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RESEARCH

Caulobacter crescentus as a biomodel to sense the UV radiation genotoxicity

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ABSTRACT

Sunlight is the main source of energy for life on our planet, but ultraviolet (UV) radiation is hazardous, damaging several biological molecules (such as lipids, proteins, and DNA). Due to the ozone layer deterioration, the scientific community is trying to understand the biological impact of UV light, with the routine use of UV lamps as experimental UV source, despite underrepresenting the real-life intensity of natural radiation. Besides, uncontrolled conditions during environmental exposures require using biomodels capable to survive and sense different biomarkers. However, no standardized or reliable biomodels for UV radiation damage sensing are available yet, against different endpoints. In this sense, *Caulobacter crescentus* is an aquatic microorganism used as a model to study cell cycle and mutagenesis. Therefore, this work was aimed to propose experimental conditions for using C. *crescentus* for the simultaneous detection of UV impact on three genotoxic endpoints: cytotoxicity, DNA damage response, and gene mutation. These endpoints were measured by the Survival assay, SOS Chromotest, and Rif^R mutagenesis techniques. The use of M2 transparent medium was used to study UV impacts, given the absorptive capacity of the complete medium (PYE). It was shown that the presence of plasmid pP3213, bearing the PimuA::lacZ transcriptional fusion, did not affect survival and mutagenesis. The use of C. *crescentus* to sense solar genotoxic effects saves resources and experimental time as simultaneously reporting multiple endpoints.

Keywords: Biodosimeter, genotoxic evaluations, Rif^R mutagenesis, SOS chromotest, colony forming units assay

RESUMEN

***Caulobacter crescentus* como biomodelo para medir la genotoxicidad de la radiación UV.** La luz solar es la principal fuente de energía para la vida en nuestro planeta, pero la radiación ultravioleta (UV) es dañina, al afectar a varias moléculas biológicas (i.e., lípidos, proteínas y ADN). Dado el deterioro de la capa de ozono, la comunidad científica intenta comprender el impacto biológico de la radiación UV, mediante el uso experimental las lámparas de luz UV y a pesar de que estas no alcanzan la intensidad real de las radiaciones naturales. Por otra parte, las condiciones incontrolables de las exposiciones ambientales, requiere de biomodelos capaces de sobrevivir y detectar diferentes biomarcadores. Sin embargo, no existen biomodelos estandarizados o confiables para medir el daño derivado de la radiación ultravioleta, contra diferentes indicadores. En este sentido, la bacteria *Caulobacter crescentus* es un microrganismo acuático usado como modelo para estudiar el ciclo celular y la mutagénesis. El propósito de este trabajo fue proponer las condiciones experimentales necesarias que permitan el uso de C. *crescentus* para la detección simultánea de tres marcadores genotóxicos: la citotoxicidad, la respuesta a daño al ADN y la mutación génica. Estos marcadores fueron medidos a través del Ensayo de supervivencia, el ensayo colorimétrico SOS y la mutagénesis Rif^R. Para estudiar el impacto de la radiación UV, se empleó el medio transparente M2, dadas las capacidades de absorción del medio completo (PYE). Se observó que la presencia del plásmido pP3213, que porta la fusión transcripcional P*imuA*::*lacZ*, no afecta los niveles de sobrevivencia y mutagénesis. El uso de C. *crescentus* para detectar los efectos genotóxicos solares ahorra recursos y tiempo experimental, ya que informa simultáneamente sobre múltiples marcadores.

Palabras clave: Biodosímetro, evaluaciones genotóxicas, mutagénesis Rif^R, ensayo SOS colorimétrico, ensayo de formación de colonias

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Introduction

There is a need for using multiple assays to evaluate the effect of DNA-damaging agents, in order to obtain reliable and complementary results. In this sense, different levels of genotoxic damage should be considered [1]. Particularly, when evaluating the DNA damaging effect of the full sprectrum of the sun's ultraviolet (UV) light, using only one genotoxic assay/endpoint per experiment limits the capacity to evaluate the influence of varied exposure conditions, some of them unable to be controlled during experimentation. Then, the number of genetic endpoints are mainly mediated by the biological model selected for the genotoxicity assay.

Some classic assays have been described to evaluate more than one genotoxicity level after a single exposure to UV light: the *Saccharomyces cerevisiae* D7 strain [2]; the SMART assay in *Drosophila melanogaster* [3]; and the micronuclei assay [4].

1. Mishra N, Srivastava R. Bacterial worth in genotoxicity assessment studies. J Microbiol Methods. 2023;215:106860.

2. Zimmermann FK, Kern R, Rasenberger H. A yeast strain for simultaneous detection of induced mitotic crossing over, mitotic gene conversion and reverse mutation. Mutat Res Genet Toxicol Environ Mutagen. 1975;28:381-8.

However, in bacteria, few assays integrate several endpoints. This is fundamental to assess environmental risks, for which natural but domesticated models could improve its detection and evaluation.

In this scenario, *Caulobacter crescentus* could be explored as a potential microorganism to evaluate UV radiation genotxicity. *C. crescentus* is an environmental gram-negative α-proteobacteria with a tightly-regulated dimorphic cell cycle, making of it an excellent model for studying cell cycle progression [5], and UV-induced DNA damage, repair, and mutagenesis. Its response against UVC radiation has been extensively studied [6-14]. There has been described the repair of pyrimidine dimers by mechanisms such as photoreactivation, nucleotide excision repair (NER), and SOS mutagenesis mediated by the *imuABC* operon [6-14]. However, further research is needed to fully understand the impact of other environmental UV wavelengths, including sunlight. Therefore, in this work, *C. crescentus* is proposed as a model microorganism for multitarget genotoxic assays. This is based on the variation of the experimental conditions employed and the analysis of the genetic characteristics of this bacterium supporting the evaluation of the UV light effects, including the use of deficient and proficient NER strains.

Materials and methods

Strains, reagents and cell growth conditions

C. crescentus wildtype cells (named NA 1000) [15] were conjugated with *Escherichia coli* S17-1 pP3213 (CC3213 promoter cloned into the pLACZ290 vector) [13] to obtain the strain NA 1000 pP3213. Strains stored at –80 ºC were recovered on plates filled with culture medium: *E. coli* in Luria–Bertani medium supplemented with tetracycline (15 mg/ mL) at 37 ºC, and *C. crescentus* in PYE medium supplemented with nalidixic acid (25 mg/mL) or tetracycline (1 mg/mL) as needed, at 30 ºC [16]. A strain with deletion of the *uvrA* gene was constructed through the Tn5 insertion previously reported (named *uvrA*$^-$) [17], and it was further conjugated (named *uvrA*$^-$ pP3213) [13].

Three endpoints from a single UV exposure were established by using a modified version of the protocol by Galhardo *et al.* [13]: cytotoxicity, DNA damage response, and gene mutation. In each experiment, a single bacterial colony was grown in liquid PYE media, under agitation at 200 rpm and 30 °C, overnight. Then, the saturated culture was 10-fold diluted and further grown until reaching 0.4 OD_{600nm} after, approximately, 3 h.

All the reagents used were analytical grade. Luria-Bertani, yeast extract, β-mercaptoethanol, antibiotics, and ONPG were obtained from Sigma-Aldrich (United States). Peptone and agar were acquired from KASVI (Brazil). Chloroform, Na_2CO_3, and salts required to prepare the M2 medium and buffers were purchased from Merck (Germany).

UV radiation conditions

A 2-mL culture sample was UV-irradiated in each 60-mm Petri dishes, uncovered. Some cells were exposed in PYE (colored) medium, and others in M2 medium (transparent) [16]. A UVC Philips TUV 15W/G15T8 germicidal lamp (mainly 254 nm) was used. The lamp energy was quantified with a UV radiometer VLX 3 W (Vilber Lourmat, Torcy, France), and the exposure time was calculated for the desired dose [18]. Non-irradiated cells were used as negative controls. Subsequent steps were done avoiding light, to prevent photorepair activation. After UV exposure, 1.5-mL samples of each culture were recovered, centrifuged, and resuspended in optimal PYE medium (1:1 v:v).

Survival test

Serial dilutions in base 100 of cells were performed and 10 µL of culture were plated in PYE medium. After 48 h of incubation at 30 ºC, viable cells were determined by counting the colony-forming units (cfu) [2, 19]. Survival (%) was calculated for each dilution by the formula:

$$\text{Survival (\%)} = \frac{\text{cfu of irradiated cells}}{\text{cfu of non-irradiated cells}} \times 100$$

SOS Chromotest

SOS induction was measured by the assay of the *imuA* promoter activity with *lacZ* transcriptional fusions [20]. A 1-mL aliquot of treated cells was incubated in PYE medium, and cells were recovered after treatment by incubation at 30 ºC under 200 rpm agitation, for 90 min. Next, OD_{600nm} was measured and 100 µL of chloroform and cells suspension (v:v) were dispensed in tubes containing 800 µL of buffer Z [20] enriched with 50 mM of β-mercaptoethanol. After quick agitation, the samples were incubated at 30 ºC in a dried bath for 5 min. Then, 200 µL of ONPG (4 mg/mL in buffer T [20]) were added to each tube, followed by agitation, and incubation for 5 min at 30 ºC. The reaction was stopped by adding 400 µL of 1 M Na_2CO_3, brief agitation, and cells were centrifuged at 10 000 rpm for 5 min. Finally, OD_{420nm} was measured and the induction of β galactosidase activity was calculated using the following equation:

$$U = \frac{OD_{420\ nm}}{OD_{600\ nm} \times t \times V}$$

where: U is β galactosidase units, t is the reaction time in minutes, and V is the cell volume in mL [20, 21].

Rif^R mutagenesis test

For mutagenesis experiments [22], 200 µL of treated cells were added to tubes containing 800 µL of PYE and grown overnight with constant shaking at 30 °C, to allow mutation fixation. Then, cells were sequentially diluted and plated on solid PYE. Non-diluted culture was also plated in PYE enriched with Rifampicin (100 µg/mL) to score Rif resistance mutant cells. After 48 h, colonies were counted, and the mutation frequencies were calculated:

$$\text{Mutation frequency} = \frac{\text{Total of mutants}}{\text{Number of viable cells} \times \text{dilution}}$$

Transmittance measurements

The absorbance capacity of different media was determined by measuring transmittance

3. Szabad J. A genetic assay for the detection of aneuploidy in the germ-line cells of *Drosophila melanogaster*. Mutat Res. 1986;164(5):305-26.

4. Kirsch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, Loock KV, *et al*. The *in vitro* MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. Arch Toxicol. 2011;85(8):873-99.

5. Barrows JM, Goley ED. Synchronized swarmers and sticky stalks: *Caulobacter crescentus* as a model for bacterial cell biology. J Bacteriol. 2023;205(2):e0038422.

6. Martins-Pinheiro M, Marques RCP, Menck CFM. Genome analysis of DNA repair genes in the alpha proteobacterium Caulobacter crescentus. BMC Microbiol. 2007;7:17.

7. Bender RA. Ultraviolet mutagenesis and inducible DNA repair in *Caulobacter crescentus*. Mol Gen Genet. 1984;197(3):399-402.

8. da Rocha RP, Paquola AC, Marques Mdo V, Menck CF, Galhardo RS. Characterization of the SOS regulon of Caulobacter crescentus. J Bacteriol. 2008;190:1209-18.

9. Alves IR, Vêncio RZ, Galhardo RS. Whole genome analysis of UV-induced mutagenesis in Caulobacter crescentus. Mutat Res. 2022;825:111787.

10. Alves IR, Lima-Noronha MA, Silva LG, Fernández-Silva FS, Freitas ALD, Marques MV, *et al*. Effect of SOS-induced levels of imuABC on spontaneous and damage-induced mutagenesis in *Caulobacter crescentus*. DNA Repair (Amst). 2017;59:20-6.

11. da Rocha RP. The SOS response of *Caulobacter crescentus* and the relationship between DNA repair mechanisms and the cell cycle progression [dissertation]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2011 [cited 2022 Oct 17]. 169 p. Available from: https://www.teses.usp.br/teses/disponiveis/42/42132/tde-21102011-164953/pt-br.php

12. Ozturk N, Kao YT, Selby CP, Kavakli IH, Partch CL, Zhong D, *et al*. Purification and characterization of a Type III photolyase from Caulobacter crescentus. Biochemistry. 2008;47(39):10255-61.

13. Galhardo RS, Rocha RP, Marques MV, Menck CFM. An SOS-regulated operon involved in damage-inducible mutagenesis in *Caulobacter crescentus*. Nucleic Acids Res. 2005;33(8):2603-14.

14. Joseph AM, Daw S, Sadhir I, Badrinarayanan A. Coordination between nucleotide excision repair and specialized polymerase DnaE2 action enables DNA damage survival in non-replicating bacteria. Elife;10:e67552.

15. Evinger M, Agabian N. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. J Bacteriol. 1977;132:294-301.

16. Ely B. Genetics of *Caulobacter crescentus*. Methods Enzymol. 1991;204:372-84.

17. Lopes-Kulishev CO, Alves IR, Valencia EY, Pidhirnyj MI, Fernández-Silva FS, Rodrigues TR, *et al*. Functional characterization of two SOS-regulated genes involved in mitomycin C resistance in *Caulobacter crescentus*. DNA repair (Amst). 2015;33:78-89.

of PYE and M2 media at 254 nm (UVC), 312 nm (UVB), and 365 nm (UVA) [23], by using a Genesis TM Series 10 spectrophotometer (Thermo Electron Corporation). The negative control was sterile miliQ water.

Immuno-slot blot

After UV irradiation in M2 medium, cells were collected and the chromosomal DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega Madison, WI, USA) and quantified with Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Inc, USA), according to the manufacturer specifications. Slot blot assays were performed only to corroborate the CPDs generated in non-irradiated and UVC-irradiated cells following the procedures described by Schuch *et al*. [18], with some modifications. Briefly, 200 ng of DNA were diluted in TE for a final volume of 100 µL for each sample. The DNA was denatured boiling for 10 minutes at 100 ºC, then immediately transferred to ice, and 100 µL of 2 M NH_4OAc was added. Samples were transferred to an Amersham Hybond-N+ (GE Healthcare Life Sciences) membrane previously pre-wetted in 1 M NH_4OAc for 5 min, using a vacuum pump and a Slot-blot apparatus (Omniphor, San Jose, CA, USA). Subsequently, the membrane was incubated in 5× SSC buffer (750 mM NaCl, 75 mM sodium citrate, Sigma-Aldrich) for 15 min, dried at room temperature for another 15 minutes, and baked for 1 h at 80 °C. Thereafter, the membrane was blocked overnight at 4 ºC in 5 % (w/v) non-fat milk prepared in PBS-T (137 mM NaCl; 2.7 mM KCl; $Na_2HPO_4 \cdot 7H_2O$ 4.3 mM; 1.4 mM KH_2PO_4, 0.1 % Tween 20) under constant shaking. The membrane was further incubated with the anti-CPD monoclonal primary antibody TDM-2 (Cosmo Bio Co., Ltd, Japan), 1:2000 diluted for 1 h at 37 °C under constant shaking. Next, following three washes with PBS-T for 5 min, the membrane was incubated for 2 h at room temperature with the secondary antibody, Peroxidase labelled anti-mouse IgG (whole molecule) R-Phycoerythrin antibody, produced in goat (Sigma- Aldrich, Inc.), diluted 1:5000. After successive washes, the membrane was revealed with Immobilon Forte Western HRP substrate (Millipore, USA). The bands were visualized using Alliance Q9 Advanced (UVITEC, Cambridge, UK) equipment and quantified using ImageJ 1.51r (National Institutes of Health, USA; http://imagej.nih.gov/ij).

Immuno-slot blot

All tests were performed using the software Statistica 14.0.0.15 (TIBCO Software Inc., USA). The experimental values were expressed as the mean ± standard error for all the measured parameters. The variables were analyzed using the Kolmogorov-Smirnov test for normality and Brown-Forsythe test for variance homogeneity, as premises for the Tukey's test. Also, a non-parametric Spearman Correlation was performed. Probability ($p < 0.05$) was considered statistically significant.

Results and discussion

A multitask approach is introduced in this work to assess UV-induced effects in survival, repair, and mutagenesis, by using NER proficient and deficient strains of *C. crescentus*. The inclusion of both lineages supports the comprehensive examination of how variations in DNA repair mechanisms influence the genotoxic outcomes. Cell survival is measured through cell division in the forming colonies assay. In fact, Rif^R mutagenesis identifies mutations in the rpoB gene (β-subunit of the RNA polymerase), which is a simple system applicable to various microorganisms [22]. At the same time, the *imuABC* operon expression can be detected in both, UV and non-irradiated, growing cells [13]. Moreover, the fusion of the *imuA* promoter and *lacZ* gene is a powerful tool to detect SOS responses to DNA damage, the SOS induction assessment requiring strain transformation with the pP3213 plasmid. The 90-min recovery period was set according to our experimental findings (data not shown) and as reported [13, 24].

Meanwhile, plasmids regulate traits crucial for bacterial survival and adaptation, in addition to their role in gene transfer [25]. In our experimental conditions, the presence of pP3213 plasmid in *C. crescentus* did not modify the number and frequency of colony forming units, neither the frequency of UVC-induced or spontaneous mutagenesis (Figure 1).

The survival, *imuA* induction, and rifampicin resistance of *C. crescentus* cells irradiated in both PYE and M2 medium for wild-type and *uvrA* deficient strains, are shown in figure 2. Remarkably, there was a substantial increase in genotoxicity levels (two-fold or higher), in comparison to the values of non-irradiated controls.

There has been previously shown that wild-type cells, irradiated up to 90 and 150 J/m^2 in PYE, decreased the colony forming capacity to 50 [17] and 10 % [15, 20], respectively, coincident with our results (Figure 2A, black line). In this sense, Da Rocha [11] reported that synchronized cultures survival of *uvrA* cells decreased to 5 % when irradiated with 15 J/m^2 in PYE media. That sharp sensitivity as compared to our findings (Figure 2D), may be attributed to the exponential phase, resulting in a more heterogeneous cell population with a greater capacity for repair through recombination [26].

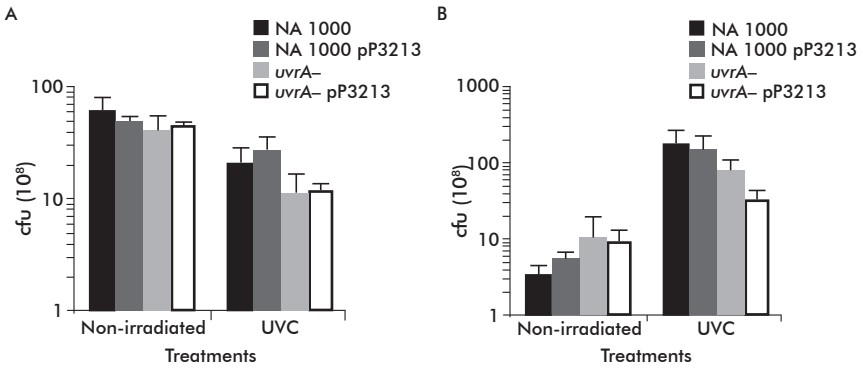

Figure 1. Effect of the presence of pP3213 plasmid in irradiated *Caulobacter crescentus* cells. Wild type and *uvrA* deficient strains were UVC-irradiated with 50 and 5 J/m^2, respectively. A) Colony forming units (cfu). B) Frequency of Rif^R mutations induced in the *rpoB* gene. The results are the mean of at least three independent experiments. Error bars represent the standard deviation. No statistical differences were found within each treatment for each treatment group, NA 1000 and *uvrA*–, respectively, by the Mann-Whitney U-test ($p < 0.05$).

18. Schuch AP, da Silva Galhardo R, de Lima-Bessa KM, Schuch NJ, Menck CF. Development of a DNA-dosimeter system for monitoring the effects of solar-ultraviolet radiation. Photochem Photobiol Sci. 2009;8(1):111-20.

19. Kirkland DJ. Chromosomal aberration tests *in vitro*: problems with protocol design and interpretation of results. Mutagenesis. 1992;7(2):95-106.

20. Miller JH. Experiments in Molecular Genetics. New York: Cold Spring Harbor Laboratory Press; 1972.

21. Miller JH. A short course in bacterial genetics: A laboratory manual and handbook for *Escherichia coli* and related bacteria. New York: Cold Spring Harbor Laboratory Press; 1992.

22. Garibyan L, Huang T, Kim M, Wolff E, Nguyen A, Nguyen T, *et al*. Use of the rpoB gene to determine the specificity of base substitution mutations on the *Escherichia coli* chromosome. DNA Repair (Amst). 2003;2(5):593-608.

23. Rosenstein BS, Weinstock MA, Habib R. Transmittance spectra and theoretical sun protection factors for a series of sunscreen-containing sun care products. Photodermatol Photoimmunol Photomed. 1999;15(2):75-80.

24. Modell JW, Hopkins AC, Laub MT. A DNA damage checkpoint in *Caulobacter crescentus* inhibits cell division through a direct interaction with FtsW. Genes Dev. 2011;25:1328-43.

25. Thompson CMA, Hall JPJ, Chandra G, Martins C, Saalbach G, Panturat S, *et al*. Plasmids manipulate bacterial behaviour through translational regulatory crosstalk. PLoS Biol. 2023;21(2): e3001988.

26. Gozzi K, Tran NT, Modell JW, Le TBK, Laub MT. Prophage-like gene transfer agents promote *Caulobacter crescentus* survival and DNA repair during stationary phase. PLoS Biol. 2022;20(11): e3001790.

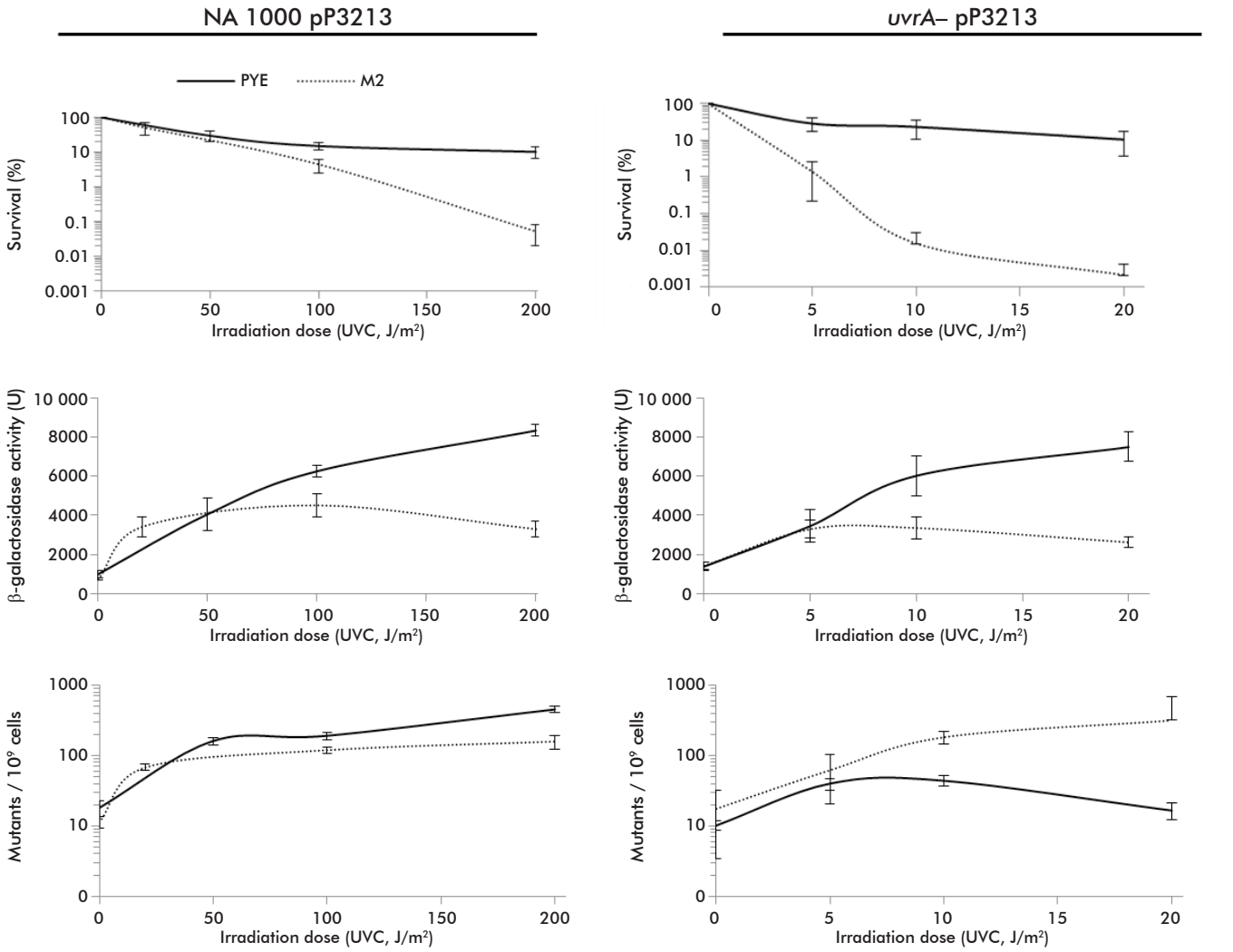

Figure 2. Effects of UVC light in *C. crescentus* wild-type and deficient cells, irradiated in different growth media PYE and M2. A, D) Survival curve. B, E) Expression of the *imuA* promoter in transcriptional fusions with the *lacZ* gene. C, F) Frequency of Rif^R mutations induced in the *rpoB* gene. Results are presented as mean ± standard error of three independent experiments.

Furthermore, the first dose select for the irradiation of wild-type cells in M2 transparent medium was lower than in PYE medium, with survival drastically decreased under these conditions (Figure 2A and D, gray line). As noted by Alves *et al*. [10] when working with NA 1000 using sterile water, changes in the irradiation process lead to varying bacterial responses, something consistent with our results. Survival reduction was more significant in the *uvrA* strain, despite receiving only one-tenth of the irradiation dose.

Regarding the β galactosidase activity units reported (Figures 2B and E, black line), they were in agreement with previous results for wild-type cells irradiated in PYE medium [11, 13]. A five-fold SOS increase was also reported by Modell *et al*. [24] when irradiating with 100 J/m^2. The induction of *imuA* in the *uvrA* background, a novel aspect introduced in this study, provided results at similar levels by using just one-tenth of the energy required for the wild type strain.

Despite, both strains were unable to trigger high levels of SOS when irradiated in M2 medium (Figure 2B and E). The lower SOS induction measured by the Slot blot assay (Figure 3) confirmed it as unrelated to the absence of CPD damage in irradiated *C. crescentus* cells in M2 medium. In fact, cells predisposed to induced death tend to exhibit a reduced SOS response. In this regard, Modell *et al*. [24] achieved comparable outcomes, showing a two- or three-fold increase in *imuA* mRNA after UVC irradiation.

Even with more variability, mutagenesis results were in the same range as previously reported for UVC-irradiated wild-type cells (Figure 2C, black line) [13, 17]. Mutation levels induced in the M2 condition did not vary as compared to PYE, also in the range reported by Alves [10] for the wild-type strain. Despite, the deficient background evidenced an unchanged mutagenesis process in irradiated PYE, which was increased with M2 upon irradiation (Figure 2F). This apparent contradiction could be

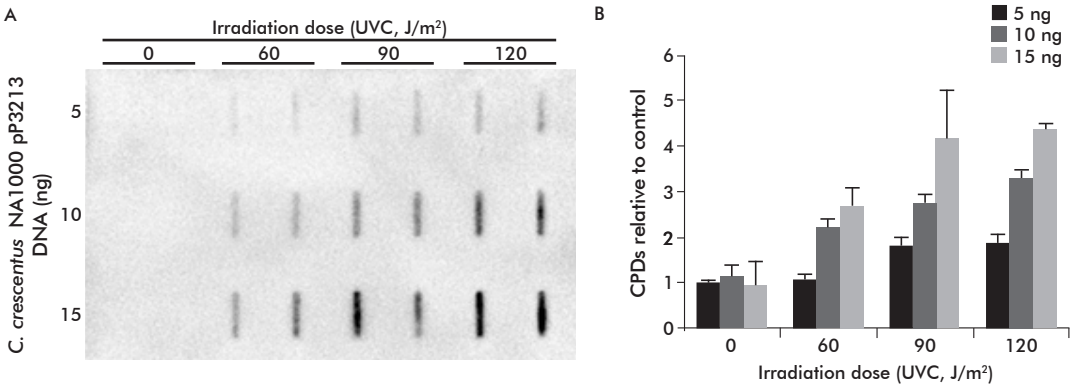

Figure 3. Immunoslot blot detection of cyclobutane pyrimidine dimers (CPDs) produced in the DNA of C. *crescentus* NA1000 pP3213 cells, irradiated with UVC in transparent M2 medium. A) Detection through the use of antibody against CPDs. B) Quantification of CPDs induction, fold increase against 5-ng of unexposed control samples.

concealed by considering that PYE is a colored-rich media, capable of absorbing part of the UVC light (Table 1), and the doses employed for these cells are lower than that for the wild-type strain. Additionally, the use of the *rpoB* gene as a mutational marker has limitations [22]. Even so, the correlation with the other endpoints was statistically significant (Table 2).

UVC variations significantly affect genetic parameters in both strains and conditions. The *imuA* induction and mutagenesis statistically increase with dosage, with a drop in survival. Additionally, biological parameters are interrelated, for instance, SOS induction is linked to both reduced survival and induced mutagenesis. As shown, the R values demonstrated that the variables did not follow a linear relationship.

Furthermore, results were statistically significant in all cases, except for the *uvrA* background. When the SOS response is functioning properly, extreme levels of cell death are not anticipated, but mutation frequency should increase, as seen in the wild-type strain. In the case of the *uvrA* strain, absence of SOS response and elevated death rates were evidenced [27]. Apoptosis-like events have been described in *C. crescentus*, triggered by the *bapE* gene [28]. According to Modell *et al*. [24], the relative amounts of *imuA*/*bapE* mRNA, 90 min after irradiating cells with 100 J/m^2, differed using PYE or M2 media during irradiation. In PYE, both genes maintained the same *imuA*/*bapE* mRNA levels, but expression was three-fold lower in M2 medium. This could suggest a preference for an apoptosis-like pathway in the M2 medium.

The findings of transmittance measurements reinforce that PYE is unsuitable for irradiation, as it absorbs various UV wavelengths, including biologically relevant UVA and UVB (Table 1). These emphasize the recommendation to use a transparent suspension during irradiation.

In summary, in this work, the usefulness of *C. crescentus* to study the genotoxic effects was established, measuring four parameters simultaneously: *imuA*:*lacZ* expression, rifampicin resistance and colony formation, and when added, CPD immunodetection. Using this approach, multiple levels of damage, repair, mutagenesis, and survival can be tested from the same hazardous exposure. While UVC lamp radiation was the agent tested, this strategy could be successfully used to evaluate the effect of exposure to other UV wavelengths and direct sunlight [29]. Furthermore, our experimental approach is potentially applicable to the study of other genotoxic stressors.

Table 1. Transmittance of irradiation media against the UV light emitted by artificial lamps in M2 and PYE cell culture media

	Culture medium transmittance	
Wavelength	M2	PYE
UVC 254 nm	100	3.5
UVB 312 nm	98	28.0
UVA 365 nm	100	63.0

Table 2. Correlation analyses between each pair of biological variables and UVC irradiation in PYE and M2 medium for wild-type and deficient cells (in parenthesis)

		M2 medium			
	Variables	UVC dose	SOS induction	Survival	Mutation frequency
PYE medium	UVC dose		0.58* (0.41*)	–0.97* (–0.94*)	0.67* (0.84*)
	SOS induction	0.86* (0.74*)		–0.58* (–0.30)	0.65* (0.35)
	Survival	–0.92* (–0.78*)	–0.84* (–0.68*)		–0.48* (–0.79)
	Mutation frequency	0.75* (0.16)	0.62* (0.02)	–0.72* (–0.28)*	

* R values followed by * indicate statistically significant transmittance values as determined by the Spearman's test ($p < 0.05$)

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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27. Kamat A, Badrinarayanan A. SOS-independent bacterial DNA damage responses: diverse mechanisms, unifying function. Curr Opin Microbiol. 2023;73:102323.

28. Bos J, Yakhnina AA, Gitai Z. BapE DNA endonuclease induces an apoptotic-like response to DNA damage in Caulobacter. Proc Natl Acad Sci U S A. 2012;109(44):18096-101.

29. Fuentes-León F, Quintero-Ruiz N, Fernández-Silva FS, Munford V, Tamayo MV, Menck CFM, *et al*. Genotoxicity of ultraviolet light and sunlight in the bacterium *Caulobacter crescentus*: Wavelength-dependence. Mutat Res Genet Toxicol Environ Mutagen. 2024;894:503727.

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