Evaluation of benefit potential of consume mushroom *Agaricus blazei* for endogenous microbiota: analysis of effect *in vivo* and *in vitro* over different bacterial lineages.

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Abstract

The *Agaricus blazei* is a mushroom widely used by various cultures mainly for medicinal purposes. This mushroom was traditionally used to treat many common diseases like atherosclerosis, hepatitis, hyperlipidemia, diabetes, dermatitis and cancer. The present study aimed to analyze *Agaricus blazei* antimicrobial activity. In order to evaluated *in vivo* activity of *Agaricus blazei* the fecal microbiota content of wistar rats was analyzed. In order to evaluate *Agaricus blazei* activity in *vitro* was analyzed the extracts effects against different bacteria's species of medical interest. The *in vivo* administration of mushroom leads to a significant reduction of microbial components when fecal contents (expressed in number of units formed colonies) are counted. However the number of anaerobic species was not affected like the aerobics species. The *in vitro* results also demonstrated that the mushroom has a good performance as bactericidal agent. However, despite its broad spectrum of action, no effect was observed against tipical endogenous microbiota species. These findings suggest the possible existence of a selective action against enteropathogens and a concomitant effect of protection of endogenous microbiota. This could be useful for strengthening barriers against intestinal infection diseases'.

Keywords: *Agaricus blazei*. Antimicrobial. Endogenous microflora.

Resumo

O *Agaricus blazei* é um basidiomiceto extensamente utilizado na alimentação humana por várias culturas, em virtude de suas propriedades, não apenas nutritivas, mas potencialmente medicinais. O presente trabalho objetivou anlisar a atividade antimicrobiana *in vivo* e *in vitro* do cogumelo *Agaricus blazei.* As avaliações *in vivo* demonstraram a atividade antibacteriana do cogumelo, levando a uma redução significativa dos componentes microbianos encontrados nas fezes de ratos wistar. Os resultados *in vitro* também demonstraram que o cogumelo possui potencial antimicrobiano contra diferentes linhagens bacterianas de interesse médico. Contudo, apesar de seu amplo espectro de ação, não foram detectados efeitos sobre as principais linhagens da microbiota endógena. Esses achados demonstram a possível existência de uma ação seletiva a enteropatógenos, concomitante a um efeito protetor da microbiota endógena, que se constitui de extrema relevância para fortalecimento das barreiras contra infecções por agentes patogênicos. Isto sugere que o consumo do cogumelo seja promissor na profilaxia de afecções intestinais.

Palavras chave: *Agaricus blazei*. Atividade antimicrobiana. Microbiota endógena.

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Introduction

The *Agaricus blazei* is a fungus of the *Basydiomicota* phylum and *Agariaceae* families, which are native to Brazil and are popularly known as sun-dried mushrooms. These are widely consumed because of their medicinal and nutritional properties, associated with the prevalent absence of adverse effects caused by their intake, which has attracted the interest of many researchers.

A. blazei consists of approximately 90% water, 2-4% proteins, 2-8% fats, 1-55% carbohydrates, and 3-32% fibers, in addition to essential amino acids, important minerals, and a low calorie content (FIRENZOULI, GORI, LOMBARDO, 2008).

According Faccin et al. (2007), this mushroom has traditionally been used to treat common disorders, including emotional and psychological stress, hyperlipidemia, and gastric digestive diseases. It also contains important anticancer, antioxidant, immunomodulator, and hypotension properties.

Extracts from A.blazei are also rich in polysaccharides, such as β-glucans, which are presumably responsible for stimulating/modulating the immune system, by inducing the secretion of various cytokines, such as IL-12, TNF-alfa, and IFN-gama, as well as activating neutrophils, macrophages, lymphocytes, and natural killer (NK) cells (HETLAND et al., 2008; ZHONG, TAI, YAMAMOTO, 2005).

These fungi are organisms classified as secondary decomposers. Therefore, in their natural habitat it is possible to find innumerable competitors, including microorganisms. Thus, it is possible that the fungi actually produce substances that perform antimicrobial actions (ZHENG et al., 2010).

The production of antimicrobial peptides is a universal characteristic of the native immune system and is present in wide range of living creatures, including bacteria, fungi, plants, and animals, whose induction paths have been relatively preserved (HOFFMAN et al., 2000). Since peptides are not the only molecules found in the Fungi kingdom, it is quite likely that *Agaricus blazei* does in fact contain bioactive properties with potential antimicrobial activity.

In this light, the present work aims to evaluate the antimicrobial activity of *Agaricus blazei*, by means of an *in vitro* antagonism test with different bacterial

lineages, including some enteropathogens, as well as the effects of the intake of this mushroom on endogenous microbiota through a series of *in vivo* experiments.

Methods

a. Preparation of extracts of *Agaricus blazei:* 1g of the mushroom was weighed and 10 mL of solution containing between 0 and 50% ethanol in a 100 Tris 100 mM buffer, pH 7.0, was prepared. After 1h of incubation at 25ºC, the extracts were centrifuged at 9000 rpm for 1h. The methanol, ethanol, propanol, and butanol extracts, after having been centrifuged, were lyophilized for 18 h and solubilized in 1 mL of water.

b. Test of antagonism *in vitro:* revealing bacteria of reference were inoculated into a liquid BHI medium and incubated for t 37ºC. After incubation, 20 µl were inoculated in 4 mL of semi-solid BHI agar. Plates containing solid BHI were divided into two parts, identified as SA (non-alcoholic – 0% ethanol) and CA (alcoholic – 50% ethanol). After, 30 µl of the extracts were applied in three applications of 10 µl each. After drying, the plates were covered with semi-solid BHI agar that had been previously inoculated with the revealing bacteria. The plates were incubated for 24 h at 37°C, and the inhibitory activity was read and recorded.

c. *In vivo* experiment: The present study used 14 healthy, male Wistar mice from the Animal Facility (Bioterium) in the Biological Sciences Institute at Universidade Federal de Minas Gerais (UFMG), with weights ranging between 160 g and 350 g. The protocol was developed contemplating four different groups, including the Control, which received saline, and three experimental groups, which received an *Agaricus blazei* tea, together with Gentamicin plus *Agaricus blazei*, by gavage. The feces of all animals were collected on the first, third, and seventh days of the experiment. For each day of collection, three serial dilutions for each animal were made and subsequently plated in selective McConkey, BHI, and MRS mediums. After, the plates were incubated at 37ºC for 24 h and the colonies which grow in each medium were counted.

Results and discussion

Evaluation of the in vitro activity of different Agaricus blazei *mushroom extracts*

The extracts of *Agaricus blazei* presented antimicrobial activity against 12 of the 15 tested strains. The better halos of inhibition were obtained through propanol extraction, which was capable of inhibiting the majority of tested strains at varying degrees. However, the aqueous extract proved to be the most appropriate for consumption and should be given priority in future tests.

Tests of *in vitro* antimicrobial activity

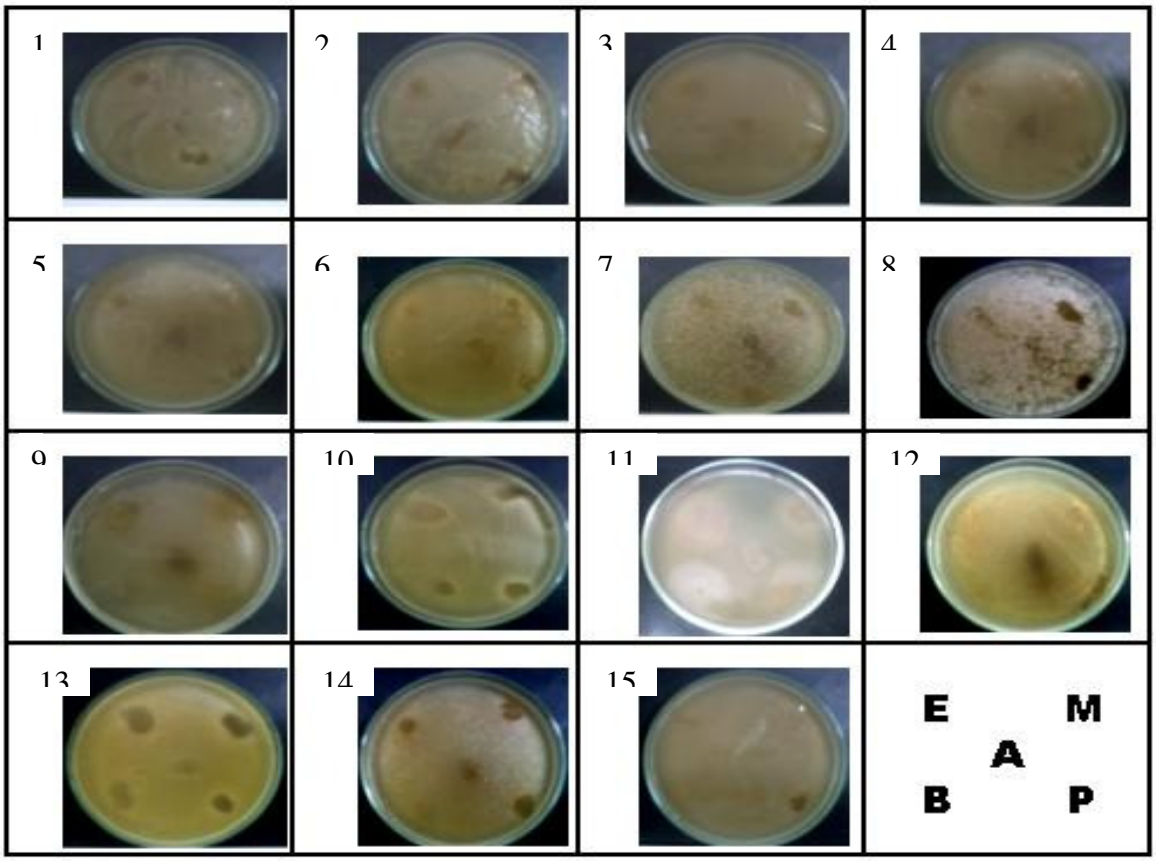
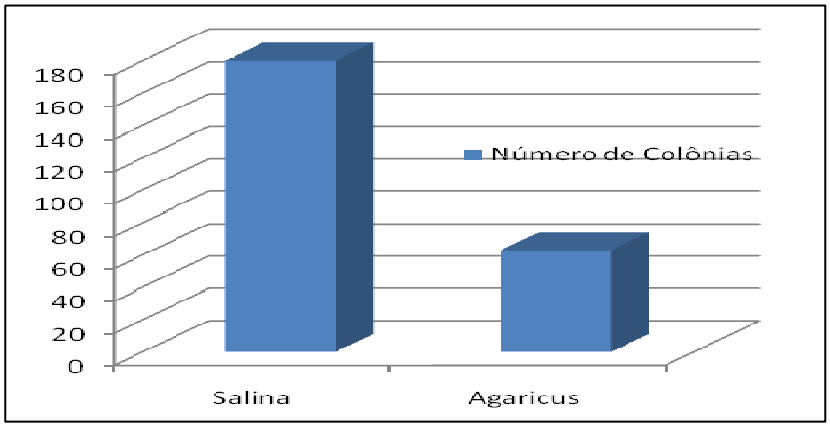


Figure 01. Tests of antimicrobial activity in the fourth experiment: Left to right, 1-*Salmonella choleraesuis, 2-Salmonella enteritidis, 3-Salmonella typhimurium, 4-Enterobacter aerogenes(1), 5-Enterobacter aerogenes(2), 6-Escherichia coli, 7-Shigella flexneri, 8-Bacillus cereus, 9-Listeria monocytogenes, 10-Micrococcus luteus, 11-Pseudomonas aeruginosa, 12-Klebsiella oxytoca, 13-Staphylococcus aureus, 14-Citrobacter freundii, 15-Proteus mirabilis.* Arrangement of the extracts in the experiment: A (aqueous extract), M (methanol), E (ethanol), P (propanol), and B (butanol); 50% alcohol extracts (v/v) in Tris 0.617M, pH 6.8. Source: Research data

Graph 01: Effect of adding *Agaricus blazei* mushroom in the diet on the fecal microbiota of *Wistar* mice.

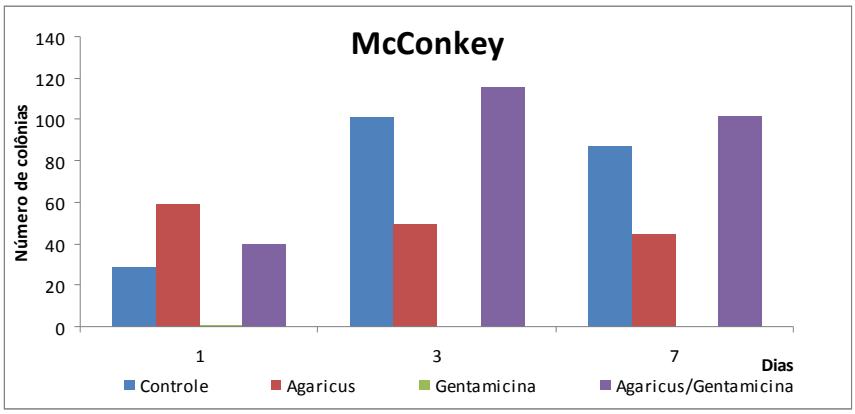


Evaluation of the bacterial population present in the fecal microbiota of Wistar mice. Group A (saline solution, via gavage), Group B (*Agaricus blazei*, via gavage). Mean of the count of colonies that grow in BHI, in three different days of the experiment (1st, 3rd, and 7th day).

Source: Research data

The mean of the colonies detected in the feces of the animals that received the *A. blazei* mushroom was approximately 60% less than the mean of the colonies detected in the control group. Also assessed were parameters such as the consumption of the food and water, but no significant change could be observed between the two groups. Graph 01 only showed data for the inspecific BHI medium. As a positive control, this experimental model used a group which received gentamicin (data not shown), which presented a highly insignificant count within the conditions described above.

Graph 02: Mean of colonies expressed in the selective and differential McConkey medium



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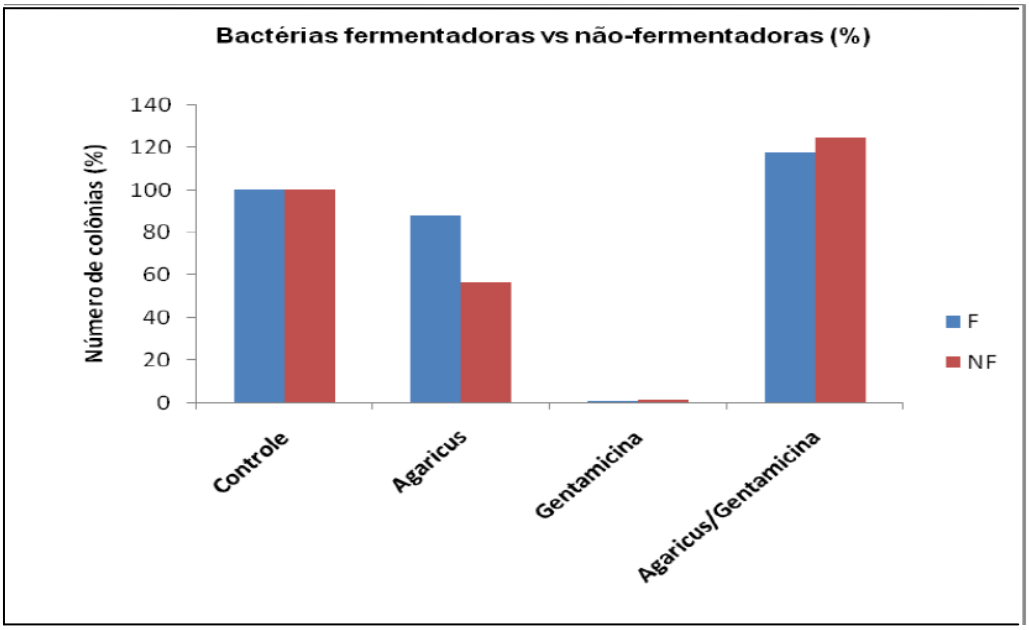
Source: Research data

Graph 02 shows an occurrence of the suppression of enterobacteria in the *A. blazei* and Gentamicin groups. The *A. blazei* group was as suppressed when compared to the control group (except on the first day) as it was when it was compared to itself over time. The Gentamicin group produced nearly complete suppression, beginning on the first day of the experiment. The *A. blazei*/Gentamicin comparison presented, paradoxically, a progressive increase in colonies form day to day, which may indicate a possible interaction among molecules from both compounds, in turn preventing their antimicrobial function.

Graph 03: Percentage of fermentative and non-fermentative colonies as compred to the control group



Source: Research data

This graph shows a greater suppression of bacteria that are unable to ferment lactose, as compared to the fermentative bacteria, in all study groups, not including the Gentamicin group, where the suppression between the two bacteria groups was similar, not the *A. blazei*/Gentamicin group, where an increased number of colonies of the two bacteria groups can be observed. This finding suggests that the *A. blazei*, when administered alone, can, in addition to suppressing non-fermentative colonies, favor the growth of fermentative bacteria, thus indicating apossible preferential antimicrobial activity over aerobic bacteria.

As the food passes through the digestive tract, undergoing different actions, as well as physical, chemical, and enzymatic processing, it is possible that compounds become activated, revealing relevant biological activity. The present study aimed to evaluate the antimicrobial action of *Agaricus blazei,* both *in vitro* and *in vivo.*

The *in vivo* assessment showed antibacterial activity from the mushroom, leading to the significant reduction of the fecal microbial components. The *in vitro* results also showed the potential antimicrobial activity of the mushroom. However, despite of their wide range of action, they do not have any effect on the main bacteria of core strains of endogenous microbiota.

These results highlighted the possible existence of selective action against enteropathogens, a concomitant protective effect stemming from endogenous microbiota, which is extremely important in strengthening defense mechanisms against infections for pathogens, suggesting the possibility that the consumption of this mushroom may well be a promising development in the preventive measures taken against intestinal tract diseases.

Findings from Bernardshaw et al. (2005) presented the positive prophylactic effects of the mushroom, also confirmed after *in vivo* testing, which showed a reduction in bacteremia and an increase in the survival rate of the animals when the *A.blazei* extract was administered orally, 24 hours after the systemic injection of serotype B *Streptococcus pneumoniae*.

Conclusion

Results from the present study confirm the antimicrobial effect of *Agaricus blazei*, both *in vitro* and *in vivo*. These findings illustrate the need for investment in research aimed at isolating the compound, present in basidiomycete, responsible for antibiotic activity.

However, further studies are warranted to promote better quantification and qualification of enterobacteria suppression caused by the *A. blazei* mushroom when administered in animals models.

Furthermore, the creation of experimental models that are different from the commonly used models can aid in shedding light on the dynamic performance of *Agaricus blazei in vivo*, as well as its interaction with other compounds of clinical

relevance, thus enabling the establishment of criteria to optimize the effects resulting from the consumption of this mushroom, in turn stimulating the expansion of its use in alternative medicine.

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