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ANTIMUTAGENIC ACTION OF *LENTINULA EDODES* AND *AGARICUS BLAZEI* ON *ASPERGILLUS NIDULANS* CONIDIA

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ABSTRACT

The antimutagenic effect of the mushrooms *Lentinula edodes* and *Agaricus blazei* was studied on conidia of *Aspergillus nidulans* when exposed to short wave ultraviolet light. Two strains of *A. nidulans* were used. For the preparation of the extracts, the fresh mushrooms were left in aqueous infusion for 12 hours and heated in a water bath for 15 min at 100ºC, and then the material was filtered. The dehydrated mushrooms were left in aqueous infusion for 12 hours and to filtrated. Both filtrates were used as extracts. *A. nidulans* conidia were incubated for three hours in water and in mushroom extracts and only after were exposed to UV light (pre-treatment). *A. nidulans* conidia were suspended in water and in mushroom extracts and immediately submitted to UV light (post-treatment). Conidial suspension in water and in mushroom extracts but without exposure to the mutagenic agent were used as controls. After mutagenic treatment, it was observed an increase in the survival rate of the *A. nidulans* and a decrease in the percentage of morphologic mutants on conidia treated with mushroom extracts. Our results demonstrated the radioprotective and antimutagenic effect of *L. edodes* and *A. blazei* mushrooms on eukaryotic cells when exposed to UV radiation.

Key words: antimutagenic, *Lentinula edodes*, *Agaricus blazei*, *Aspergillus nidulans,* shiitake

INTRODUCTION

For centuries mushrooms have been used as food by your flavor and as your nutritional and medicinal value. Some species have been included in the human diet because they possess substances beneficial to health and also as nutritional supplements. The basidiomycete *Lentinula edodes,* known as shiitake, is one of these well-studied and best-characterized mushroom species used for medicinal purposes. This species has been cultivated for centuries (14) and contains active biological compounds with antimicrobial (6,7,8), antitumoral (20,21) and antiviral activity (15,19). Antifungal, antithrombotic and anti-cholesterol effects have also been described in *L. edodes* (15,17).

Another specialty mushroom with medicinal properties, known as himematsutake (*Agaricus blazei*) enjoy increasing popularity, presents chemical compounds in its composition that enhance the immune response and homeostasis resulting in improvement of the individual physical function (10,11). Water soluble polysaccharides extracted from the fructification body of *A. blazei* presented antitumoral activity (3,9). Other properties were also analyzed by Eguchi *et al*. (5) who reported that doses of the *A. blazei* fructification body extract halted the development of hypertension and total cholesterol in rats. Eguchi *et al.* (4) described this mushroom as a functional food in the prevention and/or treatment of renal malfunctions.

In spite of the beneficial effects of mushrooms in the human diet, mutagenic activity in several species has been reported by Salmonella mutagenicity test (Ames test). By this test, mutagenic activity was detected in shiitake aqueous extracts (22).

Because of the relatively large and potentially increasing use of mushrooms as human food, and because of the few

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reports on the mutagenic or antimutagenic potential of *L. edodes* and *A. blazei* in eukaryotic cells, it was considered necessary to assess the mutagenic or antimutagenic effects of these species on eukaryotic cells.

MATERIALS AND METHODS

Aspergillus nidulans strains and growth conditions

Two strains of the ascomycete *A. nidulans* were used: 1) I Strain: *A* strain, colored green, carrier of a duplication/translocation and mitotically instable (1), and 2) II Strain: *Bio Met* strain, colored green, carrier of biochemical deficiencies for biotin and metionin synthesis. Both were cultivated at 37ºC in agar complete medium (13) and kept in this culture medium during the analyses.

Mushrooms

Shiitake mushroom, Le 10 strain, was obtained from Fungal Genetics Laboratory, CCB/State University of Londrina/PR, Brazil. Himematsutake was a comercial strain produced by farmers of region of Londrina/PR, Brazil.

Mushroom extracts

The extracts were prepared using the mushrooms in the way they are consumed: fresh and dehydrated *L. edodes* and dehydrated *A. blazei*. Fresh mushrooms were left in infusion for 12 h at the proportion of 60 g basidiocarp to 200 ml sterilized distilled water. The mixture was heated in a water bath for 15 min at 100ºC. Dehydrated mushrooms were left in infusion for 12 h at the proportion of 12 g dehydrated basidiocarp to 400 ml distilled water. All the extracts were filtered through Whatman nº 1 filter paper and later through Millipore filter (0.45 µm).

Mutagenic agent

Short wave ultraviolet light (5 Joules/m^2/s) was used as mutagenic agent (Mineralight lamp model UVSL-25, 115 volts, 60H, 0.16 Amps). The survival curves of the *A. nidulans* strains to UV light were drawn as described by Azevedo and Costa (2).

Assessment of toxicity of *L. edodes* and *A. blazei* extracts on *A. nidulans*

A. nidulans conidia were inoculated in 20 ml of agar complete medium (control) and in agar complete medium with 11% *L. edodes a*nd *A. blazei* extracts added. After seven days incubation at 37ºC, the diameter of the colonies was measured and the morphological alterations observed. Sixteen replications were performed for each treatment and the t student test ($p < 0.05$) was used for statistical analyses.

Assessment of antimutagenic action

The antimutagenic effect of the mushrooms was assessed by submitting *A. nidulans* conidia to the *L. edodes* and *A. blazei* extracts before exposure to the UV light (pre-treatment) and after exposure to the mutagenic (post-treatment). In the pre-treatment, conidia of the *A. nidulans* strains at a concentration of 10^6 conidia mL^{-1} were incubated for three hours in water (control) and in mushroom extracts. After this pre-incubation period the conidia were exposed to ultraviolet light. In the post treatment, *A. nidulans* conidia were suspended at the concentration of 10^6 conidia mL^{-1} in water and in mushroom extracts and immediately submitted to UV light treatment. After exposure to the mutagenic, the suspensions were kept in the dark at 3ºC for a period of 24 h. Then 0.1 mL of each suspension was plated in 20 mL agar complete medium in a dark room. The cultures were incubated at 37ºC without light during five days. The colonies that developed were assessed for presence of sectors and for altered morphology.

Conidial suspensions in water and in mushroom extract, but without exposure to the mutagenic agent, were used as controls.

The statistical analysis involved the Chi-square test ($p < 0.01$) with 10 replications of each treatment and the test for comparing two independent proportions with 10 replications of each treatment.

RESULTS AND DISCUSSION

Assessment of the toxicity of the *L. edodes* and *A. blazei* extracts on *A. nidulans*

No inhibitory effect (Table 1) on the mycelial growth of *A. nidulans* was observed when cultivated in presence of the extracts of mushrooms. The addition of dehydrated *A. blazei* extracts stimulated the growth of the two *A. nidulans* strains (Table 1).

Mutagenic activity assessed by the Ames/Salmonella/microsome test has been reported in several wild and cultivated

Table 1. Effect of *L. edodes* (Treatment A) and *A. blazei* (Treatment B) aqueous extract, obtained from basidiocarps on growth of two *A. nidulans* strains.

	Colony diameter (cm)	
Treatment A	I Strain	II Strain
Control	7.83 a	8.05 a
L. edodes fresh extract	7.80 a	8.11 a
L. edodes dehydrated extract	7.90 a	7.99 a
Treatment B		
Control	7.88 b	7.96 b
A. blazei dehydrated extract	8.16 a	8.09 a

Means followed by the same letter in the column within each treatment do not differ by the student test at the 5% level of significance. Mean of 16 repetitions.

mushroom species (16,22). Von-Wright *et al*. (22) described the occurrence of mutagenic compounds in the *Lactarius sp*, *Boletus edulis*, *Agaricus bisporus* and *L. edodes*. Sterner *et al.* (16) observed the occurrence of mutagenic compounds in 37 out of the 48 species tested, including *Lactarius* sp, *A. bisporus* and *Agaricus silvaticus*. These compounds are still unknown and need to be isolated and characterized so that specific tests must be carried out which can provide a safe indication of the risks involving consumption of these mushrooms by men and animals. Our results did not detect apparent mutagenic action on *A. nidulans* but it could be observed that dehydrated *L. edodes* exhibited the property of stabilizing strains that are mitotically unstable as the *A* strain of *A. nidulans*.

Fresh *L. edodes* antimutagenic activity

Aqueous extract of the fresh *L. edodes* mushrooms showed radioprotecting action. *A. nidulans* conidia treated with *L. edodes* extract before or after exposure to UV light showed an increase in their survival rate when compared with the control (Fig. 1). The fresh *L. edodes* extract protected the cells against the harmful action of the UV rays and also showed antimutagenic effect on the conidia treated. A decreased number of *A. nidulans* morphological mutants was observed after pre and post treatment with *L. edodes* extract (Table 2).

Aqueous extract of fresh *L. edodes* showed to be a powerful antimutagenic agent, suggesting the presence of compounds capable of minimizing the effects caused by the UV mutagenic agent, beneficial characteristic that should be added to the list of nutraceutical effects of shiitake.

Dehydrated *L. edodes and A. blazei* antimutagenic action

Both mushrooms species under investigation showed radioprotective and antimutagenic action on *A. nidulans* strains. Conidium survival in the *A. nidulans* strains irradiated with UV was significantly greater when treated with the aqueous extracts of these dehydrated mushrooms (Fig. 2).

Conidia of the two *A. nidulans* strains submitted to pre-treatment with dehydrated *L. edodes* and *A. blazei* extract before exposure to UV radiation showed a reduction in the mutation rate (Table 3).

These results are in agreement with those obtained by Osaki *et al.* (12) who observed *A. blazei* antimutagenic action in the Ames test. These authors obtained extracts from the fructification body of the himematsutake that were able to inhibit the mutagenic Benzo(a)pyrene (B(a)P). Linoleic acid was the antimutagenic substance purified and identified from the *A. blazei* fructification body.

Sugui *et al* (18) showed that dried *L. edodes* added to basal diet on mice reduce the frequencies of micronucleated bone marrow polychromatic erythrocytes induced by N-ethyl-N-nitrosourea.

The *L. edodes* and *A. blazei* mushrooms were shown to have substances that protect eukaryotic cells against the harmful effects of UV radiation. In addition the aqueous extract of these mushrooms also exhibited antimutagenic effect.

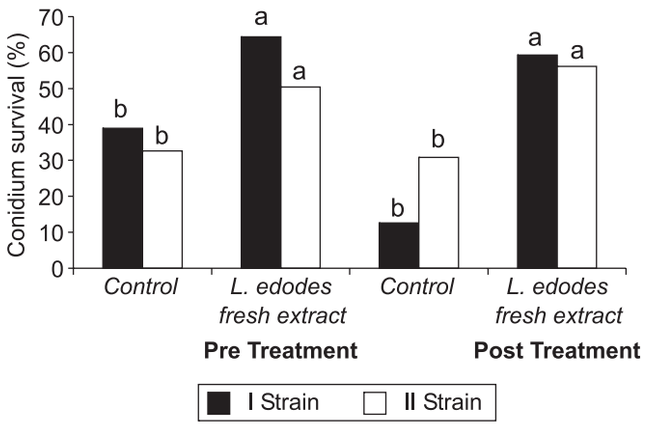

Figure 1. Radioprotector effect of fresh *L. edodes* extracts on the conidium survival of two *A. nidulans* strains submitted to irradiation with the mutagenic UV. Results represent test for comparing two independent proportions at the 5% level of significance (mean of ten repetitions). **Pre-treatment**: *A. nidulans* conidia were pre-treated with *L. edodes* extract before exposure to the UV light. **Post-treatment**: *A. nidulans* conidia were treated with *L. edodes* extract after exposure to the UV light.

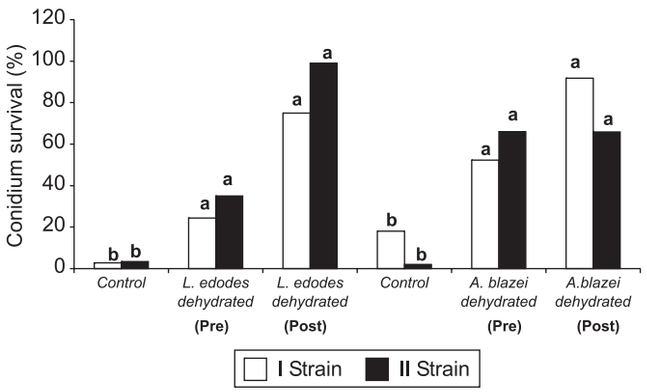

Figure 2. Radioprotector effect of dehydrated *L. edodes* and *A. blazei* extracts on the conidium survival of *A. nidulans* strains, submitted to irradiation with the mutagenic UV. Results represent test for comparing two independent proportions at the 5% level of significance (mean of ten repetitions). **Pre** (Pre-treatment): *A. nidulans* conidia were pre-treated with *L. edodes* and *A. blazei* extracts before exposure to the UV light. **Post** (Post-treatment): *A. nidulans* conidia were treated with *L. edodes* and *A. blazei* extracts after exposure to the UV light.

Table 2. Antimutagenic effect of fresh extract of *L. edodes*. *A. nidulans* conidia were treated with mushroom extract before (pre-treatment) and after (post-treatment) exposition to UV light.

			Pre-Treatment				Post-Treatment			
			I Strain		II Strain		I Strain		II Strain	
			Morphological Mutants (%)	Sectors	Morphological Mutants (%)	Sectors	Morphological Mutants (%)	Sectors	Morphological Mutants (%)	Sectors
L. edodes	Water (Control)	I	6.3 **	0	5 **	4	7.8 **	2	6.2 **	2
	Extract	I	1.3	0	0.5	0	1	0	0.7	1
	Water (Control)	W I	0	0	0.1 ns	1	0	0	0	0
	Extract	W I	0	0	0	2	0	1	0	1

I = Irradiated; W I = Without Irradiation; ** = significant; ns = not significant by the Chi-square statistic test ($p < 0.01$).

Table 3. Antimutagenic effect of dehydrated *L. edodes* and *A. blazei* extracts. *A. nidulans* conidia were treated with mushrooms extracts before (pre-treatment) and after (post-treatment) exposition to UV light.

			Pre-Treatment				Post-Treatment			
			I Strain		II Strain		I Strain		II Strain	
			Morphological Mutants (%)	Sectors	Morphological Mutants (%)	Sectors	Morphological Mutants (%)	Sectors	Morphological Mutants (%)	Sectors
L. edodes	Water (Control)	I	6.3 **	9	6.9 **	10	4.4 **	6	8.5 **	3
	Extract	I	0.15	0	0	0	0	0	0	2
	Water (Control)	W I	0	2	0.6 ns	1	0.08 ns	1	0.2 ns	0
	Extract	W I	0	3	1.1	5	0	0	0.2	0
A. blazei	Water (Control)	I	6.3 **	9	6.9 **	10	4.4 **	6	8.5 **	3
	Extract	I	1.1	0	0.3	0	0	0	0	2
	Water (Control)	W I	0 ns	2	0.6 ns	1	0.08 ns	1	0.2 ns	0
	Extract	W I	0.3	2	0.4	0	0	0	0	0

I = Irradiated; W I = Without Irradiation; ** = significant; ns = not significant by the Chi-square statistic test ($p < 0.01$).

RESUMO

Ação antimutagênica de *Lentinula edodes* e *Agaricus blazei* em conídios de *Aspergillus nidulans*

O efeito antimutagênico dos cogumelos *Lentinula edodes* e *Agaricus blazei* foram estudados sobre conídios de *Aspergillus nidulans* quando expostos à luz ultravioleta de comprimento de onda curto. Duas linhagens de *A. nidulans* foram usadas. Para o preparo dos extratos, os cogumelos frescos permaneceram em infusão aquosa por 12 horas e em seguida foram aquecidos em banho-maria por 15 min à 100ºC e a seguir o material foi filtrado. Os cogumelos desidratados foram deixados em infusão aquosa por 12 horas e a seguir filtrados. Ambos os

filtrados foram usados como extratos. Os conídios de *A. nidulans* foram incubados por três horas em água e em extrato de cogumelo e somente após foram expostos a luz ultravioleta (pré-tratamento). Conídios de *A. nidulans* foram incubados em água e em extrato de cogumelo e imediatamente submetidos à luz ultravioleta (pós-tratamento). Conídios incubados em água e em extrato de cogumelo, mas sem exposição ao agente mutagênico, foram usados como controle. Após tratamento mutagênico, observou-se um aumento na taxa de sobrevivência de *A. nidulans* e uma diminuição na porcentagem de mutantes morfológicos em conídios tratados com extrato de cogumelos. Nossos resultados demonstram o efeito radioprotetor e antimutagênico dos cogumelos *L. edodes* e *A. blazei* sobre células eucarióticas submetidas à radiação UV.

Palavras-chave: antimutagênico, *Lentinula edodes*, *Agaricus blazei*, *Aspergillus nidulans,* shiitake

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