Review Paper:

Shiitake mushroom (*Lentinula edodes*): an important macrofungus with pharmacological activities

Roy Anamika and Archana T.S.*
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara (Punjab), 144411, INDIA
*archana.26887@lpu.co.in

Abstract

Lentinula edodes is the first medicinal macrofungus to enter the realm of modern biotechnology. It is the second most popular edible mushroom in the global market which is attributed not only to its nutritional value but also to possible potential for therapeutic applications. Lentinus edodes is used medicinally for many diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, diabetes, hepatitis and regulating urinary inconsistencies.

It is the source of several well-studied preparations with proven pharmacological properties, especially the polysaccharide lentinan, eritadenine, shiitake mushroom mycelium and culture media extracts (LEM, LAP and KS-2). Antibiotic, anti-carcinogenic and antiviral compounds have been isolated intracellularly (fruiting body and mycelia) and extracellularly (culture media). Some of these substances were lentinan, lectins and eritadenine. The main aim is to discuss the therapeutic applications of this macrofungus. The potential of this macrofungus is unquestionable in the most important areas of applied biotechnology.

Keywords: Macrofungus, Antitumor mechanism, *Lentinus edodes*, Polysaccharides.

Introduction

Mushrooms are one of the important fungi belonging to the family of basidiomycetes. There are different types of mushrooms reported till now, of them some are beneficial and some are poisonous (e.g. Amanita). The poisonous mushrooms are also known as toadstool and are non-desirable as they are harmful for consumption. Mushrooms owe their popularity just not because of their delectable taste but their compositions and nutritional profiles. Mushrooms are nutrient dense and packed with antioxidants.

Here we are going to discuss a very important medicinal mushroom, *Lentinula edodes.* The shiitake mushroom is an edible mushroom native to East Asia which is mainly cultivated and consumed in many Asian countries. It is considered as a medicinal mushroom in form of some traditional mushrooms. The fungus was first described scientifically as *Agaricus edodes* by Miles Joseph Berkeley in1877. It was placed in the genus *Lentinula* by David Pegler in 1976[1,3,11,12].

Different types of edible mushrooms: There is a wide range of mushrooms found in different parts of the world. Some very common edible mushrooms are given in fig. 1.

Figure 1: Images from The Mushroom Council (http://www.mushroominfo.com/varieties/)

Morphology of *Lentinula edodes*: It is commonly consisting of a cap or pileus and a stalk or stipe but others have additional structures like veil or annulus, a cup or volva. Cap or pileus is the expanded portion of the carpophore (fuiting body) which may be thick, fleshy, membranous or corky. On the other side of pileus, gills are situated, these gills bear spores on their surface and exhibit a change in colour corresponding to that of the spores. The attachment of the gills to the stipe helps in the identification of the mushroom[2,4].

Importance of *Lentinula edodes*: These mushrooms are one of the popular mushrooms in the world and for good reason, they are known for their rich savory taste and various health benefits. Fresh shiitake mushrooms have a light woodsy flavor and aroma. They are popular for their medicinal properties and are found in powdered form in many herbal pharmacies. They are also known as black forest, black winter, brown oak, Chinese black, black mushroom etc. This mushroom is low in calories, rich in fiber as well as vitamin B. Shiitake mushroom is good for boosting the immune system, lowering blood cholestoral levels, hardening of the arteries, diabeties and as an anti-ageing agent. Also, it has promising antibacterial and antiviral effects having natural anti-cancer properties (reff-timesofindia.com)[5].

Effect of different media on the mycelial growth of *Lentinula edodes*: Mushroom cultivation is an old age practice and recent advancements in production technology have made them to cultivate under a commercial scale. Among various mushrooms cultivated, the shiitake mushroom *(Lentinula edodes)* is very well known for its medicinal values and hence its requirement in large scale production is in need. Current evaluation was done to study the mycelial growth of shiitake on different media derived from agro-residues.

Lentinula edodes is a white rot fungus that secretes a class of ligno-cellulolytic enzymes which permit it to grow on ligno-cellulosic substrates rich in lignin. Shiitake mushroom is routinely cultivated on wooden logs. Much systemic work has not been done in India with regard to this mushroom and hence there is need to carry out a research using the lignocellulosic agro-residual waste generated in agricultural fields every year[6].

Preparation of Potato Dextrose Agar (PDA) media: Potato dextrose agar (PDA) medium was prepared by using 200 g peeled potato, 20 g dextrose and 20 g agar in a litre of water. The medium was left to cool in the room before 10 ml of the PDA preparation, was dispensed into test tubes, corked and then sterilized at 121 ^{o}C and 1.5 kg/cm^2 pressure. The test tube containing the 10 ml sterilized PDA was kept in a slanted position. These slants were further maintained to inoculate fungus. To obtain the pure culture, potato dextrose agar (PDA) culture was used. A small bit/disc of tissue was collected from the pure culture of shiitake mushroom and transferred on the sterilized PDA medium under aseptic conditions. This was incubated at 25±2^{o}C for 7-10 days for sufficient mycelial growth. Pure cultures were obtained by sub culturing[7].

Growth and Antibacterial Activities in liquid Media: The highest *B.subtilis* growth inhibition was promoted by filtrates of growth media supplemented with rice bran, vermiculite or molasses *L. edodes* dry mycelial biomass in liquid culture with 0.5% added rice bran was 3.2 mg/ml, after growth for 30 days at 25^{o}C without shaking and 4.3 mg/ml under orbital shaking (150 rpm).

Table 1
Nutritional value of Shiitake mushroom[6]

	% Daily value
Total fat 0.6g	0%
Sodium 10 mg	0%
Potassium 290 mg	8%
Total carbohydrare 6.5g	2%
Dietary fiber 2.5g	10%
protin2 g	2%

	Value per 100 gm	**% daily value**		**Value per 100 gm**	**% daily value**
Vit. A	0mg	0%	Vit c	0.3mg	0%
Calcium	3mg	0%	Iron	0.4mg	2%
Vit. D	18IU	4%	Vit B6	0.159mg	15%
Vit. B12	0mg	0%	Magnesium	20mg	5%
Niacin	4mg	19%	Pantithanic acid	1.5mg	15%
Riboflavin	0.2mg	13%	manganese	0.2mg	12%
Phosphorus	112mg	11%	selenium	5.7mcg	8%
Copper	0.1mg	7%	Zinc	1mg	7%

Percent daily values are based on a 2000 caloric diet. Information compiled from the USDA National Nutrition Database for Standard Reference, (2005) and USDA Dietary Guidelines from Americans.

However, antibacterial activity detected between 20 and 24 days of incubation of stationary cultures was absent in filtrates of aerated cultures. Temperatures of 20-25ºC enhanced both growth and antibacterial activity. Optimum pH for *L. edodes* mycelial growth was 3.0-3.5, while for production of antibacterial substance(s) it was 4.5. Our results indicated that incubation conditions that enhance mycelial growth are quite different from those necessary for production of antibacterial substance(s) by *L. edodes*[8].

Recent Advances on *Lentinula edodes*

Structure and Immuno-stimulating Activities of a new heteropolysaccharide From *Lentinula edodes*: A new heteropolysaccharide, here called L2, was separated from the fruit body of *Lentinula edodes*. Chemical and physical analyses indicated that L2 has a molecular weight of 26 KDa and consists of glucose (87.5%), galactose (9.6%) and arabinose (2.8%), but it does not possess a triple-helical conformation. Further studies demonstrated that the activities of L2 exhibited high stability in wide range of pH from 4.0 to 10.0 when the thermal processing temperature was below 121 °C. Our findings revealed that a new heteropolysaccharide without triple-helical conformation from *Lentinula edodes* shows immuno-stimulating activities involving TLR2 at modest pH and thermal processing conditions, which enable to act as an active component in foods[9].

Analysis and Evaluation of Tasty Components in the Pileus and Stipe of *Lentinula edodes* at Different Growth Stages: Tasty components in *Lentinula edodes* pileus and stipe at different growth stages were studied. Mannitol, trehalose, arabitol and glucose were the main soluble polyols and sugars whereas succinic acid, malic acid and citric acid were the main organic acids. Mannitol contents were the highest in the pileus and increased at mature growth stages, although arabitol contents were the highest in the stipe and peaked at stage 5. Succinic acid contents peaked at stage 5 in the pileus and stipe during mature growth stages[10].

Threonine (sweet taste) values were the highest among all the detected amino acids followed by glutamic acid (MSG-like taste). MSG-like 5′-nucleotide contents could account for nearly 50% of the total 5′-nucleotides. Equivalent umami concentration (EUC) values of stage 5 exhibited higher levels during mature growth stages. Tasty components in the stipe were rich and EUC values were high which might be useful for further processing and by product development of *L. edodes.*

Aqueous extracts of *Lentinula edodes* exhibit high antioxidant capability and promising *in vitro* antitumor activity: Mushroom extracts are increasingly sold as dietary supplements because of several properties including the enhancement of immune function and antitumor activity. We hypothesized that soluble polar substances present in mushroom extracts may show antioxidant and anticancer properties. This report shows that Brazilian aqueous extracts of *Lentinula edodes* show inhibitory activity against the proliferation of the human tumor cell lines laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa). Cell viability was determined after using 3 different temperatures (4°C, 22°C and 50°C) for mushroom extraction. Biochemical assays carried out in parallel indicated higher amounts of polyphenols in the *L. edodes* extracts at all extraction temperatures investigated.

The scavenging ability of the 2,2-diphenyl-1-picrylhydrazyl radical showed higher activity for *L. edodes* extracts. Higher cytotoxic activity was found for the *L. edodes* extract at 22°C, with half maximal inhibitory concentration values of 0.78% ± 0.02% for Hep-2 and 0.57% ± 0.01% for HeLa. Substantial morphological modifications in cells were confirmed by Giemsa staining after treatment with either extract, suggesting inhibition of proliferation and induction of apoptosis with increasing extract concentrations. These results indicate that the aqueous extracts of Brazilian *L. edodes* are potential sources of antioxidant and anticancer compounds. However, further investigations are needed to exploit their valuable therapeutic uses and to elucidate their modes of action[11].

Antioxidant enzymes stimulation in *Aspergillus parasiticus* by *Lentinula edodes* inhibiting aflatoxin production: Biosynthesis of aflatoxins, toxic metabolites produced by *Aspergillus parasiticus* is correlated to fungal oxidative stress and cell ageing. The mechanism underlying the aflatoxin-inhibiting effect of the *Lentinula edodes* culture filtrates was studied by analysing anti-oxidant activity and β-glucan content. Mushroom β-glucans are pharmacologically active compounds stimulating anti-oxidant responses in animal cells. *L. edodes* lyophilized filtrates stimulate *A. parasiticus* anti-oxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and aflatoxin inhibition was better correlated with β-glucan content than anti-oxidant activity of the filtrates. RT-PCR analyses on treated mycelia showed a delay in the activation of *aflR* and *norA*, genes of aflatoxin cluster and a synchronous activation of *hsf2*-like, a homologue of a yeast transcription factor involved in oxidative stress responses.

The first evidence of *hsf2*-like in *A. parasiticus* and its activation during aflatoxin biosynthesis is reported. *L. edodes* filtrates could play a role as external stimulus affecting the anti-oxidant status in the fungal cell that, in turn, leads to aflatoxin inhibition. In the fungal cell, β-glucans present in the filtrates could stimulate the activation of transcription factors related to anti-oxidant response and anti-oxidant enzyme activity with a contemporaneous delay of aflatoxin genes transcription, which led to a marked reduction of aflatoxin production. This research suggests new perspectives to set suitable strategies against aflatoxins and *L. edodes* could be considered a promising tool[12].

Dissolution of Bioactive Components from Dried Fruiting Bodies of the Culinary-Medicinal Shiitake

Mushroom, *Lentinus edodes* (Agaricomycetes), during Cleaning, Soaking and Cooking: *Lentinus edodes* fruiting bodies are rich in active substances such as polysaccharides and eritadenine. Patients with gout, however, should avoid or severely limit their intake of foods containing large amounts of purine. In this study we quantitatively analyzed the polysaccharide and purine compounds dissolved from *L. edodes* fruiting bodies during cleaning, soaking and cooking. Eritadenine, adenosine, guanosine, guanosine monophosphate, adenosine monophosphate, xanthine and adenine were dissolved from *L. edodes* fruiting bodies during cleaning with tap water; their dissolution rates ranged between 3.77% and 24.30%.

Dissolution rates of polysaccharide and purine compounds *in L. edodes* fruiting bodies increased linearly with increases in the duration of soaking and cooking and adding acetic acid or $NaHCO_3$ in the soaking or cooking solutions significantly either inhibited or promoted their dissolution rates.

On the basis of these experimental results, we offer science-based suggestions for reasonable treatment of *L. edodes* fruiting bodies before eating for both patients with gout and healthy people[11].

Review of Bioactive Molecules Production, Biomass and Basidiomata of Shiitake Culinary-Medicinal Mushrooms, *Lentinus edodes* (Agaricomycetes): *Lentinus edodes* (shiitake) is a basidiomycete that has been consumed for more than 2000 years because of its nutritional value and health benefits. It has a low lipid content, high fiber content and a considerable amount of proteins; it also contains B vitamins and minerals in addition to a wide range of functional metabolites including polysaccharides, polysaccharopeptides, lectins and secondary metabolites with bioactivity e.g. lentinan, a β-(1-3)-glucan with immunomodulatory activity, among others. Extracts and pure compounds of shiitake exhibit antibacterial, antifungal, cytostatic, antioxidant, anticancer and immunomodulatory activity.

Because of these attributes, different products derived from shiitake are on the market and are sold as dietary supplements. The traditional substrate for shiitake production is oak wood, yet the search for unconventional substrates has intensified over the past three decades.

In particular, submerged cultivation of medicinal mushrooms has attracted great interest because it enables greater control of different fermentation factors to obtain products of interest. However, it is necessary to perform *in vivo* studies to determine the appropriate doses, side effects and action spectrum of different bioactive compounds and fractions as well as to improve their production in liquid media and to potentiate their activity.

We present an updated review of existing studies on the production of biomass and bioactive compounds of *L. edodes* in liquid culture and on solid fermentation for obtaining secondary mycelia and basidiomata[4-6].

Pests affecting shiitake mushroom *(lentinula edodes)*

There are some pests that are often noticed in association with shiitake mushroom production. Fortunately, there are not a large number of pests that cause serious problems. The principle problem with shiitake may be from other fungi that affect the log and shiitake mycelia.

Because the fruiting bodies grow quickly on the log and should be harvested quickly before the mushroom caps get flattened out, there is not usually a lot of time for pests to do a lot of damage to the actual mushrooms. Here are a few of the most common pests

Fungi: Many fungi can have an impact on shiitake production. There are fungi that compete with shiitake for the nutrients in the log. Fungi that create conditions that are inhospitable to shiitake (such as drying the log) and others, directly attack shiitake. Inoculate the logs in a timely manner after cutting, to provide shiitake the best chance to establish a strong colony in the logs before competing fungi do. Provide adequate ventilation and drainage and ensure the logs are in the shade and do not get too dry. If possible, keep logs off the soil and away from other possible contamination vectors. Logs that are seriously infected with disease fungi should be removed from the site.

Beetles: Some types of beetles may cause damage by digging under the bark in the larval phase, or introducing other fungi. If the bark falls off the log, it will dry out and the shiitake will die. If beetles have been a problem in the past, you might consider covering the logs with a mesh to keep beetles from laying eggs on the logs.

Soldier Beetles: These small bugs can be seen in the gills on the underside of the mushrooms. They do not harm the mushroom, but actually eat tiny mites that cannot be seen with the naked eye. Harvest mushrooms quickly while caps are still rounded. After harvest, it is possible to blow the beetles out of the mushrooms with a hair dryer.

Termites: Termites are not usually a problem, but can destroy the log. Keep logs off the soil by using pallets or something else.

Slugs and snails: Slugs and snails can be a serious problem, especially in moist weather. Prevention: Harvest mushrooms quickly while caps are still rounded. Provide adequate ventilation and drainage. Eliminate places where snails and slugs feed, shelter and reproduce such as rotting wood, leftover materials like flats and boxes and dead plant material. Decoy and trap snails with cabbage or lettuce leaves and promptly remove any snails you find. Lime and wood ash can deter slugs and snails[8].

Mammals (mice, squirrels, deer, rabbits): Some wild and domestic animals have been noted feeding on shiitake. A variety of techniques may be effective, including fences, wire screens, or other barriers or repellents. Harvest mushrooms quickly.

Conclusion

With the increasing globalization of the food trade across countries and continents, reliable identification of the geographical origin of products is critical. In this study, we describe the limitations of the current origin labeling system for non-soil-based agricultural products and suggest alternative strategies for the identification of the geographical origin of such products. An identification model based on stable isotope ratio analysis combined with discriminant analysis is used to evaluate the similarities and dissimilarities between domestic and foreign shiitake mushrooms including Chinese inoculated sawdust blocks and Chinese origin.

The results show a classification sensitivity of 92.0%, classification specificity of 91.5% and overall accuracy of 93.5%. In particular, $\delta^{15}N$ was the most important isotope marker for the identification of the origin of shiitake mushrooms. Hence, the current origin labeling system for mushroom species has to be revised to establish fair trade and avoid improper origin labeling in the global shiitake market.

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(Received 10th December 2021, accepted 15th February 2022)

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