*Advances in Biological Sciences Research, volume 15*
**Proceedings of the 10th International Seminar and 12th Congress of Indonesian Society for Microbiology (ISISM 2019)**

# An Investigation of Spawn Growth of *Pleurotus ostreatus* in Heat-Tolerant Plastic Bags Using Rice and Corn as Substrates

S Afrida[1,*] K Willard[1], Lukman[1] and Y Tamai[2]

[1] *STT Migas Balikpapan, Jl Soekarno Hatta Kilometer 8, Balikpapan 76125, East Kalimantan*
[2] *Departement of Bioscience and Chemistry, Hokkaido University, N9W9, Kita-ku, Sapporo 060-8589, Japan*
[*] *Corresponding author. Email: afrida.sitompul0214@gmail.com*

**ABSTRACT**
Indonesian mushroom farmers commonly produce *Pleurotus ostreatus* spawn in bottles. Bottles have several disadvantages: unsterilized lids, large weights, undetectable bacterial contaminations, and difficulties in removing the rest of mycelia around the edge of the bottle. Farmers rarely use plastic bags due to unfamiliarity. Therefore, in this study, we investigate growth of *P. ostreatus* spawn in heat-tolerant plastic bags using three different substrates (corn, rice, and a mixture of corn and rice). The physiological and morphological properties of mycelia growth on substrates were assessed, including growth rate, type of mycelium, and colony formation. Grain moisture is an important factor to get a successful spawn. We estimated the suitable water content of corn and rice in plastic bags was 50% and 27%, respectively. Inoculation of inoculum from potato dextrose agar (PDA) into grains was called first-generation spawn (G1). The corn medium showed the fastest growth (2 weeks), followed by the mixture of corn and rice (4 weeks), followed by the rice medium (6 weeks). Inoculation of G1 into another grain was called second-generation spawn (G2). Inoculation of G1 into G2 from corn to corn mycelia grew for 1 week, from the mixture of corn and rice on the same substrate for 2 weeks, and the slowest growth was in mycelium from rice to rice substrate for 4 weeks. An application of spawn to mushroom cultivation showed that farmers are able to expand G2 into 30-50 of sawdust fruiting body substrate. From the three types of spawn, farmers reported that they obtained good mushroom yield when the mixture of corn and rice was used as spawn. This study may be useful for new methods to generate spawn.

***Keywords:*** *White-rot fungi, Grains, HDPE, Gusseted bags, Containers.*

## 1. INTRODUCTION

According to the Directorate General of Horticulture performance report [1], mushroom production in Indonesia from 2010-2014 has decreased by approximately 20.09%. One reason was a limitation in the availability of quality spawn. Production of spawn was commonly achieved by cultivators or spawn were purchased from another cultivator. Spawn can affect the yield of mushrooms; therefore, it must have good quality. Farmers commonly use waste of ketchup bottles as container of spawn, which have several disadvantages: unsterilized lids, large weight, undetectable bacterial contaminations, and difficulties in removing the rest of the mycelia around the edge of the bottle (Figure 1 and Figure 2). Recently, some farmers have used plastic bags for spawn containers. However, the packaging can trigger the contamination of spawn because the plastic bags are only folded and tied with rubber, the resulting air circulation from outside to inside the container is exorbitant (Figure 3). Moreover, using unsterilized cotton as a plug on plastic bags has a high risk for contamination of spawn (Figure 4). The containers and packaging used by farmers required improvement to avoid high contamination of spawn. Stamets [2] has reported production of spawn using

 

grains as substrates in polypropylene plastic bags; however, the method is not fully understood.

*P. ostreatus* is part of a family of white-rot fungi that are natural decomposers. They live on dead wood and utilize wood components as nutrient sources and produce biomass-fruiting bodies that are protein rich. White-rot fungi can degrade all wood components, including cellulose, hemicellulose, and lignin due to their ability to produce a variety of hydrolytic (endo-1,4-β-glucanase, exo-1,4-β-glucanases, and xylanase) and oxidative enzymes (lignin peroxidase, manganase dependent peroxidase, and laccase) [3], also called lignocellulolytic enzymes. Moreover, degradation of wood components by white-rot fungi is an interdependent system wherein all enzymes cooperate with each other [4]. The lignocellulolytic enzymes produced by *P. ostreatus* depend on the substrate and nitrogen source [5] [6]. Grain is used globally as a spawn substrate. Corn is a common substrate used by Indonesian farmers to produce spawn because it contains carbohydrates, proteins, sugars, and vitamins [7] and yields mycelia of *P. ostreatus* that grow faster, thicker and denser. However, this substrate does not contain lignin and oxidative enzymes will likely be scarcely produced by *P. ostreatus*.

The present study was carried out to improve the quality of *P. ostreatus* spawn by selecting suitable simple local substrates, such as rice and corn, to support lignocellulolytic enzyme production. Furthermore, this study create a suitable container for the spawn to avoid contamination. The containers, high density polyethylene (HDPE) plastic bags, were more innovative, inexpensive, affordable, and easily mobilized than the containers commonly used by Indonesian farmers.

**Figure 1**. *P. ostreatus* spawn is commonly produced by Indonesian farmers using old newspapers as lids and corn as the substrate.

**Figure 2**. Spawn packaging of *P. ostreatus* in bottles using plugs made from unsterilized cotton for air circulation.

**Figure 3.** Farmers produce spawn using plastic bags that are merely folded and tied with rubber. This packaging allows high air circulation into/out of the container.

**Figure 4.** Spawn packaged in plastic bag and plugged with unsterilized cotton using corn as the substrate.

## 2. MATERIALS AND METHODS

### *2.1 Fungal source and isolation*

The isolate of *P. ostreatus* was obtained from traditional market in Balikpapan. The isolation medium used potato dextrose agar (PDA) with 39 g in 1 L of water as described by the Oxoid manual. The primary inoculum was prepared from the fresh fruiting body of *P. ostreatus*. A small piece of the mushroom tissue was removed by sterilized tweezers and placed on PDA in disposable Petri dishes for 7 days at room temperature. To purify the isolate, the mycelia was excised and transferred to another plate containing PDA and incubated at room temperature for 7 days. Sub-culturing was performed until a pure culture was obtained. The pure culture was maintained in a PDA slant culture for 1 year at room temperature. Pre-inoculum was obtained by incubating the fungus on PDA at room temperature for 10 days.

### *2.2 Design for spawn container*

A stand-up bag was designed for the spawn container. The bag must be able to withstand pressure sterilization. HDPE plastic bags were commonly found at traditional markets for hot foods. However, since the bag was not designed to stand up, we modified the original shape. The size of the HDPE plastic bag used was 12 x 25 cm and 0.03 mm in thickness. Two steps used to modify the plastic bag. Firs, the edge of the bottom of the bag was cut and folded inwardly from the left and right sides. Second, a heat press was used to make a three-dimensional gusseted bag.

### *2.3 Spawn preparation*

The spawn was prepared on corn, rice, and a mixture of corn and rice (a 1:3 corn-to-rice ratio). The grains were soaked overnight in boiling water. The rice was washed with water and then drained. The corn was washed three times and then half boiled and drained. The grains were sun dried to remove excess water and then 1% gypsum based on substrate weight was added. The grains were filled into gusseted bags at 200 g/bag and sterilized with a pressure cooker for 1.5 hours. The inoculation was carried out on the following day under aseptic conditions.

To obtain first-generation spawn (G1), the grains in the gusseted bags were inoculated with two patches (1 x 1 cm) from 10-day-old culture grown on PDA and incubated at room temperature. Second-generation spawn (G2) was expanded from G1 using the same media for each treatment; one gusseted bag of G1 was expanded into 20 bags of G2 and incubated at room temperature. The physiological and morphological properties of mycelium growth on substrates were assessed, including growth rate, type of mycelium, and colony formation. The experiment was carried out in 50 replicates.

### *2.4 Effect of grain water content on mycelial mat growth*

The effect of grain moisture on the growth rate of mycelia *P. ostreatus* was determined. The grains were sun dried until water around the surface of the grains was removed, and then the water content of grains was determined by drying at 105ºC for 24 hours. Normal water content of the grains in the plastic bags was 45-50% for corn and 27% for rice. If these two grains were mixed, the water content of the grains was 35-42%. The other water contents were made too wet and too dry compared to the normal water content. The physiological and morphological properties of mycelia growth on substrates were assessed, including growth rate, type of mycelium, and colony formation.

### *2.5 Mushroom cultivation*

The spawns were applied by farmers to mushroom cultivation. For making 1000 bags culture, the composition of the substrate was sawdust (900 kg), bran (40 kg), lime (10 kg), with 70% of water content. The substrate was sterilized for 8 hours using firewood. For spawning, 1 bag of spawn was expanded to 30-50 substrate and then incubated at 26-28ºC and 80-95% of humidity. The growth rate, type of mycelium, and visual shape of the fruiting body were assessed.

## 3. RESULT AND DISCUSSION

The lack of knowledge of local farmers regarding proper spawn production was the reason for this study. This study sought to improve the containers and substrates commonly utilized by farmers. We modified the flat shape of the HDPE plastic bags into gusseted bags (i.e., three-dimensional bags). The advantage of the gusseted bags was increased volume. Furthermore, when the bag was filled with grain, the air circulation inside the bag was more sufficient for mycelia growth from top to the bottom (Figure 5). The gusseted bags were also equipped with microporous filter patches to allow low levels of gas circulation. Mycelium consume fresh oxygen while growing, so without circulation, the bag environment will become anaerobic, wherein bacteria proliferate [7]. Flat plastic bags were a major factor leading to contamination due to poor gas circulation [2]. Stamets [2] has reported spawn production in polypropylene plastic bags using rye grain as a substrate. However, there have been no studies reporting on the use of HDPE plastic bags as spawn containers, and rice and corn as the substrates.

The growth rate of *P. ostreatus* on grains was determined when mycelium was fully colonized grain in the bag. All the grains supported the mycelia growth of *P. ostreatus*. Mycelial mat showed the fastest growth on

the corn substrate both for G1 (2 weeks) and G2 (1 week) compared to the rice and mixture of corn and rice (Table 1). The slowest growth occurred on the rice substrate; mycelial mat covered the grains on G1 and G2 after 6 weeks and 4 weeks, respectively. The growth rate of *P. ostreatus* was determined in a mixture of corn and rice for G1 and G2 and was found to be 4 weeks and 2 weeks, respectively. The type of mycelial mat was thick on the corn substrate, thin on the rice substrate, and moderate on the mixture of corn and rice (Figure 6). We suggest that the fast growth and thick layer of the mycelial mat using the corn substrate was due to the high carbohydrate and sugar content of corn [8].

Form the results, we found that water content of the grains played an important role in mycelia growth. The excess water at the bottom of bags occurred when the water content of grain was too high. It led to a greater contamination of the spawn. Mycelia growth at this water content was nonuniform, compacted, lumpy, slow, discolored (yellowish or grey), and odorous. The spawn survival was <10% when the moisture of the grain too high. The unpleasant odor may reflect the possibility that spawn contain some bacteria. On the other hand, when the moisture of the grain was too low, the mycelial mat formed fine threads, grew slowly, and often released unpleasant odor. The spawn survival for too-dry grain was <40%. The water content of the corn was around 45-50%, with ideal water content around 50%, while the ideal water content for rice was 27%. Within this water content, mycelium growth was healthy, of a pure white color, and tended to release a fragrant aroma of mushroom and sugars. The healthy mycelium spawn was tight, dense, tenacious, and held grain together. The spawn survival rate was 95-100%.

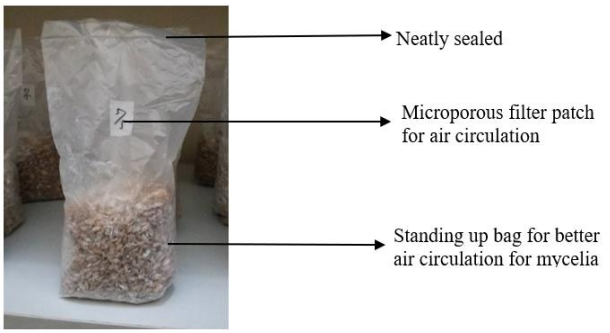


**Figure 5.** *P. ostreatus* spawn was produced in gusseted bags with a mixture of corn and rice as the substrate.


**Figure 6.** Two-week-old *P. ostreatus* grown on three substrates: corn (A), rice (B), and a mixture of corn and rice(C).


**Figure 7.** Mycelial growth of *P. ostreatus* on sawdust using corn spawn (A) and mixture of corn and rice spawn (B).

Figure 7 shows the mycelial growth of *P. ostreatus* using corn spawn and mixture spawn. Our observation of the bag cultures indicated that during active mycelial growth, decolorization on the sawdust was not visible when the corn spawn used. Meanwhile, decolorization of the sawdust was clearly visible when the mixture of corn and rice spawn used. The change of color was from dark brown (color of the sawdust) to light brown. Previously, we have studied the growth of white-rot fungi on *Acacia mangium* sawdust [10]. The results therein showed that there was a correlation between the ability of white-rot fungi to decolorize sawdust and lignin loss. Therefore, the decolorization of sawdust during active mycelial growth of *P. ostreatus* on the bag cultures indicate that degradation of lignin has occurred. According to Ejechi et al. [11], *P. ostreatus* grown on

**Table 1.** Physiological characteristic of *P. ostreatus* in different grains.

| Type of grain | Growth rate of G1 (week) | Growth rate of G2 (week) | Mycelial mat[a] |
|---|---|---|---|
| Corn | 2 | 1 | +++ |
| Rice | 4 | 2 | + |
| Mixture corn and rice | 6 | 4 | ++ |

[a] +++: thick, ++: medium, +: thin

mahogany and obeche wood blocks showed decolorization (bleaching) during decay. They hypothesized that oxidative enzymes (laccase) may be associated with the decay-retarding pigmented extractive of wood, and the lightening of the wood color caused removal of pigmented compounds. However, further study of the relationship between mycelial growth, decolorization sawdust, and lignocellulolytic enzymes produced during cultivation of *P. ostreatus* in this study is needed.

## 4.CONCLUSION

Using corn as substrate of *P. ostreatus* resulted fast growth and thick mycelium both in first- and second-generation spawn compared to rice and mixture of corn and rice. However, the second-generation spawn of mixture corn and rice was preferable by farmers because they obtained good yield.

## REFERENCES

[1] Directorate General of Horticultural 2014 *Report performance of Directorate General of Horticultural* (Ministry of Agriculture, Jakarta)
[2] Stamets P 2000 Growing Gourmet and Medicinal Mushrooms (California: Teen Speed Press)
[3] Leonowicz C Z, Matuszewska A, Luterek J, Ziegenhagen D, Wojtas-Wasilewska M, Cho N-S, Hofrichter M, Rogalski *J Fungal Genet. Biol*. **27** p 175
[4] Westermark U, Eriksson KE 1974 *Acta Chem. Scand*. **B28** p 204
[5] Membrillo I, Sanchez C, Meneses M, Favela E, Loera O 2008 *Bioresources Technol.* **99** p 7842
[6] Da Luz JMR, Nunes MD, Paes SA, Torres DP, De Silva MdCS, Kasuya MCM 2012 *Brazillian J. Microbiol.* p 1508
[7] Ogden A, Prowse K 2004 How to Make Oyster Mushroom Grain Spawn in a Simple Way *Oyter Mushroom Cultivation* (Mush World: www.Mushworld.com) Chapter 4 p 62
[8] United State Departement of Agriculture (USDA) 2018 *National Nutrient Database for Standart Reference Legacy Release* (National Agriculture Library, USA)
[9] Tsai W T, Lee M K, Chang Y M 2007 *Bioresource. Technol*. **98** p 22
[10] Afrida S, Tamai Y, Watanabe T, Osaki M 2009 *World J. Microbiol. Bioetchnol.* **25** p 639
[11] Ejechi BO, Obuekwe CO, Ogbimi AO 1996 *Int. Biodeterior. BiodegradationI.* p 199