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RESEARCH ARTICLE

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Development of a Novel Spawn (Block Spawn) of an Edible Mushroom, *Pleurotus ostreatus*, in Liquid Culture and its Cultivation Evaluation

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ABSTRACT

Mushroom cultivation has gained increased attention in recent years. Currently, only four types of spawn, including sawdust spawn, grain spawn, liquid spawn, and stick spawn, are commonly available for mushroom cultivation. This limited spawn diversity has led to difficulty in selecting suitable inoculum materials in some cultivation. In this study, three small blocks of lignocellulosic agro-wastes and one block of a synthetic matrix were prepared as support for growing *Pleurotus ostreatus* in liquid medium. Mycelium-adsorbed blocks were then evaluated for their potential as block spawn for fructification. Our results indicated that the edible fungus was adsorbed and abundantly grew internally and externally on loofah sponge and synthetic polyurethane foam (PUF) supports and also has the ability to attach and grow on the surface of sugarcane bagasse and corncob supports. The mycelia of *P. ostreatus* adhered on corncob exhibited the highest metabolic activity, while those on the PUF showed the least activity. Mycelial extension rates of block spawns made of agro-waste materials were comparable to that of sawdust spawn, but the block spawn of PUF showed a significantly lower rate. No significant differences in cropping time and yield were observed among cultivations between experimental block spawns and sawdust spawns. Moreover, the corncob block spawn maintained its fruiting potential during an examined period of 6-month storage. The developed block spawn could be practically applied in mushroom cultivation.

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Pleurotus ostreatus; mycelial adsorption; block spawn; liquid culture; mushroom cultivation

1. Introduction

Mushroom has been traditionally consumed as a food and been considered as potential nutraceuticals and medicines for centuries [1]. Recent studies revealed that mushrooms contain high levels of protein, vitamins, and minerals, but with low fat content [2]. Besides this, most mushroom species also contain high levels of bioactive polysaccharides with various physiological benefits such as antitumor and antioxidant effects, with some purified polysaccharides having been developed as a drug for cancer treatment [3]. Owing to these unique properties, mushroom cultivation is prosperous in many countries such as China, Korea, and Japan, and has become an important agricultural industry in these countries. As most agro-industrial lignocellulosic wastes such as sawdust, straw, and cottonseed hull can be utilized as substrates for culturing mushrooms, mushroom cultivation as a bioprocess has also been considered as a useful approach to reducing environmental pollution associated with the disposal of these wastes [4].

Among the large number of mushroom species currently known, *Pleurotus ostreatus*, a white-rot basidiomycete, is one of the most widely cultivated mushrooms due to its versatile attributes. These include broad substrate adaptability, rapid growth, disease resistance, short cropping cycle, high yield, and simple cultivation technique [4,5]. This edible fungus also has various biotechnological applications in areas of bioremediation [6], decolorization [7], and enzyme production [8]. As such, this species is regarded as a model fungus for mushroom study.

During mushroom cultivation, productivity is influenced by a variety of factors such as substrate nutrition and environmental conditions [9,10]. Spawn type also plays a crucial role in determining fruiting body yield [11,12]. At present, four major spawn types are available for use in mushroom cultivation [13–16]: sawdust spawn, grain spawn, liquid spawn, and stick spawn. Of these, sawdust spawn is most commonly used due to its easy preparation, low cost, and low equipment investment [16,17]. The use of grain spawn is less widespread due to its expense and high contamination rate [18]. Liquid spawn, which has been developed for more than 30 years, is gaining increased interest from mycologists and mushroom growers due to a variety of advantages, including quick preparation, less

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production space requirement, low cost, fast colonization, convenient implementation of automatic inoculation, and ease of uniform distribution in substrate [13,19,20]. Recently, stick spawn has been utilized in *Pleurotus eryngii* cultivation as it has been shown to accelerate mycelial colonization [16]. In our previous study, stalk spawn of *P. ostreatus* was developed on the agro-residue corn stalk and demonstrated promising results [21].

The diversity of spawn products remains limited. However, as a large variety of cultivation processes exist for mushroom production, and facilities vary significantly among cultivation plants, choosing suitable spawn can be extremely difficult. In this regard, it has been necessary to develop new spawns to satisfy the varying demands of the mushroom industry. The objective of this study was to develop a new spawn to meet such requirements, with desirable characteristics such as fast preparation, less production space requirement, low cost, and ease of cultivation inoculation. Based on these considerations, various lignocellulosic agro-wastes and a synthetic matrix were prepared in small blocks and used as supports for *P. ostreatus* mycelia in liquid culture. The mycelium-adsorbed blocks were evaluated as block spawn in a fruiting test. To the best of our knowledge, this study is the first report on the development and application of block spawn for mushroom cultivation.

2. Materials and methods

2.1. Supporting materials

Three lignocellulosic agro-wastes, including loofah (*Luffa cylindrica*) sponge, corncob, and sugarcane bagasse obtained locally, were used in this study. A synthetic matrix polyurethane foam (PUF) obtained from a commercial source was also obtained as a supporting material for this study.

2.2. Preparation of support blocks

To obtain regular sugarcane bagasse blocks, fresh sugarcane was peeled, cut into cubes measuring 1.5 cm^3, and squeezed carefully to remove sugar and water. Dry loofah sponge and PUF were cut into blocks with the same measurements. PUF-derived cubes were boiled in distilled water for 20 min at 80 °C, placed in methanol overnight, and finally rinsed with distilled water to remove any organic reagent residues. For corncob block preparation, after removal of grains, fresh corncob was cut into short cylinders with 1.5 cm height, and then these cylinders were further divided into four equal portions in a fan-shaped block. All the manufactured blocks were dried in an oven at 60 °C to a constant weight, and used as support in subsequent studies.

2.3. Strain and maintenance

P. ostreatus NN-1, maintained in our laboratory, was used in this study. It was cultivated on potato dextrose agar (PDA) slants at 25 °C for 7 d, stored at 4 °C, and sub-cultured at regular intervals of four weeks.

2.4. Inoculum preparation

P. ostreatus NN-1 was inoculated on PDA agar slants and incubated at 25 °C for 7 d. The slant was squeezed into about soybean-size agar plugs with a self-designed inoculated rake, and 10 pieces of the agar plugs of mycelium were inoculated into a 250 mL flask with 80 mL liquid medium comprised of (in g/L): 35 glucose, 5 peptone, 4 yeast extract, 1 KH_2PO_4, and 0.5 $MgSO_4$, which was modified based on that of Tang and Zhong [22]. The flasks were incubated at 28 °C for 5 d with shaking speed of 150 rpm in a rotary incubator. The final culture (containing approximately 6.3 g dry biomass/L) was homogenized in a sterile blender at 10,000 rpm for 20 s and used as inoculum in subsequent studies.

2.5. Liquid culture conditions for block spawn development

To test the influence of support number on the mycelial adsorption and growth, blocks of different supports ranging to 12 pieces were individually added to 250-mL flasks containing 72 mL liquid medium (with ingredients as described above). Flasks without the addition of support were run as the control. After being autoclaved and cooled, 8 mL of the homogenized mycelium suspension was inoculated. Culture conditions were 28 °C, 150 rpm, with a cultivation period of four days. At the end of the four-day period, free and adsorbed biomass was determined.

To obtain mycelium-covered blocks as block spawn for use in a fruiting test, the smallest number of blocks attaining a complete mycelial adsorption was used in flask cultures. Namely, for loofah sponge- and PUF-supplemented cultures, six pieces of blocks were used in each flask. This number rose to nine blocks for the sugarcane bagasse- and corncob-supplemented cultures. Mycelium-covered blocks were harvested by filtration on sterile gauze and utilized as block spawn. To evaluate the suitability of block spawn for long-term use, they were maintained at 4 °C and used in subsequent fruiting tests.

2.6. Development of sawdust spawn control

A substrate composed of 78% sawdust, 20% wheat bran, and 2% gypsum (dry weight basis) with 63% moisture content was used. Each polypropylene bag (30 cm × 15 cm) was filled with 750 g wetted substrate. These bags were autoclaved at 121 °C for 2 h. After cooling, bags were inoculated with a mycelial agar plug (1 cm^2) cut from 7-d-old PDA slants and incubated at 25 °C and 60% relative humidity in the dark. After the mycelium completed colonization, the solid cultures were utilized as sawdust spawn.

2.7. Evaluation of substrate colonization

Cottonseed hull basal substrate (60% cottonseed hull, 18% sawdust, 20% wheat bran, and 2% gypsum, based on the dry weight) with 65% moisture content was used. Then, 35 g of this wetted mixture was uniformly packed in glass tubes (25 mm × 200 mm) to a depth of 14 cm. After sterilization at 121 °C for 1 h, each tube was inoculated by placing a piece of block spawn on the surface of the substrate using sterilized tweezers. These tubes were cultivated at 25 °C for 14 d for all developed block spawns. Tubes inoculated with sawdust spawn cubes (1.5 cm^3 volume) were used as the control. Mycelial colonization distance was measured according to the methodology described by Zervakis et al. [23]. The mycelium extension rate was calculated by dividing the measured colonization distance by cultivation time (14 d) and expressed as mm/d.

2.8. Substrate formulation, spawning, and incubation

Polypropylene bags (30 cm ×15 cm) were separately packed with 750 g moistened cottonseed hull basal substrate and enclosed with plastic rings and vent caps. Sterilization occurred at 121 °C for 2 h. After cooling, one piece of block spawn or cube-shaped sawdust spawn (1.5 cm^3 volume) was seeded on the surface of the substrate using sterile tweezers as a tool. Incubation was undertaken at 25 °C and 70% relative humidity in the dark. The time required for spawn running was recorded. For all tested spawns, 30 replicate bags were prepared.

2.9. Fruiting, harvest, and determination of biological efficiency

Matured bags were transferred into a fruiting room, where the temperature and relative humidity were controlled at 15 °C and 85–95%, respectively. The photoperiod was 12 h/d with light density of 1000 lux, and aeration was performed twice daily. Mushrooms were harvested from the bags when the mushroom cap surfaces were flat to slightly uprolled at the cap margins [24]. Only first flush mushroom was harvested and weighted before the spent substrate was discarded. Biological efficiency was calculated as described by Rodriguez Estrada et al. [25].

Biological efficiency (%) = Average weight of fresh mushroom harvested per bag/weight of dry matter per bag ×100.

2.10. Quantification of metabolic activity

The tetrazolium salt 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT), commonly employed for fungal activity assay [26], was used for staining and activity assay in this work. The mycelium-covered blocks were harvested on sterile gauze and washed with distilled water. Washed blocks were stained in 0.9% sterile saline with 0.5 mg/mL MTT in glass tubes at a temperature of 30 °C for 2 h with gentle shaking. Then, reduced products of MTT within the mycelia were extracted using 95% ethanol at 30 °C for 2 h, and centrifuged at 8000×g for 10 min. The absorbance of the supernatants obtained was measured at 570 nm with 95% ethanol as the blank. The metabolic activity of mycelia on the loofah sponge was assumed to have a level of 100% in this study, and then the relative metabolic activity of mycelia on other tested supports was compared under the same conditions.

2.11. Measurement of biomass

Biomass was estimated as described by Feng et al. [27]. For free biomass determination, the culture broth in each flask was collected and centrifuged at 8000×g for 10 min. The mycelial pellet obtained was washed three times with distilled water, harvested, and dried at 60 °C to a constant weight. For the determination of adsorbed biomass, the mycelium-adsorbed blocks were harvested by filtration on gauze and gently washed to avoid the detachment of adsorbed mycelia, then dried at 60 °C until a constant weight. Adsorbed biomass was obtained by the weight difference between the original dry weight of supports and the measured weight.

2.12. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The data were analyzed by a one-way analysis of variance and means showing statistical differences were subjected to Duncan's multiple-range test using the SPSS version 17.0 program (SPSS Inc., Chicago, IL). Differences were considered significant at $p < .05$.

Figure 1. Different mycelia-adsorbed blocks of *P. ostreatus* before and after staining. A, corncob; B, loofah sponge; C, sugarcane bagasse; D, PUF. The staining was carried out with an MTT concentration of 0.5 mg/mL at 30 °C for 2 h.

Table 1. Free and adsorbed biomass formed in final flask cultures of *P. ostreatus* with different supports for different numbers of support blocks and their calculated growth capacity.

			Dry mycelial weight (g/L)		
Support	Number of blocks/flask	Weight of support (g)/flask	Free	Adsorbed	Growth capacity (mg/g support)
Corncob	3	0.81 ± 0.07	5.15 ± 0.38	0.54 ± 0.03	53.33 ± 2.96
	6	1.74 ± 0.13	3.22 ± 0.13	1.22 ± 0.16	56.10 ± 7.36
	9	2.29 ± 0.19	0	1.64 ± 0.21	57.29 ± 4.07
	12	3.46 ± 0.21	0	1.88 ± 0.33	43.46 ± 7.63
Sugarcane bagasse	3	0.18 ± 0.02	5.34 ± 0.27	0.37 ± 0.07	164.44 ± 31.11
	6	0.40 ± 0.02	4.19 ± 0.16	0.86 ± 0.05	172.00 ± 10.00
	9	0.58 ± 0.03	0	1.12 ± 0.13	154.48 ± 17.93
	12	0.83 ± 0.05	0	1.36 ± 0.22	131.08 ± 21.20
Loofah sponge	3	0.12 ± 0.03	1.73 ± 0.16	5.32 ± 0.37	1934.54 ± 134.54
	6	0.21 ± 0.02	0	5.90 ± 0.39	2247.61 ± 148.57
	9	0.36 ± 0.04	0	6.84 ± 0.51	1520.00 ± 113.33
	12	0.45 ± 0.03	0	7.66 ± 0.84	1361.78 ± 149.33
PUF	3	0.06 ± 0.01	1.28 ± 0.23	4.23 ± 0.55	5640.00 ± 733.33
	6	0.13 ± 0.02	0	5.13 ± 0.65	3156.92 ± 400.00
	9	0.18 ± 0.03	0	6.17 ± 0.53	2742.22 ± 235.56
	12	0.25 ± 0.02	0	7.03 ± 0.44	2249.60 ± 140.80
Without	0	0	6.24 ± 0.26	–	–

Data are means ± standard deviation of three independent flask cultures.

3. Results

3.1. Mycelial growth of P. ostreatus on various supports in liquid cultures

P. ostreatus grew internally and externally on the loofah sponge and PUF (Figure 1). For the corncob and sugarcane bagasse supports, the mycelia firmly adhered to the surface and exhibited abundant growth. Free mycelial biomass in cultures with the addition of any of the four supports decreased as their supplementation amount was increased, while adsorbed biomass increased as the addition amount increased (Table 1). When the number of added loofah sponge and PUF blocks reached six or above in each flask, all of the mycelia were adsorbed. To obtain the same level of adsorption for sugarcane bagasse and corncob, nine blocks were required.

Total mycelial biomass (including free and adsorbed) decreased when three to six sugarcane bagasse and corncob blocks were added to cultures, and this decline was more dramatic in cultures with nine blocks added. This coincided with complete mycelial adsorption, i.e., no visible free mycelia present in the broth (Table 1). Addition of more than nine blocks resulted in a slight increase in the total biomass. In the case of using the porous supports (loofah sponge and PUF), the total biomass obtained in the flasks containing three blocks was lower than that of the control, and a drastic decline was clearly observed in flasks containing six blocks coinciding with complete mycelial adsorption. Afterward, the

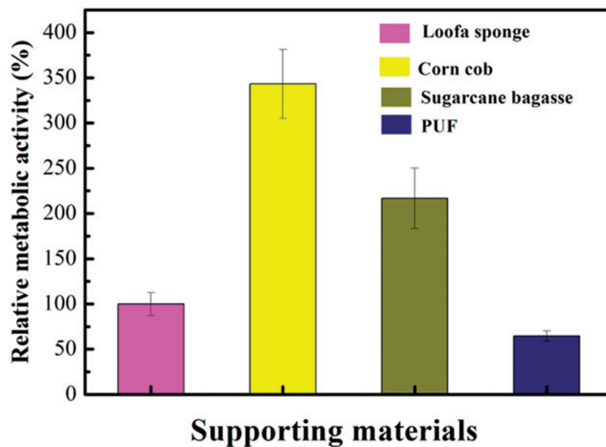

Figure 2. The relative metabolic activity of adsorbed mycelia on different supports. The metabolic activity of adsorbed mycelia on loofah sponge was assumed to have a level of 100%, and the relative metabolic activity of mycelia on other supports was compared under the same conditions. Values are means ± standard deviation of three samples (three blocks per sample for staining).

total biomass began to increase with increasing block number.

Mycelial growth capacity with loofah sponge and corncob blocks peaked at the same number of blocks at which complete mycelial adsorption occurred (six blocks for loofah sponge and nine blocks for corncob per flask) (Table 1). Beyond this level, a decrease in growth capacity was observed. In contrast, for sugarcane bagasse, mycelial growth capacity increased up until six blocks per flask were added, despite not attaining complete mycelial adsorption. It then decreased with further block additions. Regarding the synthetic matrix (PUF), growth capacity decreased with increased block number throughout the tested range. To summarize, mycelial growth capacity was highest when using the PUF supports, followed by loofah sponge, sugarcane bagasse, and corncob, respectively.

3.2. Variation in the metabolic activity of adsorbed mycelia on different supports

The metabolic activity of adsorbed *P. ostreatus* mycelia on the corncob supports was the highest (343.11% relative to the control), followed by the activity of mycelia on the sugarcane bagasse (216.87%) and on the loofah sponge (no difference relative to control) supports (Figure 2). The adsorbed mycelia on the PUF supports showed the lowest activity, with only 64.63% of the activity measured on the control.

3.3. Comparison of colonization rate between various block spawns and sawdust spawn

Block spawns consisting of corncob, sugarcane bagasse, and loofah sponge showed similar linear mycelium extension rates to that of the sawdust spawn, as tested in glass tubes (Table 2). Meanwhile, PUF block spawn gave a significantly lower ($p < .05$) extension rate relative to that of sawdust spawn.

Table 2. Comparison of mycelium growth rate between different block spawns and sawdust spawn of *P. ostreatus* in glass tubes.

Spawn type	Supports	Linear growth rate (mm/d)
Block spawn	Corncob	6.24 ± 0.13ab
	Sugarcane bagasse	6.13 ± 0.09a
	Loofah sponge	6.16 ± 0.12a
	PUF	5.47 ± 0.08b
Sawdust spawn	–	6.25 ± 0.16ab

Data are means ± standard deviation of three independent replicates; values followed by the same letters within each column are not significantly different ($p > .05$).

3.4. Comparison of the main fruiting parameters among various block spawns and sawdust spawn

Table 3 lists the three main fruiting parameters of the four block spawns and sawdust spawn. There were no statistical differences ($p > .05$) in colonization duration (range: 34.38–36.12 d), fruiting body yield (range: 180.22–186.21 g/bag), and biological efficiency (range: 68.65–70.94%) between the four block spawns and the control.

3.5. Effect of storage time on the fruiting of corncob block spawn

Storage time had minimal effect on fruiting for corncob block spawn, and there were no statistical differences ($p > .05$) in spawn running time, yield, and biological efficiency among cultivations using the corncob block spawn at different storage times (Table 4).

4. Discussion

Cell immobilization is a commercially important and useful technique and can be applied to plant, algal, and microbial cells for production of useful metabolites or for other purposes [28–32]. Currently, four major methods are available for immobilization: entrapment, adsorption, covalent binding, and cross-linking [33]. For the immobilization of mushroom cells, adsorption is commonly adopted due to its simple procedure, lack of necessity for special treatments, and reduction of diffusion limitation [33,34]. Various natural and synthetic materials like PUF, nylon sponge, and loofah sponge have frequently been used as supports [34–36] for this procedure.

In this study, the four tested supports were demonstrated to be capable of effectively adsorbing the mycelia of *P. ostreatus* and supporting its growth in

Table 3. Comparison of the main fruiting parameters of *P. ostreatus* between different block spawns and sawdust spawn.

Spawn type	Supports	Spawn running period (d)	Yield (g/bag)	Biological efficiency (%)
Block spawn	Corncob	34.57 ± 1.06a	186.21 ± 12.24a	70.94 ± 4.66a
	Sugarcane bagasse	35.36 ± 1.24a	181.55 ± 12.08a	69.16 ± 4.60a
	Loofah sponge	35.61 ± 1.33a	184.03 ± 11.27a	70.10 ± 4.29a
	PUF	36.12 ± 1.25a	180.22 ± 15.15a	68.65 ± 5.77a
Sawdust spawn	–	34.38 ± 0.85a	184.26 ± 8.63a	70.19 ± 3.29a

Data are means ± standard deviation of thirty replicates; values followed by the same letters within each column are not significantly different ($p > .05$).

Table 4. Data on the main fruiting parameters of cultivation using the corncob block spawn at different storage times.

Storage time (months)	Spawn running period (d)	Yield (g/bag)	Biological efficiency (%)
0	34.57 ± 1.06a	186.21 ± 12.24a	70.94 ± 4.66a
1	33.48 ± 1.54a	180.52 ± 10.05a	68.77 ± 3.83a
2	35.29 ± 1.10a	176.13 ± 14.26a	67.10 ± 5.43a
3	33.14 ± 0.85a	178.82 ± 9.56a	68.12 ± 3.64a
4	34.81 ± 1.43a	175.67 ± 12.32a	66.92 ± 4.69a
5	33.63 ± 1.53a	184.74 ± 9.27a	70.38 ± 3.53a
6	34.44 ± 1.31a	176.38 ± 13.10a	67.19 ± 4.99a

Data are means ± standard deviation of thirty replicates; values followed by the same letters within each column are not significantly different ($p > .05$).

a liquid medium. Despite corncob and sugarcane bagasse having been utilized as supports for immobilization of several white-rot fungi [37,38], their use as supports for immobilization of *P. ostreatus* mycelia has yet not been reported. A significantly higher mycelial growth capacity was observed for the loofah sponge and PUF supports than for the corncob and sugarcane bagasse supports, which was mainly due to the high adsorption surface area caused by high pore volume in the former two supports [36,39]. However, the higher adsorbed biomass did not lead to faster mycelial colonization, indicating that the mycelial colonization of block spawns mainly depends on their surface mycelia. Another important observation was that a higher total biomass was always observed in cultures with the loofah sponge support than with PUF support when the same number of support blocks was used. Higher biocompatibility of loofah sponge with cells compared to that of PUF may account for this.

Higher activity levels were detected on the mycelia grown on the corncob and sugarcane bagasse supports as oppose to those grown on the loofah sponge and PUF supports. Two possible explanations are available. First, because mycelial adhesion mainly occurred on the surface of corncob and sugarcane bagasse supports, it may mean that the easy accessibility of dissolved oxygen and nutrients by the mycelial cells from the broth promoted their activity. Conversely, the mycelia abundantly grew both on the surface and inside the pores present on the loofah sponge and PUF supports, causing a saturation of *P. ostreatus* filaments. This saturation may have served as a barrier for oxygen and mass transfer, thereby decreasing the mycelial cell activity. A similar mechanism has been observed in the decline in gluconic acid production by immobilized *Aspergillus niger* due to the saturation of the mycelia inside and on the PUF [40]. Second, the corncob and sugarcane bagasse as supports would likely provide some nutrients for mycelial growth due to their composition, thereby strengthening mycelial activity. The support serving as a source of carbon and energy for adsorbed mycelia has been reported in previous studies [41].

This study proved that the performance of the four block spawns in terms of cropping time and mushroom yield was comparable with that of the sawdust spawn in cultivation. This result is important for the development of block spawn as a new spawn for industrial applications. The biological efficiency obtained in this study using the four block spawns was nearly equal to that of the research carried out by Gaitán-Hernández and Salmones [42] and was significantly higher than those obtained by Obodai et al. [14] and Wang et al. [43]. This could be attributed to the differences in the mushroom strains and substrate used, or due to differences in environmental conditions [9,24,42].

Among the four supports used, corncob is a low cost and easily obtained material, it is, therefore, considered to be the most suitable matrix to be used in block spawn development. To be an ideal mushroom spawn for practical use, it should have the following attributes: easy and fast preparation, low space requirement, low cost, fast mycelium growth, and high fruiting body yield. Ideally, it could also be used after long periods of storage with little to no loss of productivity. This study demonstrated that the prepared corncob block spawn met this requirement and by maintaining its fruiting performance even after a six-month storage time. On the basis of these findings, it can be concluded that the developed corncob block spawn has the potential to be

an excellent inoculum for mushroom cultivation. Additionally, in order to facilitate inoculation, developing a spawning system for this type of spawn may be required.

During mushroom cultivation, spawn that can be easily and uniformly distributed into substrates is generally considered desirable, because a higher spawned area or volume can accelerate the rate at which mycelium cover the substrate, thereby reducing the likelihood of contamination by pathogens such as mold. As block spawn developed in this study cannot exceed its biomass volume when inoculated, this may be a shortcoming associated with this type of spawn. Despite this, as a variety of cultivation processes exist for mushroom production, and cultivation facilities vary significantly among different mushroom plants, the block spawn developed here may be suitable for use in some cultivation cases. Moreover, as most mushroom species can be adsorbed and grown using the same method as *P. ostreatus*, their block spawns can be readily prepared, and may also be suitable for cultivation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Ali MA, Mehmood MI, Nawaz R, et al. Influence of substrate pasteurization methods on the yield of oyster mushroom (*Pleurotus* species). Pak J Agri Sci. 2007;44:300–303.
[2] Sadler M. Nutritional properties of edible fungi. Nutr Bull. 2003;28:305–338.
[3] Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol. 2002;60:258–274.
[4] Sάnchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Appl Microbiol Biotechnol. 2010;85:1321–1337.
[5] Girmay Z, Gorems W, Birhanu G, et al. Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. AMB Express. 2016;6:87.
[6] Rigas F, Papadopoulou K, Philippoussis A, et al. Bioremediation of lindane contaminated soil by *Pleurotus ostreatus* in non- sterile conditions multilevel factorial design. Water Air Soil Pollut. 2009;197:121–129.
[7] Asgher M, Shah SAH, Ali M, et al. Decolorization of some reactive textile dyes by white rot fungi isolated in Pakistan. World J Microbiol Biotechnol. 2006;22:89–93.
[8] Flores C, Casasanero R, Trejo-Hernández MR, et al. Production of laccases by *Pleurotus ostreatus* in submerged fermentation in co-culture with *Trichoderma viride*. J Appl Microbiol. 2010;108: 810–817.
[9] Sher H, Al-Yemeni M, Bahkali AHA, et al. Effect of environmental factors on the yield of selected mushroom species growing in two different agro ecological zones of Pakistan. Saudi J Biol Sci. 2010; 17:321–326.
[10] Xu F, Li ZM, Liu Y, et al. Evaluation of edible mushroom *Oudemansiella canarii* cultivation on different lignocellulosic substrates. Saudi J Biol Sci. 2016;23:607–613.
[11] Sainos E, Díaz-Godínez G, Loera O, et al. Growth of *Pleurotus ostreatus* on wheat straw and wheat-grain-based media: biochemical aspects and preparation of mushroom inoculum. Appl Microbiol Biotechnol. 2006;72:812–815.
[12] Mamiro DP, Royse DJ. The influence of spawn type and strain on yield, size, and mushroom solids content of *Agaricus bisporus* produced on non-composted and spent mushroom compost. Bioresour Technol. 2008;99:3205–3212.
[13] Frieal MT, McLoughlin AJ. Production of a liquid inoculums/spawn of *Agaricus bisporus*. Biotechnol Lett. 2000;22:351–354.
[14] Obodai M, Cleland-Okine J, Vowotor KA. Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. J Ind Microbiol Biotechnol. 2003;30:146–149.
[15] Jo WS, Rew YH, Choi SG, et al. Effect of various sawdusts and logs media on the fruiting body formation of *Phellinus gilvus*. Mycobiology. 2007;35: 6–10.
[16] Zhang RY, Hu DD, Ma XT, et al. Adapting stick spawn reduced the spawn running time and improved mushroom yield and biological efficiency of *Pleurotus eryngii*. Sci Hortic. 2014;175: 156–159.
[17] Wang LQ, Li YF, Liu DH, et al. Immobilization of mycelial pellets from liquid spawn of oyster mushroom based on carrier adsorption. HortTechnology. 2011;21:82–86.
[18] Rosado FR, Kemmelmeier C, Da Costa SM. Alternative method of inoculums and spawn production for the cultivation of the edible Brazilian mushroom *Pleurotus ostreatoroseus* SING. J Basic Microbiol. 2002;42:37–44.
[19] Kawai G, Kobayashi H, Fukushima Y, et al. Effect of liquid mycelial culture used as a spawn on sawdust cultivation of shiitake (*Lentinula edodes*). Mycoscience. 1996;37:201–207.
[20] Ma L, Lin YQ, Yang C, et al. Production of liquid spawn of an edible mushroom, *Sparassis latifolia* by submerged fermentation and mycelial growth on pine wood. Sci Hortic. 2016;209:22–30.
[21] Liu SR, Zhang WR, Kuang YB. Production of stalk spawn of an edible mushroom (*Pleurotus ostreatus*) in liquid culture as a suitable substitute for stick

spawn in mushroom cultivation. Sci Hortic. 2018; 240:572–577.

[22] Tang YJ, Zhong JJ. Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharide and ganoderic acid. Enzyme Microb Technol. 2002;31:20–28.

[23] Zervakis G, Philippoussis A, Ioannidou S, et al. Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. Folia Microbiol (Praha). 2001;46:231–234.

[24] Yang WJ, Guo FL, Wan ZJ. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. Saudi J Biol Sci. 2013;20:333–338.

[25] Rodriguez Estrada AE, Jimenez-Gasco M, Royse DJ. Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay. Bioresour Technol. 2009;100: 5270–5276.

[26] Levitz SM, Diamond RD. A rapid colorimetric assay of fungal viability with the tetrazolium salt MTT. J Infect Dis. 1985;152:938–945.

[27] Feng J, Feng N, Zhang JS, et al. A new temperature control shifting strategy for enhanced triperpene production by *Ganoderma lucidum* G0119 based on submerged liquid fermentation. Appl Biochem Biotechnol. 2016;180:740–752.

[28] Liu YK, Seki M, Tanaka H, et al. Characteristics of loofa (*Luffa cylindrica*) sponge as a carrier for plant cell immobilization. J Biosci Bioeng. 1998;85: 416–421.

[29] Iqbal M, Zafar SI. Bioactivity of immobilized microalgal cells: application potential of vegetable sponge in microbial biotechnology. Lett Appl Microbiol. 1993;17:289–291.

[30] Iqbal M, Zafar SI. Vegetable sponge as a matrix to immobilize micro-organisms: a trial study for hyphal fungi, yeast and bacteria. Lett Appl Microbiol. 1994;18:214–217.

[31] Santos DT, Sarrouh BF, Rivaldi JD, et al. Use of sugarcane bagasse as biomaterial for cell immobilization for xylitol production. J Food Eng. 2008;86: 542–548.

[32] Zhu HJ, Wang WH, Liu JH, et al. Immobilization of *Streptomyces thermotolerans* 11432 on polyurethane foam to improve production of acetylisovaleryltylosin. J Ind Microbiol Biotechnol. 2015;42: 105–111.

[33] Wan-Mohtar WAAQI, Abd Malek R, Harvey LM, et al. Exopolysaccharide production by *Ganoderma lucidum* immobilised on polyurethane foam in a repeated-batch fermentation. Biocatal Agric Biotechnol. 2016;8:24–31.

[34] Prasad KK, Mohan SV, Bhaskar YV, et al. Laccase production using *Pleurotus ostreatus* 1804 immobilized on PUF cubes in batch and packed bed reactors: influence of culture conditions. J Microbiol Biotechnol. 2005;43:301–307.

[35] Haapala A, Linko S. Production of *Phanerochaete chrysosporium* lignin peroxidase under various culture conditions. Appl Microbiol Biotechnol. 1993; 40:494–498.

[36] Samir AM, Mahmoud AK. Biosynthesis of gibberellic acid from milk permeate in repeated batch operation by a mutant *Fusarium moniliforme* cells immobilized on loofah sponge. Bioresour Technol. 2009;100:374–379.

[37] Mohammadi A, Enayatzadeh M, Nasernejad B. Enzymatic degradation of anthracene by the white rot fungus *Phanerochaete chrysosporium* immobilized on sugarcane bagasse. J Hazard Mater. 2009; 161:534–537.

[38] Zhang A, Wang G, Gong G, et al. Immobilization of white rot fungi to carbohydrate-rich corn cob as a basis for tertiary treatment of secondarily treated pulp and paper mill wastewater. Ind Crop Prod. 2017;109:538–541.

[39] Silvério SC, Moreira S, Milagres AM, et al. Laccase production by free and immobilized mycelia of *Peniophora cinerea* and *Trametes versicolor*: a comparative study. Bioprocess Biosyst Eng. 2013;36: 365–373.

[40] Mukhopadhyay R, Chatterjee S, Chatterjee BP, et al. Production of gluconic acid from whey by free and immobilized *Aspergillus niger*. Int Dairy J. 2005;15:299–303.

[41] Mazmanci MA, Ünyayar A. Decolourisation of reactive black 5 by *Funalia trogii* immobilised on *Luffa cylindrical* sponge. Process Biochem. 2005;40: 337–342.

[42] Gaitán-Hernández R, Salmones D. Obtaining and characterizing *Pleurotus ostreatus* strains for commercial cultivation under warm environmental conditions. Sci Hortic. 2008;118:106–110.

[43] Wang SX, Xu F, Li ZM, et al. The spent mushroom substrate of *Hypsizigus marmoreus* can be an effective component for growing the oyster mushroom *Pleurotus ostreatus*. Sci Hortic. 2015;186: 217–222.