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Assessment of the cytotoxic, genotoxic and mutagenic effects of the commercial black dye in *Allium cepa* cells before and after bacterial biodegradation treatment

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HIGHLIGHTS

- BDCP metabolites generated by bacterial biodegradation induce cellular alterations.
- BDCP products generated by bacterial degradation are more toxic than the original BDCP.
- Nuclear buds and binucleated cells indicate genotoxic effects.
- BDCP and its metabolites induce high rates of cell death on *Allium cepa* cells.

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ABSTRACT

The present study evaluated the cytotoxic, genotoxic and mutagenic actions of different concentrations (50 and 200 μg/L) of BDCP (Black Dye Commercial Product) used by textile industries, before and after bacterial biodegradation, by the conventional staining cytogenetic technique and NOR-banding in *Allium cepa* cells. Differences in the chromosomal and nuclear aberrations and alterations in the number of nucleoli were observed in cells exposed to BDCP with and without the microbial treatment. The significant frequencies of chromosome and nuclear aberrations noted in the tests with bacterially biodegraded BDCP indicate that the metabolites generated by degradation are more genotoxic than the chemical itself. Losses of genetic material characterize a type of alteration that was mainly associated with the action of the original BDCP, whereas chromosome stickiness, nuclear buds and binucleated cells were the aberrations that were preferentially induced by BDCP metabolites after biodegradation. The significant frequencies of cell death observed in the tests with biodegraded BDCP also show the cytotoxic effects of the BDCP metabolites. The reduction in the total frequency of altered cells after the recovery treatments showed that the test organism *A. cepa* has the ability to recover from damage induced by BDCP and its metabolites after the exposure conditions are normalized.

1. Introduction

Among the different classes of synthetic dyes, the azo dyes have been most widely used, particularly in the textile sector (Kunz et al., 2002). These dyes have one or more azo groups (−N=N−) associated with one or more aromatic rings in their structures (Wong and Yuen, 1996).

The pollution caused by effluents that contain azo dyes can cause serious impacts in the environment (Hao et al., 2000). Organisms exposed to azo dyes have shown different cellular alterations, such as chromosome aberrations and micronucleated cells, which demonstrate the genotoxic and mutagenic effects of the product (Caritá and Marin-Morales, 2008; Phugare et al., 2011; Ventura-Camargo et al., 2011; Fernandes et al., 2015). According to Umbuzeiro et al. (2005), assays performed with *Salmonella* using water samples from a river that receives effluent from a textile

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industry that uses BDCP point to a mutagenic action of this dye. Alves de Lima et al. (2007) showed the mutagenic and carcinogenic potential of a textile azo dye processing plant effluent.

According to Jadhav et al. (2007), Chengalroyen and Dabbs (2013) and Ventura-Camargo and Marin-Morales (2013), different methods for treating effluents that contain azo dyes have been used in an attempt to minimize the problems associated with this type of contamination. The use of biodegradation for the treatment of azo dyes is a process that has been indicated as having great potential for success. However, there is a need to establish an efficient biological treatment that considers the types of enzymes that are able to degrade certain azo dyes (Kunz et al., 2002; Jadhav et al., 2007; Saratale et al., 2011) because they are relatively resistant to biological degradation processes (Martins et al., 2003; Oliveira et al., 2007) due to their complex chemical structure and high light stability (Kim and Shoda, 1999).

Several bacterial species are used for the degradation of azo dyes (Hu, 1998; Saratale et al., 2011; Singh et al., 2015). Under anaerobic conditions, azo dyes can be degraded into recalcitrant aromatic amines (Weber and Adams, 1995). Treatment with bacteria under aerobic conditions is generally efficient at completely mineralizing aromatic amines (Isik and Sponza, 2003; Saratale et al., 2011), although some studies have shown that some azo dyes are more resistant to bacterial attack under aerobiosis conditions (Hu, 1998). Oliveira et al. (2007) showed that BDCP is recalcitrant even under aerobic conditions because components of this compound were detected in effluent samples that were treated by activated sludge systems.

Higher plants are recognized as important biological materials for detecting genotoxic aberrations caused by environmental pollutants (Yi and Meng, 2003). The species *Allium cepa* constitutes one of the pioneer materials in the study of chromosomal aberrations caused by the action of chemical agents (Levan, 1938) and has been used as an efficient test organism because it presents a genetic pattern that is suitable for genotoxicity tests (Fiskesjö, 1985; Fernandes et al., 2009; Leme et al., 2008; Hoshina and Marin-Morales, 2009; Ventura-Camargo et al., 2011). A study performed by Caritá and Marin-Morales (2008) showed the mutagenic potential of certain samples of industrial effluents contaminated by BDCP as well as other pollutants by applying the chromosomal aberration test in *A. cepa*. Another study (Ventura-Camargo et al., 2011) showed the genotoxic potential of different concentrations of BDCP on *A. cepa* meristematic cells.

Chromosomal staining techniques have been successfully applied to chromosomal analyses in plants (Tuna et al., 2004) and may provide information about the DNA composition and its arrangement along chromosomes, enabling a more detailed analysis of the structural organization of chromosomes (Kim et al., 2002). Staining with silver nitrate has been used to identify the number of nucleoli and possible active sites of NOR in different plant species (Carvalho and Guerra, 2002; Ventura-Camargo et al., 2011; Mazzeo and Marin-Morales, 2015). The positions of NORs helps researchers gain a better understanding of the chromosomal alterations that were established in each karyotype (Mazzeo and Marin-Morales, 2015). Thus, in cases in which chromosomal aberrations are induced by genotoxic agents, this technique may provide particular sensitivity by easily diagnosing the probable sites of action of these agents and determining the cell aberrations they caused. Ventura-Camargo et al. (2011) showed that BDCP can act in chromosomal regions of *A. cepa* that were or were not associated with rDNA sites using the NOR-banding technique.

Considering that azo dyes and their related products generally have the potential to cause serious damage in the genetic material of exposed organisms and that their mechanisms of action on DNA need to be further investigated, the aim of this study was to analyze the cytotoxic, genotoxic and mutagenic potential of the commercial black dye in root cells of *A. cepa* before and after the bacterial biodegradation treatment using the conventional cytogenetic staining technique (chromosomal and nuclear aberrations test) and AgNOR-banding.

2. Material and methods

2.1. Chemicals tested

We tested the commercial black dye (BDCP - Black Dye Commercial Product), which is an azo dye composed of 3 dyes that belong to the nitro-aminobenzene group (Umbuzeiro et al., 2005): C.I. Disperse Blue 373 ($C_{21}H_{21}BrN_6O_6$; CAS n° 51868-46-3), C.I. Disperse Violet 93 ($C_{18}H_{19}BrN_6O_5$; CAS n° 268221-71-2) and C.I. Disperse Orange 37 ($C_{17}H_{15}Cl_2N_5O_2$; CAS n° 13301-61-6).

2.2. Treatment solutions

The concentrations of the commercial black dye (50 μg/l and 200 μg/l) were determined by pilot tests to allow viable development of the cytogenetic techniques used in this study. It is important to consider that the concentrations tested are close to those detected in environmental samples derived from a textile industry that used BDCP, as studied by Oliveira et al. (2007).

2.3. Test organism

The assays were performed using seeds of a single variety of *A. cepa*.

2.4. Bacterial biodegradation treatments

The biodegradation treatments were performed using a pool of heterotrophic bacteria obtained from a biological wastewater treatment plant. This bacterial pool was added to the Sabouraud liquid culture medium. The microorganisms were previously incubated in a 35 °C incubator for 3 days. Then, tests were performed in sterilized test tubes, in which the final volume was of 10 mL for each tube, as follows:

- *Blank Tests* - Blank 1: 8.0 mL of culture medium + 2.0 mL of distilled water; Blank 2: 7.9 mL of culture medium + 100 μL of bacterial suspension + 2.0 mL of distilled water.
- *Tests with the commercial black dye* - Concentration of 50 μg/L: 7.9 mL of culture medium + 100 μL of bacterial suspension + 1.5 mL of distilled water + 0.5 mL of the stock solution of 100 mg/L BDCP; concentration of 200 μg/L: 7.9 mL of culture medium + 100 μL of bacterial suspension + 2.0 mL of the stock solution of 100 mg/L BDCP. The stock solution was prepared in distilled water.

The microorganisms were incubated at 35 °C for four more days. Subsequently, all of the tubes were autoclaved at 1 atm for 10 min. After this period, the germination assay was performed using *A. cepa* seeds. All tests were performed in duplicate.

2.5. A. cepa bioassays

A. cepa seeds (100 per dish) were germinated at room temperature (21 ± 4 °C) in Petri dishes covered with filter paper soaked in ultrapure water. When the roots reached 1.5 cm in length, they were transferred to Petri dishes covered with filter paper soaked in 50 μg/l and 200 μg/l BDCP. Tests with and without the bacterial treatment were conducted. The roots of the negative control