and degrees of genotoxicity. Furthermore, the focus of this study is not only to obtain genotoxicity of a suspected chemical but also to predict the potential mode of genotoxicity through the use of different stress promoters fused to the *lux* genes. Since the genotoxicity detection and classification is an important subject in the field of novel drug/chemical screening (Rusling et al., 2007), the toxicity screening of newly synthesized chemicals has become an important issue to ascertain safety in the food industry to take measures for early warning purposes. In this study, therefore, a series of seven different genotoxicity biosensing bacteria were employed to classify the genotoxicity of model compounds based on their responses to these chemicals. Specific biosensor responses to model genotoxic chemicals enabled us to characterize and classify biosensor responses that not only determined genotoxicity but also potentially elucidate mode of DNA damage that may be caused by an unknown chemical.

2. Materials and methods

2.1. Strains construction

Construction of the recombinant luminescent bacteria was performed using the genomic DNA of an *E. coli* wild-type strain, RFM443 (*strR*, *glaK2*, *lac*Δ*74*) as template (Drolet et al., 1995). For genotoxicity biomarker selection, DNA microarray experiments were performed as described in Supporting Information (SI) Section I (a). Promoters, primer sets and construction of recombinant bioluminescent strains are all described in SI Section I (b) (Table S1).

2.2. Culture conditions and chemical test protocol

All genotoxicity responsive strains were cultured under identical conditions at 37 °C, except DPD2794, which grows at an optimum temperature of 30 °C. Culture conditions and procedures for 96-well plate luminometer assays using various model toxicants are described in SI Section I (c) (Table S2).

2.3. Data analysis

All tests were performed in triplicates and conducted simultaneously for error analysis. The data values were plotted and the standard deviations are shown as the errors bars. The maximum relative bioluminescence (Max. RBL) defined as the maximum ratio of the bioluminescence of the induced cells to that of the control under standard assay conditions (at the same time point), was used to analyze the expression levels. The characteristics of each biosensing cell as summarized using the following biosensor properties: (a) minimum detectable concentration (MDC), which is the minimum concentration of the test chemical at which the cells respond by at least a 2-fold induction, (b) maximum response time (MRT) is the time required to reach the maximum relative bioluminescence level (Max. RBL), (c) Max. RBL and (d) dynamic concentration range.

To differentiate the responses of the biosensor strains, a common statistical analysis tool, EPCLUST (expression profile data clustering and analysis) was applied mainly to partition the data set for clustering analysis. The EPCLUST was performed using the MDC values with the help of a web-based tool (http://ep.ebi.ac.uk/EP/EPCLUST/). The topological relationships were elucidated using the order of similarity by average linkage (UPGMA) clustering based upon correlation-measured distance (uncentered).

3. Results and discussion

3.1. Construction of DNA damage specific biosensors

Five biomarker candidates were selected from the results obtained by DNA microarray analysis conducted in this study. The promoter elements of these five biomarker genes (*recN*, *sbmC*, *sulA*, *dinI* and *alkA*) were fused to the bacterial lux-operon to construct DNA damage specific recombinant bioluminescent bacteria. These, along with two other well known genotoxicity sensing strains, such as DPD2794 (*recA*-lux) (Min et al., 1999) and BBTNrdA (*nrdA*-lux) (Hwang et al., 2008) were combined into a group of seven biosensor strains for comparison and classification of the genotoxicity caused by model chemicals (see SI Section II).

3.2. Response characteristics of seven DNA damage biosensors to four model chemicals

Four chemicals, such as MMC, MNNG, 4-NQO, and Nal were selected as models that are known to cause genotoxicity. Each of these chemicals represent different mode for causing genotoxicity, which enabled us to probe the responses of each of the seven biosensing cells. Mitomycin C (MMC) is a bioreductive alkylating agent that undergoes metabolic reductive activation, and has various oxygen tension-dependent cytotoxic effects on cells (Abraham et al., 2006). Our result showed that all biosensor strains responded to MMC except the strain harbored *alkA-lux* fusion (Fig. 1a). MMC exposure resulted in dose-dependent bioluminescent responses with the strains harboring the *recA*-, *recN*-, *sbmC*-, *dinI*-, *sulA*-, and *nrdA*-lux fusions. Similarly, exposure of the cells to Nal and 4-NQO also resulted in the induction of bioluminescent responses by these six out of the seven strains (Fig. 1b and c). The strain carrying *alkA*-lux was not induced by Nal or 4-NQO suggesting that these chemicals caused genotoxity, but not strictly by alkylation. However, all seven strains responded when the cells were treated with MNNG, a DNA-methylating agent (Wyatt and Pittman, 2006). Expectedly, the strain carrying the *alkA*-lux fusion showed a significant induction in its bioluminescent response (392.5-fold) after the MNNG exposure (Fig. 1d).

It is to be noted that each of these compounds caused DNA damage by a different means. For example, nalidixic acid (Nal) acts as quinolone antibiotic and inhibits bacterial DNA gyrase or the topoisomerase IV enzyme (Emmerson and Jones, 2003) while 4-NQO generates the superoxide radical in *E. coli* and this explains the highly potent ability of 4-NQO to induce oxidative DNA damage, which contributes to tumor promotion (Kanojia and Vaidya, 2006). Therefore, both Nal and 4-NQO are distinct category of chemicals that induce DNA damage, but not through the alkylation, which is consistent to our result where the *alkA*-lux did not respond. The distinct responses of seven strains with the model genotoxic chemicals are summarized in Table S4 and Table S5 (SI) according to four parameters, such as (a) MDC, (b) MRT, (c) Max. RBL (Min et al., 1999) and (d) dynamic concentration range.

Based on the above result, it is clear that though most genes are involved in the SOS response, their expression pattern was distinct to the different chemicals. This can be attributed to differences in the mechanisms by which the chemical compounds may cause DNA damage, as well as the expression properties of each of the genes involved.

3.3. Grouping biosensors based on the MDC and genotoxicity typing

To overcome conventional bacterial genotoxicity assays, such as the Ames test which indicates the mutagenicity of a chemical, but does not allow identifying the potentially specific mode by which a

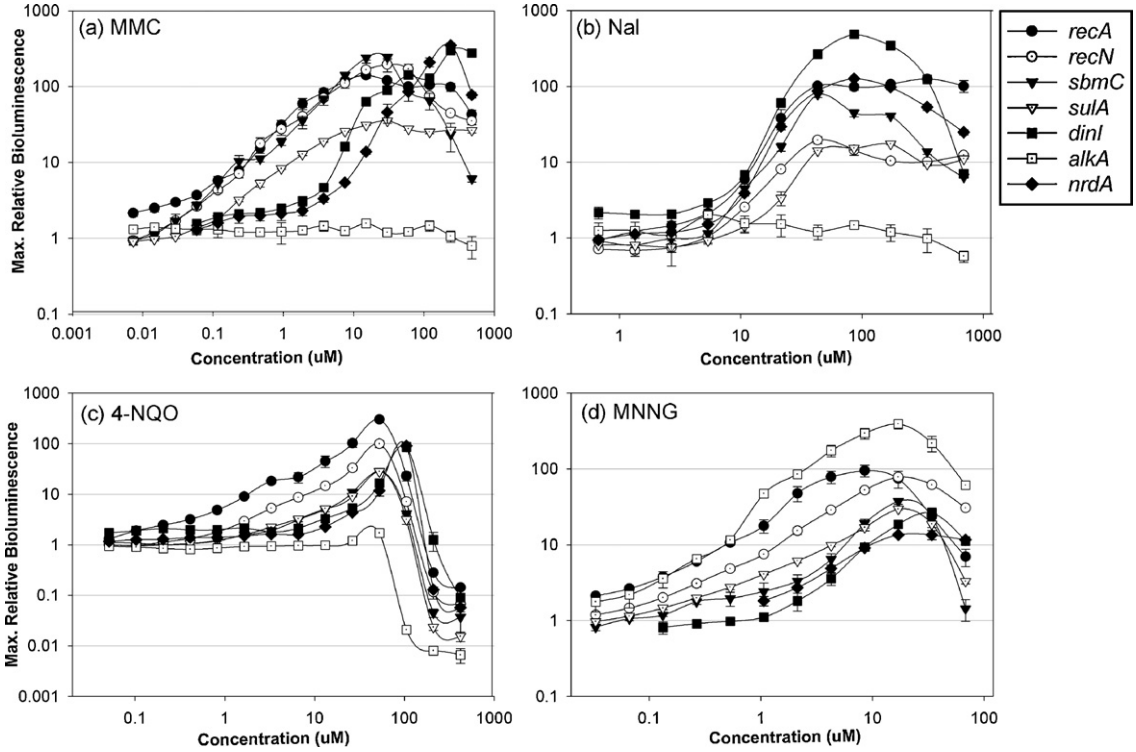

Fig. 1. Maximum relative bioluminescence seen with the seven biosensor strains after exposure to various concentrations of the genotoxins: (a) Mitomycin C (MMC), (b) Nalidixic acid (Nal), (c) 4-Nitroquinoline N-oxide (4-NQO) and (d) 1-methyl-1-nitroso-*N*-methylguanidine (MNNG).

chemical causes genotoxicity (Ku et al., 2007). MDC can be the most important parameter for specific biosensor/s to detect toxicant in the real environment, because it represents detection ability of a biosensor for low concentrations of toxicants. As well, this value can be used as an indicator of the assay's capability (Carbaugh, 2003). Therefore, we used the MDC as criteria for classification of the biosensors by exploiting the characteristic responses of each gene-lux fusion to a particular chemical.

First, biosensor strains were exposed to a series of model genotoxins and MDC for each strain with respect to toxicants was determined (Table S4 in SI). A clustering tool was employed that utilized MDC values under standard conditions and the biosensor strains were classified (see Section 2 and SI). As a result three groups of biosensors were obtained (Fig. 2, Groups A–C). The first group of strains comprised *nrdA*, *sbmC* and *dinI* that were responsive to the DNA damage through cascade response (Fig. 2, Group A). Here, the *nrdA* gene is responsible for encoding ribonucleoside–diphosphate reductase I, an enzyme essential for DNA synthesis. Despite the fact that *nrdA* being not involved in SOS response, studies have shown that it is strongly responsive to mutagenic agents (Hwang et al., 2008) and UV (Courcelle et al., 2001). The results obtained in this study consistently showed that *nrdA* was actively involved in response to DNA damage caused by model chemicals, which was also evidenced by its expression ratio from DNA microarray analysis (*nrdA* $\log_2$ ratio = 4.038 or ~16-fold induction against Nal) (Table S3 of SI). While both *sbmC* and *dinI* are involved in the SOS response genes. The sensitivities and responses of *sbmC* and *dinI* for detecting model chemicals were similar to *nrdA*, as against to their partner SOS response genes (*recA*, *recN*, and *sulA*) in terms of MDC, MRT, Max. RBL and dynamic concentration range (Tables S4 and S5 in SI). The physiological role of *sbmC* is to inhibit DNA gyrase, that is shown to be elusive (Chatterji et al., 2003). Because *sbmC* protects the cells from toxins by interfering DNA binding by gyrase (Chatterji and Nagaraja, 2002; Chatterji et al., 2003). The role of DinI is distinct in that, it indirectly affects RecA (Cox, 2007; Yasuda et al., 2001). Both *sbmC* and *dinI* are involved in SOS response categories. Therefore, clustering analysis using MDC showed that *sbmC*, *dinI* and *nrdA* are classified under one group (Group A), representing DNA damage cascade response.

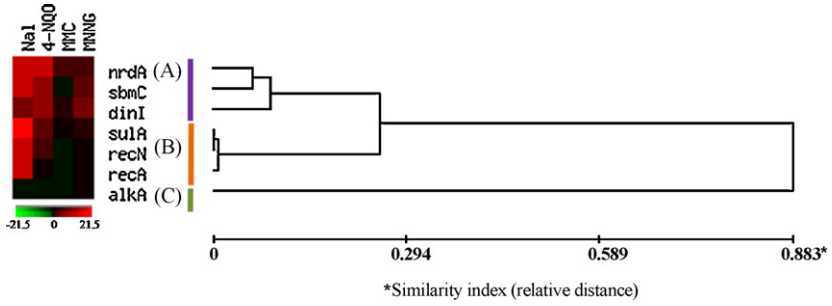

Fig. 2. Clustering results based on the minimum detectable concentration (MDC) using the responses from each of the strains. The clustering results are clarified based on the representative genotoxicity action in each group—Group A: DNA damage cascade response, Group B: General DNA damage response, and Group C: Methylation specific response.

Likewise, the genes that were responsive to general DNA damage were categorized within Group B by clustering analysis using MDC values for various genotoxins. These include the DNA repair genes (also a SOS response), such as *recN*, *recA* and *sulA* (Fig. 2, Group B). Each gene in Group B (*recA*, *recN* and *sulA*) has a different function in *E. coli* as a response to DNA damage. The *recA* gene is well known and encodes for the main regulatory protein of the SOS regulon genes, which includes over 20 genes in *E. coli* (Movahedzadeh et al., 1997). The *recN* gene is one among the SOS response genes (Skaar et al., 2002). The last SOS-related gene characterized in this category was *sulA*, as stated previously, its protein is involved in controlling cell division when the cells are exposed to DNA damage. In this study, all three of these genes fell under one cluster (group). All three of these genes were significantly induced by each of the genotoxins tested (4-NQO, MNNG, MMC, and Nal). Therefore, this group can be regarded as a response for the general DNA damage.

Further, clustering analysis showed a third and a last group that contained only one gene which was distinct and responsive to the damage caused specifically by alkylation (*alkA*) (Fig. 2, Group C). Unlike the strain carrying *alkA*-lux that was specifically induced by only MNNG, the other five strains were induced to other genotoxins in addition to MNNG (Table S4 in SI). Therefore, the clustering results showed that all other genes are separated and distinct to that of *alkA* in their response behaviors and therefore, this category potentially represents the DNA-damage specifically through methylation.

The biosensor strains were initially treated with various concentrations of test chemical and monitored the effect of concentration with respect to exposure time (0–4 h). The data presented in Fig. 1a–d shows the responses of biosensor strains at maximum response time (MRT) of chemical exposure. It is the chemical exposure time at which the cells showed maximum response with respect to the concentration (Table S4 in SI Section). The response of each biosensor strain for a particular chemical varied with respect to the MDC as well as MRT. Therefore, the response curves, particularly of *sbmC* and *nrdA* in Fig. 1a varied that was dependent on the characteristic response of biosensor strains against different test chemicals. Thus, the dynamic concentration range of chemicals was determined to be varying with respect to biosensor strains as shown in Table S5. Here, much emphasis was given on the dose response behavior at a MRT (Fig. 1), and considered the MDC at a MRT as a signature for each strain that allowed predicting the mode of genotoxicity.

Finally, three genotoxicity groups were derived from the gene function and the MDC, which is one possible characteristic for estimating the assay activity (Davidian et al., 1988). Based on the above results, we propose the three potential groups, namely, (A) DNA damage cascade response, (B) general DNA damage response (genes belonged to SOS response) and (C) methylation specific response that may be used for determining the potential mode of an unknown genotoxin.

3.4. Application of grouped biosensors to other chemicals

Three genotoxicity groups (A–C) were applied for deciphering genotoxicity with four other chemical classes that includes DNA damage, oxidative stress, endocrine disrupting chemicals (EDCs) and phenolic compounds.

3.4.1. Response to other DNA damage chemicals

Tests were conducted using three DNA damage agents that were not considered during clustering analysis, such as methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS) and hydroxyurea (HU). These chemicals were tested with all seven biosensors to determine their genotoxicity categories. MMS and EMS known to cause DNA damage by DNA alkylation (Beranek, 1990). Our result showed that MMS predominantly induced the strain carrying *alkA*-lux, which was consistent to the response seen with MNNG (Figs. 1d and 3a). The other strains were also showed significant induction to MMS. However, a distinct response was seen with EMS, where *recA*-followed by *alkA-lux* were strongly induced and there was no significant response by the strain carrying *nrdA*- and *dinI-lux* (Fig. 3b). The differential responses of strains to EMS were possibly due to the chemical nature and permeability of EMS. Therefore, as expected, MMS and EMS induced DNA damage by methylation but these also induced genotoxicity through different routes as evidenced by the responses with other strains. It is clear from our results that MMS and EMS induced biosensors of all three groups designating their strong genotoxic nature. Similarly, hydroxyurea (HU), an inhibitor of DNA replication (Shechter et al., 2004) induced all genes under Groups A and B but not Group C (*alkA*), suggesting that HU induce genotoxicity but not through alkylation (Fig. 3c).

3.4.2. Response of genotoxicity biosensors to oxidative stress chemicals and EDCs

A series of oxidative stress inducing chemicals were tested for their potential ability to induce genotoxicity (Fig. S1a-f in SI). Paraquat (MV), benzyl viologen (BV) and ethyl viologen dibromide (EVD) are structural analogs that produce superoxide radicals (Lee et al., 2007). These chemicals induced *recA*-, *nrdA*- and *dinI*- in a dose-dependent manner with at least 3-fold of induction but at relatively higher concentrations. For example, Max. RBL from DPD2794 for MV, BV, and EVD was 4.54 mM, 0.49 mM and 4.5 mM, respectively (Fig. S1a-c in SI). Therefore, from the above results, it is possible to identify that redox chemicals, such as MV, BV and EVD at higher concentrations can cause indirect DNA damage possibly via SOS cascade system.

Ethidium bromide (EtBr), on the other hand, was selected for this study, since it was found previously to cause DNA damage through an indirect mechanism, including the finding that EtBr also induce oxidative stress (Ahn et al., 2004). The results showed that only *recA*-lux responded to EtBr with only >2-fold induction (Fig. S1d in SI). In contrast to EtBr, cadmium chloride ($CdCl_2$) also responsible for production of reactive oxygen species (Stohs et al., 2001) but it did not induce any DNA damage (Fig. S1e in SI). Interestingly, our results showed that H_2O_2 induced all of genes found in Groups A (*nrdA*, *sbmC* and *dinI*) and B (*recA*, *recN* and *sulA*) (Fig. S1f in SI). However, at high concentration of H_2O_2, these strains showed quick loss of bioluminescence (Fig. S1f in SI), which was probably because of lack of oxygen for cells or cell death. Not surprisingly, previous reports also showed that H_2O_2 led to a strong induction of *recA* (Min et al., 1999). Therefore, though H_2O_2 is a known oxidative stress agent, such chemicals can also induce genotoxicity as evidenced by responses obtained from Group A and B strains.

Three well-known EDCs were selected, such as styrene, 17-β-estradiol and bisphenol A to test using these biosensor panels. Our results showed there was no response by any of the strains to EDCs tested, except *recA*, which responded to only styrene exposure (Fig. S1g-i in SI). This result was consistent to a previous report that styrene exposure leads to a secondary DNA damage via oxidative stress (Vodicka et al., 2006). Therefore, such a unique response of biosensors can be a signature for styrene like compounds.

3.4.3. Response to phenolics

Phenolic compounds, such as phenol, 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP) and 2,4,5-trichlorophenol (2,4,5-TCP) were used as candidates to test the responses of the panel of seven biosensors. Phenolic compounds have ability to cause membrane damage (Choi and Gu, 2001). The responses of *recA* with phenolics were previously found to occur by pseudo-effects and

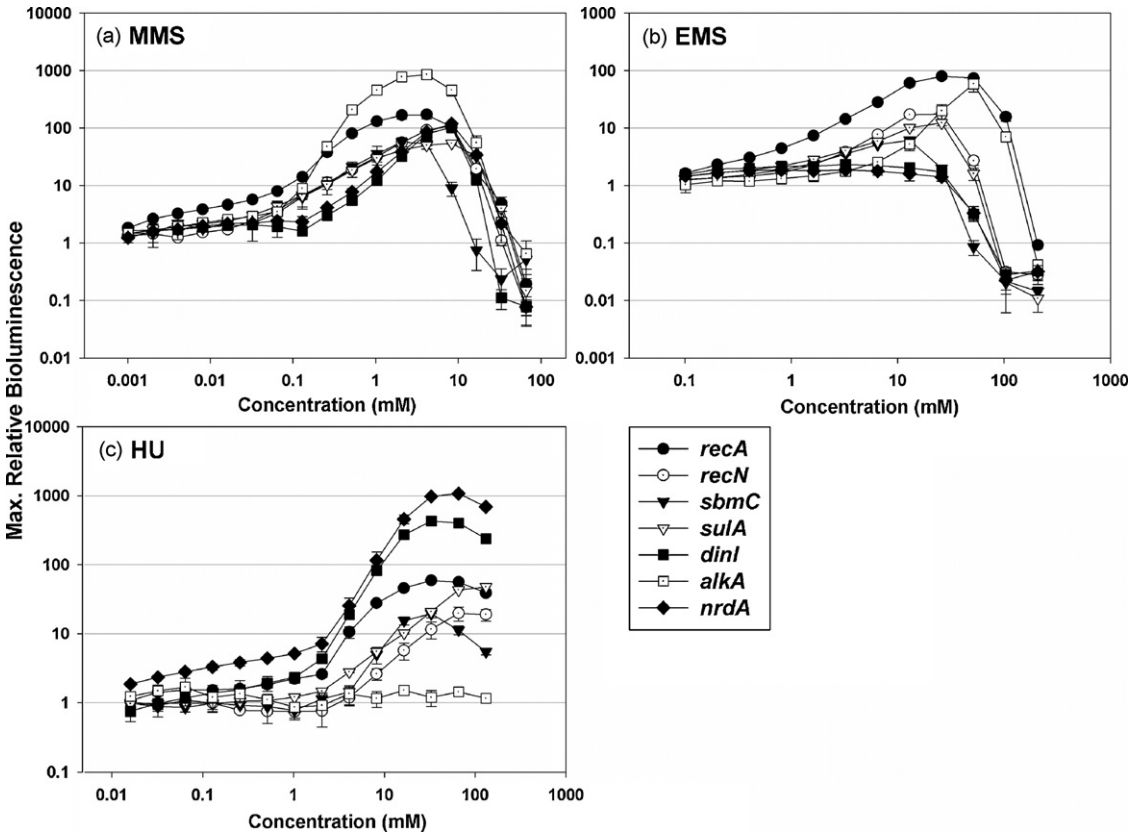

Fig. 3. Responses of seven biosensor strains to other DNA damage chemicals: (a) Methyl methanesulfonate (MMS), (b) Ethyl methanesulfonate (EMS), (c) Hydroxyurea (HU) that induce DNA damages by different modes.

that it is not a true response (Gu et al., 2000). Therefore, we have excluded the responses of *recA*-strain to phenolics in Supporting Information Figures S2a-d. However, results showed that phenolics did not show genotoxicity except with moderate response by *alkA*-strain against 2,4-DCP (2.9-fold) (Fig. S2c in SI). Based on this result, it seems likely that phenolics did not cause genotoxicity with exception that 2,4-DCP mildly induced DNA damage through induction of an *alkA* gene. With the help of genotoxicity classification and by using groups of signature bacterial biosensors, phenolics generally do not cause genotoxicity and if occurred, it may be weak

Table 1
Genotoxicity test in this study and comparison with the Ames test.

Chemical group	Chemicals	DNA damage cascade (Group A)			General DNA damage (Group B)			Alkylation (Group C)	Mutagenicity (Ames)	
		BBTNrdA	BBTSbmC	BBTDinI	DPD2794	BBTRecN	EBSulA	EBalkA	Ames test	
		nrdA	*sbmC*	*dinI*	*recA*	*recN*	*sulA*	*alkA*	Result	*Ref.*
DNA damage agents	Methyl methanesulfonate (MMS)	+	+	+	+	+	+	+	+	1
	Ethyl methanesulfonate (EMS)	–	+	Δ	+	+	+	+	+	1
	Hydroxyurea (HU)	+	+	+	+	+	+	–	+	1
Oxidative stress	Paraquat	–	–	+	+	–	–	–	+	1
	Ethidium bromide (EtBr)	–	–	–	Δ	–	–	–	+ (w/S9)	1
	Benzyl viologen dichloride (BV)	Δ	–	Δ	+	–	–	–	NA	NA
	Ethyl viologen dibromide (EVD)	Δ	–	Δ	+	–	–	–	NA	NA
	Cadmium chloride	–	–	Δ	–	–	–	–	–	1
	Hydrogen peroxide	+	+	+	+	+	+	–	±	1
Endocrine disrupting chemicals	Styrene	Δ	–	–	+	–	–	–	±	2
	17-β Estradiol (E2)	–	–	–	–	–	–	–	NA	NA
	Bisphenol A	–	–	–	–	–	–	–	–	2
Phenolics	Phenol	–	–	Δ	–	–	–	–	–	1
	2-Chlorophenol	–	–	–	–	–	–	–	–	1
	2,4-Dichlorophenol	–	–	–	–	–	–	+	–	1
	2,4,5-Trichlorophenol	–	–	–	–	–	Δ	–	NA	NA

Note: +, Positive; –, Negative; ±, Positive in one report, negative in another report; Δ, nearly 2-fold expression; NA, not available.
Reference 1: Reifferscheid and Hell (1996).
Reference 2: Choi et al. (2004).

through phenolic derivatives, such as 2,4-DCP possibly through indirect alkylation by an unknown route. Likewise potential mode of genotoxicity of unknown chemicals can be evaluated using three genotoxicity groups based on the responses of a series of biosensors.

These strains, and groups, can be used in conjunction with each other to provide more accurate view into the potential mechanisms by which a compound causes genotoxicity. For instance, the *recA* strain was found to be induced by all of the compounds that lead to DNA damage, even those that generate oxidative radicals, like H_2O_2. Moreover, it was always the most sensitive strain. Therefore, DPD2794 (*recA*) can be used to determine if a sample has genotoxic characteristics and induced alone among all of the strains will indicate that the sample is either mildly genotoxic (too low for the other strains to respond) or damage is resulting from the generation of hydroxyl radicals, such as by H_2O_2.

Further, we summarized and compared our approach with popularly known Ames assay for similar set of chemicals used in this study (Table 1). It is clear that both types of assays showed no significant differences, especially with *recA*- and *dinI*-lux. In addition, we observed that the strains differently responded to oxidative stress inducing chemicals and EDCs (Fig. S1 in SI). This was possibly because of the differential genotoxicities caused by chemicals through different mode of action. Therefore, an additional advantage of our approach is that, it allows detecting potential mode of oxidative DNA damage, such as that of structure analogs of paraquat or styrene.

Genotoxicity caused by any chemical agent is an intrinsic chemical characteristic that modifies or alters the structure or function of the genetic material. Pre-screening of novel chemical candidates and environmental contaminants for genotoxic properties has become an important issue. Screening includes an analysis of mutagenicity in bacteria and clastogenicity in cultured mammalian cells (Witte et al., 2007). A previous study analyzing 177 chemicals demonstrated that the specificity of the bacterial-based tests (Ames) was reasonable (73.9%), but the tests based on mammalian cells were found to have low specificity (below 45%) (Kirkland et al., 2005). This low specificity highlights the importance of understanding the mechanism by which genotoxicity may be induced, and it is possible to apply this biosensor panel for predicting the genotoxic mode of unknown chemicals.

4. Conclusions

This study demonstrates that with the help of bacterial promoter elements as biomarkers and the development of biosensors, it is possible to predict the potential mode of genotoxicity caused by chemicals. This genotoxicity assay has the potential to be a useful tool in the analysis of unknown chemicals or novel drugs that are of health and environmental concerns. Although the Ames tests and the Comet assay still need for screening carcinogenesis and mutagenesis in many other fields, presented assay has a benefit over those assays, since it aids in predicting the mechanisms by which a compound leads to genetic aberrations. Furthermore, although the number of genes used in this study was limited to seven, this assay can be expanded using other biosensors, which will aid in the specificity and reliability of the results and may even expand the scope of these strains to include genes which are specific for the oxidative damage of cellular DNA.

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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.2009.08.025.

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