Chapter 13
Cultivation and Utilization of Shiitake Mushroom

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Abstract Shiitake (*Lentinula edodes*) is the third most commonly cultivated edible mushroom species in the world. It has attracted people's attention with its medical properties as well as taste and nutritional value. Shiitake which has been known and used in Chinese medicine for more than 2000 years is now considered a great resource for modern clinical and pharmacological research. This mushroom contains many biologically active compounds (polysaccharides, lentinan, LEM and KS–2, ergosterol, nucleic acid derivatives, water-soluble lignins, eritadenine, etc.) which possess different medicinal effects such as antitumor, immunomodulatory, hypocholesterolemic, antibacterial, antifungal, anti–inflammatory and antioxidant. The chapter presents an overview of the research on the shiitake mushroom including its taxonomy, cultivation techniques, biotechnological approach, functional compounds and medicinal properties.

Keywords *Lentinula edodes* · Medicinal mushroom · Lentinan · Eritadenine · Anticancer

13.1 Introduction

Shiitakae (*Lentinula edodes)* is the third most commonly cultivated edible mushroomin the world, ranking just behind *Agaricus bisporus* and *Pleurotus* spp. representing about 17% of worldwide production (Zervakis and Koutrotsios 2017).

Although it is also known by different names such as the oakwood mushroom, the golden oak mushroom and the black forest mushroom (USA), xiang–gu and dong–gugo (China), lectin (France) in different parts of the world, today the name shiitake is the most widely used name for this mushroom. "Shiitake" name was derived from two words, "shii" (shii tree (*Castanopsis cuspidata* (Thunb.) Schottky)) and "take" (mushroom in Japanese), so shiitake means "mushroom of the shii or oak tree" in Japanese.

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The shiitake mushroom has been a symbol of healthy and youth for thousands of years in Far Eastern culture. Although shiitake production started in China and Japan, today there is also a significant increase in the production and consumption of shiitake in other parts of the world.

Until the mid-1980s, Japan that grown shiitake on natural logs was the main producer of shiitake in the world, while China became the major producer of shiitake in a short time with the development of sawdust-based techniques. According to the data of China Edible Mushrooms Association, in 2015, shiitake mushroom production accounted for 20% of the total edible mushroom production in China (Li et al. 2018), and this amount is also estimated to be approximately 98% of worldwide shiitake production (Yamanaka 2017).

Shiitake is a mushroom species that take people's attention with its high nutritional value and taste. Besides nutritional value, shiitake is one of the most widely known medical mushrooms in the world. The shiitake mushroom was known and used in classical Chinese medicine since more than 2000 years ago (Mizuno 1995). Shiitake contains several bioactive compounds, including polysaccharides, lentinan, LEM and KS–2, ergosterol, nucleic acid derivatives, water-soluble lignins and eritadenine. Shiitake has immune modulating, anticarcinogenic and antitumor, antioxidative, antilipidemic, hepatoprotective, antiviral, antibacterial and antiparasitic effects in relation to these bioactive compounds contained in fruitbody and mycelium.

Shiitake can be consumed directly as fresh and dried food as well as health supplements. Nowadays, different types of supplements obtained from shiitake are sold as capsules, tonics or tablet in many of countries in the world (Bisen et al. 2010).

The chapter focuses on cultivation and medicinal properties of shiitake which is an edible and medicinal mushroom. The aim of this article is to gather and summarize available information on the shiitake mushroom, including its taxonomy, enzyme production, cultivation techniques, functional compounds and medicinal properties.

13.2 Geographic Distribution

Lentinula genus includes five morphologically defined species that were identified on the basis of morphology characteristics and geographical distribution by Pegler (1983) (Table 13.1). However, through subsequent research, it was determined that the phylogenetic relationships of *Lentinula* genus were more complex than the Pegler's would suggest, and apart from these five species, new *Lentinula* species were also identified (Mata and Petersen 2000; Hibbett 2001; Mata et al. 2001).

Shiitake (*L. edodes*) grows naturally throughout Southeast Asia, but the exact limits are uncertain. Samgina (1981) reported that *L. edodes* was found in Kazakhstan. This report notes that the mushroom was found on conifer wood. But *L. edodes* is usually grow on *Quercus, Castanopsis and Lithocarpus* (Pegler 1983). Therefore, the species idetification may have been incorrect.

L. edodes was first identified as *Agaricus edodes* by Miles Joseph Berkeley in 1877. Then, Singer placed shiitake in *Lentinus* genus in 1936. David Pegler suggested

Table 13.1 Geographical distribution of *Lentinula* genus (Pegler 1983)

Species	Geographic distribution
Lentinula boryana (Berk. & Mont.) Pegler	Central America, northern South America, and the Gulf Coast states of North America
Lentinula guarapiensis (Speg.) Pegler	Paraguai
Lentinula edodes (Berk.) Pegler	North-east Asia
Lentinula lateritia (Berk.) Pegler	Southeast Asia and Australasia (except New Zealand
Lentinula novaezelandieae (Stev.) Pegler	New Zealand

transferring this species to genus *Lentinula* based on microscopic observations in 1975. Molecular phylogenetic studies also support relocation of shiitake from the genus *Lentinus* to genus *Lentinula* (Molina et al. 1992; Hibbett and Vilgalys 1993; Hibbett and Donoghue 1996). Today, shiitake is classified in the genus *Lentinula*, the family *Tricholomataceae*, the order *Agaricales* and the subphylum of *Basidioimycotina*. But even now, the shiitake is often still being misspelt as *Lentinus edodes* (Berk.).

Shiitake is a fleshy gilled mushroom. The mushroom produces white-colored spores and white mycelia. Shiitake pileus that are light tan to dark brown is convex to applanate, and size of pileus ranges from 5 to 25 cm. The stipe is usually attached to the pileus centrally. Deep cracks can occur that reveal the underlying white tissue on pileus when shiitake grown on hardwood logs.

Shiitake is a saprophytic white-rot fungi that has the ability to enzymatically degrade cellulose, lignin and other macromolecules (Asgher et al. 2008). In the nature, this mushroom grows in cutting or dead logs particularly of the oak family (*Quercus* spp.) and various deciduous or broad-leaved trees in warm and humids regions (Royse 1997).

13.3 Nutritional Properties of Shiitake

Shiitake has high nutritional content as well as excellent flavor. This high-quality mushroom has important nutrients including dietary fiber (Mattila et al. 2002), minerals (George et al. 2014), vitamin B_{12} (Bito et al. 2014) and vitamin D (Jasinghe and Perera 2006), while it does not have vitamins A and C.

Also, the shiitake mushroom represents an excellent protein supplement. Dried shiitake contains a level of protein comparable to that of several different types of meat. In addition, shitake has 18 different amino acids and almost ideal ratios of eight essential amino acids (Turło et al. 2008). The essential amino acid content of shiitake is better than soybeans, meat, milk or eggs (Vetter 1995), especially they are rich in arginine and lysine (Liu and Bau 1980).

Dietary fiber plays an *important* role in preventing type 2 diabetes, insüline resistance, obesity, hypertension and some type of cancers (Galisteo et al. 2008). The dietary fiber content of shiitake is significantly higher than meats, whereas its fat content is much lower. Moreover, 77.7% of fatty acid content of shiitake consist of unsaturated fatty acids (Bisen et al. 2010). High dietary fiber and unsaturated fatty acid content of shiitake may help protect against cardiovascular diseases by lowering cholesterol values.

13.4 Major Active Compounds Isolated from Shiitake

13.4.1 Lentinan

Lentinan (β–(1→3)–D–glucan) is a polysaccaride isolated from the fruiting bodies or mycelium of shiitake. It is situated in cell wall and has a high molecular weight. The estimated molecular weight of lentinan was reported as 400– 800 × 10^3 Da by Ooi and Liu (2000). The chemical structure of lentinan is consisting of five β–(1 → 3)–D–glucopyranoside in a linear linkages and two β–(1 → 6)–D– glucopyranoside branches in side chains. This structure results in a right-handed triple-helical form (Wang et al. 2020). The biological activity is assossiated with the position of the glucose molecules in the helix structure (Surenjav et al. 2006). The molecular weight of polysaccades has also an influence on the immune stimulating effect as well as the degree of branching and chain conformation of them (Kulicke et al. 1997).

The lentinan displays numerous bioactivities such as immunomodulator and antitumor (Chihara et al. 1970; Zheng et al. 2005), antivirus (Guo et al. 2009), stimulating the expression of cytokines (Kupfahl et al. 2006) and hypocholesterolemic (Gu and Belury 2005). It does not have direct cytotoxic effects on cancer cells, instead it displays antitumor activity by strengthening the host immune system (Chihara et al. 1970) Moreover, lentinan has been recognized as a promising compound for the formulation of new functional foods and nutraceuticals due to its minimal side effects in addition to medicinal properties mentioned above. The sulfated derivatives of lentinan also exhibit bioactivities similar to lentinan such as strengthening the immune system (Guo et al. 2009).

13.4.2 Lem

Lentinula edodes mycelium (LEM) is a bioactive substance derived from powdered mycelia of shiitake harvested before fructification. The major active compound od LEM is a heteroglycan protein conjugate, being a protein-bound polysaccaride and largely composed of sugar (44%) and protein (24.6%) (Sugano et al. 1982). In addition to heteroglycan protein complex in the structure of LEM, various nucleic acid derivatives, thiamine (vitamin B1), riboflavin (vitamin B2), ergosterol and eritadenine (Breene 1990), water-soluble lignins (Suzuki et al. 1990) and KS–2 (Fujii et al. 1978) are also presented.

LEM extract has thus been used as a medicinal food for at least 30 years in Japan (Yoshioka et al. 2012). LEM has been determined to have some bioactivity such as antioxidant (Akamatsu et al. 2004), hepatoprotective (Watanabe et al. 2006; Yoshioka et al. 2012), immunoregulatory activity and anticancer (Sugano et al. 1982; Kojima et al. 2010) activity. The antitumor activity of LEM is also thought to be associated with strengthening the host immune system rather than direct cytotoxicity, similar to lentinan. Macrophages are vital components of immune system, and LEM may play a significant role in macrophage stimulation. This macrophage activation is thought to be related to the antitumor and immunoregulatory activity of LEM (Morinaga et al. 1992).

13.4.3 Ks–2

KS–2 is a peptide–polysaccharide complex isolated from shiitake. KS–2 is consist of a-linked mannose and a small amount of peptide which is composed of serine, threonine and alanine with residual amounts of the other amino acids (Bisen et al. 2010). KS–2 polysaccharides possess antitumor and antiviral properties, and the molecular weight of KS–2 was reported as between 6.0×10^4 and 9.5×10^4 (Fujii et al. 1978).

13.4.4 Eritadenine

Eritadenine (2(R),3(R)–dihydroxy–4–(9– adenyl)–butyric acid), also known as lentinacin or lentysine, is a nucleic acid derivative produced mainly by shiitake. It was isolated from shiitake first time by Chibata et al. (1969) and Rokujo et al. (1970). Eritadenine is considered to be one of the major active substances accountable for hypocholesterolemic activity of shiitake (Sugiyama et al. 1995; Shimada et al. 2003; Enman et al. 2008; Bisen et al. 2010). To date, a number of studies (Sugiyama et al. 1995) have reported on the mechanism by which eritadenine exerts its cholesterol and triglyceride lowering properties, but the detailed mechanism is not yet completely explained.

13.4.5 Lectins

Lectins are carbohydrate-binding proteins (glycoproteins) of non-immunoglobulin origin, with an ability to produce cell agglutination (Dixion 1981). Various biological activities of mushroom lectins such as immunomodulatory and anti-tumor (Wang et al. 1996; Zhang et al. 2010), antivirus (Li et al. 2008) antifungal (Chandrasekaran et al. 2016), antibacterial (Chandrasekaran et al. 2016) and hypotensive (Wang et al. 1996) have been reported. However, there are few studies on the lectins of shiitake mushrooms (Jeune et al. 1990; Wang et al. 1999; Vetchinkina et al. 2008). Extracellular lectin activity of shiitake grown in submerged cultures was reported in some studies (Tsivileva et al. 2005; Wang et al. 1999; Vetchinkina et al. 2008).

The saline extract of the fruiting bodies of shiitake contains an amount of lectin that represents about 10% of the protein content of the fruitbody (Li et al. 2018). Lectin activity of fruiting bodies of shiitake is usually higher that that of lectins of mycelia. Mitogenic effects of lectin isolated from shiitake in human and murine were determined by Jeune et al. (1990) and Moon et al. (1995). A lectin isolated from shiitake has ability to agglutinate L1210 cell lines and HeLa cells (Moon et al. 1995).

13.4.6 Lentin

The lentin, a antifungal protein, isolated from the fruiting bodies of shiitake, exhibiting strong antifungal activity. It also showed an ability of inhibit proliferation of HIV–1 reverse transcriptase and leukemia cells (Ngai and Ng 2003).

13.4.7 Ergesterol

Ergosterol, (ergosta–5,7,22–trien–3β–ol) is a sterol found in cell membranes of mushrooms. It is abundant in shiitake as most of edible mushrooms. Ergosterol is provitamin form of D_2, and ultraviolet irradiation can convert these bioactive sterols to vitamin D_2 (Jasinghe and Perera, 2006; Morales et al. 2017). Shiitake mushrooms containing about 0.5% ergosterol (dry weight) were able to produce 400 IU of vitamin D per gram after being exposed to a fluorescent sunlamp (Breene 1990). Although mushrooms are traditionally dried under the sun, today this process is mostly performed in mechanical dryers.

Vitamin D_2 content of shiitake mushroom can be increased up to 5 times by exposure to direct sunlight for 3 h/day. Exposure to sunlight also increases the free amino acid content of the fruitbodies, making them sweeter and less bitter (Kiribuchi 1991).

13.5 Shiitake Cultivation

Shiitake is a saprotrophic mushroom, which means that they obtain the nutrients they need by decomposing various lignocellulosic waste. The ability of shiitake in converting complex lignocellulosics into simple organic compounds has been allowed many agricultural waste to be used in the cultivation of shiitake. In the commercial production of shiitake mushroom, two different techniques are applied, namely natural log cultivation and bag cultivation techniques in the world.

13.5.1 Natural Log Cultivation Technique

Cultivation of shiitake on natural logs began in far east almost a thousand years ago (Chang and Miles 2004). The first traditional cultivation method used natural logs, usually from the oak family such as shii tree (*Castanopsis cuspidata*) under outdoor conditions. This method has been the most common cultivation method until the mid-1980s.

Shiitake can be grown on many kind of hardwood and softwood trees, but oak (*Quercus*) are the most widely used species in natural log cultivation. Although logs from hardwood trees have longer fruiting period, harvest starts later than those of softwood logs. One of the most important points in the selection of logs is that shiitake mycelia colonize easily on the sapwood. Sapwood is living portion of log and contains polysaccharides needed for mycelial development, whereas colonization is difficult in the heartwood, a dead portion. For this reason, logs with a wide sapwood portion should be preferred while selecting for the production of shiitake.

Spawn running period in the log cultivation of shiitake may take 6–18 months, whereas cultivation cycle takes approximately 6 years depending on spawn, tree species, log size, moisture content of logs and climate factors, etc. Maximum biological efficiency is around 33%. Approximately 75% of the total yield is obtained the 2nd and 3rd years (Royse 2001).

Production of shiitake on log has been steadily declining with the development of sawdust-based techniques that has the shorter crop cycle and quick return of the money invested. However, the natural log technique has also some advantages such as requiring less care and labor, less susceptibility to microorganisms, rich in flavor and bioactive content. High molecular weight polysaccharide contents of shiitake mushrooms grown on logs are higher than those of fruitbodies grown on bags (Brauer et al. 2002).

13.5.2 *Bag Cultivation Technique*

After the middle of the 80s, a new method that uses plastic bags which are filled with lignocellulosic substrates has gradually replaced the traditional system on tree logs (Chang and Miles 2004). The time between the start and end of the crop cycle of the bag cultivation system is approximately 3 months, which corresponds to approximately 6% of the that of the log system. Moreover, in this technique, the biological efficiency is on average 75 to 125%, depending on the substrates used (3 times higher than the log system). The bag cultivation technique has advantages such as a shorter cultivation cycle, higher yields and year-round mushroom production, even if a bag cultivation technology needs relatively high initial investment cost of installation.

13.5.2.1 Growing Substrates

Although hardwood sawdust is the most commonly substrate for shiitake cultivation, various studies have shown that different agricultural or agro-industrial by-products that are locally abundant and cheaper such as cotton straw (Levanon et al. 1993), wheat straw and corn cobs (Philippoussis et al. 2003), sunflower hulls (Curvetto et al. 2002), hazelnut husk (Özçelik and Pekşen 2007), chickpea straw, sunflower head residue, alfalfa hay, corn stalk (Atila 2019a) may be alternative substrates for shiitake cultivation.

Supplementation of sawdust with millet, rice bran, sugarcane molasses, maize powder, rye, soy flour, grape pomace has improved mushrooms yields considerably (Royse 1996; Rossi et al. 2003; Royse and Sanchez 2007; Moonmoon et al. 2011; Atila 2019b), and but the use of alternative substrates rich in phenolic content such as olive press cake (Gregory and Pohleven 2014 and green walnut husk (Atila 2019b) seem to affect negatively mycelia growth and yield of shiitake. The situation could be associated with the presence of phenolic compound inhibiting mycelium growth and fructification.

13.5.2.2 Substrate Preparation

Optimal environmental conditions and choosing the suitable substrates are essential for success of shiitake cultivation. The first step of shiitake production is the preparation of the growing medium. Substrate preparation is based on process involving shredding, wetting, mixing and sterilization.

If sawdust is used as basal substrate in the preparation of the growing medium, no shredding is required. However, straw and other agricultural wastes such as corn cobs, cotton stalk and corn stalk need to be shredded into pieces 3–5 cm in length to facilitate disinfection and bagging process. Then, the substrates are soaked in water 6–12 h at room temperature and drained. Shiitake mycelium needs nitrogen sources as well as carbon. Therefore, several supplements rich in nitrogen such as bran, soybean flour and maize powder are added to basal substrate. Overuse of supplements may cause the growth of some competitive organisms such as green mold *(Trichoderma* sp). After the mixing process is completed, the moisture of the growing medium is adjusted to 60–65% and filled in bags. Substrate disinfection is an important stage for maximum yield and quality in shiitake production. Autoclave sterilization method is generally used for substrate disinfection. However, it is difficult to use this method by some farmers due to the high cost of production and the need for expensive equipment. For this reason, steam sterilization has been recommended by some researchers (Mata and Savoie 1998; Savoie et al. 2000). However, this method is not commercially available.

13.5.2.3 Spawning Substrate and Incubation

After cooling, sterilized bags are inoculated with spawn at 1–5% (w/w) ratio in sterile environment. Incubation period must be carried out at 25 ºC ± 2 with a 12-hour light and 12-hour dark cycle. Unlike the other commercial mushroom species, in the production of shiitake, vegetative phase takes place in two stages, spawn run and browning. At the end of the spawn running period, the entire surface of the substrate turns from white to brown, and a hard hipha crust forms on the surface of the substrate, indicating that mycelium is ready for fructification. Although it is not a general rule, after the incubation period, generally the plastic bags are removed, the substrate blocks are soaked or sprinkled with cold water for fruiting induction, and then, bags are transferred to the production room at 17 to 19 ºC with a humidity of 90%. Moreover, lighting should be provided 12 h daily. Mushrooms should be harvested when they are turgid and before the pileus extends fully. Harvest may be done by hand, grasping the mushrooms at the base and turning them slightly so that they can be removed without physical damage. After obtaining the first harvest, blocks can be rehydrated to induce a second flush by soaking them in water for 12 h (Gaitán-Hernández and Mata 2004).

13.5.2.4 Yield

Shiitake mushrooms yield better in substrates containing moderate amounts of N, hemicellulose and lignin and with a low cellulose: lignin ratio (Atila 2019a). Biological efficiency differs considerably by ranging from 2.8 to 124.1% in sawdust-based substrates (Diehle and Royse 1996; Pire et al. 2001; Atila 2019b), up to 99.3% in straw-based substrates (Gaitan-Hernandez and Mata 2004; Philippoussis et al. 2007; Elisashvili et al. 2015) or from 102 to 112% in sunflowers hulls (Curvetto et al. 2002) (Table 13.2).

Table 13.2 Biological efficiency of shiitake grown on different substrates

Substrate	Biological efficiency (%)	References
Sawdust (maple and Birch) Rice bran, millet	6.11–124.1	Diehle and Royse (1986)
Cotton straw (CS) Cotton straw + wheat straw (CWS)	CS–46 CWS–82	Levanon et al. (1993)
Sawdust Wheat bran, white millet, rye, CO_3	59.1–99.6	Royse (1996)
Sugarcane baggase, Sugarcane leaves Pineapple crown	36.3–133.0	Salmones et al. (1999)
Various formulations of rice straw, chestnut sawdust, pinus sawdust, soyflour, rice, barley, maize flour, some chemical fertilizer	42.3–59.5	Morais et al. (2000)
Different types of sawdust	2.8–52.3	Pire et al. (2001)
Sugarcane bagasse + rice bran (25–30%)	98.42–99.84	Rossi et al. (2003)
Oak sawdust, wheat straw, corn cobs	19.44–54.17	Philippoussis et al. (2003)
Pasteurized Wheat Straw	24.8–55.6	Gaitan-Hernandez and Mata (2004)
Sunflower hulls + wheat bran	102–112	Curvetto et al. (2002)
Different formulations of corn cob, Euclyptus sawdust and rice bran	18.88–43.87	Eira et al. (2005)
Vineyard pruning, barley straw, wheat straw	37.02–93.25	Gaitan-Hernandez et al. (2006)
Different mixtures of wheat straw (WS), corn-cobs (CC), and oak wood sawdust (OS) and millet, wheat bran, soybean flour	41.07–80.64	Philippoussis et al. (2007)
HH alone and its mixtures with wheat straw (WS), beech wood-chip (BWC) and wheat bran (WB) in different ratios	43.73–87.73	Özçelik and Pekşen (2007)
Wheat straw Oak sawdust Rye, wheat bran, white millet	80.4–98.9	Royse and Sanchez (2007)
Sawdust with 10%–40% of rice bran wheat bran and maize powder	53.5–153.3 g/500 g substrate	Moonmoon et al. (2011)

(continued)

Table 13.2 (continued)

Substrate	Biological efficiency (%)	References
Various formulations of oak sawdust, corn cobs, wheat straw, maize stubble, chopped cardboard, cotton waste, peanut husk, wheat kernels, wheat bran, rice meal, $MgSO_4$, $CaCO_3$, thiamine, urea	4.9–61	Martínez-Guerrero et al. (2012)
Sawdust (S) and straw (St) with different ratio of wheat bran (Wb)	S + Wb = 7.20 − 81.0 St + Wb = 11.8 – 66.8	Sharma et al. (2013)
Pasteurized wheat straw	66–320	Gaitan-Hernandez et al. (2014)
Chickpea straw, sunflower head residue, alfalfa hay; corn stalk	20.1–51.0	Atila (2019a)
Oak sawdust, grape pomace, green walnut hulls, tea wastes, olive press cake	38.2–70.7	Atila (2019b)

13.6 Medicinal Properties and Usage

Shiitake has been considered as a medicament for the inhibition and treatment of many diseases in the Orient for thousands of years. Today, medicinal properties of the shiitake have been also confirmed by a large number of high-quality scientific researches involving in vitro, in vivo and clinical studies. Extracts from shiitake, entire or part of fruit body, have been stated as having anti-cancer and antitumor, anti-hypercholesterolemic, hypoglycemic, hepatoprotective, antioxidant and antimicrobial (Table 13.3). In the following sections, major published laboratory and clinical studies on the some medicinal properties of shiitake were summarized.

13.6.1 Antitumor and Immunostimulating Activity

Cancer is the second leading cause of death globally. Although treatment methods such as chemotherapy and radiotherapy are widely used in cancer treatment, they are not always effective and often cause a number of side effects. Therefore, alternative methods of treatment, such as medicinal mushrooms, have attracted great attention of people all over the world.

Shiitake is a mushroom species known for its antitumor properties. Antiproliferative activities of this mushroom against prostate cancer, gastric cancer, breast cancer, colon carcinoma, lung cancer, skin cancer lines have been explored, and promising results have been obtained from many clinical and experimental trials carried out in shiitake (Vere White et al. 2002; Ng et al. 2002; Gu and Belury 2005; Fang et al.

Table 13.3 Biologically active constituents isolated from shiitake and their medicinal properties

Major Bioactive Compounds	Source	Extract	Pharmacological effects	Applications		References
				In vitro	In vivo	
Lentinan	Fruitbody	Ethanol	Immunomodulator		Mice	Chihara et al. 1970
	Fruitbody	5% NaOH–0.05% NaBH4	Immunomodulator	A colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method	Rats	Zheng et al. (2005)
	Mushroom fruiting bodies, mushroom spores and mushroom cultured broth	Ethanol	Hypocholesterolemic	mouse skin carcinoma cell line, CH72, and the non-tumor cell line, C50 cultured in modified Eagle's Minimal Essential Medium		Gu and Belury (2005)
	Fruitbody	Boiling water and ethanol	Antiviral		Chickens	Guo et al. (2009)
	Fruitbody		Antitumor		Mice	Ng and Yap (2002)
LEM	Culturated mycelia of shiitake	Ethanol	Antiviral	Cultured medium		Tochikura et al. (1988)
	Culturated mycelia of shiitake	Hot water and ethanol	Antioxidant Hepatoprotective	İnvitro	Dimethyl nitrosamine-injured mice	Akamatsu et al. (2004)
	Mycelia	Hot water	Hepatoprotective	primary cultured rat hepatocytes		Watanabe et al. (2006)
	LEM extract powder	Hot water and ethanol (50%)	Hepatoprotective	primary cultures of rat hepatocytes exposed to CCl4		Yoshioka et al. (2012)
	Mycelia	Water	Anticancer		Rats	Sugano et al. (1982;)

(continued)

Table 13.3 (continued)

Major Bioactive Compounds	Source	Extract	Pharmacological effects	Applications		References
				In vitro	In vivo	
	Cultured mycelia	Hot water	Anticancer, immunoregulatory activity	In vitro		Kojima et al. (2010)
KS-2	Cultured mycelia	Hot water	Antitumor		Mice	Fujii et al. (1978)
Eritadenine	Dry mushroom	Ethanol	Hypocholesterolemic		Rats	Chibata et al. (1969)
	Fruitbody		Hypocholesterolemic		Rats	Rokujo et al. (1970;)
	Fruitbody		Hypocholesterolemic		Rats	Sugiyama et al. (1995;)
	Pure eritadenine		Hypocholesterolemic		Rats	Shimada et al. (2003)
	Mycelia	Methanol	Hypocholesterolemic	Submerged culture		Enman et al. (2008)
Lectin	Fruitbody	Physiological saline	Antitumor		Animals and human	Moon et al. (1995)
	Mycelium, brown mycelial film, primordium and fruitbody	Water	Hemagglutinating activity	Several agar media		Tsivileva et al. (2001)

(continued)

Table 13.3 (continued)

Major Bioactive Compounds	Source	Extract	Pharmacological effects	Applications		References
				In vitro	In vivo	
O-sulfonated α-D-glucan	Fruitbody	5% NaOH/0.05% NaBH4	Antitumor		Mice	Unursaikhan et al. (2006)
	Fruitbody	Ethanol	Antiviral		Tobacco plants infected tobacco mosaic viruses	Wang et al. (2015)
Mycelial extracts	Mycelia	Water	Antiviral	Culture media		Sasaki et al. (2001)
LEP	Fruitbody	Aqueous and ethanol extracts	Antiviral	Poliovirus, Bovine herpesvirus cells grown in Dulbecco's Modified Eagle Medium (DMEM)		Rincão et al. (2012)
Chitosan	Stripe of fruitbody	Aqueous NaOH	Antioxidant	The conjugated diene method		Yen et al. (2007)

2006; Ina et al. 2013; Zhang et al. 2018). Biologically active substances isolated from shiitake exhibit numerous mechanisms of anticancer activity such as the inhibition of angiogenesis (Deocaris et al. 2005), stimulation of the cancer cells for apoptosis (Fang et al. 2006) or retarding the development of tumors (Ng et al. 2002).

Lentinan, known as immunomodulatory and anticancer agents, is the most broadly studied compound in the shiitake mushroom. The antitumour effect of lentinan is attributed to stimulation of the immune response in various investigations (Chihara et al. 1970; Fujii et al. 1978; Ina et al. 2013). Moreover, lentinan is reported to trigger hematopoietic stem cells, macrophages and natural killer cells (Akramiene et al. 2007). Similarly, macrophage achieved from the KS–2 treated mice. KS–2 strongly inhibited tumor growth in mices who administered orally in both doses between 1 and 100 mg/kg (Fuji et al. 1978).

Antitumor properties of shiitake are not only due to polysaccharides, and a lectin isolated from shiitake was an agglutinin of tumor cell lines tested by L1210 and HeLa cells (Moon et al. 1995). Arginine, a substance used in the supplement of cancer patients, is also abundant in shiitake (Eghianruwa et al. 2011). IA–a (a glycogen-like structure) and IA–b (arabinoxylan-like polysaccharide) isolated from LEM stimulate cytokine production and phagocytosis in RAW264.7 cells (Kojima et al. 2010). The *O*–sulfonated α–D–glucan of shiitake has higher antitumor activity than those of the native glucan (1 → 3)–α–D –glucans and the antitumor activity of the native glukans against S–180 can be enhanced by *O*–sulfonation of these glukans (Unursaikhan et al. 2006).

Several researcher reported that use of the combination of lentinan and chemotherapy drugs has inhibited proliferation and induced apoptosis than use of chemoterapy drugs alone (Zhao et al. 2013; Liu et al. 2015; Sun et al. 2015). Clinical researches have revealed that lentinan is effective in prolonging survival in patients with stomach, ovarian or colorectal cancer (Borchers et al. 1999; Fujimoto et al. 2006) and prevents side effects such as nausea and asthenia, which are common in chemotherapeutic treatment of D. Moreover, administration of lentinan in combination with Bacillus Calmette–Guerin (BCG) vaccine which is used against tuberculosis induces activation of immune cells in the lung tissue (Drandarska et al. 2005).

13.6.2 Hypocholesterolemic Activity

Coronary artery disease (CAD) is the first cause of death worldwide. The most important risk factors of the disease are hypercholesterolemia, obesity, diabetes, high triglycerides and low density lipoprotein cholesterol (LDLc) levels, hypertension and cigarette smoking as well as genetic factors (Abdel-aziz and Mohamed 2013).

The ability of shiitake to lower cholesterol was described for the first time by Kamiya et al. (1969). The major active hypocholesterolemic component in the shiitake mushroom is a adenosine derivative eritadenine. (Takashima et al. 1973). In addition to eritadenine, nucleic acid compounds extracted from shiitake were found to be inhibitors of platelet agglutination (Sugiyama et al. 1995).

Eritadenine can reduce cholesterol level in plasma and expedite lipid accumulation in the liver by removing it from the circulations. LDL is converted into high-density lipoprotein (HDL) cholesterol which is beneficial for the human system in the liver (Kabir and Kimura 1989). Another suggestion regarding the activity of eritadenine is that high dosages of eritadenine may break down the secretion of very low density lipoprotein (VLDL) and reduce cholesterol by lowering the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) in liver microsomes (Sugiyama et al. 1995). However, the mechanism that reveals the cholesterol-lowering effect of eritadenine has not been fully elucidated. Eritadenine signafically reduced serum cholesterol, phospholipids and triglycerides, both in intact rats and in animals fed a high-fat diet (Rokujo et al. 1970). The eritadenine content in the shiitake mushrooms was in the range 3.2–6.3 mg/g dried mushrooms (Enman et al. 2007). A diet containing eritadenine (0.005%) caused a 25% reduction in total cholesterol within 1 week (Chibata et al. 1969). The use of eritadenine 10–21 mg/kg/day in male and female rats decreased the atherogenic index (TC/HDL) in rat sera (Morales et al. 2018). Although the hypocholesterolemic effect of eritadenine has been investigated in several studies on rats (Sugiyama et al. 1995; Shimada et al. 2003), there are few human studies in the literature (Suzuki and Ohshima 1976).

The experimental and clinical data show that shiitake has beneficial effects on lowering low-density lipoproteins, total cholesterol and triglycerides, as well as in preventing the diseases such as arterial hypertension and high blood sugar levels which are effective in the development of cardiovascular diseases. (Kabir and Kamura 1989; Yang et al. 2002).

13.6.3 Antioxidant Activity

Oxidative stress occurs as a result of an imbalance caused by increased reactive oxygen species (ROS) and/or decreased antioxidant defense systems of the body. Numerous studies have demonstrated that oxidative stress plays a role in the emergence and development of some diseases such as atherosclerosis (Kattoor et al. 2017), muscle wasting (Moylan and Reid 2006), hypertension (Higashi et al. 2002), neurodegeneration (Brown 2005) and stroke (Cherubini et al. 2005).

Methanolic extract of shiitake is a promising alternative for use as an antioxidant (Sasidharan et al. 2010). LEP (polysaccharides isolated from shiitake), which can act as an antioxidant, may play a role in healing oral ulceration. LEP administration, in rats with oral ulceration, significantly was increased activities of serum antioxidant enzymes, whereas it was decreased levels of serum, mucosal interleukin–2 (IL–2) and tumor necrosis factor alpha (TNF–a) (Yu et al. 2009). Moreover, the administration of LEP can stimulate the expression of genes encoding antioxidant enzymes, reducing the increased oxidation stress-induced feeding by high-fat diet in rats. In addition, this treatment would decrease expression of VCAM–1mRNA of thoracic aorta endothelial cell in rats (Xu et al. 2008). Another substance that has strong antioxidant and anti-inflammatory properties in the shiitake mushroom is ergothioneine, and it is an amino acid analog (Jang et al. 2016).

Heat treatment significantly increases the antioxidant activities of shiitake mushrooms. The antioxidant activity of raw shiitake mushroom is increased about 2.0–fold by heat treated at 121 °C for 30 min (Choi et al. 2006). Although freeze drying is suggested for the protection of eritadenine and protein content of fruitbody, hot air drying at 50 °C has been proposed for the stability or formation of total phenolics in shiitake (Zhang et al. 2013).

13.6.4 Hepatoprotective Activity

Shiitake has direct protective effects on hepatocytes. Methanolic extract of shiitake fruitbody can protect liver cells from paracetamol-induced liver damage, with its antioxidative effect on hepatocytes, thus reducing or eliminating the harmful effects of toxic metabolites of paracetamol. Administration of shiitake extract at a dose of 200 mg/kg for seven days to paracetamol-induced hepatatoxic mice reduces the activity of serum enzymes and bilirubin, resulting in significant hepatoprotective effects (Sasidharan et al. 2010). A significant reduction in liver injury was also noted when mice with severe liver damage were fed vitamin D–enriched shiitake mushroom extracts (Drori et al. 2016).

Not only the fruit bodies of shiitake, but also its mycelia has hepatoprotective activity. Polyphenolic compounds contained in the L.E.M. seemed to be responsible for the protective effect (Watanabe et al. 2006). Oral administration of the extracts of LEM has the protective effect against CCl_4 (Chen 2012) and D–galactosamine (Watanabe et al. 2006) induced hepatic injury in rats. Hot water and ethanol extracts of L.E.M. repress the development of liver fibrosis induced by dimethylnitrosamine (DMN) and inhibit proliferation and morphological change of isolated rat hepatic stellate cells (HSCs) (Akamatsu et al. 2004). LEM containing anti-oxidation and anti-inflammation activities might be used for alleviating side effects of chemotherapy and preventing the progression of liver cancer for patients with chronic hepatitis (Yagi 2012).

13.6.5 Antimicrobial Activity

Antibiotics are widely used as therapeutic agents in the treatment of many diseases. But, with increasing bacterial resistance to antibiotics, plants and mushrooms with antibacterial activity have attract attention. The superior abilities of fungi in improving host immunity can be very useful in fighting infection. Shiitake contains several compounds such as lentinan, which have the ability to stimulate humoral immunity to help prevent bacterial infections that are resistant to antibiotics (Markova et al. 2003; Hatvani 2001).

Table 13.4 Antimicrobial activity of shiitake in vitro

Sources	Target organisms	References
Aqueous extracts of dry and fresh mushroom	*Bacillus subtilis, Esherichia coli*	Casaril et al. (2011)
Chloroform, ethylavetate and water extracts of dried mushroom	*Streptococcus* spp., *Actinomyces* spp., *Lactobacillus* spp., *Provotella* spp., *Porphyromonas* spp.	Hirasawa et al, (1999)
The culture filtrate after 18–25 days of cultivation of shiitake	*Bacillus subtilis*	Ishikawa et al. (2001)
Mushroom extract	*Aspergillus ochraceus and Penicillium verrucosum*	Ricelli et al. (2002)
Lentin isolated from fruitbody of shiitake	*Physalospora piricola, Botrytis cinereal, Mycosphaerella arachidicola*	Ngai and Ng, (2003)
Mycelial extracts of shiitake	*Helminthosporium euphorbiae, Helminthosporium sp, Fusarium solani and Phomopsis sojae*	Sasaki et al. (2001)
Mushroom extract	*Aspergillus parasiticus, Aspergillus flavus*	Reverberi et al. (2011)

Extracts and pure compounds of shiitake exhibit high levels of antimicrobial activity, including the antibacterial and antifungal action. The effect of shiitake on the growth of various bacteria and fungi was revealed by several authors (Hirasawa et al. 1999; Ishikawa et al. 2001; Sasaki et al. 2001; Ricelli et al. 2002; Ngai and Ng 2003; Reverberi et al. 2011; Casaril et al. 2011) in vitro studies (Table 13.4).

13.6.6 Antiviral Activity

Various extracts of shiitake mushroom and some polysaccharides isolated from shiitake have been suggested as sources of potential antiviral agents. Aqueous and ethanol extracts and polysaccharide (LeP) from shiitake are effective in the replication of poliovirus type 1 (PV–1) and bovine herpes virus type 1 (BoHV–1) (Rincão et al. 2012). Improvement was observed in liver function tests in patients with cornic hepatitis B and seropositive for hepatitis B (Hbe) antigenemia who consumed 6 g of LEM orally daily for 4 months, while some patients undergo a change from HBeAg-positive to anti-HBe positive (Amagase 1987). A laccase isolated from fresh fruitbody of shiitake mushroom exhibited inhibitory activity to HIV–1 (Sun et al. 2011). LEM and ethanol extract of LEM blocked the HIV virus at the initial stage of its development (Tochikura et al. 1988). Clinical and in vitro studies shown that LEM has ability of inhibition of HIV infection of cultured T-cells. LEM increased the T-cell count in

HIV patients with AIDS symptoms from 1250/mm^3 to 2550/mm^3, and the symptoms were much improved after 60 days (Izuka 1990). Administration of aqueous extracts from shiitake in dose of 0.4–2 mg/mice protected mice against lethality induced by the herpes simplex type 2 virus (Razumov et al. 2013). The feeding of influenza virus-infected mice for 2 weeks with a mixture of glucans obtained mycelial mushroom powders of Shiitake significantly reduced the clinical symptoms of infection. (Vetvicka and Vetvickova 2015). The possibility of using shiitake polysaccarides and other compounds in the control and prevention of viral infections that affect plants and animals was also reported (Sasaki et al. 2001; Wang et al. 2015).

13.6.7 Dosage and Toxicity

It is important to know the efficacious and safety doses of dietary supplements and nutraceuticals in order to benefit effectively from them. The recommended dose is 6–16 g for dried shiitake and about 90 g for fresh fruitbody (Liu and Bau 1980). One study on mice showed that the daily intake of 100 mg/kg of shiitake mushroom could have potential health-improving effects (Grotto et al. 2016). Since an aqueous extract of shiitake fruitbody reduces the activity of blood platelets in the process of coagulation, especially people who are taking blood thinners should be careful when using shiitake or water-soluble fractions (Yang and Jong 1989).

The doses of compounds isolated from shiitake such as lentinan are lower than that of mushroom consumption. Lentinan has been found safe to be administered to humans by IV injection in a dose range of 1–5 mg/day once or twice a week, and greater doses can cause immune suppression (Taguchi et al. 1982; Aoki 1984). In the early stages of AIDS or chronic hepatitis, the best dose of LEM was recommended between 2–6 g per day in 2 or 3 divided doses orally, while the dose may be reduced to 1/2–1 g per day once the disease becomes more stable (Sharon 1988). Lentinan and LEM have no known serious side effects (Aoki 1984). Some people may experience minor side effects or allergic reactions, known as shiitake dermatitis, caused by glucan lentinan (Nguyen et al. 2017).

13.7 Biotechnological Approach

13.7.1 Submerged Liquid Fermentation with Shiitake

Submerged liquid fermentation (SLF) techniques are used in different areas such as liquid spawn production, enzyme production and biomass production for pharmaceutical and nutraceutical applications.

Several researchers reported that liquid has a shortened spawn running period and a higher yield in comparison with the grain spawn (Kawai et al. 1996; Leatham and Griffin 1984; Lee et al. 2019). On the other hand, thanks to the liquid spawn technology, mycelia can be stored for a long time (Zilly et al. 2011). But there may be some problems in use of liquid spawn such as degeneration and mutation (Itävaara 1993). Submerged liquid cultivation can also be a promising alternative method for bioactive molecules to be obtained in a shorter time, with a higher amount and with less risk of contamination (Harvey et al. 2001; Tepwong et al. 2012). Moreover, various enzyme activities of shiitake have been determined in submerged liquid cultures (Buswell et al. 1996; Nagai et al. 2002). It is possible to eliminate the toxicity of wastewaters by using this feature of shiitake mycelia (D'Annibale et al. 2004). The medium composition and environmental parameters are crucial for optimal biomass, enzyme or metabolite production (Enman et al. 2008; Lee et al. 2019).

13.7.2 Utility of Spent Shiitake Mushroom Substrate

The total production of cultivated mushrooms in the world was approximately 34 million tons in 2013, and shiitake accounted for 7.48 million tons (Royse et al. 2017). Every kg of mushroom produced 5 kg of wet mass SMS (Medina et al. 2012). When 35–40% of the waste compost is calculated as dry matter, the resulting spent shiitake mushroom substrate (SSMS) in 2013 can be estimated about 13 million tons.

Shiitake mycelia secrete various enzymes capable of breaking down polyphenols, including lignin peroxidase, Mn-dependent peroxidase and laccase (Asgher et al. 2008). SSMS contains plenty of shiitake mycelia and can biodegrade organic xenobiotic compounds found in soil and water and adsorb some pollutants (Ahlawat and Sing 2009). This allows the use of SSMS in some biotechnological applications easily and cheaply. The biotechnological areas, in which SSMS can be used, are reviewed in the following section.

13.7.2.1 Bioremediation

The extracellular enzyme system of shiitake developed unique non-specific enzyme systems with the ability to attack not only lignin but also a broad spectrum aromatic compounds as well as some non-aromatic organopollutants such as pentachlorophenol (Okeke et al. 1993), 17α–ethinylestradiol (Eldridgea et al. 2017) and 2,4–dichlorophenol (Tsujiyama et al. 2013). The use of shiitake cultures for these remediation of contaminated soils could be less expensive and beneficial for human health and environment. Moreover, the biological degradation of pollutants using shiitake spent substrates would reduce the cost of disposal.

13.7.2.2 Dye Wastewater Degradation

A large amount of dye wastewaters, which are harmful to the environment and human health, are released into the environment from paint factories and other dye-using industries. Although some methods are applied to dispose of these wastewaters, they are expensive applications (Moreira et al. 2000). Biodegradation of colored wastewater by ligninolytic enzymatic system of white rot fungus appears to be an attractive alternative. Use of shiitake in the degredation processes has a important potential. Several strains of shiitake also have been reported as the dye- or colored material-degrading organisms (Hatvani and Mecs 2002; Boer et al. 2004). On the other hand, olive oil mill water (OMWW) has inhibitor effects of on plant growth, microbial activity and soil properties (Rusan et al. 2016; Mekki et al. 2013). The main cause of the harmful effects of OMWW is considered to be the presence of high concentrations of phenolic compounds (Azam et al. 2002). Some of strain of shiitake show high performance for treatment of OMW, resulting a significant decrease in the color and phenolics concentration of OMWW (Lakhtar et al. 2010).

13.7.2.3 Ethanol Production

In the traditional production of bioethanol, which attracts attention as an alternative fuel today, lignocellulosic materials with high sugar and starch content such as straw, corn, sugar cane, potatoes are used (Watanabe et al. 2010; Belal 2013; Patni et al. 2013). However, uses of most of these products as food cause significant cost increases.

Shiitake growing medium is prepared from various agricultural and forest wastes. SSMS remaining after mushroom production contains high amount of lignin. The use of this waste material with high lignocellulosic content as a raw material in ethanol production reduces the cost (Asada et al. 2011). The producing ethanol from SSMS has been demonstrated in the several studies (Asada et al. 2011; Hiyama et al. 2016; Xiong et al. 2019). However, further studies are needed to develop technologies that will integrate mushroom and biofuel production.

13.8 Future and Perspective

Shiitake is a type of mushroom that is appreciated not only for its unique flavor and nutritional value, but also for its health benefits. Despite these superior properties, some problems with the production and medicinal use of this mushroom need to be addressed in order for shiitake to reach its deserved place.

Although shiitake is widely consumed in some countries, the amount of consumption in some countries is very low or absent. The reasons for low consumption are high prices, lack of familiarity with the mushroom species and low production quantities. Increasing local production and developing new technologies to get higher yield of shiitake can lead to a decrease in the retail price. Moreover, shiitake production can be a good source of income for farmers, especially living in rural areas of less developed countries, and limited resources can be used beneficially.

In the rural areas, the biggest problem for the farmers could be the supply of growing substrate for shiitake cultivation. In countries where the shiitake production sector does not improve, it is not possible for farmers to obtain the growing substrates from a supplier. On the other hand, it is difficult to prepare the growing medium by himself, because the disinfection of the growing medium in the cultivation of shiitake is carried out by autoclave sterilization method and the establishment and implementation costs of this method are high. Therefore, it is important to develop alternative disinfection methods in order to expand cultivation of shiitake in rural areas. Selection of strains adapted to different substrates and disinfection methods is critically important for prolonged crop cycle and high mushroom yield. Moreover, researches for the selection and breeding of genotypes with high production capacity at high temperatures will be of great benefit in reducing energy consumption in tropical regions and summer production.

Consumers are increasingly interested in functional foods that are proven to help improve human health. Some bioactive substance of shiitake are especially effective at strengthening the immune system and lowering cholesterol and used as active compounds in the development of various functional foods. Although in vitro and in vivo studies conducted with identified bioactive compounds isolated from shiitake offer exciting results for this compounds to be qualified as a functional food and source of potential drugs, more information is required about the therapeutic effects of bioactive ingredients. Further studies, including clinical trials, to determine dose ranges that are both safe and useful in the treatment or prevention of diseases are required to obtain maximum benefit from bioactive compounds without negative consequences. Moreover, detailed studies on the chemical structures and mechanisms of action of some less researched bioactive substances isolated from shiitake may contribute to the determination of the new medicinal properties of the fungus.

Although the content of bioactive substance of shiitake may be affected by the strain, growing substrates and culture conditions, there are no protocols yet to ensure standardization of the quality and quantity of bioactives. For this reason, it is one of the conditions that should be taken into consideration that approved standard production protocols may be required to guarantee the quality and effectiveness of fungal products to be used for pharmaceutical applications. The production of high-quality products of standard quality and the provision of sustainable production under controlled conditions should be determined as the most important targets. The submerged liquid fermentation method appears to be much more useful in achieving these targets.

On the other hand, factors such as temperature and humidity applied in the cultivation of mushrooms provide optimum conditions for the development of harmful fungi species, bacteria and pests. For this reason, it is common to use pesticides to solve the problems related to diseases and pests that are frequently encountered in mushroom production. Considering the negative effects of these pesticides on human health, it should be preferred to apply controlled and certified production systems such as organic agriculture or good agricultural practices in the production of mushrooms that will be used for medical purposes. Another solution proposal in this regard is to use a submerged culture instead of fruitbody to produce bioactive metabolites for pharmaceutical applications. Mushroom mycelium is known to be very rich in bioactive content. While it is possible to produce mycelium without using chemicals in the production of submerged mycelia culture, it will be much easier to set this type of production to standards.

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