

Minireview

Bacterial genotoxicity bioreporters

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Summary

Ever since the introduction of the *Salmonella typhimurium* mammalian microsome mutagenicity assay (the 'Ames test') over three decades ago, there has been a constant development of additional genotoxicity assays based upon the use of genetically engineered microorganisms. Such assays rely either on reversion principles similar to those of the Ames test, or on promoter–reporter fusions that generate a quantifiable dose-dependent signal in the presence of potential DNA damaging compounds and the induction of repair mechanisms; the latter group is the subject of the present review. Some of these assays were only briefly described in the scientific literature, whereas others have been developed all the way to commercial products. Out of these, only one, the *umu*-test, has been fully validated and ISO- and OECD standardized. Here we review the main directions undertaken in the construction and testing of bacterial-based genotoxicity bioassays, including the attempts to incorporate at least a partial metabolic activation capacity into the molecular design. We list the genetic modifications introduced into the tester strains, compare the performance of the different assays, and briefly describe the first attempts to incorporate such bacterial reporters into actual genotoxicity testing devices.

Introduction

The increasing need to assay and monitor the potential genotoxic effects of an ever-growing number of chemicals and environmental samples is countered by the logistic, economical and ethical constraints imposed by the use of animal-based test systems. Consequently, ever since the introduction of the revolutionary *Salmonella typhimurium* mammalian microsome mutagenicity assay (the 'Ames test') over three decades ago (Ames *et al.*, 1973), continuous efforts have been directed towards the development, improvement and implementation of additional bacterial-based genotoxicity assays. One group of these assays is based on the same principle of the Ames test, in that they quantify the reversion rate from a defined mutation back to the wild-type (Biran *et al.*, 2009; Reifferscheid and Buchinger, 2009). The present review will not discuss this group but rather concentrate on assays that employ genetically engineered microorganisms, 'tailored' to generate a quantifiable signal that reflects the genotoxic potency of the tested sample. Such assays possess several significant advantages including rapid response times, high reproducibility, facility of use and low operational cost. Yet, bacterial-based assays cannot carry out the complex biochemical reactions collectively known as 'metabolic activation'; these take place mainly in mammalian liver cells, in which xenobiotics may be transformed into genotoxic forms. Herein, we review the main directions undertaken in the construction and testing of bacterial-based genotoxicity bioassays, including the attempts to incorporate at least a partial metabolic activation capacity into the molecular design.

The promoter–reporter concept

As will be discussed below, several genetic engineering approaches have been employed over the years in the construction of bacterial reporter strains that respond to the presence of genotoxic compounds. Many of these share the same basic principle: the fusion of a gene promoter, known to be activated by the presence of genotoxic chemicals, to a gene or a group of genes the activity of which can be monitored quantitatively, preferably in real time (Belkin, 2003). The gene promoter acts as the sensing element, which – upon activation – drives the transcription of the downstream reporter gene(s).

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Consequently, the gene promoter will dictate the response spectrum of the construct and, to some extent, its sensitivity. The reporter genes determine the nature of the generated signal (bioluminescence, fluorescence, etc.) and thus also the instrumentation required for its acquisition. The host cell, the third major component in the construction of a genotoxicity reporter strain, is selected for ease of genetic manipulation, for its relevance, and – most importantly – for its effects on detection sensitivity and threshold.

Sensing elements

In the selection of sensing elements to be used for the construction of genotoxicity reporter systems, the most promising candidates are promoters of genes involved in DNA repair. Such genes, induced in response to either actual DNA damage or to the presence of DNA damaging agents, are mainly part of one of two inducible systems: the *recA*-dependent, *lexA*-controlled SOS response and the *recA*-independent, *ada*-controlled adaptive system induced in response to alkylation damage of DNA. The latter system responds to the presence of methylated phosphotriesters generated by DNA alkylation that activate the *ada* gene product which, in turn, triggers the transcription of genes such as *ada*, *alkA*, *alkB* and *aid* (Volkert, 1988; Volkert *et al.*, 1989).

The SOS response is under the control of the LexA protein that binds to the SOS box in the promoter region of the regulon genes, repressing their expression. De-repression occurs when the RecA protein binds to single-stranded DNA at replication forks that are blocked by DNA damage, forming RecA-ssDNA nucleoprotein filaments (Courcelle and Hanawalt, 2003; Janion, 2008). Once bound to DNA, the RecA protein changes conformation and acts as a co-protease in the cleavage of LexA, thus allowing transcription of the SOS genes (Little, 1991; Janion, 2001; Giese *et al.*, 2008). Among these are genes such as *uvrA*, *recA*, *recN* or *umuDC*, responsible for DNA repair, and others such as *sulA*, that couple DNA damage to cell division (D'Ari, 1985; Janion, 2001). Expression of a given SOS gene depends on the specific LexA-binding properties of its promoter, determined by the sequence of the LexA-binding sites (SOS boxes), their number and their arrangement (Lewis *et al.*, 1994; Fernández de Henestrosa *et al.*, 2000; Norman *et al.*, 2005).

SOS promoters

Several gene promoters from over 30 known SOS regulon genes induced upon DNA damage were used for the construction of genotoxicity sensors, as is briefly described below.

umuDC. The two proteins coded by this operon, UmuD and UmuC, are induced under DNA damage conditions by the LexA- and RecA-dependent transcriptional upregulation of the SOS regulon. They first form a UmuD₂C complex, which acts as a checkpoint inhibitor of cell division until repair can address the original DNA damage signal. After RecA/ssDNA-mediated UmuD cleavage, these proteins form a (UmuD')₂C complex (DNA polymerase V), which carries out the error-prone replication of damaged DNA (SOS mutagenesis; for review see Sutton *et al.*, 2000).

The first description of a *umuC*–*lacZ* fusion coded on plasmid pSK1002 in *S. typhimurium* for the detection of genotoxic agents was published by Oda and colleagues (1985), and has since been recognized as the 'umu-test'. The *S. typhimurium* strain carrying this fusion (TA1535) has undergone several modifications including excision repair deficiency (*uvrB*), an *rfa* deletion which increases permeability to many chemicals, and a deletion of the natural *lac* operon. The *umu*-test was standardized according to the German Institute of Standardization (DIN 38415-3) and the International Standardization Organization (ISO/CD 13829). It is now a part of the set of tools available to authorities and researchers for the investigation and monitoring of genotoxicity of environmental samples. The system was adapted to a 96-well microtiter plate format (Reifferscheid *et al.*, 1991) and has been used, for example, to detect a wide range of carcinogenic mutagens (Oda *et al.*, 1985; Nakamura *et al.*, 1987; Shimada and Nakamura, 1987; McDaniels *et al.*, 1990; Reifferscheid and Heil, 1996), as well as genotoxic activity in disinfectants (Sakagami *et al.*, 1988), complex mixtures (Whong *et al.*, 1986; Hamer *et al.*, 2000), environmental pollutants (Bihari *et al.*, 1990), river waters and industrial wastewaters (Reifferscheid *et al.*, 1991; Ehrlichmann *et al.*, 2000; Dizer *et al.*, 2002).

sulA (*sfiA*). The SulA protein, produced in large amounts during the SOS response, halts cell division in *Escherichia coli* by binding to the tubulin-like GTPase, FtsZ (Higashitani *et al.*, 1995). It has been used as a sensing element for genotoxicity detection in several cases, most notably in the colorimetric SOS chromotest (Quillardet *et al.*, 1982), commercialized in 1984. The *E. coli* PQ37 tester strain used in the SOS chromotest harbours a *sfiA*::*lacZ* fusion and carries a deletion of the normal *lac* region, so that β-galactosidase activity is strictly dependent on *sfiA* expression. Similarly to the *umu*-test bacterium it is mutated in the *uvr*-system (*uvrA*) to hinder DNA repair, and in *rfa* to increase cell wall permeability (Quillardet and Hofnung, 1985). A different colorimetric assay based on a plasmid-borne *sulA*::*lacZ* fusion in *S. typhimurium* TA1538 was proposed by El Mzibri and

colleagues (1996), who also described a procedure that includes metabolic activation based on S9-mix.

recN. Another *E. coli* SOS gene promoter fusion that has been developed into a commercial product (VITOTOX™) is based on the *recN* gene, coding for a protein that is involved in double stranded DNA break repair. The *E. coli* and several *S. typhimurium* strains (TA 98, TA 100 and TA104) are used as bacterial hosts. A multi-copy plasmid harbouring a fusion of the *recN* promoter to the *Vibrio fischeri luxCDABE* genes drives the emission of light in response to the presence of DNA damaging agents (van der Lelie et al., 1997), allowing real-time monitoring of the bacterial response. The VITOTOX™ test strains were evaluated with a variety of chemicals (van der Lelie et al., 1997; Verschaeve et al., 1999; Westerink et al., 2009) as well as river water (Vijayashree et al., 2005), ground water (Verschaeve, 2002) and air samples.

recA. The promoter of the RecA recombinase gene, which plays a key role in the SOS response by its co-protease activity on the LexA repressor, has served as the basis of several genotoxicity sensors. Nunoshiba and Nishioka (1989; 1991) described the colorimetric *E. coli* 'Rec-lac test', based on the GE94 strain that carries the *recA-lacZ* fusion, and its DNA repair-deficient derivative strains such as KY946 (*uvrA*), KY945 (*recA*) and KY943 (*lexA*). The system was tested against 4-Nitroquinoline-*N*-oxid (4-NQO), *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), mitomycin C (MMC) and UV radiation, as well as with hydrogen peroxide, formaldehyde, *tert-butyl* hydroperoxide, cumene hydroperoxide and streptonigrin.

A different reporter system, the *V. fischeri luxCDABE* operon, was used by Vollmer and colleagues (1997) to generate several *E. coli* reporter strains, one of them (DPD2794) carrying a *recA':luxCDABE* fusion on the multi-copy plasmid pUCD615 (Vollmer et al., 1997). As in other constructs carrying this 5-gene complement, these bioluminescent fusions allowed real-time visualization of the transcriptional responses induced by DNA damage, without the need for cell-free enzyme assays or the exogenous addition of luciferase substrates. To make full use of these advantages, Polyak and colleagues (2000) have alginate-immobilized a similar *recA':lux* harbouring strain to the tip of an optic fibre, the other end of which was connected to a photon counter. The instrument allowed a real-time determination of genotoxicity by dipping the bacteria-clad fibre end into a sample.

The *recA* gene promoter of the radiation resistant bacterium *Deinococcus radiodurans*, characterized by an extremely efficient DNA repair capabilities, was fused to the EGFP gene, generating a genotoxicity and radioactivity bioreporter that can persist in extremely geno-

toxic conditions (Gao et al., 2008). A real-time concentration-dependent fluorescent response to γ -radiation and to MMC was demonstrated.

cda. The colicin D gene *cda*, a constituent of the ColD plasmid (Frey et al., 1986), also served as a basis for a bioluminescent genotoxicity sensor using *Photobacterium leiognathi luxCDABE* as a reporter. This 'SOS *lux*' test responded sensitively to diverse genotoxins such as MMC, MNNG, nalidixic acid (NA), dimethylsulfate (DMS), H₂O₂, CH₂O, UV and γ -radiation (Ptitsyn et al., 1997). This assay has later been combined with the GFPuv-based Lac-Fluoro test to generate a combined toxicity-genotoxicity sensor (Baumstark-Khan et al., 2001).

Four different SOS promoters (*recA*, *umuCD*, *sulA* and *cda*) were compared by Norman and colleagues (2005) using the same fluorescent reporter (*gfpmut3**, in plasmid pANO1). The differences between the constructs were evaluated after exposure of cells harbouring the fusion plasmids (MG1655/pANO1::SOS promoter) to the known genotoxicant *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG). A *tolC* mutation enhanced the sensitivity to this chemical, the only genotoxic agent tested in this study. Performance of the *cda*-based sensor in response to MNNG clearly surpassed the other three with respect to the SOS-induction factor, as a result of high rates of gene expression combined with a low background activity of the *cda* promoter. Thus, *cda* promoter was selected for the further development of the GenoTox test (Østergaard et al., 2007).

Non-SOS promoters

alkA. Several bacterial DNA protection and repair systems that are independent of the SOS regulon have been described, one of which, most efficiently induced by alkylating agents, has been generally termed the 'adaptive response'. Several genes of this system have been characterized, including *alkA*, which encodes a repair glycosylase (*N*3-methyladenine DNA glycosylase II; Volkert, 1988). A promoter of this gene has been fused by Vollmer and colleagues (1997) to the *V. fischeri luxCDABE* genes. The construct displayed a very strong response to the alkylating agent MNNG, the magnitude of which was enhanced by very low background bioluminescence; the responses of an equivalent *lacZ* fusion were much more moderate.

nrdA. The expression of the *E. coli nrdA* gene, which encodes for a ribonucleoside diphosphate reductase, is strongly affected by DNA damage, induced, for example, by UV exposure, but is independent of LexA (Courcelle et al., 2001). The *nrdA* promoter was fused by Hwang and colleagues (2008) to *Photobacterium luminescens*

luxCDABE genes. The *E. coli* strain BBTNrdA carrying this plasmid-borne fusion responded to the DNA damaging agents NA, MMC, MNNG, 4-NQO and hydrogen peroxide, but not to other oxidants or phenolic compounds (Hwang *et al.*, 2008).

Reporter systems

The spectrum of reporter systems available for monitoring gene expression by transcriptional fusions is continuously expanding, as is the instrumentation for signal detection and quantification. Colorimetric, fluorescent, bioluminescent and electrochemical detection of genotoxicity have been described, and are briefly outlined below.

Colorimetric and electrochemical (*lacZ*, *phoA*, *uidA*)

The β -galactosidase gene, *lacZ*, has been used as a gene expression reporter for several decades. The most common substrates employed for assaying the activity of this enzyme are *o*-nitrophenyl β -D-galactopyranoside (ONPG) and 5-bromo-4-chloro-3-indolyl β -D-galactoside (X-gal) for colorimetric detection, 4-methylumbelliferyl- β -D-galactopyranoside (MUG) for fluorimetry, 1,2-dioxetane substrates for luminescence, and *p*-aminophenyl- β -D-galactopyranoside (pAPG) for electrochemical analysis. The advantages of colorimetric assays lie in their simplicity and rapidity, but the need for improved sensitivity, faster response times, a broader dynamic range and the capability of real-time monitoring has led to a continuous search for alternatives (Jain and Magrath, 1991). The *umu*-test (Oda *et al.*, 1985), SOS chromotest (Quillardet *et al.*, 1982), *sulA*-test (El Mzibri *et al.*, 1996) and Rec-*lac* test (Nunoshiba and Nishioka, 1991) were all developed using *lacZ* as the reporter and ONPG as the substrate. Several remedies have been proposed to overcome interferences by colored samples, such as the inclusion of a washing step after the exposure of the bacteria to the samples (Nakamura *et al.*, 1987; Pal *et al.*, 1992), or a post-treatment dilution and re-incubation (McDaniels *et al.*, 1990; Reifferscheid *et al.*, 1991). The latter procedure was reported to enhance the sensitivity of the *umu*-test to genotoxicants in environmental samples in a high-throughput microtiter plate system (Hamer *et al.*, 2000). Oda and colleagues (2004) achieved higher sensitivity by using a different substrate, chlorophenol red- β -D-galactopyranoside (CRPG). The red reaction product following cleavage by the β -galactosidase has a longer life time than *o*-nitrophenol, the ONPG reaction product. Similar modifications were also introduced to the SOS chromotest (Ohta *et al.*, 1984). The original colorimetric procedure of the assay (Quillardet *et al.*, 1982) was successfully changed to a fluorimetric one by using the fluorescent substrate 4-methylumbelliferyl- β -D-galactopyranoside (MUG) (Fuentes *et al.*, 2006).

A different approach was proposed by Matsui and colleagues (2006), who provided the *umu*-test bacteria (TA1535/pSK1002) with the substrate pAPG, the end product of which (*p*-aminophenol) can be monitored electrochemically. This approach, utilized earlier for other bacterial sensor systems (Biran *et al.*, 1999; 2000; Paitan *et al.*, 2003; Schwartz-Mittelmann *et al.*, 2003), requires the addition of an external substrate but does not involve lysis or permeabilization of the cells. Similarly to bioluminescence, it is thus suitable for continuous online measurement of enzymatic activity, even in turbid solutions and under anaerobic conditions (Badihi-Mossberg *et al.*, 2007). Matsui and colleagues (2006) have demonstrated this by scanning electrochemical microscopy (SECM) in a specialized glass biochip configuration, using 5 nl cell aliquots immobilized in collagen gel. Overall, lower limits of detection of 2-aminofluorene (2-AF), MMC and 2-aminoanthracene (2-AA; +S9-mix) were obtained by the microbial chip as compared with the conventional *umu*-test, but it should be noted that the definition of the detection limit was different and exposure times were longer. Buchinger and colleagues (2009) demonstrated the applicability of chrono-amperometric detection using screen printed electrodes for assaying the activity of the *umu*-test bacterial strain (TA1535/pSK1002). The effect of the S9-mix on the measurement was evaluated and no interference was found. The response of the *umu*-test strain to IQ, metabolically activated with an S9-mix, was monitored both electrochemically and with the ISO-standardized colorimetric detection, revealing a good correlation between the induction factors calculated for both methods.

Bioluminescence (*lux*, *luc*)

The reaction by which photons are released by a biological reaction is shared by numerous groups of organisms, including bacteria, protozoa, fungi, insects and fish. In all cases the reaction is catalysed by an enzyme generically referred to as luciferase, which oxidizes a substrate known as luciferin; however, the chemical and enzymatic nature of both entities vary greatly, depending on the organism from which the system is derived. The two bioluminescent systems most commonly used as reporters of gene activation are of bacterial and insect (firefly) origin. Firefly luciferase, coded by the *luc* gene, is a 62 kDa monomeric protein, and its activity is oxygen- and ATP-dependent. Its luciferin, benzothiazoyl-thiazole, has to be added externally when *luc* is used as a reporter gene. Bacterial luciferase catalyses the oxidation of a reduced flavin mononucleotide (FMN_{H2}) by a long-chain fatty aldehyde to FMN and the corresponding fatty acid, in the presence of molecular oxygen. All bacterial luciferases are heterodimeric proteins composed of two subunits, α

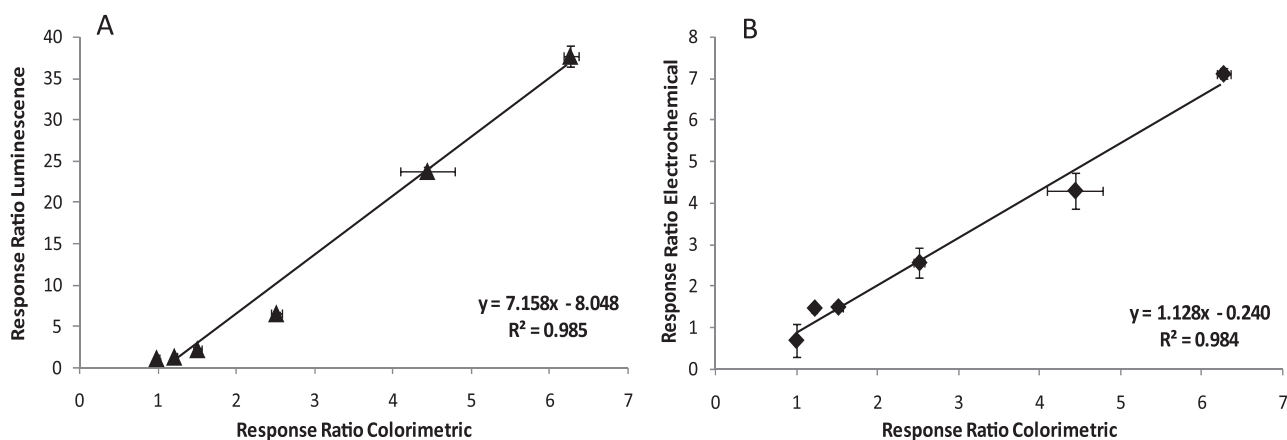


Fig. 1. Correlations between the activation of the *sulA* promoter fused to different reporter systems, comparing colorimetric (*sulA*::*phoA*) detection with either (A) bioluminescent (*sulA*::*luxCDABE*) detection or (B) electrochemical (*sulA*::*phoA*) detection. Response ratio – fold induction over the non-induced control.

(40 kDa) and β (37 kDa), encoded by the *luxA* and *luxB* genes of the *lux* operon. Three other genes in this operon (*luxCDE*) encode the synthesis and recycling enzymes of the fatty acid aldehyde (Meighen, 1993). Constructs carrying just *luxAB* are sufficient to generate a bioluminescent signal, but necessitate the external addition of a long-chain aldehyde. The commonly used luciferases of *V. fischeri* and *Vibrio harveyi* have limited upper temperatures of 30°C or 37°C respectively. In recent years, *Photobacterium luminescens lux* genes are thus often used due to the higher upper temperature limit (45°C) of their gene products (Meighen and Szittner, 1992).

The non-invasive protocol using *lux* fusions allows real-time reporting of the transcriptional activation of the monitored gene promoters. As described above, the VITOTOX™ test uses the *V. fischeri luxCDABE* operon under the control of *recN* promoter (van der Lelie *et al.*, 1997; Verschaeve *et al.*, 1999), and the SOS *lux* test employs the *luxCDABFE* operon of *P. leiognathi* under control of the *cda* gene promoter of the plasmid ColD (Ptitsyn *et al.*, 1997). Vollmer and colleagues (1997) have fused the *E. coli recA*, *uvrA* and *alkA* gene promoters to *V. fischeri luxCDABE*. Further modifications to the same system (Davidov *et al.*, 2000; Rosen *et al.*, 2000) included integration of the *recA*::*lux* fusion into the *E. coli* chromosome, a change of the reporter system to *P. luminescens lux*, and the use of either *S. typhimurium* or a *tolC E. coli* mutant as alternative hosts. Application of the *P. luminescens* reporter, which allowed a working temperature of 37°C, resulted in a more rapid response to various genotoxic chemicals and UV.

The *luxCDABE* genes of *V. fischeri* were also fused to the *recA* promoter of *Pseudomonas aeruginosa* (Elasri and Miller, 1998). As a soil and freshwater bacterium, *P. aeruginosa* was presented as a good candidate to serve as a sensor for the state of natural bacterial communities

of both pristine and polluted habitats. Light production in response to UV exposure was monitored in this strain as part of a study of UV effects on natural bacterial populations.

To increase the sensitivity of the *umu*-test and to expand its detection capabilities, two groups independently replaced its β -galactosidase reporting gene by either bacterial (Justus and Thomas, 1998) or insect (Schmid *et al.*, 1997) luciferase. In both cases, improvements in performance were reported, including enhanced sensitivity, improved signal to noise ratios, stronger signals and a better neutralization of color interferences.

In an attempt to compare between bioluminescence, colorimetric and electrochemical detection, a single gene promoter, *sulA*, was fused to either *P. luminescens luxCDABE* (Yagur-Kroll *et al.*, 2009) or to the *E. coli* alkaline phosphatase gene *phoA*, the activity of which was monitored both colorimetrically (with *p*-nitrophenyl phosphate as a substrate) or electrochemically with *p*-aminophenyl phosphate. Figure 1 displays the correlation between the three assays, comparing colorimetric detection to either bioluminescence (Fig. 1A) or electrochemical detection (Fig. 1B). While the correlations demonstrate the validity of all three reporters, the higher dynamic range of the bioluminescence and electrochemical measurements provide a more reliable data over a much broader range of genotoxicant concentrations.

Fluorescent protein genes

The highly stable green fluorescent protein, GFP, of the jellyfish *Aequorea victoria* was the first fluorescent protein the gene of which was utilized as a molecular reporter (Chalfie *et al.*, 1994). It was soon followed by additional fluorescent protein genes isolated from various marine organisms as well as by mutated forms with improved

performance (Crameri *et al.*, 1996; Tsien, 1998; Matz *et al.*, 1999; Fradkov *et al.*, 2000; Nagai *et al.*, 2002; Wiedenmann *et al.*, 2004; Shaner *et al.*, 2007). The GFP protein has a high quantum yield and can be expressed in both prokaryotic and eukaryotic systems with no need for a substrate or cofactor (Kain and Kitts, 1997). To increase the sensitivity of assays based on the GFP reporter system, several green fluorescent protein mutants were constructed (Cormack *et al.*, 1996; Crameri *et al.*, 1996; Heim and Tsien, 1996; Welsh and Kay, 1997).

Arai and colleagues (2001) have modified the *umu*-test by replacing the *lacZ* gene with a DNA fragment encoding for EGFP (enhanced green fluorescent protein). This construct was tested in *E. coli* strain KY706 with 3 µg ml⁻¹ 4-NQO, a concentration that strongly induced β-galactosidase activity in the *umu*-test. Detection sensitivity of the GFP reporter system became similar to that of β-galactosidase only after the introduction of additional modifications to the plasmid. These included utilization of tandem *lacUV5* and chimeric *trp/umu* promoters, and coexpression of the *E. coli* *recA5327* mutant. An additional construct that harbours the fusion of the *umuDC* promoter to the *gfp* gene was generated by Justus and Thomas (1999), who reported an overall performance inferior to that of the *lacZ*-based assay.

Other fluorescent genotoxicity sensors were constructed by fusing the *gfp* and *gfpmut3* reporting genes to the *recA* promoter (Kostrzynska *et al.*, 2002). GFPmut3 is a mutant that is approximately 20 times more fluorescent than wild-type GFP and only weakly excited by UV light (Cormack *et al.*, 1996). The use of the wild-type *gfp* yielded dose-dependent but weak responses, while with *gfpmut3*, the detection thresholds for MMC, MNNG, NA, hydrogen peroxide and formaldehyde were comparable to the SOS chromotest (Quillardet *et al.*, 1982), the *umu*-test (Nakamura *et al.*, 1987) and the SOS *lux* test (Ptitsyn *et al.*, 1997).

The three fluorescent protein genes coding for EGFP, GFPuv and DsRed [a red fluorescent protein derived from the sea anemone *Discosoma* sp. (Matz *et al.*, 1999)] were similarly fused to the *recA* promoter (Sagi *et al.*, 2003), and the responses to nalidixic acid were compared with a luminescent *recA::lux* strain. Performance was usually poorer compared with bioluminescent *recA*-based reporters: lag times were longer and detection thresholds were higher, unless incubation times were very long. The *recA::DsRed* plasmid, hosted in *E. coli* UTL2, was used in order to monitor antigenotoxic activity of plant extracts that exhibited some protection against MMC, NA and hydrogen peroxide (Bartolome *et al.*, 2006).

The use of fluorescent proteins as reporters has been characterized as superior in terms of stability but inferior to enzyme-based reporters in terms of sensitivity and response kinetics (Hakkila *et al.*, 2002; Sagi *et al.*, 2003).

Norman and colleagues (2006) have demonstrated that these drawbacks can be circumvented by the use of flow cytometry, displaying a response threshold of strain *cda::gfpmut3* to MNNG of 5 nM, 10-fold lower than the minimal detectable concentration (MDC) of the *umu*-test (Reifferscheid *et al.*, 1991). Moreover, the experimental procedure enabled the detection of MMC in spiked soil.

Cytotoxicity controls

As samples or chemicals of suspected DNA damaging activity are also likely to be cytotoxic, genotoxicity assays often incorporate suitable controls to neutralize or correct for the effects that cell damage or cell death may exert on assay results. One simple measure is an optical determination of cell growth in parallel to assaying reporter gene activity (Baun *et al.*, 1999). However, this solution is limited, as optical density does not necessarily reflect the viability of a cell suspension. A different approach is based on the inclusion of an additional, constitutive reporting strain or enzyme which serves as a 'light off' sensor: a decrease in its signal indicates a toxic effect of the sample. This approach, for example, was adopted in the VITOTOX™ test that introduced a constitutive light-producing strain with a *lux* operon under the control of the strong promoter, *pr1* (Verschaeve *et al.*, 1999).

The SOS *lux* test similarly incorporated a cytotoxic reporting strain harbouring a constitutive *lac*-GFPuv plasmid in the same *S. typhimurium* host strain (Baumstark-Khan *et al.*, 2001). In a further development of this system a SWITCH plasmid was added, combining the SOS *lux* plasmid pPLS-1 and the LAC-Fluoro plasmid pGFPuv (Baumstark-Khan *et al.*, 2005).

Using a different approach, the tester strain in the SOS chromotest was made constitutive for alkaline phosphatase synthesis (Torriani and Rothman, 1961). This enzyme, non-inducible by DNA-damaging agents, is assayed in parallel to β-galactosidase and the ratio of the two activities is taken as a measure of the specific activity of β-galactosidase (Quillardet *et al.*, 1982).

The toxicity of a sample can also be evaluated with promoters that are induced by a broad spectrum of environmental insults and are thus good indicators of toxic cellular stress, such as the promoter of the *grpE* gene, a component of the chaperone network in *E. coli* (Van Dyk *et al.*, 1994; de Marco *et al.*, 2007). The use of two strains, one harbouring the plasmid *recA::GFPuv* and the other *grpE::lux* allowed an assessment of the toxicity of the sample along with its genotoxicity (Sagi *et al.*, 2003). A dual-function toxicity/genotoxicity bioreporter system was reported by Hever and Belkin (2006) who described a plasmid containing both *recA::EGFP* and *grpE::DsRed* fusions. A somewhat different double reporter concept was demonstrated by Mitchell and Gu (2004), who

presented a strain containing a fluorescent genotoxicity reporter fusion (*recA*::*GFPuv4*) and a bioluminescent oxidative stress reporter (*katG*::*luxCDABE*).

Detection performance

As described in detail above, numerous genotoxicity bioassays based on genetically engineered bacteria have been presented over the years. While some of them, such as the *umu*-test and the SOS chromotest, have undergone intensive validation and were tested against hundreds of compounds, others have only been briefly described along with their responses to a very limited range of chemicals. Quite clearly therefore pending further validation of the latter group, the validated tests are of a much higher value for routine testing and their results merit higher credibility. Detailed reports of an extensive testing of these assays and their comparison to the Ames test can be found in Nakamura and colleagues (1987), Reifferscheid and Heil (1996), and Quillardet and Hofnung (1993). The *umu*-test has been standardized and accepted for wastewater quality testing (ISO/CD 13829, DIN 38423-5).

Table 1 summarizes reported detection thresholds of selected genotoxicants exhibited by many of the assays described in the present review. Even a brief glance at Table 1 reveals that of all the tested systems, only two, the *umu*-test and the SOS chromotest, were challenged with the required spectrum of genotoxic chemicals necessary to demonstrate their applicability to environmental testing. All others were only preliminarily challenged with a very limited number of compounds. In fact, Table 1 only lists chemicals that have been tested by at least one bioassay in addition to the *umu*-test and the SOS chromotest; it thus does not contain the detection thresholds for hundreds of other compounds that have been reported for these two tests (Quillardet *et al.*, 1982; 1985; Ohta *et al.*, 1984; Oda *et al.*, 1985; Nakamura *et al.*, 1987; von der Hude *et al.*, 1988; Quillardet and Hofnung, 1993; Reifferscheid and Heil, 1996). Based on this limited comparison it may also be observed that detection thresholds vary greatly between the different assays, sometimes by several orders of magnitude. Other factors such as response times, detection spectra or facility of use that have not been compared in Table 1 confer additional advantages to some of the reporter strains.

Further comparison of the performance of some of these bioassays has been performed in several hands-on workshops conducted in Belgium (Mol TECHNOX; Corbisier *et al.*, 2000; Baumstark-Khan *et al.*, 2007) and in the USA (Eilatox-Oregon; Hakkila *et al.*, 2004; Meriläinen and Lampinen, 2004; Pancrazio *et al.*, 2004; Pedahzur *et al.*, 2004). In addition to highlighting differences in response characteristics, such workshops help

to emphasize the difficulties encountered when taking a newly developed test out of the lab and into the field and emphasized the merits of utilizing standard testing protocols for performance validation.

Sensitivity enhancement and expansion of response spectrum

Very early in the short history of genetically engineered bacterial reporters, it became apparent that simple promoter-reporter fusions may be sufficient to demonstrate the applicability of the concept for genotoxicity testing, but that additional molecular manipulations are required in order to turn them into efficient tools for routine use. Such manipulations have taken several forms, including modification of the sensing elements, introduction of mutations into the reporter strains for enhanced sensitivity and permeability, and the incorporation of metabolic activation capabilities.

Table 2 lists some of the genetic manipulations introduced into the *E. coli* or *S. typhimurium* host strains and their reported effects. The modifications can be divided into two classes: deficiencies that reduce the ability of the cells to defend against DNA damaging agents, and new or enhanced capabilities of bacterial cells to metabolically activate pre-genotoxic compounds, thus at least partially mimicking the metabolic pathways such compounds may undergo in mammalian systems.

Introduction of host strain mutations for enhanced sensitivity

As listed in Table 2, several mutations have been introduced into genotoxicity reporter strains to enhance their sensitivity and thus lower their detection thresholds. While some mutations, such as *tag* or *oxyR*, have only been reported once (for the SOS chromotest; Quillardet and Hofnung, 1993), others have become almost a prerequisite in microbial genotoxicity reporters. Most notable in the latter group are *uvrAB* mutants deficient in excision repair, and *rfa* mutations that, by increasing membrane permeability, allow the build-up of higher intracellular concentrations of the tested chemicals (Makela *et al.*, 1974; Ames *et al.*, 1975).

Altering the sensing element: manipulation of regulatory sequences

Molecular manipulations of the DNA fragment harbouring the sensing promoter element in order to improve the bacterial response to target chemicals have also been described. One of the first examples is the VITOTOX™ strain (van der Lelie *et al.*, 1997). In addition to the wild-type *recN* promoter, two different mutant promoters were

Table 1. Published detection thresholds of selected chemicals by genetically engineered bacterial genotoxicity reporters.

Promoter Reporter gene(s)	umu-test	SOS chromotest	SOS lux test (2), (3), (18), (21)	VITOTOX™ (24), (26), (29)	suIA-test (4)	recA::lux (10), (28)	nrdA::lux (6)	recA::gfp (7)	cdsA::gfp (13)	recA::DsRed (1)
Host strain	<i>umuCD</i> <i>lacZ</i> <i>S. typhimurium</i> TA1535	<i>suIA</i> (<i>stfA</i>) <i>lacZ</i> <i>E. coli</i> PQ37	<i>Cda</i> (<i>ColD</i>) <i>luxCDABFE</i> <i>P. leiognathi</i> <i>S. typhimurium</i> TA1535	<i>recN</i> <i>luxCDABE</i> <i>V. fischeri</i> <i>S. typhimurium</i> TA104	<i>suIA</i> <i>lacZ</i> <i>S. typhimurium</i> TA1535	<i>recA</i> <i>luxCDABE</i> <i>V. fischeri</i> <i>E. coli</i> RFM443	<i>nrdA</i> <i>luxCDABE</i> <i>P. luminescens</i> <i>E. coli</i> RFM443	<i>recA</i> <i>gfpmut3</i> <i>E. coli</i> C600	<i>Cda</i> (<i>ColD</i>) <i>gfpmut3</i> <i>S. typhimurium</i> TA1535	<i>recA</i> <i>DsRed2</i> <i>E. coli</i> UTL2
Compound	MDC (µM)									
Fungal toxins and antibiotics										
MMC	0.01 (12)	0.016 (19)	4.3×10^{-3}	0.046	5.3×10^{-3}	2.9×10^{-4}	0.93	0.012	9.1×10^{-3}	0.011
Doxorubicin	1.1 (12)	0.41 (11)	0.9		0.43					
NA	2.4 (12)	4.6	4.6	0.69			10.77	3.57	1.02	3.01
Bleomycin	0.04 (12)	22.5 (11)	0.35							
Esters										
MMS	150 (22)	63 (19)		10	117					
EMS	1.8×10^5 (12)	10^4 (27)		2061	3414					
DMS	300 (12)	6.7 (19)	7.5							
Nitroso-, nitro-										
DEN	5×10^4 (12)	199 (19)	0.6	2349	1.5×10^4	0.33	1.06	0.763	0.16	
MNNG	3.0 (5)	0.45 (27)		0.40	1					
NPAHs										
4-Nopd	32 (14)	ND (16)		10.4						
3-NFA	0.04 (15)	0.25 (8)		0.06						
4-NQO	0.1 (12)	0.02 (19)	0.042	3.16	0.018		13.1			
Furazolidone	0.22 (17)	< 0.44 (16)		0.002						
HAs										
2-AA	1 (22)	3 (27)	1.08		0.037				0.08	
2-AF	0.4 (22)	< 274 (27)		1.1						
PAHs										
B[a]P	4 (12)	2.33 (19)		3.6	0.58	5×10^{-5}				
Fluoranthene	ND (12)	ND (9)		15.3						
Chrysene	65 (12)	< 87 (16)		21.9						
Acridine	12 (12)	75 (19)								
Acridine orange	0.5 (12)	0.7 (27)		0.68						
ICI 191	46 (12)	ND (16)								
9-aminoacridine										
Miscellaneous										
H ₂ O ₂	1322 (12)	1000 (27)	50	59	84	23.5	1470	286	24.7	588
CH ₂ O	623 (12)	> 1000 (27)	6500					305	141	
Epi	649 (12)	3300 (27)		1383						
EtBr	127 (12)	1165 (20)				0.32				
Metals										
K ₂ Cr ₂ O ₇	258 (12)	68 (25)	0.68	14						
CdCl ₂	ND (23)	ND (20)		ND		0.99				

MDC, the lowest concentration at which the response is systematically over twice the background; ND, not detected; MMC, mitomycin C; NA, naldixic acid; MMS, methyl methanesulphonate; EMS, ethyl methanesulphonate; DMS, dimethylsulfoxide; DEN, diethylnitrosamine; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; 4-Nopd, 4-nitro-o-phenylenediamine; 3-NFA, 3-nitrofluoranthene; 4-NQO, 4-nitroquinoline-N-oxide; 2-AA, 2-aminoanthracene; 2-AF, 2-aminofluorene; B[a]P, benzo[a]pyrene; H₂O₂, hydrogen peroxide; CH₂O, formaldehyde; Epi, epichlorohydrin; EtBr, ethidium bromide. Numbers in parentheses indicate the following references: 1 – Bartolome et al. (2006), 2 – Baumstark-Khan et al. (2001), 3 – Baumstark-Khan et al. (2007), 4 – El Mizbiti et al. (1996), 5 – Hamer et al. (2000), 6 – Hwang et al. (2008), 7 – Kozłowska et al. (2002), 8 – Mersch-Sundermann et al. (1991), 9 – Mersch-Sundermann et al. (1992), 10 – Min et al. (1999), 11 – Müller and Janz (1992), 12 – Nakamura et al. (1987), 13 – Norman et al. (2005), 14 – Oda et al. (1985), 15 – Oda et al. (1992), 16 – Ohta et al. (1984), 17 – Pat et al. (1992), 18 – Pititsyn et al. (1997), 19 – Quillardet et al. (1982), 20 – Quillardet and Hofnung (1985), 21 – Rabbow et al. (2002), 22 – Reifferscheid et al. (1991), 23 – Reifferscheid and Heil (1996), 24 – van der Leije et al. (1997), 25 – Venier et al. (1988), 26 – Verschaeve et al. (1989), 27 – von der Hude et al. (1988), 28 – Vollmer et al. (1997), 29 – Westerink et al. (2009).

Table 2. Molecular modifications introduced into genotoxicity reporter strains to enhance sensitivity, expand the response spectrum and incorporate metabolic activation capabilities.

Manipulation	Test	Strain	Modified capabilities	Effect	Reference
<i>uvrAB</i> mutation	SOS chromotest <i>umu</i> -test GenoTox SOS <i>lux</i> VITOTOX™ <i>sulA</i> -test Rec-lac test SOS-Chromotest	PQ37/ <i>sfIA</i> :: <i>Mud</i> (<i>Ap lac</i>) <i>cts</i> TA1535/ <i>umuDC</i> :: <i>lacZ</i> TA1535/ <i>cdA</i> :: <i>gfp</i> TA1538/ <i>cdA</i> :: <i>lux</i> TA104/ <i>recN2-4</i> :: <i>lux</i> TA1538/ <i>sulA</i> :: <i>lacZ</i> KY946 ϕ (<i>recA-lacZ</i>) PQ243/ <i>sfIA</i> :: <i>Mud</i> (<i>Ap lac</i>) <i>cts</i>	Deficiency in nucleotide excision repair	Increased sensitivity toward certain genotoxicants	Ames <i>et al.</i> (1973), Baumstark-Khan <i>et al.</i> (2001), Nunoshiba and Nishioka (1991), El Mzibri <i>et al.</i> (1996), Oda <i>et al.</i> (1985), Quillardet <i>et al.</i> (1982), Østergaard <i>et al.</i> (2007)
<i>tag</i> mutation	Rec-lac test SOS-Chromotest	PQ243/ <i>sfIA</i> :: <i>Mud</i> (<i>Ap lac</i>) <i>cts</i>	Inactivation of the constitutive 3-methyl-adenine DNA glycosylase I	Response to lower concentrations of alkylating agent as MNNG, MMS, etc.	Costa de Oliveira <i>et al.</i> (1987), Quillardet and Hofnung (1993)
<i>oxyR</i> mutation	SOS chromotest	PQ300/ <i>sfIA</i> :: <i>Mud</i> (<i>Ap lac</i>) <i>cts</i>	Depletes the oxidative stress responses under the control of OxyR transcription regulator	More sensitive to various classes of peroxides and compounds generating peroxides	Muller and Janz (1992), Quillardet and Hofnung (1993)
<i>rfa</i> mutation	SOS Chromotest <i>umu</i> -test GenoTox SOS <i>lux</i> VITOTOX™ <i>sulA</i> -test	PQ37/ <i>sfIA</i> :: <i>Mud</i> (<i>Ap lac</i>) <i>cts</i> TA1535/ <i>umuDC</i> :: <i>lacZ</i> TA1535/ <i>cdA</i> :: <i>gfp</i> TA1538/ <i>cdA</i> :: <i>lux</i> TA104/ <i>recN2-4</i> :: <i>lux</i> TA1538/ <i>sulA</i> :: <i>lacZ</i>	Mutation in the core enzymes of lipopolysaccharide (LPS) biosynthesis. Incomplete LPS composed of the ketodeoxyoctanoate-lipid core.	Higher permeability to substances, especially important with larger hydrophobic genotoxins.	Ames <i>et al.</i> (1973), El Mzibri <i>et al.</i> (1996), Oda <i>et al.</i> (1985), Østergaard <i>et al.</i> (2007), Verschaeve <i>et al.</i> (1999), Quillardet and Hofnung (1985), Rettberg <i>et al.</i> (2001)
<i>tolC</i> mutation	GenoTox SOS <i>lux</i> <i>recA</i> :: <i>lux</i> SOS <i>lux</i> <i>umu</i> -test	N43/ <i>cdA</i> :: <i>gfpmut3</i> PB3/ <i>cdA</i> :: <i>lux</i> DE112/ <i>recA</i> :: <i>lux</i> KT1008/ <i>umuD</i> :: <i>luc</i> NM1011/ <i>umuDC</i> :: <i>lacZ</i>	Inactivation of the efflux, outer membrane transporter-TolC	Limited efflux capability, increases sensitivity to genotoxins	Davidov <i>et al.</i> (2000), Norman <i>et al.</i> (2005), Rettberg <i>et al.</i> (2001), Maehana <i>et al.</i> (2004)
<i>S. typhimurium</i> NR overexpression	<i>umu</i> -test	NM2009/ <i>umuD</i> :: <i>lacZ</i>	High nitroreductase activity	Highly sensitive towards many nitroarenes as 2-NF, 1-NP, etc.	Oda <i>et al.</i> (1992)
<i>S. typhimurium</i> O-AT overexpression	<i>umu</i> -test GenoTox	TGO2/ <i>cdA</i> :: <i>gfp</i>	Thirteen-fold higher isoniazid- <i>N</i> -acetyltransferase activity	High sensitivity toward nitro- and dinitro-containing compounds, as well as arylamines, aminoazo and HAs.	Oda <i>et al.</i> (1993), Oda <i>et al.</i> (1995)
<i>S. typhimurium</i> O-AT and NR overexpression	<i>umu</i> -test GenoTox	NM3009/ <i>umuDC</i> :: <i>lacZ</i> TGO2/ <i>cdA</i> :: <i>gfp</i>	High O-AT and NR activity	Increased sensitivity to aromatic amines and nitroarenes with/without external MA (S-9)	Oda <i>et al.</i> (1995), Oda <i>et al.</i> (2004), Østergaard <i>et al.</i> (2007)
Human <i>N</i> -ATs (NAT1 and NAT2) expression in <i>S. typhimurium</i>	<i>umu</i> -test	NM6001/ <i>umuDC</i> :: <i>lacZ</i> NM6002/ <i>umuDC</i> :: <i>lacZ</i>	<i>N</i> -acetyltransferase 1 or <i>N</i> -acetyltransferase 2 activity	Increased sensitivity to aromatic amines and heterocyclic aromatic amines	Oda <i>et al.</i> (1999)
Human CYP1A2 and NADPH-P450 reductase expression in <i>S. typhimurium</i>	<i>umu</i> -test	OY1001/ <i>umuDC</i> :: <i>lacZ</i>	7-Ethoxyresorufin <i>O</i> -de-ethylation and NADPH-cytochrome <i>c</i> reductase activity	Detection of some carcinogenic HAs, without the addition of metabolic activation system (S-9)	Aryal <i>et al.</i> (1999)
Human CYP1A2 and NADPH-P450 reductase with O-AT expression in <i>S. typhimurium</i>	<i>umu</i> -test	OY1002/ <i>umuDC</i> :: <i>lacZ</i>	7-Ethoxyresorufin <i>O</i> -de-ethylation and NADPH-cytochrome <i>c</i> reductase activity, joint with high O-AT activity	More sensitive to HAs than the previous strain, with ought external MA. Detects the mutagens APNH and APH.	Aryal <i>et al.</i> (2000), Oda <i>et al.</i> (2004)

CYP1A2, cytochrome P450 1A2; O-AT, *O*-acetyltransferase; NR, nitroreductase; *N*-AT, *N*-acetyltransferase; HA, heterocyclic amines; MA, metabolic activation; APNH, aminophenylharman; APH, aminophenylharman.

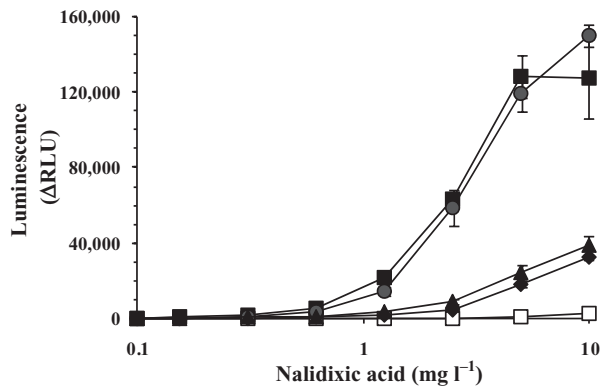


Fig. 2. Effects on reporter performance of several manipulations of the promoter region of a *sulA::lux* construct in *E. coli*. Data represent the increase in bioluminescence (in relative light units) following a 60 min exposure to different nalidixic acid concentrations. (□) wild-type; (◆) a mutation near the *lexA* binding site; (▲) a tandem *sulA-recA* promoter; (●) a modification of the -35 consensus sequence; (■) a promoter-containing fragment extending into the *sulA* ORF. Activity is reported as the difference in luminescence intensity (in arbitrary relative light units, RLU) between the induced and non-induced reactions.

constructed and tested alone and in combination: a deletion of one of the LexA binding sites, and a 'promoter up' mutation where a consensus nucleotide was introduced in the -35 region. Each of the single mutants was superior to the wild-type in at least one respect, but the double mutation resulted in poorer performance. The influence of addition/subtraction of *lexA* binding sites on reporter gene expression in the SOS promoter::*uidA* fusion under genotoxic stress was examined with several SOS promoters (*umuD*, *sulA*, *recA* and *recN*) (Dreier *et al.*, 2002). Highest signals were produced by constructs that either harboured an additional *lexA* binding site in the *sulA* promoter that overlapped the -35 promoter region, or that lacked one of the two *recN* LexA binding sites. Another successful effort was reported by Arai and colleagues (2001), who improved the performance of a *umuDC::gfp* construct by replacing the wild-type -35 promoter sequence with the -35 sequence of the highly active *trp* gene promoter.

Further exploration of different approaches for enhancing the sensing performance of a *sulA::luxCDABE* fusion by manipulation of the promoter region was carried out by Yagur-Kroll and colleagues (2009). Four independent strategies were used: modifying the length of the DNA segment containing the promoter region, introduction of random mutations by a directed evolution process, insertion of site-directed mutations into the -35 and -10 regions, and promoter duplication (Fig. 2). Two manipulations had the most dramatic effect: extending the promoter-containing fragment into the *sulA* open reading frame and a modification of the -35 consensus site. A mutation near the *lexA* binding site and adding an additional *recA* promoter improved performance to a lower

extent. Such manipulations, however, may be promoter-specific and thus not necessarily universal in nature.

Introduction of metabolic activation enzymes

To at least partially alleviate the restriction of metabolic activation potential in prokaryotes and correspondingly reduce the dependency on external metabolic activation by rodent-derived cytochrome P450 (S9) preparations (Malling, 1971; Ames *et al.*, 1973), several attempts have been made to genetically engineer bacterial cells to incorporate some of the enzymatic activities involved in the activation process of xenobiotics. Some of these efforts are summarized in Table 2, clearly demonstrating the viability and potential of this approach. Nevertheless, while the study of single enzymes is a necessary step in improving our understanding of the activation of promutagens to mutagens (Oda *et al.*, 1999; Muckel *et al.*, 2002), it should be remembered that the CYP P-450 is composed of a diverse group of enzymes that are not very likely to be engineered together into a single reporter strain in the near future.

Devices incorporating bacterial genotoxicity reporters

Several reports describe the incorporation of genetically engineered bacteria into specially designed hardware to generate dedicated genotoxicity biosensors. Polyak and colleagues (2000) have immobilized the *E. coli* strain DPD1718 (Davidov *et al.*, 2000; Rosen *et al.*, 2000) that contains a chromosomally integrated *recA::lux* fusion in sodium alginate onto the tip of an optical fibre. The luminescent signal induced in the bacteria by the presence of genotoxicants was collected by the fibre and electronically amplified. Sensor strains embedded in alginate retained their sensitivity following a 2-month incubation, but at the cost of a significantly delayed response (Davidov *et al.*, 2000). A biosensor composed of a high-density living bacterial cell array was fabricated by depositing single *E. coli* cells carrying a *recA::gfpmut2* into a microwell array formed on one end of an imaging fibre bundle (Kuang *et al.*, 2004). Each fibre in the array had its own distinct light pathway, allowing thousands of individual cell responses to be monitored simultaneously with both spatial and temporal resolution. An active sensing lifetime of more than 6 h and a shelf-life of 2 weeks were demonstrated. An on-chip whole-cell genotoxicity bioassay was developed (Tani *et al.*, 2004), using a three-dimensional microfluidic network system composed of one perforated microwell chip bound in two microchannel chips, in which the sensor cells were immobilized in agarose. The bioluminescent responses to MMC of the sensor strain, SOSLuc, in a wild-type *E. coli* or its *tolC* deficient derivative

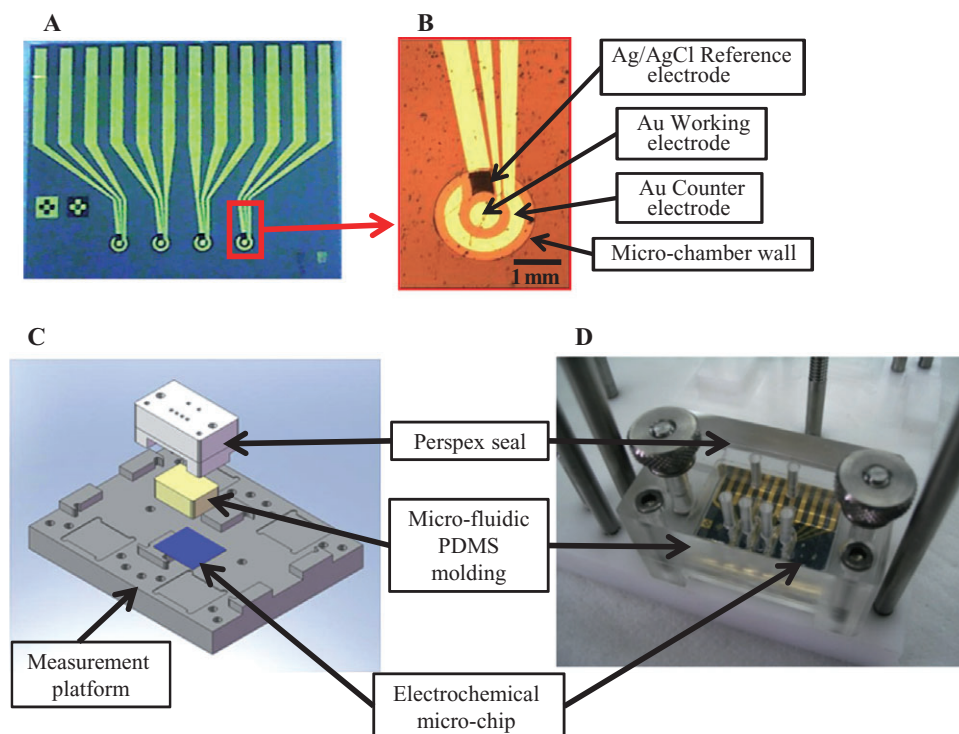


Fig. 3. A. A silicon-based micro-chip comprised of four electrochemical micro-chambers (2 mm in diameter). B. Close-up view of a single three-electrode electrochemical micro-chamber. C. Layout of the measurement platform comprised of a Perspex seal, micro-fluidic PDMS moulding, and an electrochemical micro-chip. D. The assembled micro-fluidic electrochemical chip system.

were measured by a charge-coupled camera (Maehana *et al.*, 2004). The immobilized cells were stable for at least 1 week at 4°C. Following further optimization, the detection limits of model hydrophobic and hydrophilic genotoxicants were comparable to those obtained by the reference method used (Maehana *et al.*, 2006)

Electrochemical mutagen screening based on the *umu*-test was performed on a microbial chip combined with a SECM device (Matsui *et al.*, 2006). The microbial chip was fabricated by embedding 5 nl of collagen-immobilized genetically engineered *S. typhimurium* strain (TA1535/pSK1002) in a microcavity on a glass substrate. β -Galactosidase activity was monitored by electrochemical determination of the concentration of *p*-aminophenol (pAP), the hydrolysis product of pAPG. Recently, a novel microelectro-mechanical system (MEMS)-based micro-chip incorporated in a micro-fluidic system was constructed and characterized by Ben-Yoav and colleagues (2009) (Fig. 3). The activity of minute volumes (down to 2.5 nl) of a *S. typhimurium* strain carrying a *umuC*::*lacZ* fusion and an *E. coli* strain harbouring a *sulA*::*phoA* fusion was measured after exposure to IQ and NA respectively. The presence of genotoxic materials was detected by chrono-amperometry using the substrates pAPP (for the *phoA* reporter) and pAPG (for the *lacZ* reporter), gen-

erating a significant signal after 3 min with MDCs of 0.31 and 42 μ M for IQ after metabolic activation and NA respectively.

Although such biosensors open potential horizons for field applications and high-throughput screening systems, their current status requires additional development before they become available for routine use. Such future development should clearly take into account the necessity of including metabolic activation as an integral part of the process, as well the need for significant improvement in long-term maintenance of cell viability and activity.

Summary and outlook

Genetically engineered bacterial sensor systems play a central role in effect-directed analysis of environmental contaminants and the assessment of the DNA damaging potential of chemicals. The basis for a successful construction of bacterial sensors consists of three elements: (i) a bacterial tester strain that offers an appropriate genetic background facilitating high permeability for chemicals, an appropriate DNA-repair capacity and negligible background activity of the reporter gene; (ii) a sensitive promoter that offers well-balanced repressor binding properties for sensitive induction of the reporter

gene; and (iii) a sensitive, fast responding reporter system with a broad dynamic range and preferably the capability of real-time monitoring.

The discovery and the understanding of the bacterial SOS-system, a regulatory pathway that is mainly responsible for inducible DNA repair and induced mutagenesis in bacteria, opened diverse possibilities for genetically tailoring bacteria for the specific, sensitive and fast detection of genotoxic contaminants. The fusion of SOS gene promoters to reporter genes that generate quantifiable signals allow to easily detect the genotoxic potency of a sample. This review describes the current stage of development of the sensing systems by discussing the molecular, biochemical and physico-chemical characteristics of the different promoters and suitable reporter genes based on colorimetric, luminometric, fluorimetric and electrochemical detection.

The potential of future sensor developments with enhanced external and/or internal metabolic competence is immense. Against the background of a worldwide increasing freshwater demand, and the need for reclamation of process water as drinking water resource, bacterial sensors can play a crucial role in risk minimization. To fully reach this objective, additional progress needs to be made in several directions including enhancement of sensitivity, expansion of the response spectrum, stabilization of the more sensitive reagents and, most importantly, the introduction of broad-spectrum metabolic activation capabilities into the sensor strains.

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