INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF BASIDIOMYCETES *Lentinula boryana* **AND** *Lentinula edodes*

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ABSTRACT

Two species of basidiomycetes, *Lentinula boryana* and *Lentinula edodes*, were evaluated for their antibacterial activities, biomass production and growth in two different culture media. The basidiomycete *L. boryana* occurs naturally in the Brazilian territory whilst *L. edodes* grows in Asia. Mycelia from each species were incubated in liquid media for 28 days and vacuum-filtered. *L. boryana* showed the largest biomass production in both culture media when compared to *L. edodes*, which presented significant differences in growth when cultivated in different culture media. Antibacterial activity of the two species was evaluated against 10 bacterial species, six of them being of clinical importance. Both basidiomycetes *L. boryana* and *L. edodes* showed antibacterial activity against *B. cereus* and *S. aureus*, although only *L. edodes* was active against *S. mutans.*

Key words: Basidiomycetes, *Lentinula boryana*, *Lentinula edodes* (shiitake mushroom), antibacterial activity.

INVESTIGAÇÃO DA ATIVIDADE ANTIBACTERIANA DOS BASIDIOMICETOS *Lentinula boryana* **and** *Lentinula edodes*

RESUMO

Duas espécies de basidiomicetos, *Lentinula boryana* e *Lentinula edodes*, foram avaliados quanto à atividade antibacteriana, crescimento e produção de biomassa em dois diferentes meios de cultivo. O basidiomiceto *L. boryana* apresenta ocorrência natural no território brasileiro, enquanto que *L. edodes* ocorre prevalentemente na Ásia. Os micélios de cada uma das espécies foram cultivados em meio líquido, por 28 dias e posteriormente filtrados sob vácuo. O *L. boryana* apresentou a maior produção de biomassa em ambos os meios de cultivo utilizados, quando comparado ao *L. edodes*, o qual apresentou diferenças significativas em seu desenvolvimento quando cultivado nos diferentes meios. A atividade antibacteriana das duas espécies foi avaliada contra dez espécies bacterianas, seis delas de relevante importância clínica. Ambos os basidiomicetos *L. boryana* e *L. edodes* mostraram atividade antibacteriana contra *B. cereus* e *S. aureus*, porém, somente *L. edodes* apresentou atividade contra *S. mutans*.

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INTRODUCTION

Basidiomycetes are widely used as a esquisite type of food due to their pleasant taste and, most specially, due to their nutritional and medicinal properties. They contain very important proteins, carbohydrates and mineral salts, besides being a very good source of fibers. They are able to sinthesize a great amount of secondary metabolites that present antitumoral, antiviral, anti-inflamatory, antithrombotic, citostatic and hipoglicemic activities. (BREENE, 1990; CHANG & BUSWELL, 1996; BRIZUELA et al., 1998; WASSER & WEIS, 1999; KÜES & LIU, 2000; SUAY et al., 2000).

Lentinula edodes (Berk.) Sing. is a fungus that decomposes wood by utilizing lignin, cellulose and hemicellulose as carbon sources (HIBBETT et al., 1995; CHANG & BUSWELL 1996). It forms fruiting bodies in mild temperatures in the range of 15ºC to 20ºC with relative humidity above 80%, making it the world's second most cultivated mushroom species, naturally distributed in Japan, Southeast Asia and Australasia (PRZYBYLOWICZ & DONOGHUE, 1990; SHIMOMURA et al., 1992). In Brazil its cultivation was established in the Southern and Southeast regions because of the appropriate climate conditions, employing *Eucalyptus* spp. logs and sawdust to grow the fruiting bodies (PRZYBYLOWICZ & DONOGHUE, 1990; ISHIKAWA et al., 2001; ISHIKAWA et al., 2003).

Lentinula boryana (Berk. & Mont.) Pegler, has being found in Brazil and other subtropical and tropical countries of the Americas. However being an edible mushroom, its cultivation in large scale has not developed as yet, and little information is available concerning potential medicinal uses (PEGLER, 1983; GUZMÁN et al., 1993). Both fungi *L. boryana* e *L. edodes* belong to the Basidiomycota division, Agaricales order, Tricholomataceae family (GUZMÁN et al., 1997). These two species are characterized by possessing dense hiphae in the basidiocarp with sinuous lamels anexed that protrude from the steep, their colour fading as they become aged (HIBBETT, 1992). These morphological similarities between *L. boryana* and *L. edodes* (shiitake mushroom), have brought forth many studies focusing on the biochemistry, physiology, ecology and philogeny of *L. boryana*, in order to successfully cultivate this fungus (GUZMÁN et al., 1993; HIBBETT et al., 1995; GUZMÁN et al., 1997; MATA & PETERSEN, 2000).

As a consequence of the facts reported above, this research work had three main objectives: i) to establish parameters of micelial development and micelial behaviour of *L. boryana* and *L. edodes* in laboratory conditions; ii) to study the production, *in vitro*, of therapeutic metabolites; iii) to assess the antibacterial spectra of these metabolites against clinically important bacteria.

MATERIALS AND METHODS

MICROORGANISMS

Two species of basidiomycetes were assayed: *L. boryana*, collected in Morro do Canal located in the outskirts of the city of Piraquara, state of Paraná, in july, 12, 2003, and *L. edodes*, purchased from a market place in Curitiba, state capital of Paraná, Brazil. Bacterial species used as target organisms to assess the antimicrobial activities of the mushroom's metabolites are listed in Table 1.

ISOLATION IN CULTURE MEDIA

Small tissue fragments of basidiocarps were excised from each species of the Basidiomycetes. The tissue fragments were placed in Petri dishes containing Potato Dextrose Agar (Difco) and incubated at 25ºC to obtain enough micelial biomass, that was transferred to glass tubes with Potato Dextrose Agar and incubated at room temperature.

PRODUCTION OF THE EXSICATA

Basidiocarps of both species were placed in heated cabinets, with temperature ranging from 40ºC-45ºC, in order to dehydrate properly. Exsicata from *L. boryana* was sent to the Instituto de Botanica in São Paulo, Brazil, where it was found that its spores were somewhat larger (8,4-9,8 µm by 2,8-4,2µm) than those described by Pegler (1983) which were 5-6µm by 2-3,5µm, Q=2,00, according to Dr. Marina Capelari in a personal communication. This specimen is being maintained at the Herbário-FUEL of the

Universidade Federal de Londrina, state of Paraná, Brazil.

ASSESSMENT OF MICELIAL GROWTH

Micelial growth of *L. boryana* and *L. edodes* was determined by cultivation in Petri dishes containing Potato Dextrose Agar and Sabouraud Dextrose Agar (Difco). Micelial agar discs with 5mm in diameter from each of the basidiomycetes were used as inocula in the experiments. All assays were performed in quintuplicate.

CULTIVATION IN LIQUID MEDIA

Five micelial discs (5mm in diameter) of the basidiomycetes were transferred to each one of six 250 mL Erlenmeyers flasks, containing 100mL of Malt extract-Soy peptone broth (3g of malt extract Biobrás and 0,3g of soy peptone Merck). The flasks were sterilized and the pH adjusted to a value of 6,0, prior to inoculation. The inoculated flasks were incubated at 25ºC for 28 days, without agitation and in the dark. Afterwards, all micelia were vacuum-filtered and the filtrates stored in plastic capped flasks at minus 10ºC to be further used in the antimicrobial activity assays.

DETERMINATION OF MICELIAL DRY WEIGHT

Biomass of micelia dehydrated at 110ºC for 12 hours were then weighted in analytical scales.

ANTIBACTERIAL ACTIVITY

For this assay, the agar diffusion method in Petri dishes containing three sterile stainless steel cylinders was used. Bacterial species of clinical importance were inoculated in 5mL of Brain Heart Infusion Broth (BHI,Difco) and incubated at 35ºC for 18 hours. The resulting bacterial suspensions had their concentrations adjusted to a 0,5 value of the MacFarland scale, with sterile saline solution. Afterwards, Petri dishes containing Mueller Hinton Agar (Difco) were inoculated in triplicate with the bacterial suspensions to be tested, in accordance with the protocols of the National Committee for Clinical Laboratory Standards (2000).

Following appropriate growth of the bacteria to be tested, three sterile stainless steel cylinders were disposed in each of the Petri dishes, and 250 µL of the micelial filtrates were asseptically poured into them. The Petri dishes were then maintained at 4ºC for 12 hours to allow proper diffusion of the compounds contained in the filtrates, and then incubated at 37ºC for 18 to 24 hours. Antimicrobial activity of each of the compounds was determined by measuring the zone of inhibition formed around the steel cylinders.

RESULTS AND DISCUSSION

L. boryana showed a rapid mycelial growth in Potato Dextrose Agar and Sabouraud Dextrose Agar, reaching the border of the Petri dish in seven days (Fig. 1).

L. edodes showed a slower growth in Sabouraud Dextrose Agar, taking it twenty days to reach the border of the Petri dish (Fig.1). *L. boryana* cultivated in Peptone Malt Extract Broth produced a larger amount of micelial biomass than *L. edodes* in the same medium and growth conditions (Fig.2).

Results of the antibiosis assays showed quite clearly that filtrates from mycelia of *L. boryana* inhibited growth of *B. cereus* and *S. aureus*, whilst filtrates from *L. edodes* were active against *Bacillus cereus*, *S. aureus* and *S. mutans* (Table 2).

Ishikawa *et al*. (2001), reported data similar to the results obtained in this work, showing antibacterial action of *L. edodes* against *B. cereus*, *S. aureus* and *S. epidermidis*. Komemushi *et al*. (1996) reported that *L. edodes* inhibited growth of Gram-positive and Gram-negative bacteria. Hirasawa *et al*. (1999) and Shouji *et al*.(1999) reported activity of *L. edodes* against *S. mutans* and *S. sobrinus*, which are two of the most important causative agents of dental cavities in man. Shouji *et al*.(1999) also showed reduction of dental decay in guinea pigs after treating them with extracts obtained from dehydrated shiitake mushroom.

Amongst important antimicrobial compounds isolated from *L. edodes* (shiitake) the following deserve to be cited: i) Cortineline, that is effective against Gram-positive bacteria (PRZYBYLOWICZ & DONOGHUE, 1990); ii) Lentinamicin, (octa-2,3-dien-5,7-diin-1-ol), identified as the main compound produced by *L. edodes* mycelia, effective against bacterial

pathogens in man (KOMEMUSHI et al., 1996; ISHIKAWA *et al*., 2001[b]); iii) Lentinan, a polissacaride reported to be active against resistent *Mycobacterium tuberculosis*, *B. subtilis*, *S. aureus*, *Micrococcus luteus*, *Candida albicans* and *Saccharomyces cerevisiae* (WASSER & WEISS,1999).

Antimicrobial activity of *L. edodes* against *S. epidermidis* and *S. sobrinus* cited in literature was not demonstrated in this work. This discrepancy can be explained by genetic variability at the intraspecific level (SUAY et al., 2000), thus explaining differences in the production of bioactive metabolites found among 35 co-specific isolates of *L. edodes* (ISHIKAWA *et al*.,2001); also, it may explain significant differences in bioactivity found in 10 isolates of *Flammulina velutipes* (ISHIKAWA, 2001) and in some groups of fungi other than Basidiomycetes (ROSA et al. 2003, SUAY et al. 2000).

These facts show the utmost importance of careful manipulating the cultures in order to isolate and maintain different strains of basidiomycetes without contamination. Other relevant cultivation factors, such as pH of culture media, substrate and nutrient composition, incubation time periods, light and darkness periods, aeration and agitation of micelia, could adversely affect growth and induce undesired variability in strains of Basidiomycetes.

This research work reported the unique production, *in vitro*, of antibacterial metabolites by *L. boryana* and *L. edodes*. The antibiosis assays demonstrated that compounds produced by both Basidiomycetes inhibited growth of clinically relevant Gram-positive bacteria. Data gathered in the experiments show the necessity to expand this line of research, in order to further characterize and identify the bioactive metabolites, specially those produced by *L. boryana*.

AKNOWLEDGEMENTS

To Dr. Marina Capelari, departamento de Micologia e Liquenologia, Instituto de Botânica de São Paulo for the identification of *L. boryana* and to CNPq for financial support throughout the research.

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Table 1 – Microorganisms tested in the antimicrobial activity assays.

Gram-positive bacteria	**Strain**
Bacillus cereus	ATCC 11778
Bacillus subtilis	ATCC 6633
Enterococcus faecalis	ATCC 19433
Staphylococcus aureus	ATCC 25923
Staphylococcus epidermidis	ATCC 12228
Streptococcus mutans	CCT 3440
Streptococcus sobrinus	ATCC 27607
Gram-negative bacteria	**Strain**
Escherichia coli	ATCC 25922
Pseudomonas aeruginosa	ATCC 27853
Salmonella typhimurium	ATCC 14028

Table 2 – Antimicrobial activity of the filtrates obtained from cultures of *L. boryana* and *L. edodes* against Gram-positive and Gram-negative bacteria.

	L. boryana	*L. edodes*
Gram-positive bacteria		
Bacillus cereus	+	+
Bacillus subtilis	-	-
Enterococcus faecalis	-	-
Staphylococcus aureus	+	++
S. epidermidis	-	-
Streptococcus mutans	-	+
S. sobrinus	-	-
Gram-negative bacteria	*L. boryana*	*L. edodes*
Escherichia coli	-	-
Pseudomonas aeruginosa	-	-
Salmonella tiphymurium	-	-

+ = inhibition zone diameter from 10 to 20 mm;
++ = inhibition zone diameter from 20 to 30 mm;
- = absence of inhibition zone.

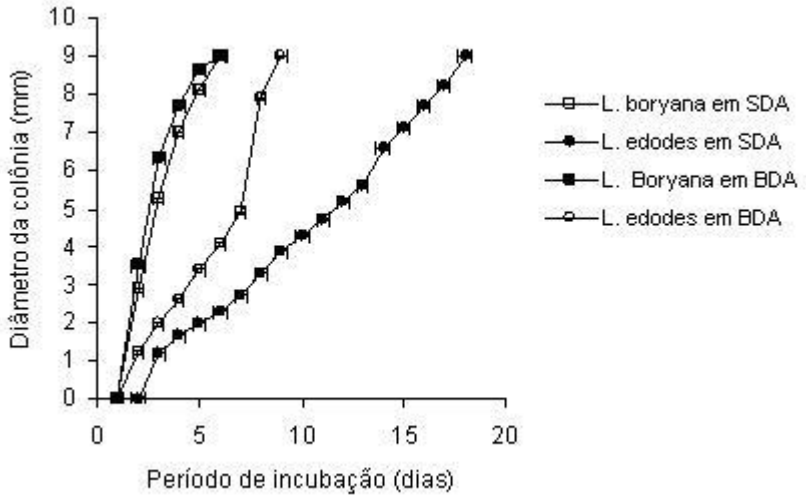

Figure 1 – Mycelial development of *L. boryana* and *L. edodes* in PDA and SDA media.

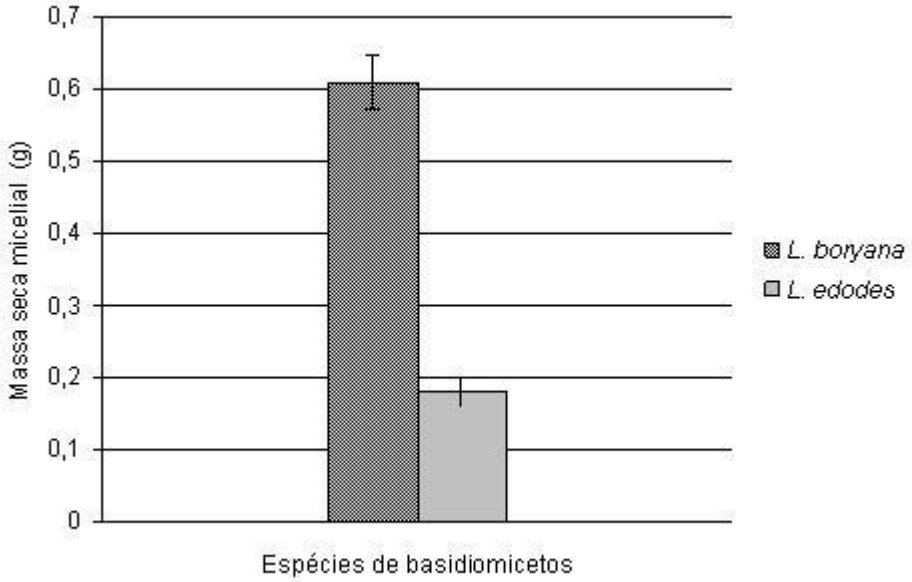

Figure 2 – Dried mycelial biomass of *L. boryana* and *L. edodes* developed in MEP broth medium (100 mL), at 25°C for 28 days, in triplicate.