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Research Article

Effect of Dimethyl Sulfoxide on the Bioluminescence Expression of the Inducible SOS-*Lux* gene Biosensor *E. coli* C600 pPSL-1 and *E. coli* DPD1718 elicited by Mitomycin-C

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Abstract

The microbial SOS *lux* gene test was designed for quick screening of environmental mutagens. The assay isbuilt on the receptor-reporter principle, with SOS as receptor sensitive to DNA damage and a bioluminescence system expressing detectable and measurable light signal. This bioassay was aimed at evaluating the effects of dimethyl sulfoxide (DMSO) on the bioluminescence expression of inducible SOS *lux* gene biosensor *E. coli* C600 pPSL-1 and *E. coli* DPD1718 elicited by Mitomycin C (a known mutagen). The bacterial culture of *E. coli* C600 pPSL-1 and *E. coli* DPD1718 at the mid-exponential growth phase were exposed to different concentrations of MMC (0, 10, 25, 75 and 100 ng/ml respectively) and with DMSO (0, 0.5, 1.0, 2.0 and 5.0 % respectively) for each MMC concentration). *E. coli* C600 pPSL-1 bioluminescence demonstrated a dose-dependent increase in SOS response that peaked at 3 hr post-MMC exposure, whereas *E. coli* DPD1718 bioluminescence demonstrated a gradual increase in bioluminescence which can be distinguished statistically same time. Above 1% DMSO concentrations significantly reduced the bioluminescence expression of *E. coli* C600 pPSL-1 and *E. coli* DPD1718. DMSO concentration above 1% caused significant reduction in bioluminescence in both microbial sensors. Hence, DMSO can cause additional toxicity in terms of hydrophobic compounds mobility across cell membrane of test subjects in toxicity evaluation and its role be screened meticulously.

Keywords: Dimethyl Sulfoxide; Bioluminescence; Mitomycin C; *E. coli* C600 pPSL-1; *E. coli* DPD1718

Introduction

Cells of different species have been used to evaluate the toxicity and mutagenicity of numerous environmental stressors such as the UV, ionising radiations, pollutants, heavy metals and products of pharmaceutical interest. These environmental stressors and other xenobiotics contribute to increased reactive oxygen species (ROS) production implicated in the cause of oxidative stress and mutagenesis in cells and tissues [1]. The different approaches developed to screen for ROS and mutagenicity coverstumour induction in animals [3], human somatic mutation test of the HPRT gene in peripheral blood cells [24], and induction of chromosome aberrations [15], fluorogenic probes [17], oxidative damages of lipids and protein, and nucleic acids [9]. Such assays are generally time consuming and less cost-effective and provide limited information on the mechanisms of genotoxicity.

These limitations have led to the evolution of new short-term *in vitro* bioassays designed to detect potential carcinogenic compounds. They are based on the inference that DNA damages are the cause of carcinogenesis [1,2,11]. Among these short-term assays is the Ames *Salmonella* reverse mutation assay which have been used to detect various kinds of mutagens [10]. The Ames *Salmonella* mutation assay was validated in studies of

hundreds of chemicals and about 90% of the carcinogens tested were mutagenic [21]. In response to DNA-damaging agents, a set of functions known as SOS-response are induced which include synthesis of a number of proteins such as RecA and UmuC/D proteins related to mutagenesis as observed in SOS-chromotest [20]. The SOS-induction potency has been correlated with Ames reverse mutation test for a number of tested mutagens [19].

Of the current strategies involved in the screening of mutagenicity, the use of luminescent protein biosensors offers an alternative and ideal situation, because the reporter measurements are nearly almost instantaneous, exceptionally sensitive and quantitative, and typically there is no endogenous activity in the host to interfere with quantification [22].

The luminescence is a product of luciferase genes cloned from bacteria, firefly and *Renilla* and have been usedas markers of gene expression [8,25]. Bacterial luciferase is a dimeric enzyme found mostly in marine bacteria [6,12]. The luciferase is generated from the redox reaction involving the reduced flavin mononucleotide (FMNH2) and a long chain aliphatic aldehyde to yield FMN, carboxylate and blue light (Figure 1). A complete cassetes of *lux*-operon consists of *luxCDABE*. *luxA* and *luxB* codes for the α and β subunits of the enzymes [7] while *luxC*, *luxD* and *luxE* genes code for the fatty acid reductase complex needed to recycle the reaction product back to aldehyde substrate. The recycling is possible through the normal homeostasis of the bacterial cells. Thus, autonomous expression of luminescence can be attained in bacterial cells with the expression of the *lux*-operon [5,21].

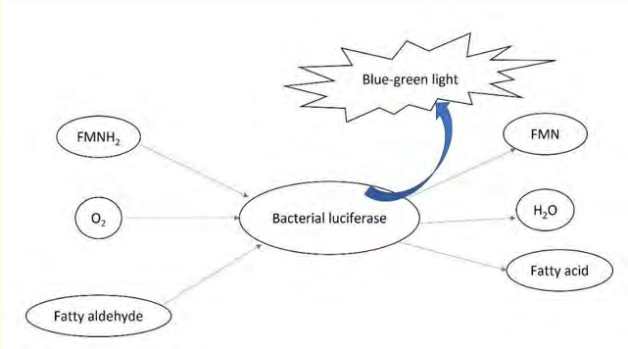

Figure 1: Schematic representation of bacterial bioluminescence reaction.

Among the inducible *SOS-lux* gene biosensors developed is *E. coli* C600 pPSL-1 and *E. coli* DPD1718. *E. coli* C600 pPSL-1 bioassay is based on recombinant plasmid pPLS-1 carrying a promoterless *luxCDABE* genes of *Photobacterium leiognathi* downstream with a strong SOS-dependent *col* promoter [18]. *E. coli* DPD1718 contains a chromosomally integrated fusion of *recA* promoter *to Photorhabdus luminescens luxCDABE* genes into *lacZ* locus of *E. coli* 1692 [5,26]. The inducible *lux* gene bioassay is time effective as result can be available within few minutes and light signals can be evaluated without disruption of the cells [18].

Inducible *SOS-lux* gene *E. coli* C600 pPSL-1 and *E. coli* DPD1718 have been used to screen known hydrophilic chemical genotoxins such as hydrogen peroxide (H2O2) and nalidic acid [18]. However, to screen hydrophobic compounds, an agent of solubilisation is required to transport the chemical subject across the cell membrane of biosensors. Therefore, to avoid interference of the solvent in the bioassay,there is need to screen for the safe permissible level of the solvent in the bioassay. This study examined the safe threshold of DMSO in the bioluminescence expression of inducible SOS-*lux* gene *E. coli* C600 pPSL-1 and *E. coli* DPD1718 stimulated with MMC, a known chemical mutagen.

Materials and Methods

Chemicals and laboratory consumables

Luria-Bertani medium (LB), Oxoid bacteriological agar, Ampicillin (CAS No. 69-53-4), Chloramphenicol (CAS No. 56-75-7), Mitomycin C (CAS No. 50-07-7), Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, UK.

Batch culture preparation and Optimal growth phase

All microbiological assays were conducted under aseptic conditions in the laminar flow hood. The biosensors were sub-cultured from the glycerol stock, with the aid of sterile plastic inoculation loop onto LB agar plates supplemented with the antibiotics ampicillin (50 µg/ml) for *E. coli* C600 pPSL-1, and chloramphenicol (30 µg/ml) for *E. coli* DPD1718 and incubated at 30^0C for 48 h. The sub-cultures were prepared by streaking out a single colony using a sterile wire loop from the first plates onto fresh LB plates supplemented with the appropriate antibiotics as above. The sub-cultured plates were incubated at 30^0C.

An overnight culture from a single colony of the sub-cultured plate was inoculated into 10 ml of LB media in a 30-ml McCartney Universal bottle containing appropriate antibiotics and incubated in orbital shaker incubator set at 25°C for *E. coli* C600 pPSl-1 and 37°C for *E. coli* DPD1718 for a minimum of 15 h at 200 rpm. Following the overnight incubation, the optical density was measured using the spectrophotometer (Camspec Ltd) set at 600 nm. An aliquot of 1 ml from the overnight culture was transferred into a 250-ml Erlenmeyer conical flask containing a pre-warmed LB media in triplicate and incubated at 25°C (for *E. coli*C600 pPSL-1) and 37°C (for *E. coli* DPD1718) at 200 rpm and supplemented with their respective selective antibiotics till it reached the mid-exponential growth phase (6th hr for *E. coli* C600 pPSL-1 and 8th hr for *E. coli* DPD1718).

Effect of DMSO on bioluminescence expression of Inducible SOS-*lux* gene biosensor *E. coli* C600 pPSL-1 and *E. coli* DPD1718 co-treated with MMC

From the culture at mid-exponential growth phase, 0.1 ml aliquot of biosensor culture was inoculated into a3-ml luminometer cuvette each containing 0.9 ml of MMC treatments. The MMC treatment were in 3 levels designed to give 0, 10, 25 and 100 ng/ml final concentration, respectively. Each level of MMC were treated with DMSO to a final concentration of 0, 0.5, 1, 2 and 5% (v/v) in triplicate. The luminometer cuvettes were incubated in a benchtop orbital shaker set at 25°C (*E. coli* C600 pPSL-1) and 37°C (*E. coli* DPD1718), 200 rpm. The bioluminescence was measured after 3 hr period using a benchtop JADE luminometer. The data were presented as line graphs and analysed using the analysis of variance (Anova) (with repeated measures when necessary) to determine the impact of DMSO on the bioluminescence expression of *SOS-lux* gene *E. coli* C600 pPSL-1 and *E. coli* DPD1718.

Results

Both DMSO treatments and different concentrations of MMC had a similar effect on *E. coli* DPD1718 and *E. coli* C600 pPSL-1. After 3 hours of exposure to MMC, the mid-exponential growth phase of *E. coli* DPD1718 and *E. coli* C600 pPSL-1 produced significant ($p < 0.05$) optimal bioluminescence (data not presented). *E. coli* DPD1718 produced a higher background bioluminescence value (RLU) compared to *E. coli* C600 pPSL-1 and bioluminescence when exposed to concentrations of MMC (Figure 2). At 5% DMSO, both *E. coli* DPD1718 and *E. coli* C600 pPSL-1 strains produced significantly lower SOS-lux expression at 10, 25, and 100 ng/ml when compared to the control. DMSO concentrations above 1% cause a significant ($p < 0.05$) reduction in SOS-*lux* gene expression. Therefore, the SOS-*lux* gene biosensors *E. coli* DPD1718and *E. coli* C600 pPSL-1 are presented as potential tools in the genotoxicity assessment of hydrophobic compounds that require DMSO as an agent of solubilization.

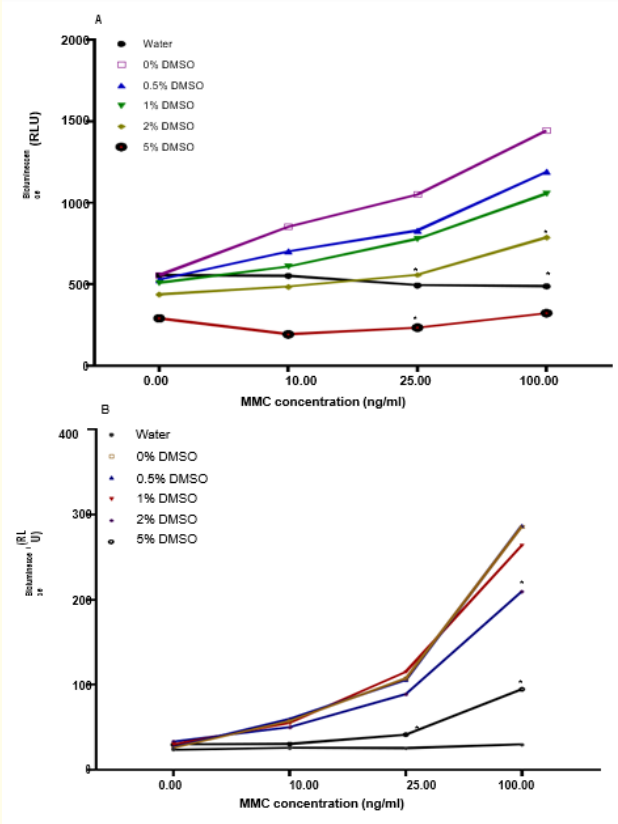

Figure 2: Bioluminescence expression of *E. coli* DPD1718 (A) and *E. coli* C600 pPSL-1 (B) co-treated with different concentrations of Mitomycin C and DMSO for a 3-hr period. Data are presented as mean ± standard deviation of the triplicate measurement of bioluminescence. (* = represents a significant ($p \leq 0.05$) reduction of bioluminescence in comparison with MMC treatments only).

MMC concentrations: 0 (no MMC), 10 ng/ml (the concentration of MMC across the DMSO treatments and same goes for 25, and 100 ng/ml).

DMSO (%): 0.0, 0.5, 1.0, 2.0 and 5.0% (v/v).

Discussion and Conclusion

This work examines the effects of DMSO on a well-characterised and genetically regulated luminescent bacterial reporter system, the inducible SOS-*lux* gene in *E. coli* C600 pPSL-1 and *E. coli* DPD1718. The inducible SOS-*lux* gene *E. coli* C600 pPSL-1 and *E. coli* DPD1718 bioassay is specific for the detection of soluble genotoxins like other SOS-based bacterial tests, such as the umu test [16] and SOS-chromotest [19]. The SOS *lux* test makes use of the fact that mutagenesis in *E. coli* cells exposed to chemical mutagens involves SOS function, which is stimulated by DNA damage such as changes in bases and DNA strand breaks [20]. The SOS bioassays specifically detect soluble environmental genotoxic substances and have been demonstrated to detect some mutagens [18]. The spectrum of damages induced by mutagens ranges from DNA base modifications [22], alkylations, strand breaks, and intrastrand cross-linking [4], and all these damages can initiate an SOS response. The SOS *lux* bioassay test has a sensitivity as low as a nanomolar (10 ng/ml) (Figure 2).

MMC is readily soluble and elicits SOS responses via expression of dose-dependent bioluminescence in *E. coli* C600 pPSL-1 and *E. coli* DPD1718. DMSO is the commonly used solvent to dissolve poorly soluble drugs in permeation assays in cell culture studies [14,28]. DMSO concentrations below 1% DMSO were determined to be the safest threshold (Figure 2).

Therefore, concentrations above 1% can impair SOS-*lux* gene expression by likely limiting the cascades of enzymatic processes leading to the production of luminescence in the tested *E. coli* strains. This finding corroborates the findings of [28]. DMSO concentrations above 1% cause reduced bioluminescence expression, which can be linked to toxicity. SOS-*lux* gene bioluminescence expression is a biochemical process driven by enzymes. Therefore, DMSO concentrations above 1% have the propensity to alter the luciferase enzymatic activities, leading to reduced bioluminescence expression in *E. coli* C600 pPSL-1 and *E. coli* DPD1718. Studies by [13,23] corroborated this finding, in which DMSO above 1% caused a decrease in light-emitting luciferase enzyme (PLG2) and Caspase-Glo 3/7 assays.

The SOS-*lux* gene bioluminescence expression of *E. coli* DPD1718 at 5% was below the background bioluminescence (Figure 2A) and can be seen as a clear case of toxicity. This is different from the case of *E. coli* C600 pPSL-1 (Figure 2B), where bioluminescence expression was still higher than the background value. However, in both test models, DMSO presents a clear case of toxicity on the inducible SOS-*lux* gene biosensors and its applicability in the screening of hydrophobic molecules with a high octanol-water partition coefficient. We acknowledge the fact that each test model responds differently to DMSO and may range from 0.1% [28] to 10% [14]. We recommend screening the tolerance level of each test model in research requiring the use of DMSO as an agent of solubilisation.

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