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Domestication of a wild edible and nutritious mushroom *Lentinus tigrinus* from Pakistan

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ABSTRACT

Lentinus tigrinus is a significant edible and medicinal mushroom. It was collected from University of the Punjab, Lahore under a *Morus* tree. For the domestication of this wild significant species, its three parameters i.e., culturability, spawn production and cultivation potential were assessed using different synthetic culture media and substrates. Completely randomized design was used to determine the parameters and all the effects were evaluated in triplicates. Among these different media used, maximum mycelial growth where observed on the potato dextrose agar (PDA) at 35°C, followed by the compost extract agar (CEA) medium, malt extract agar (MEA), glucose peptone agar (GPA), and Saboraud dextrose agar (SDA). Spawning material was prepared on rice, sorghum and barley grains by the cultured mycelium on PDA medium. Sorghum grains were found as the appropriate medium for spawn production of this fungus. Cultivation potential and biological efficiency was assessed on the different substrates i.e., pure rice straw, pure sawdust, pure tea-waste, mixture of sawdust and rice straw, mixture of tea waste and sawdust and mixture of tea waste and rice straw. Mixed substrate of sawdust and rice straw at 30°C showed the maximum yield. Tea-waste medium was used as the casing material and proved very effective. These results indicated that *Lentinus tigrinus* exhibit the growth potential and its domestication can compete with nutritional and medicinal peculiarities of one of the most cultivated species, *L. edodes*.

Keywords: Biological efficiency, culturability, cultivation potential, spawn.

RESUMO

Domesticação do cogumelo selvagem comestível e nutritivo *Lentinus tigrinus*, do Paquistão

Lentinus tigrinus é um importante cogumelo comestível e medicinal. Foi coletado na Universidade de Punjab, Lahore, sob uma amoreira. Para a domesticação desta espécie silvestre, foram avaliados sua culturabilidade, capacidade de reprodução e potencial de cultivo, usando diferentes meios sintéticos de cultura e substratos. O delineamento experimental foi inteiramente casualizado em triplicata. Entre os diferentes meios de cultura utilizados, o crescimento micelial máximo foi observado no agar batata dextrose (PDA) a 35°C, seguido pelo meio compost extract agar (CEA), malt extract agar (MEA), glucose peptone agar (GPA) e agar Saboraud dextrose (SDA). O material de reprodução foi preparado em grãos de arroz, sorgo e cevada usando micélio cultivado em meio BDA. Grãos de sorgo constituíram o meio adequado para a multiplicação desse fungo. O potencial de cultivo e a eficiência biológica foram avaliados nos diferentes substratos, ou seja, palha de arroz pura, serragem pura, resíduo de chá puro, mistura de serragem e palha de arroz, mistura de resíduo de chá e serragem e mistura de resíduo de chá e palha de arroz. O substrato misto de serragem e palha de arroz a 30°C apresentou o máximo rendimento. O meio de resíduos de chá foi usado como material de revestimento e provou ser muito eficaz. Esses resultados indicaram que *Lentinus tigrinus* apresenta potencial de crescimento e sua domesticação pode competir com as peculiaridades nutricionais e medicinais de uma das espécies mais cultivadas, o shiitake (*Lentinus edodes*).

Palavras-chave: Eficiência biológica, culturabilidade, potencial de cultivo, reprodução.

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Mushrooms are rich in protein, vitamins and minerals and also popular as food due to its unique flavor. Their cultivation is gaining popularity worldwide because of economic, medicinal and nutritional contribution. Around 3000 species are primarily edible mushrooms and about 700 species considered as healthy therapeutic mushrooms (Niazi & Ghafoor, 2021). The genus *Lentinus* belongs to the Polyporaceae family, Basidiomycota phylum that are grown in groups or solitary in upland and lowland area during the months May to October. They occur in boreal, temperate, subtropical and tropical regions, however, are widely reported from the southeastern Asia, including Indonesia, Thailand, Laos, Peninsular Malaysia, Borneo, Philippines, China and India (Bolhassan *et al.*, 2012).

Most of the *Lentinus* species are edible and also renowned for their therapeutic effects (Dulay *et al.*, 2014). *Lentinus* species are also recommended during the pandemic covid 19 as they strengthen the immune system. They also exhibit the effective antiviral and anti-inflammatory peculiarities (Shahzad *et al.*, 2020). This genus imparts significant contribution in natural ecosystems as wood decomposers and shows potential for seasonal food,

medicine and alternative income mainly in southeastern Asia and southern Africa (Njouonkou *et al.*, 2013). As edible and medicinal macrofungal resource, *Lentinus* species contain proteins, carbohydrates, sugars, fiber, lipids, and minerals, and exhibit antioxidant, antibacterial, and anti-hyperglycemic activities (Dulay *et al.*, 2014).

Morphologically, *Lentinus* species are characterized by tough round pileus, saw-toothed edges, white to yellowish underside gills and scaly stipe of fruiting bodies. Few species of *Lentinus* have been reported as domesticated strains in which *L. sajor-caju* is first recorded, followed by *L. tigrinus* (Dulay *et al.*, 2012), *L. squarrosulus* (Leon *et al.*, 2017), *L. strigosus*, and most recent *L. swartzii* (Dulay *et al.*, 2017). Different countries like India, Japan, China and Korea started the artificial mushroom cultivation of *Lentinula* spp. shiitake (*Lentinus edodes*) placed at the second position among the major six cultivated mushrooms in the world, which account 25% of the world mushroom production (Stamets, 2000). One of the big advantages of domestication is that there are the huge chances of radioactive contamination in wild form which can be overcome by the cultivation in maintained conditions (Falandysz *et al.*, 2015).

Lentinus tigrinus is an edible wood-rotting basidiomycete with leathery flesh, strong aroma, and taste that makes it applicable in gourmet preparations (Dulay *et al.*, 2012). This basidiomycetous mushroom is often seen growing on fallen logs in the forest from May to September (Dulay *et al.*, 2017). It is reported frequently in tropical regions, especially in southeastern Asia and has essentially a north temperate distribution (Bolhassan *et al.*, 2012). It exhibits high amounts of proteins, carbohydrates, fibers and minerals (Dulay *et al.*, 2012). It also possesses hypoglycemic effect, antioxidant potential and antibacterial activity (Dulay *et al.*, 2012). *L. tigrinus*, due to both nutritional and therapeutic peculiarities, can meet the demand of food of a growing population. In addition to nutrient and taste, fruiting at relatively high temperature (25-30°C) is also a big motivation for the growers (especially of high temperature countries) to cultivate it at large scale. Instead of all these peculiarities, cultivation practice of *L. tigrinus* is not common, its farming conditions optimization requires more research and refinement. Its domestication is only frequently reported from Philippines, although it is native to many countries of the world. It is a highly nutraceutical and pharmaceutically effective species, therefore it is significant to understand the vital aspects of its culture growth and successful fruiting body production. Pakistan's climatic conditions (tropical to temperate) favor the natural growth of *L. tigrinus* but its domestication was never tried before in Pakistan. In the present study, first time culturing and cultivation conditions of the native Pakistani *L. tigrinus* were studied and first-time tea waste-based substrates were used for the cultivation of *L. tigrinus* and proved effective.

The aim of this study was to collect and identify a wild edible strain of *L. tigrinus* and optimize the culture and development conditions by the usage of locally accessible media and cheap lignocellulosic substrates. This research work will ultimately lead the in practice of different combinations of the lignocellulosic substrates and media for the culture growth and fruiting of this nutraceutical effective species (*L. tigrinus*). Its domestication can compete with the nutritional peculiarities of the widely growing edible fungal species like button and oyster strains.

MATERIAL AND METHODS

Sampling and identification

Basidiomata of the *L. tigrinus* was collected under a *Morus* tree from the Hostel Area, University of the Punjab, Lahore, Punjab, Pakistan. The collected specimens were photographed using an Android camera and identified macro-microscopically and phylogenetically accordingly to the already reported literature (Karunarathna *et al.*, 2011). This specimen was submitted in the Herbarium, Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH27920).

Experiment layout

All the experiments i.e., identification, culturability, spawn production and cultivation potential were carried out in Fungal Biology and Systematics Research lab, Institute of Botany, University of the Punjab, Lahore. The experiments were arranged in a complete randomized design with three replications per treatment.

Evaluation of culturability of *L. tigrinus*

Culturability of *L. tigrinus* was assessed according to the method described by Niazi & Ghafoor (2022). Small tissues from inner unexposed part of the fruiting bodies of *L. tigrinus* were placed onto five different nutrient agar media i.e., malt extract agar [2% MEA: agar 20 g, malt extract 20 g dissolved into 1000 mL dH_2O (distilled water)], glucose peptone agar medium (2% GPA: 20 g peptone, 20 g dextrose, 5 g NaCl, 15 g agar dissolved into 1000 mL dH_2O), potato dextrose agar (2% PDA: thin potato slices 200 g, glucose 20 g, agar 20 g per liter of dH_2O), Saboraud dextrose agar (2% SDA: 15 g agar, 40 g dextrose, 10 g peptone dissolved into 1000 mL dH_2O) and compost extract agar (2% CEA: 20 g agar,10 g glucose dissolved into 1000 mL wheat straw water based filtrate). Inoculated Petri plates were sealed with parafilm and incubated at various temperatures i.e., 20°C, 25°C, 30°C, 35°C, and 40°C. Mycelial growth characteristics (growth rate, density, texture, color) were observed for up to 30 days on regular basis. The diameter of the mycelium extension rate and surface mycelium density (abundant, moderate and scarce) was measured using a transparent ruler and visual observation at the same time (between 10:00 AM and 11:00 PM). Completely randomized design was used to determine the culturability potential on five different media at five different temperatures. Each effect was determined in triplicates. The mushroom cultures were deposited in University of the Punjab, Lahore Culture Collection as (LAH012021C).

Spawn production

Niazi & Ghafoor's (2022) described methodology was used to prepare

spawn. For spawn preparation, cereal grains i.e., sorghum (*Sorghum bicolor*), rice (*Oryza sativa)* and barley (*Hordeum vulgare*) were washed and soaked for overnight, boiled for half an hour and excess water from grains was removed by spreading the grains on blotting paper while maintaining the moisture content of 65% in the grains. The moisture contents of the grains were calculated using the formula as follows: Moisture content (%) = [(initial weight - dry weight)/ initial weight] × 100. 1 L filter jars were filled with 500 g boiled grains, supplemented with gypsum (2 g) and lime (1 g) and then autoclaved. Spawns were prepared by inoculating mycelial discs from pure PDA culture on sterilized grains in a laminar air flow cabinet. Inoculated grains were incubated at 35°C. Effect of grains on production of spawning material was determined in triplicates.

Substrate production

Rice straw, sawdust and tea waste were used as the raw materials. Dried rice straw collected from the field area of University of the Punjab, Lahore, sawdust of *Morus* species collected from the furniture shop, while tea-waste collected from the Hostel Canteens of University of the Punjab, Lahore. Tea waste is just the left-over residue of the tea (water containing tea) after usage. Six types of substrates were prepared in the cemented sterilized room. Three were of pure types i.e., pure rice straw, pure sawdust and pure tea-waste while three were of mixed type with equal ratio i.e., mixture of sawdust and rice straw, mixture of tea waste and sawdust and mixture of tea waste and rice straw. For substrate production (pure and mixed types), raw materials were sprinkled with water and made pile of them, 65% moisture (determined through moisture meter) maintained during the substrate production process of ten days. Piles were turned on every second day, chicken manure and urea (one fourth of the substrates (25 g/1 kg) were added as supplements for nitrogen source on the second and last turning while gypsum (15 g/kg) was added and thoroughly mixed before the pasteurization process. When substrates were prepared, they were filled in polypropylene bags and autoclaved for 3 to 4 hours for sterilization purpose. Polypropylene bags of 20 x 15 cm were used and 800 g of the substrates were filled in each bag.

Spawning

Sterilized substrate bags on cooling were inoculated with the spawn prepared on sorghum grains (proved efficient for the spawning material) at the rate of 30 g per 800 g bag at the sterilized surface and bag mouths were loosely tied with the rubber bands and incubated at different temperatures i.e., 20°C, 25°C, 30°C, 35°C, and 40°C. Completely randomized design was used for spawning on six different substrates at five different temperatures. Experiment was performed in triplicates.

Spawn running

Spawn running period on the different substrates at different temperatures was observed. During the spawn running, relative humidity of 70% was maintained by humidifier and ventilation fan at different incubation temperatures. When the spawn running was almost completed, casing of autoclaved tea-waste (tea containing water) was done manually to maintain the moisture of the substrates. After pinhead emergence, bags were transferred to the cropping room with 85% relative humidity (determined by the temperature humidity meter), maintained by continuous ventilation.

Biological efficiency

Biological efficiency of different types of substrates (fresh weight basis) was observed as per 800 g of the substrate bags.

Statistical analysis of the data

Completely randomized design was used to determine the different parameters i.e., culturability, spawn production and cultivation potential. All treatments were evaluated in triplicates and two-way analysis of variance was applied to determine the significant differences between different treatments. SPSS software package (version 21) was used for the statistical analysis. Data are also expressed as mean value ± S.E.

RESULTS AND DISCUSSION

Screening the most effective culture medium and optimization of temperature

Mycelial growth of any mushroom or fungi always depends on the suitable culture medium used for culturing in the laboratory. Mycelial texture and growth pattern of *L. tigrinus* was evaluated at different temperatures on different nutrient agar media. Mycelium extension pattern of *L. tigrinus* was continuous and flat while texture of

Figure 1. A= basidiomata of *Lentinus tigrinus*; B-F= cultures on different nutrient agar media at 35°C after 15 days of inoculation; B= on PDA; C= on CEA; D= on GPA; E= on MEA; F= on SDA. Scale bar: A= 2 cm, B-F= 1 cm. CEA= compost extract agar; PDA= potato dextrose agar; MEA= malt extract agar; DAS= Saboraud's dextrose agar; GPA= glucose peptone agar. Lahore (Pakistan), University of Punjab, 2020.

mycelial colony was cottony on all the media and at temperatures tested for the optimization of vegetative growth condition of this significant mushroom species. Its mycelial colony showed same white color from the start to end day of incubation (30-day duration). Fibrillar growth of mycelium was observed since start of the inoculum of mushroom tissue on all the media. Mycelial density was moderate to abundant on the different media. Dulay *et al.* (2020) observed thick mycelial density of *L. tigrinus* on the majority of carbon sources used for its culturability. Mensah & Obodai (2014) found the same white color of mycelial colony for one strain of *Lentinus squarrosulus* during the whole period of incubation.

Amongst the different media utilized for the culturing of *L. tigrinus,* PDA proved the most efficient medium in terms of mycelium extension rate (10.9±0.057 mm/day) and density that may be due to availability of required nutrients for the growth of *Lentinus tigrinus* in the PDA medium, while Saboraud dextrose agar medium was found as the least appropriate for the mycelial growth (2.96±0.033 mm/day) of this species (Figure 1). The current results were in agreement with Dulay *et al.* (2021) who scrutinized the potato dextrose agar medium (PDA) as the favourable one for the mycelial growth of *Lentinus swartzii*. These results were also in concurrent with Dulay *et al.* (2012), who studied different broth media for basidiospores germination of *L. tigrinus* and concluded the potato sucrose broth as the best medium. However, our findings were contrasted with the of Leon *et al.* (2017), who found the maximun mycelial growth rate and density of *L. sajor-caju* on the coconut water gelatin (CWG) medium rather than potato based medium.

Vegetative growth of mushrooms requires a definite range of temperature for propagating due to its effect on metabolic reactions. Most of the basidiomycetous species grow in a wide range of temperatures, however the best temperature was revealed between 20 and 30°C (Nwokoye *et al.* 2010). Different temperatures were evaluated for the cultural growth of *L. tigrinus*, while 35°C was found as the suitable temperature on all the culturing media tested for culturability. At 40°C, mycelium growth remains suppressed in terms of density and growth rate. Our results were in agreement with Leon *et al.* (2017), who evaluated the temperature suitability for the mycelial growth of *L. sajor-caju* at various temperatures and found 28-33°C as the most suitable. Zervakis *et al.* (2001) found 30°C as the suitable temperature for linear mycelium growth of *L. edodes*.

Figure 2. Spawn production of *Lentinus tigrinus* on A= sorghum grains; B= rice grains; C= barley grains at 35°C after 17 days of inoculation. Scale bar: A-C= 1cm. Lahore (Pakistan), University of Punjab, 2020.

Figure 3. A= Spawned compost; B= pinheads and C= fruiting body of *Lentinus tigrinus*. Lahore (Pakistan), University of Punjab, 2020.

Table 1. Mycelium extension rate (mm/day) of *Lentinus tigrinus* at different temperatures on different media after the 2nd day of inoculation**.** Lahore (Pakistan), University of Punjab, 2020.

Types of media	Mycelium extension rate (mm/day)					
	20°C	25°C	30°C	35°C	40°C	P-value
PDA	5.96±0.088	7.33±0.088	8.86±0.088	10.9±0.057	9.4±0.057	<0.001
CEA	5.8±0.057	6.86±0.088	7.93±0.066	8.86±0.088	7.96±0.033	<0.001
GPA	4.88±0.069	6.4±0.057	6.96±0.033	7.96±0.033	7.93±0.066	<0.001
MEA	3.9±0.057	4.99±0.057	5.86±0.088	7.43±0.066	6.83±0.088	<0.001
SDA	2.6±0.057	2.9±0.066	2.93±0.88	2.96±0.033	2.30±057	<0.001
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	

Values given are mean ± standard error. Media type and temperature have significant impact over mycelium growth rate ($p<0.001$). Moreover, the joint effect of media and temperature has also a significant impact over mycelium extension rate ($p<0.001$). CEA= compost extract agar; PDA= potato dextrose agar; MEA= malt extract agar; DAS= Saboraud's dextrose agar; GPA= glucose peptone agar.

Table 2. Days required to complete spawn running period of *L. tigrinus* on different substrates at variable temperatures. Lahore (Pakistan), University of Punjab, 2020.

Types of substrates	Days required to complete spawn running period				
	20°C	25°C	30°C	35°C	40°C
Sawdust & rice straw	23.86±0.088	21.9±0.057	19.23±0.617	22.96±0.033	28.9±0.057
Pure rice straw	27.86±0.088	26.9±0.057	22.86±0.088	25.9±0.057	30.83±0.088
Tea waste & rice straw	27.9±0.057	27.6±0.033	25.90±0.057	29.9±0.057	Not initiated
Tea waste & sawdust	29.86±0.088	28.96±0.033	27.83±0.088	34.9±0.057	Not initiated
Pure sawdust	29.9±0.057	29.6±0.033	27.88±0.092	33.91±0.060	Not initiated
Pure tea waste	30.83±0.088	30.6±0.057	28.96±0.030	34.29±0.303	Not initiated

Values given are mean ± standard error. Substrate types and temperature have significant impact over spawn running time ($p<0.001$). Moreover, the joint effect of substrates and temperature has also a significant impact over spawn running time ($p<0.001$).

Table 3. Comparison of yield (g/800 g) of *L. tigrinus* obtained after harvesting all appeared flushes from pure and mixed substrates at 30°C. Lahore (Pakistan), University of Punjab, 2020.

Types of substrates	Biological efficiency (fresh weight basis)			
	1st flush yield (g)	2nd flush yield (g)	3rd flush yield (g)	Total yield (g)
Sawdust & rice straw	100.9±0.057	100.4±0.057	90.9±0.057	292.2±0.057
Pure rice straw	89.8±0.088	87.86±0.088	80.9±0.057	258.56±0.088
Tea waste & rice straw	85.9±0.057	79.9±0.057	Not appeared	165.8±0.057
Tea waste & sawdust	78.4±0.057	77.9±0.057	Not appeared	156.3±0.033
Pure sawdust	77.86±0.033	76.9±0.057	Not appeared	154.76±0.057
Pure tea waste	70.46±0.088	Not appeared	Not appeared	70.46±0.088

Values given are mean ± standard error. Substrate types have significant impact over the total yield ($p<0.001$).

Mycelium of the different species of tropical areas such as *L. swartzii* (Dulay *et al.*, 2021), and *L. squarrosulus* (Leon *et al.*, 2017) showed the same response. However, our results were contrasted with the of Quaicoe *et al.* (2014), who evaluated the optimum temperature for mycelial growth of *L. edodes* and found 25°C as the most suitable. All media used for the evaluation of culturability of *L. tigrinus* were proved to be supportive for its growth but PDA remained the best option at 35°C temperature. Mycelium extension rate (mm/day) on different media at different temperatures was significantly different (Table 1).

Spawn production

Healthy seeds of mushrooms (grain spawn) are the initiator of the good spawn running and successful fruiting body production. Colonization rate of the active mycelium (cultured on the PDA medium) on cereal grains (sorghum, rice and barley) was assessed at 35°C. Mycelium colonized more quickly on *Sorghum bicolor* grains (19.1±0.057 days) at 35°C followed by *Oryza sativa* (22.4±0.088 days) and *Hordeum vulgare* (26.98±0.044 days) grains (Figure 2). Full spawning material was ready on sorghum grains after 19 days of inoculation of mycelium. The dense growth of *L. tigrinus* on sorghum grain is due to their moisture and nutrient composition. Cuevas *et al.* (2009) used sorghum grains for formation of spawning material for *Lentinus sajor-caju* and revealed it as the most suitable substrate. Sorghum grains were also reported to be a good spawning material for *L. squarrosulus* (Oghenekaro *et al.* (2009). Leon *et al.* (2017) investigated the spawn preparation efficiency of *L. squarrosulus* on different types of cereal grains. They found sorghum grains as the best spawn production medium. However, our results were not consistent with the results reported by Dulay *et al.* (2012). They experimented rice seeds for spawn production of *L. tigrinus* and found it appropriate. Our findings showed that *L. tigrinus* mycelia could efficiently grow in any type of grain spawning materials; so, it would be easy to propagate the mycelia of this mushroom for mass production.

Determination of efficient lignocellulosic substrates in term of cultivation potential

Various factors like light, temperature, and humidity of the incubation room effect the spawn running time of mushrooms mycelia. Spawning material prepared on sorghum grains was used to evaluate the mycelial running time on six different substrates (three were of pure type and three mixed substrates) at various temperatures viz., 20°C, 25°C, 30°C, 35°C, and 40°C. At 30°C, the lowest spawn running period (days) was observed on the rice straw + sawdust substrate (19.23±0.617) followed by the rice straw substrate (22.86±0.088) (Table 2). Our findings were corresponding with Krupodorova *et al.* (2019). They obtained the highest biomass of *Lentinus edodes* at 26-28°C.

Further, cultivation potential (fruiting and yield) was evaluated on six types of substrates at 30°C. These were pure wheat straw, mixture of sawdust and wheat straw, pure sawdust mixture of tea waste and sawdust, pure tea-waste, and mixture of tea waste and wheat straw. The mixture of sawdust and rice straw proved to be the best substrate for the cultivation of *L. tigrinus* at

30°C in terms of fruiting and yield (292.2±0.057 g/800 g) followed by rice straw substrate (258.56±0.088 g/800 g) (Table 3 and Figure 3).

As far as the efficiency of substrates were concerned, our results were similar to the findings of Dulay *et al.* (2012); they recorded the highest biological efficiency of 15.93% of *L. tigrinus* in 2 parts of sawdust + 8 parts of rice straw substrate formulation. Shahtahmasebi *et al.* (2017) demonstrated that the wild strain of *L. tigrinus* successfully produce normal mushrooms at a biological efficiency of 56.66% in sawdust-based substrates enriched with wheat bran. Our research was also closely related to the findings of Dulay *et al.* (2021) in which they experimented the combination of rice straw and sawdust at various temperatures as the suitable medium for the cultivation potential of *Lentinus* species. Tea-waste was used for the first time as the growing medium for the cultivation of *L. tigrinus* and proved significant. Atila (2019) revealed the tea-waste as the most economic and suitable substrate for better yield of *L. edodes*.

In this study, tea-waste was used as the casing material for the cultivation of *L. tigrinus* that also proved to be a suitable and economical casing material. Peyvast *et al.* (2007) investigated the mixture of tea waste and traditional peat as the finest casing material for the maximum yield production of *Agaricus bisporus*. The current study elucidated the domestication of wild edible *L. tigrinus* from Pakistan for the first time. Sawdust + rice straw substrate at 30°C proved the best conditions for the fruiting of *Lentinus tigrinus*. However, the tea waste medium was used for the first time as the growth medium of *L. tigrinus* and was found to be suitable. Nevertheless, different combinations of the substrates and medium should be investigated in more detail to enhance the yield and biological efficiency of this sister species of one of the most widely cultivated species of *L. edodes*.

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