DNA-REPAIR DEFICIENT STRAINS *CHLAMYDOMONAS*
REINHARDTII AS GOOD TOOLS FOR ENVIRONMENTAL
TOXICOLOGY

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Abstract

The aim of the present study was to compare stress response of *Chlamydomonas reinhardtii* strains with different DNA repair capability to zeocin in order to develop a set of endpoints for the revealing of very low levels of xenobiotics. Four *C. reinhardtii* genotypes – 137C (wild type); CW15 (cell-wall-less); UVS-10 (*recombination*-repair deficient); UVS-14 (*mismatch*-repair deficient) and different endpoints were applied – micro-colonies survival assay, malondialdehyde (MDA) and intracellular H₂O₂ quantities, carotenoids content, double-strand DNA breaks (DSBs) induction. Based on the cell survival, strains could be arranged as follows: WT > CW > UVS10 ~ UVS14. The strongest oxidative stress, measured as the highest MDA and H₂O₂ contents, was evaluated for UVS-14 genotype. The highest quantity of DSBs induced was measured after zeocin treatment with low doses (2–10 µg/ml) for UVS-10 genotype ($P < 0.01$). Based on the data reported, a short-term bioassay based on *C. reinhardtii* with DNA repair-deficient genotypes is proposed to be successfully used in genotoxicity screening of low doses xenobiotics – micro-colonies assay on both strains (UVS-10 and UVS-14); measurement of the kinetics of MDA and H₂O₂ content on strain UVS-14, and primary induced DSBs levels on strain UVS-10.

Key words: *Chlamydomonas reinhardtii*, genotype susceptibility, DSBs induction, oxidative stress, zeocin

Introduction. Increased human activity in different areas of life – agriculture, industry, and urbanization, has resulted in release of huge amount of xenobiotics in the environment [1]. An important aspect of environmental genotoxicology is the development of highly sensitive bioindicator and biomarker systems for the

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detection of bioavailability of low doses xenobiotics in biota, including photosynthetic organisms such as primary producers [2].

In our previous works, we used *C. reinhardtii* as a test system for toxic and/or genotoxic screening and risk assessment of polluted soil [3] and air [4]; γ -rays or radiomimetics [5,6], heat treatment [7], etc. Additionally, *C. reinhardtii* was used as a model organism to study mechanisms of genotype resistance/susceptibility to oxidative stress [8]. Previously it was shown by us that DNA repair deficient strains UVS-10 and UVS-14 are more susceptible to cadmium induced oxidative stress compared to DNA repair proficient genotypes [9]. Pronounced antioxidant and HSP70B responses to paraquat-induced oxidative stress were also observed in two paraquat-susceptible *C. reinhardtii* genotypes [10], which provoked us to convey future experiments in order to clarify whether these genotypes could be used for the purposes of genotoxicology.

Based on our previous findings, here we hypothesized that DNA repair deficient genotypes UVS-10 and UVS-14 are probably more susceptible to oxidative stress and could be successfully used for revealing of very low levels of inducers of oxidative stress. To test our hypothesis, the antibiotic zeocin (InvivoGen) was chosen as a model genotoxic agent due to two main reasons: well-known mode of action of zeocin and long-term experience in our lab with zeocin.

Materials and methods. Two DNA repair proficient genotypes: wild type (WT) 137C and cell-wall-less CW15, described in [10] and two DNA repair deficient genotypes: *recombination*-repair deficient UVS-10 and *mismatch*-repair deficient UVS-14, described in [9] were used. Cell suspensions (4–5 days old) were treated with zeocin (2, 6, 10, 20, 60, 100, 150 and 300 $\mu\text{g}/\text{ml}$) for 1 min. Concentrations that induce LD₅₀ were calculated [9]. MDA and H₂O₂ quantities [9] and carotenoids contents were measured [9] 1 h and 4 h after zeocin treatment. The fraction of induced DSBs was measured by CFGE as described in [6].

Experiments were repeated at least three times from independently grown algal cultures. Student's *t*-test for statistical assessment of results (exposure vs. control) was used. One-way ANOVA with Tukey multiple comparison tests were performed to compare mean differences among genotypes (GraphPad Prism 5 software). Two-way ANOVA with Bonferroni post-tests were applied to determine how a response was affected by genotype, concentration and interaction between them (GraphPad Prism 5 software).

Results and discussion. A dose-dependent increase of the genotoxic effect of zeocin was demonstrated (data not shown). Its strength was found to depend on the genotype and probably on the morphological characteristics of the cells (presence or absence of cell wall). Based on the susceptibility towards zeocin the strains could be arranged as follows: DNA-repair deficient > cell-wall-less > WT. Both DNA-repair deficient genotypes manifested approximately equal highest susceptibility to zeocin based on the concentrations inducing 50% lethality (LD₅₀ $\sim$ 2.5 $\mu\text{g}/\text{ml}$) (Fig. 1).

Fig. 1. LD₅₀ induced by different zeocin concentrations in *C. reinhardtii* genotypes 137C, CW15, UVS-10 and UVS-14. Mean data are from three independent experiments. Error bars represent standard errors of mean values. Statistically significant differences between: 137C and UVS-10 ($P < 0.0001$), 137C and UVS-14 ($P < 0.0001$), 137C and CW15 ($P < 0.0001$), CW15 and UVS-10 ($P < 0.0001$), CW15 and UVS-14 ($P < 0.0001$). Two-way ANOVA with Bonferroni post-tests showed that the factor genotype contributed the most on the total variance of the LD₅₀ values – 99.27% ($F = 2177.68$, $df = 3$; $P < 0.0001$)

On the other hand, LD₅₀ for the cell-wall-less genotype was calculated as 9.63 µg/ml and as 25.12 µg/ml for the WT. The higher susceptibility of the cell-wall-less genotype to zeocin in comparison with the WT could be explained by the lack of cell wall. Our results confirmed the suggestions of [11] that the cell wall is one of the cellular defense pathways against radiomimetic bleomycin. Moreover, in our previous findings different susceptibility to other genotoxic agents – paraquat and CdCl₂ was also found [9,12].

Statistically significant increase in the MDA and H₂O₂ contents after the treatment with the lowest tested zeocin concentration (2 µg/ml) was measured for all genotypes (UVS-10, UVS-14, CW15) except the WT (Fig. 2). Based on the results for MDA, measured one hour after the treatment, well-expressed dose dependence was observed only for the repair-deficient strains UVS-10 and UVS-14. The levels were statistically significantly higher than those in CW15 ($P < 0.05$) and 137C ($P < 0.01$). Non-significant differences in the MDA content were measured between the 1st and the 4th hour after the treatments for all genotypes ($P > 0.05$). The most sensitive strain was found to be UVS-14.

Concerning the other endpoint – H₂O₂ content, such dependence was evaluated for UVS-10, UVS-14 and CW15 again one hour after the treatment. The levels remained similar between the 1st and the 4th hour after the treatment with low doses zeocin (2 and 6 µg/ml) for all genotypes ($P > 0.05$). The genotype with

Fig. 2. Effect of zeocin on MDA (A), H_2O_2 (B) and carotenoids (C) contents in *C. reinhardtii* genotypes 137C, CW15, UVS-10 and UVS-14, 1 h and 4 h after the treatment. Mean data are from three independent experiments. Error bars represent standard errors of mean values. Where no error bars are evident, errors were equal to or smaller than the symbols. Two-way ANOVA with Bonferroni post-tests showed that the MDA and H_2O_2 quantities were statistically significantly affected mostly by the factor zeocin concentration (MDA – $F = 122.93$; $df = 4$; $P = 0.0001$; H_2O_2 – $F = 175.54$; $df = 4$; $P = 0.0001$), followed by the factor – genotype (MDA – $F = 85.13$; $df = 7$; $P = 0.0001$; H_2O_2 – $F = 117.72$; $df = 7$; $P = 0.0001$)

the most pronounced increase in the levels of MDA and H_2O_2 was the *mismatch*-repair deficient one ($P < 0.01$; $P < 0.001$), indicating strongest zeocin-induced oxidative stress response in UVS-14 genotype. This result is in correspondence with our already reported data that the genotype UVS-14 shows the strongest oxidative stress response to another genotoxin – $CdCl_2$ [9]. Our results are in good agreement with the findings of [13,14].

Carotenoids, playing a substantial role in countering oxidative stress in plants [15], were chosen as a third marker for the evaluation of zeocin-induced oxidative stress. It is known that higher levels of constitutive or induced antioxidants are in a correlation with greater resistance in plants to different types of environmental

oxidative stress [10]. In our experiments, all genotypes have approximately similar constitutive levels of carotenoids (data not shown). No differences were observed depending on the doses in the range of low zeocin concentrations 2–10 µg/ml (Fig. 2). The carotenoids content was statistically significantly increased only after the treatment with the highest zeocin concentration tested (100 µg/ml) in the *mismatch*-repair deficient genotype one hour ($P < 0.01$) and four hours ($P < 0.0001$) after the treatment. Therefore, based on these data, it could be speculated that in the present research, carotenoids content is not sensitive enough as an endpoint for detection of the effect of low doses of zeocin.

As a next step, DSB induction was used as a molecular marker for the evaluation of DNA susceptibility. Our results revealed approximately equal DSBs levels despite the dose in the DNA repair deficient genotypes after zeocin treatment with concentrations in the range 10–100 µg/ml (Fig. 3A). The DSBs levels were around 2-fold higher than those measured and reported for WT by [6] and [16].

In order to evaluate the suitability of this molecular marker for our purpose, low doses of zeocin (2–10 µg/ml) were tested on both DNA repair deficient genotypes and cell-wall-less genotype. The highest induced DSBs levels were measured in *rec*-repair deficient genotype (UVS-10) (Fig. 3B). The well-expressed increase of DSBs in the *rec*-repair deficient genotype comparing with the levels induced in the *mismatch*-repair deficient genotype suggests that UVS-10 is more sensitive

Fig. 3. Induction of DSBs, measured as the mean fraction of DNA released (FDR value) during electrophoresis, in *C. reinhardtii* genotypes: UVS-14, UVS-10 and CW15 after zeocin treatment in two concentration ranges: (A) – 10–100 µg/ml zeocin; (B) – 2–10 µg/ml. Mean data are from three independent experiments. Error bars represent standard errors of mean values. Where no error bars are evident, errors were equal to or smaller than the symbols. (A): Two-way ANOVA with Bonferroni post-tests showed that the zeocin-induced DSBs levels were significantly dependent on the genotype ($F = 58.44$, $df = 3$; $P < 0.0001$), the zeocin concentration ($F = 63.09$, $df = 8$; $P < 0.0001$) and the interaction between them ($F = 3.43$, $df = 24$; $P < 0.0001$). (B): Two-way ANOVA with Bonferroni post-tests showed that zeocin-induced DSBs levels were statistically significantly affected by the factors genotype ($F = 54.22$, $df = 3$; $P < 0.0001$), zeocin concentration ($F = 18.76$, $df = 5$; $P < 0.0001$) and the interaction between them ($F = 5.62$, $df = 10$; $P < 0.0001$).

to zeocin-induced DSBs. Our results are in agreement with the findings of [17,18] for the high susceptibility of the *rec*-repair deficient *C. reinhardtii* genotype to different chemicals inducing DNA strand breaks. The authors suggested that a gene responsible for *recombination* repair has an important role in repair of DNA-damages induced by MNNG and phenylcarbamic acid esters in *C. reinhardtii*. Our results are in accordance with the findings of [19], who reported higher sensitivity of *mismatch*-repair proficient tumour cells vs. *mismatch*-repair deficient tumour cells to BLM-induced DSBs recombination (HR) by *mismatch*-repair.

Conclusions. Here a set of endpoints based on two DNA repair deficient strains of *C. reinhardtii* is presented. It is shown that UVS-10 *rec*-repair deficient and UVS-14 *mismatch* repair deficient strains are good enough for the revealing of a bioactivity of low doses xenobiotics. Several endpoints are recommended: micro-colonies survival assay; kinetics of MDA and H₂O₂; DSBs induction. Both strains UVS-10 and UVS-14 could be successfully involved in genotoxicology for the revealing of low doses contaminants with unknown mode of action.

REFERENCES

- [1] KUMAR V., S.K. SHAHI, S. SINGH (2018) Bioremediation: an eco-sustainable approach for restoration of contaminated sites, In: Microbial Bioprospecting for Sustainable Development (eds J. Singh et al.), Singapore, Springer, 115–136.
- [2] POGÁNYOVA A., K. MIČIETA, J. DUŠIČKA (2019) Genotoxic assessment of selected native plants to differentially exposed urban ecosystems, J. Environ. Sci. Pollut. Res., **26**(9), 9055–9064.
- [3] DIMITROVA M., E. DIMOVA, Z. MITROVSKA, V. KAPCHINA-TOTEVA, S. CHANKOVA (2007) Testing of polluted soil samples for genotoxic potential using *Chlamydomonas reinhardtii*, Alg. Stud., **123**, 111–121.
- [4] DIMITROVA M., Z. MITROVSKA, V. KAPCHINA-TOTEVA, E. DIMOVA, S. CHANKOVA (2009) Testing of polluted air samples for genotoxic potential using *Chlamydomonas reinhardtii*, C. R. Acad. Bulg. Sci., **62**(1), 57–62.
- [5] CHANKOVA S. G., P. E. BRYANT (2002) Acceleration of DNA-double strand rejoining during the adaptive response of *Chlamydomonas reinhardtii*, Radiat. Biol. Radioecol., **42**, 600–603.
- [6] CHANKOVA S. G., E. DIMOVA, M. DIMITROVA, P. E. BRYANT (2007) Induction of DNA double-strand breaks by zeocin in *Chlamydomonas reinhardtii* and the role of increased DNA double-strand breaks rejoining in the formation of an adaptive response, Radiat. Environ. Biophys., **46**, 409–416.
- [7] CHANKOVA S. G., N. YURINA, E. DIMOVA, O. ERMOHINA, Y. OLESKINA et al. (2009) Pretreatment with heat does not affect double strand breaks DNA rejoining in *Chlamydomonas reinhardtii*, J. Therm. Biol., **34**, 332–336.
- [8] CHANKOVA S. G., N. YURINA (2012) In: Radiobiology and Environmental Security (eds C. Mothersill, V. Korogodina, C. Seymour), Dordrecht, Springer, 19–30.
- [9] DIMITROVA M., D. MITEVA, Z. MITROVSKA, S. CHANKOVA (2014) Cadmium induced stress response in different genotypes of *Chlamydomonas reinhardtii*, C. R. Acad. Bulg. Sci., **67**(7), 943–950.

- [10] CHANKOVA S., E. DIMOVA, Z. MITROVSKA, D. MITEVA, D. MOKEROVA et al. (2014) Antioxidant and HSP70B responses in *Chlamydomonas reinhardtii* genotypes with different resistance to oxidative stress, *Ecotoxicol. Environ. Saf.*, **101**, 131–137.
- [11] AOUIDA M., O. TOUNEKTİ, A. LEDUC, O. BELHADJ, L. MIR et al. (2004) Isolation and characterization of *Saccharomyces cerevisiae* mutants with enhanced resistance to the anticancer drug bleomycin, *Curr. Genet.*, **45**, 265–272.
- [12] DIMOVA E., M. DIMITROVA, D. MITEVA, Z. MITROVSKA, S. CHANKOVA (2008) Paraquat induced adaptive response in *Chlamydomonas reinhardtii* strains depends on the genotype, *C. R. Acad. Bulg. Sci.*, **61**(7), 911–918.
- [13] KAUR S., M. ARORA, A. K. GUPTA, N. KAUR (2012) Exploration of biochemical and molecular diversity in chickpea seeds to categorize cold stress-tolerant and susceptible genotypes, *Acta Physiol. Plant*, **34**(2), 569–580.
- [14] MANDHANIA S., S. MADAN, V. SAWHNEY (2006) Antioxidant defense mechanism under salt stress in wheat seedlings, *Biol. Plant.*, **50**, 227–31.
- [15] MELEGARI S. P., F. PERREAULT, R. H. R. COSTA, R. POPOVIC, W. G. MATIAS (2013) Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*, *Aquat. Toxicol.*, **142**, 431–440.
- [16] DIMOVA E., M. DIMITROVA, D. MITEVA, Z. MITROVSKA, N. YURINA et al. (2009) Does single-dose cell resistance to the radio-mimetic zeocin correlate with a zeocin-induced adaptive response in *Chlamydomonas reinhardtii* strains?, *Radiat. Environ. Biophys.*, **48**, 77–84.
- [17] MIADOKOVÁ E., S. PODSTAVKOVÁ, Z. ČERVENÁK, D. VLČEK (1994) Different responses of repair-deficient strains of *Chlamydomonas reinhardtii* to UV and MNNG treatments, *Biologia (Bratislava)*, **49**, 633–637.
- [18] MIADOKOVÁ E., K. ŠEPAKOVÁ, S. PODSTAVKOVÁ, D. VLČEK (1995) Effects of 3-(2-alkoxyphenyl)carbamoyloxy) chinuclidum chlorides on repair-deficient strains of *Chlamydomonas reinhardtii*, *Biol. Plantarum*, **37**(1), 15–19.
- [19] VERNOLE P., A. MUZI, A. VOLPI, A. S. DORIO, A. TERRINONI et al. (2009) Inhibition of homologous recombination by treatment with BVDU (brivudin) or by RAD51 silencing increases chromosomal damage induced by bleomycin in mismatch repair-deficient tumour cells, *Mutat. Res./Fund. Mol. M.*, **664**(1–2), 39–47.

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