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Dye detoxification by *Lentinula edodes* INCQS 40220

Detoxificação de corante por *Lentinula edodes* INCQS 40220

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ABSTRACT

Lentinula edodes belongs to the group of fungi known as ligninolytic fungi, due to its ability to degrade the aromatic structure of the lignin. Textile dyes also have aromatic structure, and after microbial degradation, some of them can be transformed into toxic compounds, when compared to the original structure, representing risk to environment and human health. Therefore, the potential of the fungus *L. edodes* INCQS 40220 to decolorize and to detoxify reactive red 198 (azo dye), reactive blue 214 (azo dye) and reactive blue 21 (cooper phthalocyanine dye) in a mixture (MXD), was evaluated. After 14 days of incubation, total decolorization in liquid media was obtained. The fungal treatment of MXD did not present toxic effects towards blood human cells. The genotoxicity of MXD, assessed by Comet assay, was efficiently reduced to 61% and in the Ames test presented negative response for mutagenicity for *Salmonella enterica* serovar *Typhimurium* TA97, TA98, TA100 and TA102 strains. The results indicated *L. edodes* INCQS 40220 efficiency on decolorization and genotoxicity reduction of a mixture of different dyes. Therefore, based on these results, obtained under laboratory conditions, *L. edodes* INCQS 40220 has potential for textile effluent treatment.

KEYWORDS: Decolorization; Fungi; Textile Dyes; Toxicity; Genotoxicity; Mutagenicity

RESUMO

Lentinula edodes pertence ao grupo de fungos conhecidos como fungos ligninolíticos, devido a sua habilidade de degradar estruturas aromáticas da lignina. Corantes têxteis também possuem estruturas aromáticas e, após a degradação microbiana, alguns destes corantes podem ser transformados em compostos tóxicos quando comparados à estrutura original, representando risco à saúde ambiental e humana. Portanto, o potencial do fungo *L. edodes* INCQS 40220 para descolorir e detoxificar a mistura de vermelho reativo 198 (corante azo), azul reativo 214 (corante azo) e azul reativo 21 (corante ftalocianina de cobre) foi avaliada (MXD). Após 14 dias de incubação, foi obtida descoloração total em meio líquido. O tratamento fúngico de MXD não apresentou efeitos tóxicos em células sanguíneas humanas. A genotoxicidade de MXD, avaliada por meio do Ensaio Cometa, foi eficientemente reduzida para 61% e, no teste de Ames, apresentou resposta negativa à mutagenicidade para *Salmonella enterica* serovar *Typhimurium* TA97, TA98, TA100 e TA102 strains. Os resultados indicaram eficiência de *L. edodes* INCQS 40220 na descoloração e redução de genotoxicidade na mistura de diferentes corantes. Portanto, com base nos resultados obtidos em condições de laboratório, *L. edodes* INCQS 40220 tem potencial para o tratamento de efluente têxtil.

PALAVRAS-CHAVE: Descoloração; Fungos; Corantes Têxteis; Toxicidade; Genotoxicidade; Mutagenicidade

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INTRODUCTION

The textile industry is responsible for the production and release of many toxic compounds into the environment as wastewater. Textile dyes are the main problem, since their metabolites can be mutagens and carcinogens[1].

Exposition to contaminated water with textile dyes, and with their possible toxic, mutagenic and/or carcinogenic metabolites, as aromatic amines, could be a risk to human and animal health[2]. The first known cause of bladder cancer described in humans was the result of a exposure to industrial dyes arylamines[3].

The contamination of water resources from textile effluents, when dyes are neither photo or thermally degraded, nor biodegraded, can lead to its accumulation in the environment as recalcitrant xenobiotics[2].

The bioremediation, a technology that uses microorganisms to degrade pollutants and to remediate environmentally impacted areas, has been an alternative for treating this kind of effluents[4]. Methods of color removal from textile effluents are often highly expensive and not efficient, and can still result on an environmental impact because the toxic compounds remaining in the effluent.

Infinity of methodologies using different organisms has been proposed for treating effluents, including fungi[5]. Studies have demonstrated that several white-rot fungi are capable of oxidizing various types of synthetic textile dyes[6,7]. The great fungal capacity to degrade many aromatic compounds is possible through their nonspecific enzymes, such as laccase, manganese peroxidase and lignin peroxidase[8].

The fungus *Lentinula edodes* (popularly known as shiitake) is the second most popular edible mushroom on the global food market and belongs to the group of white-rot fungi. This fungus has the ability to degrade environmentally persistent pollutants such as textile dyes[9,10,11].

Studies of decolorization have been associated to toxicological evaluations to ensure the protection of environmental health, since the decolorization obtained by some methods using biological systems do not guarantee toxigenic and mutagenic reduction after dye biotransformation[12].

Textile dyes are recognized as toxic to different organisms[13,14,15]. The Ames Test, that uses strains of the bacteria *Salmonella enterica* serovar *Typhimurium*, is well-recognized for evaluating different compounds and environmental samples, expressing mutagenicity following OECD[16]; besides it, the Comet assay, using human blood, is indicated to detect damage on the DNA, a genotoxicity assay[17].

Considering the importance of identifying effective and sustainable treatments to reduce impacts caused by textile dyes on the environment and on the human health, the present study tested the ability of fungus *L. edodes* INCQS 40220 to decolorize the mixture of three reactive textile dyes. Toxicological effects after fungal treatment were additionally studied by genotoxicity and mutagenicity tests.

MATERIAL AND METHODS

Textile dyes

Reactive red 198 and reactive blue 214 dyes were chosen as representatives of azo dyes intensively used in the textile industry; reactive blue 21 was selected as a representative of phthalocyanine dyes.

Lentinula edodes culture condition

L. edodes INCQS 40220 (CCT 4519) was obtained from the Culture Collection of Microorganisms of Reference in Health Surveillance from the National Institute for Quality Control in Health, INCQS/Fiocruz, Brazil.

The fungus was inoculated in the center of a Potato Dextrose Agar plate – PDA (20% Potato Broth, 2% Dextrose, 2% agar), containing a mixture (MXD) of the following dyes: reactive red 198 (CAS no. 145017-98-8) 66.6 mg L^{-1}, reactive blue 214 (CAS no. 141255-32-5) 66.6 mg L^{-1} and reactive blue 21 (CAS no. 131257-19-1) 66.6 mg L^{-1}, at a total dye concentration of 200 mg L^{-1}, in order to observe the degradation halo, as well as to adapt the fungus metabolism to the textile dyes and to obtain standardized inoculum for the liquid media inoculation. Then, the cultures in solid media were incubated at 28°C and after 5 days, 9 agar disks (5mm diameter) were transferred from the colony margin, where the cells are younger, to conical flasks containing 150 mL of Potato Dextrose Broth – PDB (20% Potato Broth, 2% Dextrose). The cultures were incubated for 2 days at 28°C on a rotary shaker (140 rpm), seeking to obtain fungal biomass; afterwards, 33.3 mg L^{-1} of each dye was added to the cultures, reaching 100 mg L^{-1} of total dye concentration[18,19]. The culture media (PDB) with MXD but without *L. edodes* INCQS 40220; and the PDB without MXD or *L. edodes* INCQS 40220 were incubated under the same conditions described above, and were used as controls. All assays were conducted in triplicate, in the dark.

Decolorization assay

Following the addition of textile dyes (MXD) to the liquid media, aliquots of the fungal culture were collected at cultivation times 0, 2, 5, 7 and 14 days and were then centrifuged at 10000 rpm for 10 min; the supernatants were diluted 1:10 with distilled water and were measured spectrophotometrically using a Shimadzu UV-1601 spectrophotometer (Kyoto, Japan). The uninoculated flask containing the MXD was used as a control regarding the decolorization; the uninoculated medium without MXD was used as blank. The color removal was reported as percentage of decolorization (%) = $(A_b - A_a)/A_b$ X 100, where A_b is the absorbance of the initial dye solution (day 0) and A_a is the absorbance at cultivation time (2, 5, 7 and 14 days)[20]. Results were expressed as mean of three different cultures.

Toxicity determination

The samples, obtained from the liquid media cultures after 14 days of incubation, were filtered through polyethersulfone

membrane 0.22 µm; and the filtrated samples were used for the toxicological assays.

Genotoxicity assay: For Comet Assay (Single Cell Gel Electrophoresis - SCGE), the cell viability test was first conducted. A volume of 100 µL heparinized human blood cells from a human male donor (healthy non-smoker, aged 31) was incubated at 37ºC for 2 h with (i) positive control methyl methanesulfonate (MMS), (ii) negative controls: cells without treatment (cells control); solvent controls (distilled water and PDB), (iii) filtrate of MXD in PDB without *L. edodes* INCQS 40220 (PDB + MXD), (iv) the solution of possible metabolites generated by *L. edodes* INCQS 40220 (PDB + MXD + *L. edodes*). The cells (200 cells) were analyzed through microscopic observation by **fluorescence microscope** (Nikon, model EFD3 - Japan) after addition of 50 µl ethidium bromide EtBr 8 µg mL^{-1} in phosphate buffered saline (PBS) and fluorescein diacetate (FDA 30 µg mL^{-1}) solution to determine the percentage of cell viability. Viable cells appear green-fluorescent, whereas orange-stained nuclei indicate dead cells[21]. For Comet Assay, the heparinized whole blood cells were treated in duplicate and incubated at 37ºC. MMS (160 µM) was used as a positive control. After 2 h, 120 uL of 0.5% Low Melting-Point Agarose (LMPA) in PBS at 37°C was mixed to 10 uL of MXD before and after the fungal treatment with *L. edodes* and to the controls, and was immediately pipetted onto a 1,5% Normal Melting-Point Agarose (NMPA) pre-coated slide and dried overnight. Following literature[22,23], four slides per treatment were prepared (two slides per culture). The slides were placed on a glass plate for 3 min in the refrigerator (4-5ºC) to allow complete polymerization of the agarose, and then immersed in freshly made cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% N-lauroylsarcosine sodium salt, 10% DMSO and 1% Triton X-100) at 5ºC for 2 h in order to liberate DNA. The slides were then rinsed with distilled water. Next, the slides were placed in a horizontal electrophoresis tank with 300 mM NaOH and 1mM Na_2EDTA at pH 13 for 20 min, allowing salt equilibration and further DNA unwinding before electrophoresis at 25 V, 300 mA for 20 min. The slides were then washed three times (5 min per wash) with 0.4 M Tris (pH = 7.5) at room temperature and fixed with absolute ethanol for 10 min; they were then left at room temperature to dry[22,23].

The dried slides were stained with EtBr (20 µg mL^{-1}) and examined microscopically (400X magnification) using a fluorescence microscope (Nikon, model EFD3 – Japan). All slides were coded and blindly examined. A total of 200 cells per sample (50 cells per slide) were scored visually according to the intensity of the fluorescence in the tail into four classes: 0 (undamaged, i.e. no visible tail), 1 (lightly damaged), 2 (moderately damaged) and 3 (maximally damaged, i.e. very small head of comet and most of DNA in tail). The DNA damage score per 200 cells, expressed as Total Arbitrary Units (TAU), was calculated according to the formula: TAU = (0 x No. of comets class 0) + (1 x No. of comets class 1) + (2 x No. of comets class 2) + (3 x No. of comets class 3). Thus, the total score for 200 comets (TAU) could range from 0 (all undamaged) to 600 (all maximally damaged). The results of the different treatment groups were compared using Students' one-tailed *t*-test, establishing a statistically significant response when $p \leq 0.05$[24].

Mutagenicity Test: The *Salmonella enterica* serovar *Typhimurium* strains TA97, TA98, TA100 and TA102, as described by Maron & Ames[25], were provided by Dr. B. N. Ames, University of California, Berkeley, CA (USA). A mix of metabolizing enzymes known as S9 fraction (19.1 mg L^{-1} protein), prepared from livers of Sprague-Dawley rats pre-treated with a polychlorinated biphenyl mixture (Aroclor 1254), was purchased from Molecular Toxicology Inc. (Moltox™, USA). The S9 metabolic activation mixture (S9 mix) was prepared according to Maron & Ames[25] for the Ames test. Mutagenicity of the (i) filtrate of MXD, (ii) the solution of possible metabolites generated by *L. edodes* INCQS 40220 in the absence of MXD (PDB + *L. edodes*), (iii) the solution of possible generated metabolites by *L. edodes* INCQS 40220 in the presence of MXD (PDB + MXD + LE), and (iv) the respective controls (PDB, Distilled water - H_2O)were evaluated using the procedure described by Maron & Ames[25] with some adaptations from Aiub et al.[26] with TA97, TA98, TA100 and TA102 strains. The assay mixture consisted of 100 µL of samples, 500 µL of S9 mix (19.1 mg L^{-1} total proteins), or the same volume of 0.2 M sodium-phosphate buffer (pH 7.4) for experiments without S9 mix, and 100 µL of the bacterial suspension (2 x 10^9 cells/mL) and 2 mL of top agar (0.6% agar, 0.6% NaCl, 50 µM L-histidine, 50 µM biotin, pH 7.4, 45ºC) was poured onto a plate with minimal media agar (1.5% agar, Vogel-Bonner E medium, containing 2% glucose). This final mixture was incubated at 37ºC for 72 h, and the *His*$^+$ revertant colonies were counted. Positive controls for assays were: 4- NQO (1.0 µg/plate), for TA97, TA 98 and TA102; Sodium Azide (SA) (0.5 µg/plate) for TA100, either in the absence or in the presence of S9 mix. The tests were conducted in triplicate and considered positive for mutagenicity when: (a) the number of revertants colonies in the test assay was at least twice the number of spontaneous revertants (mutagenicity induction, MI ≥ 2) (b) and the response of variance analysis (ANOVA) presented $p \leq 0.05$.

An aliquot (100 µL) from the Ames test tubes was used to evaluate their cytotoxic effects (Aiub et al.[26]), diluted (10^2 cells/mL) and poured on the Louria Bertani plates. The samples were considered toxic when the survival was less than 70% when compared to control test. All the experiments were conducted in duplicate.

RESULTS

Decolorization assay

After 5 days of incubation, *L. edodes* INCQS 40220 grown on solid medium PDA enriched with the mixture of the three dyes (MXD) attained a growth of 7.0 cm, producing a degradation halo of 5.5 cm diameter (Figure 1), demonstrating decolorization of about 80%. In liquid media, after 5 days of incubation in the presence of the MXD, the ability of *L. edodes* INCQS 40220 to decolorize this mixture of dyes, measured spectrophotometrically, was about 94.3% (Figure 2). After 14 days of incubation, the decolorization attained 100% and was followed by the disappearance of the entire visible wavelength, including the characteristic peak of MXD of 621 nm, as demonstrated by spectrophotometric analyses (Figure 3).

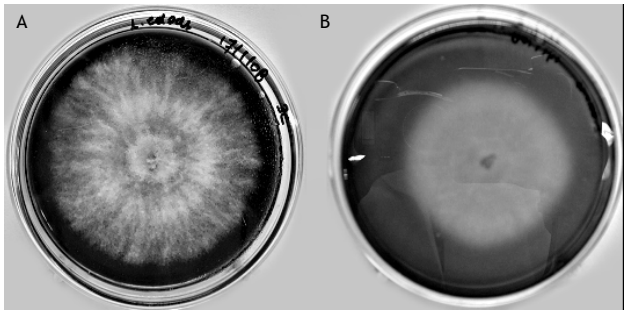


Figure 1. Decolorization in solid media PDA supplemented with 100 mg L^{-1} of MXD. Halo of degradation obtained after 5 days by *L. edodes* INCQS 40220. A – front plate from which the colony of 7.0 cm diameter can be observed; B – reverse plate from which the degradation halo of 5.5 cm diameter can be observed.

Toxicity determination

Genotoxicity

None of the tested samples were toxic according to the viability test performed before the Comet Assay, presenting viability higher than 90% (Table 1). The results of genotoxicity using Comet Assay are presented in Figure 4. The MXD before the treatment, in the absence of *L. edodes* INCQS 40220 (PDB + MXD), was considered genotoxic (132 TAU) when compared to the solvent control (PDB) that presented 27 TAU ($p < 0.001$). MXD after fungal treatment (PDB + MXD + Le) had its genotoxicity significantly reduced ($p < 0.05$) to 52 TAU, representing 61% of reduction. The positive control 160 µM MMS showed a clear and significant ($p < 0.001$) genotoxic effect (547 TAU), in comparison with the solvent control H_20 (18 TAU).

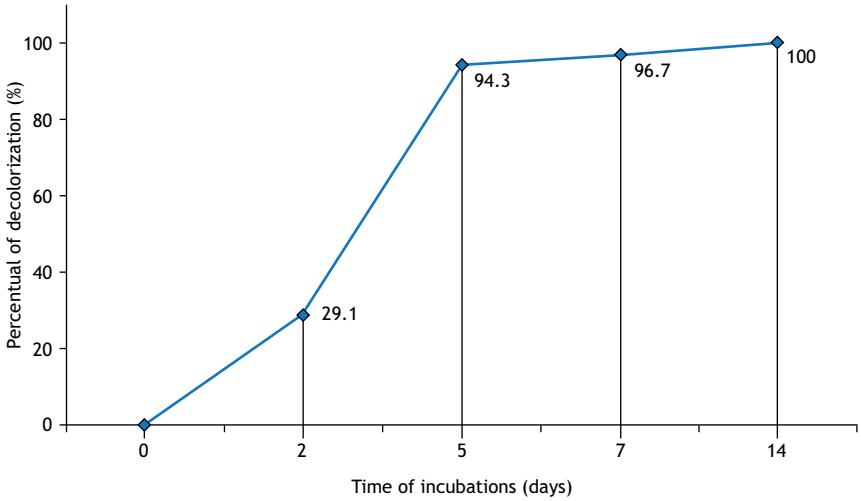


Figure 2. Percentage of decolorization of MXD by *L. edodes* INCQS 40220 in liquid media PDB after 0, 2, 5, 7 and 14 days of incubation.

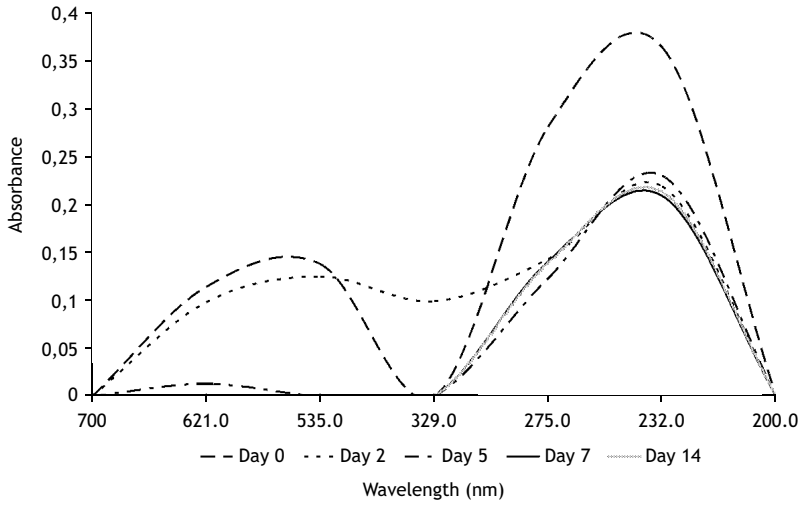
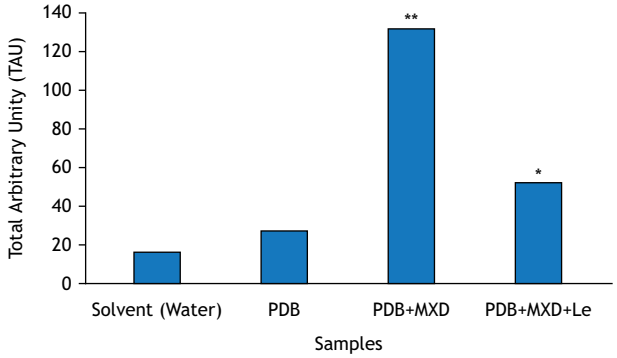


Figure 3. Wavelength of 700 to 200 nm of MXD treatment by *L. edodes* INCQS 40220 after 0, 2, 5, 7 and 14 days of incubation.

Table 1. Viability assay with blood human cells to Comet Assay.

Samples	Viability cells (%)
Cells control	100
Water	100
PDB	100
PDB + MXD	100
PDB + MXD + Le	100
Positive control	98,5

PDB: Culture media Potato Dextrose Broth; MXD: mixture of dyes; Le: *L. edodes* INCQS 40220. Positive control methyl methanesulfonate (MMS).



* statistically significant ($p < 0.05$); ** high significant ($p < 0.001$); positive control - MMS - 547 TAU.

Figure 4. Results from Comet Assay, where: the negative controls were H_2O and culture media PDB; the samples tested were PDB + MXD (before treatment by *L. edodes* INCQS 40220) and PDB + MXD + Le (after treatment by *L. edodes* INCQS 40220).

Mutagenicity

Table 2 presents the values of mutagenicity and cytotoxicity obtained before and after the fungal treatment of MXD, in the presence of *S. typhimurium* TA97, TA98, TA100 and TA102 strains; in the absence of S9 mix (-S9); or in the presence of S9 mix (+S9), respectively. The cytotoxic response was observed in the presence of PDB and PDB + MXD, just for TA102 strain, which was indicated by a decrease in the number of colonies (Table 2). In the presence of metabolization (+S9), a clear cytotoxic effect was observed for TA97 and TA98 strains in PDB+Le, PDB + MXD and in PDB + MXD + Le (Table 2).

For all the evaluated conditions (PDB, PDB+Le, PDB + MXD and PDB + MXD + Le), in the absence of metabolization (-S9) no mutagenic responses were observed (Table 2).

DISCUSSION

A study conducted by Minussi et al.[9] already demonstrated the efficiency of *L. edodes* CCT 4519, which is the same strain of the present study [*L. edodes* INCQS 40220 (CCT 4519)], decolorizing different dyes individually, including reactive blue 19 (500 mg L^{-1}), reactive red 195 (500 mg L^{-1}), reactive yellow 145 (250 mg L^{-1}) and reactive black 5 (500 mg L^{-1}), on solid media (PDA). They could observe a complete decolorization of reactive blue 19, reactive red 195 and reactive yellow 145 after 7, 9 and 10 days, respectively. However, the most recalcitrant dye, reactive black 5, was 75% decolorized after 22 days of incubation.

In the present study, the mixture of reactive red 198, reactive blue 214 and reactive blue 21 at a total concentration of 200 mg L^{-1} was decolorized by *L. edodes* INCQS 40220 on the same solid media previously used (PDA) after 5 days of incubation (Figure 1), corroborating the results earlier obtained by Minussi et al.[9] Therefore, after many years of preservation, about 10 years, *L. edodes* INCQS 40220 (CCT 4519) has maintained its degradation capacity.

In liquid media, another strain of *L. edodes* CCB-42 had also efficiently and completely decolorized 10 different types of textile dyes (amido black, congo red, trypan blue, methyl green, remazol brilliant blue R, methyl violet, ethyl violet and Poly R478), individually, at 200 mg L^{-1} after 18 days of incubation[11].

In the current study, *L. edodes* INCQS 40220 was able to decolorize 100% of the mixture dyes in liquid media, at a total dye concentration of 100 mg L^{-1} within 14 days of incubation (Figure 2) demonstrating, therefore, efficiency for this mixture dye degradation.

The effluents from textile industries, even after treatment, can remain toxic and mutagenic; nevertheless, they are released into the environment anyway[27,28]. Consequently, the inefficiency of this process leads to the need of toxicological assays after treatment being conducted[1,29]. Therefore, MXD after the treatment by *L. edodes* INCQS 40220 was analyzed regarding its genotoxicity and mutagenicity.

Prior to the Comet assay, the viability test demonstrated that the medium PDB with MXD was not toxic to human blood cells (Table 1). Besides, just the PDB with MXD before fungal treatment was genotoxic in the Comet Assay (Figure 4). In an earlier study, Sumathi et al.[30], also using the Comet assay, detected genotoxicity in samples that contained textile dyes.

In the present investigation, *L. edodes* INCQS 40220, when in contact with MXD, demonstrated anti-genotoxicity potential (Figure 4), corroborating previous results with another *L. edodes* strain (LE 96/17), which reduced the genotoxicity of another compound, methyl methanesulfonate[31].

Therefore, besides *L. edodes* INCQS 40220 efficiency decolorizing dyes in a mixture (MXD), the fungus is also efficient to reduce the genotoxicity of the mixture, demonstrating a potential for bioremediation applications.

Regarding the results from the Ames test, the present work could not demonstrate a mutagenicity reduction of MXD by the treatment with *L. edodes* INCQS 40220, since neither MXD before nor after fungal treatment, showed mutagenicity to all the *S. typhimurium* strains tested (Table 2). Some dyes with specific characteristics in its structure contribute to different response among the organisms in toxicological assays[32], as observed in the current study in the Ames test and the Comet assay. Another assumption is that the non-mutagenicity of the mixture is related

Table 2. Induction of *His*+ revertants in *S. typhimurium* strains TA97, TA98, TA100 and TA102 by PDB, PDB and *L. edodes* INCQS 40220, and MXD before and after the treatment by *L. edodes* INCQS 40220, without metabolic activation (-S9) and with metabolic activation (+S9).

Strains	Samples	-S9			+S9		
		MI[a]	His+ [b]	% Survival[c]	MI[a]	His+ [b]	% Survival[c]
TA97	0	1.0	109	100	1.0	282	100
	PDB	1.0	103	100	0.9	268	100
	PDB+Le	1.4	144	100	0.6	164	49.2*
	PDB + MXD	1.2	122	100	0.7	188	55.5*
	PDB + MXD + Le	0.9	113	100	1.4	254	28.6*
	PC	2.3	250	NT	4.6	1297	NT
TA98	0	1.0	32	100	1.0	39	100
	PDB	1.5	51	100	1.2	49	100
	PDB+Le	1.1	54	100	0.4	22	76.4
	PDB + MXD	1.1	36	100	0.9	47	31.9*
	PDB + MXD + Le	1.2	42	100	0.9	44	31.9*
	PC	5.8	183	NT	3.0	120	NT
TA100	0	1.0	134	100	1.0	134	100
	PDB	0.9	118	100	0.7	89	94.3
	PDB+Le	1.0	122	100	1.4	124	93.9
	PDB + MXD	0.9	102	100	1.1	96	100
	PDB + MXD + Le	1.2	127	100	0.9	90	70.7
	PC	10.6	1410	NT	8.4	1123	NT
TA102	0	1.0	229	100	1.0	189	100
	PDB	0.8	175	75,5	0.7	136	100
	PDB+Le	1.2	205	100	1.6	219	79
	PDB + MXD	0.3	55	75,5	1.0	132	75.6
	PDB + MXD + Le	0.3	55	100	0.9	126	88.4
	PC	3.5	801	NT	7.0	1320	NT

Le: L. edodes INCQS 40220; MXD: mixture of dyes; NT: Not Tested; PC: positive control as described in Material and Methods; PDB: Culture media Potato Dextrose Broth.

* Toxic samples.

[a] Mutagenic index (MI): number of His + revertants induced in the sample/number of spontaneous His + revertants in the negative control.

[b] Number of His+ revertants/plate: mean values of at least three experiments.

[c] Survival proportion calculated in relation to the negative control (destilled water). The dose was considered toxic when percentage of survival was smaller than 70%.

to the presence of cooper, considering that the analyzed dye mixture is constituted by two different structures of dyes, azo and cooper phtalocyanine. This supposition is based on a review conducted by Chung et al.[33] concluding that complexation with a metal ion may produce non-mutagenic responses or may decrease the mutagenicity of some dyes, and that there is a need of more investigations regarding this aspect. In fact, similar observations were already made in an earlier study[32], stating that the complexation with copper ions diminishes mutagenicity of dyes. Tigini et al.[15] also did not find mutagenic effects in four simulated wastewaters to TA 98 and TA100, and justified the mutagenicity absence by the sulphonic groups present in dye molecules that decrease the amine mutagenic effects.

The cytotoxicity observed for TA97 and TA98 strains in the presence of S9 mix, before and after fungal treatment of MXD (Table 2), indicated that metabolic activation products can produce frameshift mutation, leading to cytotoxic effects, even prior to entrance in cells. The action of cytochrome P450, present in the S9 mix, probably generated more toxic products than the original compounds in these bacteria strains. These results were different from those previously observed with human blood cells, where all the tested samples were genotoxic (Figure 4). Therefore, there was again a difference of toxic response regarding the tested organisms. This can be explained since blood cells are not metabolic competent cells.

All the used strains of *S. typhimurium* in the Ames test are genetically modified to evaluate different ways to detect substances that are capable to attack DNA, including reactive oxidative species (ROS). According to Mortelmans & Zeiger[33], the strain *S. typhimurium* TA102 was developed to detect chemicals that may induce oxidative damage, as ROS. In the current study all the samples with *L. edodes* INCQS 40220 present (Table 2) the toxicity; it was possibly influenced by ROS, which is normally produced by basidiomycetes fungi during lignocellulose biodegradation[36], since the induced ROS acts in G:C base pairs. The present results demonstrated that TA97 and TA98 strains were more sensible than TA100 and TA102 to the analysed samples (Table 2).

L. edodes produces several compounds that exert inhibitory effects on bacteria[37]. It is not clear, however, the relation between the chemical structure of the toxic agent after decolorization by live organisms, and their toxic effect to organisms that have different sensitivity, indicating that the absence of color do not necessarily indicate absence of genotoxicity and/or mutagenicity.

CONCLUSION

Lentinula edodes INCQS 40220 proved to be efficient to decolorize a mixture of dyes and to reduce their genotoxicity. This study nevertheless demonstrated the importance of conducting further work on the degradation of different types of dyes by this fungus, including those associated to heavy metals, such as cooper, which could influence the complete decolorization of the dye mixture and, possibly, of the toxicity results. In addition, this study proved that it is necessary to test textile effluent after biological treatments with different toxicological assays, due to the fact that they may give different toxicological responses, and in order to verify the efficiency of the biological treatment applied in reducing the impact of textile dyes on human and animal health.

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