Heterologous induction of a predicted promoter sequence for paraquat-inducible genes of *Chromobacterium violaceum* in response to paraquat compound

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(With 1 Figure)

The *Chromobacterium violaceum* is a Gram-negative, free-living betaproteobacterium that dominates a variety of ecosystems in tropical and subtropical regions. Notably, several refined mechanisms related to remarkable and exploitable adaptability have been revealed in the genome prospecting of this bacterium, including biological responses to oxidative stress by predicting of paraquat-inducible proteins (Brazilian National Genome Project Consortium, 2003). In the cited study, two open reading frames (ORFs) for paraquat-inducible proteins were identified during genome annotation analyses of *C. violaceum* ATCC 12472, demonstrating a high similarity to sequences of paraquat-inducible genes (*pqi* genes) previously characterized in the *Escherichia coli* bacterium (Farr and Kogoma, 1991). The paraquat-inducible genes are drastically modulated by acting of several oxidizing agents, unleashing a complex cellular response to oxidative stress in a great variety of bacterial strains to minimize deleterious effects of the superoxide radical-generating compounds on the maintenance of the cellular homeostasis (Hungria et al., 2004). Thus, the purpose of the present study was to functionally evaluate the influence of the paraquat compound on the heterologous induction of the predicted promoter sequence for paraquat-inducible genes revealed during genome annotation analyses of the *C. violaceum* bacterium.

Initially, specific primers were designed by using computational program (http://www.idtdncom/SciTools/SciTools.aspx) to flank target sites situated between ORFs CV2550 and CV2551, corresponding to the promoter sequence of paraquat-inducible genes predicted during *C. violaceum* genome annotation analyses. Sequences of the forward (5'–CGT**GAATTC**TAATGGCAGACCGACATCAG–3') and reverse (5'–GGT**AGATCT**TTTCGTGCGGGTGCTGTTTC–3') primers were constructed according to Sambrook and Russell (2001), containing sites-specific DNA cleavage for restriction enzymes *Eco*RI and *Bgl*II, respectively (bold and underlined bases). Genomic DNA of *C. violaceum* ATCC 12472 isolated from saline solution and phenol-chloroform extraction, was amplified in PCR buffer at a final concentration of 1× (20 mM Tris-HCl pH 8.4, 50 mM KCl), 0.4 mM dNTPs, 2.5 mM magnesium chloride, 1.2 µM of specific primers and 1 unit of the Platinum® *Taq* DNA Polymerase enzyme (Life Technologies) at a final volume of 25 µL. The amplification reactions comprehended denaturation at 95 °C for 30 s, annealing at 64 °C for 45 s and extension at 72 °C for 45 s, totaling 35 cycles. The 388 bp amplicon was ligated into broad host range cloning vector pMP220 that contains the *E. coli lacZ* gene without a promoter (Spaink et al., 1987). Competent *E. coli* S17 strains were transformed by electroporation for insertion of the conjugative vector fused to the promoter of interest, as established by Sambrook and Russel (2001), followed by cellular growth of the bacterial isolates onto Luria-Bertani (LB) agar and 12.5 µg/mL tetracycline at 37 °C.

The heterologous induction of the promoter sequence of *pqi* genes of *C. violaceum* was evaluated in response to paraquat compound by measuring the expression levels of the β-galactosidase enzyme in the presence of the ONPG reagent (ortho-nitrophenyl-β-D-galactopyranoside) (Sigma-Aldrich), as proposed in detail by Miller (1972). The expression assays of the β-galactosidase enzyme were carried out from 100 µL of saturated culture of *E. coli* cells diluted in 4.9 mL of LB medium containing 12.5 µg/mL tetracycline and maintained at 37 °C under aeration conditions. To achieve an OD_{600nm} reading of 0.25 (approximately two hours of incubation), the paraquat dichloride hydrate compound (Sigma-Aldrich) was added to the bacterial inoculums at the final concentrations of 50 and 100 µg/mL, remaining for five more hours under same conditions for induction and activation of the promoter of interest. *E. coli* cells carrying exclusively the conjugative vector without insertion of the pqi promoter sequence

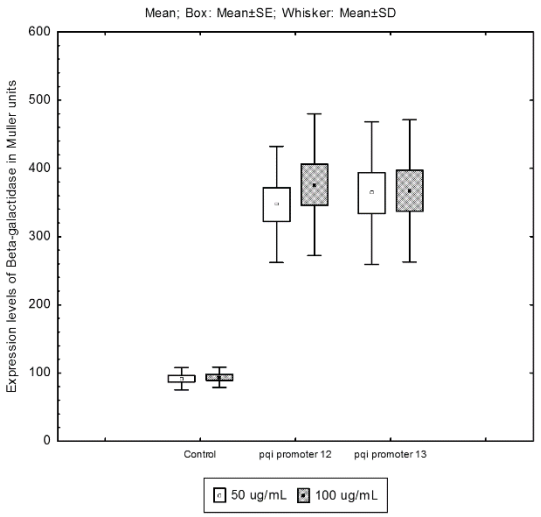

Figure 1. Expression levels of the β-galactosidase enzyme induced by heterologous activating the predicted promoter sequence for paraquat-inducible genes of *Chromobacterium violaceum* exposed to paraquat compound at distinct final concentrations (50 and 100 µg/mL). Results are presented as means, standard deviations (SD) and standard errors of the means (SE).

were employed as control group. Data were statistically analyzed by using software STATISTICA/W statistical package version 10.0 (Statsoft, Tulsa, OK, USA) from hierarchical linear model and analysis of variance, where p value <0.05 was considered statistically significant.

Irrespective of the concentration tested, the paraquat compound provoked increases in the expression levels of the β-galactosidase enzyme in *E. coli* strains carrying the predicted promoter sequence for *pqi* genes of *C. violaceum* fused to the *lacZ* gene, where significant values of the enzyme were 3.5 to 4-fold higher in response to paraquat than that observed in the control group ($p<0.05$) (Figure 1). Alternatively, the growth of wild *C. violaceum* colonies was monitored onto LB agar plates containing paraquat compound at the same final concentrations employed in the expression assays of the β-galactosidase enzyme, resulting in estimated values ranging of 10^6 to 10^8 colony-forming units per milliliter.

Based on the significant effect of the paraquat compound on the activation of the promoter sequence of *pqi* genes of *C. violaceum* as well as the expressive number of colony-forming unit per milliliter of *C. violaceum* cells grown in the presence of this reagent, the results presented herein experimentally confirm the inherent existence of regulatory DNA motifs inducible by a potent superoxide radical-generating agent in the genome of *C. violaceum*. Thus, the findings described in the present study represent the first reports in the literature charactering the influence of the paraquat oxidant compound on the heterologous induction of a predicted promoter sequence for paraquat–inducible genes of the *C. violaceum* bacterium.

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