The influence of malachite green on the level of transcriptional expression of the laccase and DyP-peroxidase genes of the *Azospirillum brasilense*

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Key message. Malachite green (MG), a widely-used and recalcitrant dye, has been confirmed to be carcinogenic and mutagenic against many organisms. Herein, we were aimed at the investigation of the hypothetic role of ligninolytic bacterial enzymes similar to fungal ones in the degradation of synthetic dyes. A multiple increase in the laccases and DyP-peroxidases genes expression level was recorded by RT-qPCR for bacteria of the genus *Azospirillum* in the presence of MG.

Keywords: *Azospirillum*, biodegradation, malachite green, laccase, DyP-peroxidase, RT-qPCR, relative gene expression.

Triphenyl methane cationic dye MG is widely known as coloring agent in food and textile industries, as an antiseptic drug in clinics, etc. The MG molecules can be involved into the food circuits with possible subsequent carcinogenic, mutagenic and teratogenic effects on humans. Traditional pollutants cleanup methods are based on the removal of unwanted materials through cyclic sedimentation and filtration steps, followed by chemical treatments such as flocculation, neutralization. Alternatively, biological processes are very promising owing to their cost-effectiveness, low sludge production, environmental safety etc.

Bacteria of the genus *Azospirillum* are able to produce a complex of phenol-oxidizing enzymes, which may be considered as efficient degraders of recalcitrant biopolymers such as MG. This extracellular non-specific enzyme system composed by laccases (Lcc) and high redox peroxidases, such as lignin peroxidase, manganese peroxidase, and new representatives like dye-decolorizing peroxidases (DyP), which function together with H₂O₂-producing oxidases. Herein we performed a comparative study of the listed above phenol oxidase complex enzymes-coding genes expression profiles of the *azospirillum*, under cultivation in the presence of MG and in standard conditions. The bioinformatics amino acid sequences search for the putative homologous of Lcc and DyP enzymes was performed based on the comparison of annotated in GenBank (NCBI, USA) data using the Local Alignment Tool algorithm. The obtained data was then translated into the nucleic acid format and served as DNA templates sequences for further PCR-based examination. Then oligonucleotide primers set for Lcc and DyP genes identification were originally designed using the Vector NTI software product (Invitrogen, USA). The genomic DNA (gDNA) was extracted from *azospirillum* bacterial suspensions with MG added to the growth media, the non-treated bacteria served as blanks in all experiments. The matching of designed primers for the desired templates (gDNAs) were firstly checked by conventional PCR using a T-100 thermocycler (Bio Rad, USA) and an agarose-gel ethidium bromide staining detection. Then the cDNA library was obtained from the gDNAs by a standard reverse-transcription (RT) reaction using random oligo-dT primers and a commercial RT kit (Syntol, Russia) following the manufacturers instructions. The RT-qPCR reactions were performed in an CFX-96 thermocycler (Bio Rad, USA), with a SYBR green amplicon detection. A multiple increase in the Lcc and DyP genes normalized relative expression level in response to the addition of MG, referred to the house-keeping genes (gyrase A, glyase A, recombinase A and pykase A) expression value was evaluated using a standard CFX Manager software (Bio Rad, USA). The estimated more significant increase in DyP expression upon exposure to MG suggests that these enzymes play the main role in the biodecolorization of triphenylmethane dyes by *azospirillum*.