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Characteristics of sawdust cultivation of *Lentinula edodes* with different methods of spawn inoculation

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ABSTRACT: This study was carried out to investigate the management characteristics and growth performance of *L. edodes* from the cooling stage to incubation. Bags of different heights and weights are available for bagging. When the medium size of 17x13 cm was used and the size of the inoculation hole was changed from 1/3 to 2/3, the browning period was shortened to 30 days. Mycelial growth was evaluated according to the cooling temperature after sterilization. It was observed to be the highest at 122 mm/15 days at 10°C and 114 mm/15 days and 117 mm/15 days at 15°C and 20°C, respectively. The contamination rate of the sawdust media before inoculation was measured as 0, 4.5x10, $1.3x10^2$, $4.0x10^3$ cfu at 5°C, 10°C, 15°C, and 24°C respectively. The average of $1.6x10^8$ colony forming units (cfu) of microorganisms was observed in the sawdust that had been piled for six months outdoors. In summer, the sawdust has to be used immediately after mixing. The sterilized medium had an average of $4x10^3$ cfu of microorganisms at 24°C and 1.3×10^2 cfu at 15 °C. After 15 days of inoculation in vitro, the growth conditions of the sawdust was the best at 132 mm, followed by grain and liquid. When inoculated with liquid spawn, the moisture content of the substrate should be adjusted between 50% and 55% in advance.

KEYWORDS: Bag type, Incubation, *Lentinula edodes*, Shiitake, Spawn

Introduction

Shiitake(*Lentinula edodes*) is a medicinal mushroom that contains lentinan, an anti-cancer agent, and eritadenin, which lowers cholesterol levels(Park *et al*., 2011). It is also a mushroom that can decompose aromatic compounds and restore contaminated soil (Stamet, 2005). Domestic shiitake mushroom production has been on the decline since 2010. In 2014, it recorded a total of 25,350 tons; 943 tons of dry matter and 18,456 tons of raw mushrooms (Shin, 2015).

In the case of sawdust cultivation, its cultivation period is short while the recovery rate is high. There is an advantage of having a small labor force and as a result, the number of farmers who convert to shiitake sawdust cultivation is increasing (Seo *et al*., 2008). Due to the spread of sawdust cultivation methods, with imports of Chinese sawdust medium, the cultivation mode of harvest and shipment within 1-2 months has been increasing. The amount of sawdust media imported from China increased from about 6,000 tons in 2006 (Kim *et al*., 2009) to 13,491 tons in 2013 and 23,759 tons in 2014. However, the safety management standards for imported mushroom medium and raw materials of medium are not so clear.

With the help of the experienced farmers, the effects of different types of sawdust cultivation from the process of cooling, inoculation and incubation which directly affect and relate to yield and income have been determined. The process of sawdust cultivation based on their skill and knowledge was also demonstrated.

To contribute to the development of the mushroom industry and to revitalize the successful farming activities of domestic farmers, this research was carried out. The best practices and the management knowledge in the sawdust cultivation process of the excellent farmers were discovered and shared, thereby contributing further to the improvement of the farmers'

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household income through the high quality, low-cost but high-yield shiitake production.

Materials and Methods

The Study Area The study was conducted at the Department of Mushroom of Korea National College of Agriculture and Fisheries.

The Strain The strains used in this study were Sanjo 701 that were grown on PDA medium for 10 days and used as test strains in oak sawdust of Erlenmeyer flasks. This process was repeated three times.

Substrate preparation The check substrate contains oak sawdust which is 100% sawdust that are 3 mm in size. While the other substrate contains a combination of sawdust, 3 mm in diameter with a mixture of 5% woodchips, 5 mm in size and rice bran (80:20). These woodchips were soaked in advance for one week in winter and four days in summer. The oak sawdust containing 5% of wood chips and the rice bran were mixed at 80:20 (v / v) setting moisture content to 55%, while the volumetric weight was constantly adjusted to a glass column (30 mm × 200 mm) with 1.4 bulk density.

Sterilization The substrate was sterilized at 121°C for 40 minutes, cooled to 10, 15 and 20°C. Pressure steam autoclave sterilizer was used under normal and high pressure sterilization. This is done to reduce contamination during the process of inoculation and improve its physical properties then inoculated with sawdust spawn.

Inoculation The substrate is inoculated with mushroom spores or spawn after which fungal fibers known as mycelium begin to grow.

Incubation This is the time after inoculation and before the mycelium has fully colonized the substrate. This is the time at which the fungus has not yet consolidated its hold on the substrate. During the stage, the nutritious substrate is more susceptible to contamination. Most often, colonization of highly nutritious spawn substrates is completed in an enclosed sterile environment. Growth rate and density of mycelium were examined by 5 day intervals basis at 70% humidity and 22±1°C temperature.

Contamination determination To determine the amount of contamination of the sawdust media, 1g sample was taken from the medium prior to inoculation having various temperatures (5, 10, 15, 24 ° C). Having mixed with 10ml of distilled water, it is homogeneously mixed by homogenizer. In order to identify the source of contamination, it was thoroughly determined by the dilution plate method on the PDA medium (Fuse, G., *et al*., 1984).

Growth rate and density according to inoculated type of spawn The substrate was sterilized at 121 °C for 40 minutes, cooled to 15°C. Sawdust spawn was inoculated with 1, 2, and 3 g, respectively. The grains were inoculated with 1, 2, and 3 grams, respectively. The liquid were inoculated with 1,2,3 cc. Growth rate and density of mycelium were examined by 5 day intervals basis at 70% humidity and 22±1°C temperature.

Medium preparation The culture completion period, mycelial density and biological efficiency were investigated by preparing medium having a height of 17 cm and a width of 13 cm with weight of 1.5 kg. The culture depth, mycelial density and biological efficiency were examined by treating the depth of the medium with 1/3 to 2/3 of the original depth.

Results and Discussion

Mycelial growth and density depending on the incubation period and browning

When the height is maintained at 17 cm and the width at 13 cm, the browning and incubation period was shortened to 30 days. On the other hand, when the hole of the medium (inoculation hole) was changed from 1/3 to 2/3, the gas release during incubation was facilitated and ventilation was increased.

When lowering the height to 17 cm and widening the width to 13 cm, the incubation period of 120 days was shortened to 90 days while the mycelial density increased from 2 (++) to 3 (+++), and the biological efficiency was increased from 20 to 30% (Table 1).

When the pores are drilled into the medium by 2/3, the gas is easily discharged, the ventilation rate is increased and the growth rate is accelerated.

Table 1. Mycelial growth and density depending on the shortening of incubation and browning

	Medium Size(cm)			Incubation Period(days)	Mycelial Density[a]	Biological Efficiency(BE)[b](%)
	H	W	D			
Control	22	10	1/3	120	++	18
Treatment	17	13	2/3	90	+++	32

[a] : +(weak), ++(normal), +++(excellent)
b : BE = weight of harvested fresh mushroom

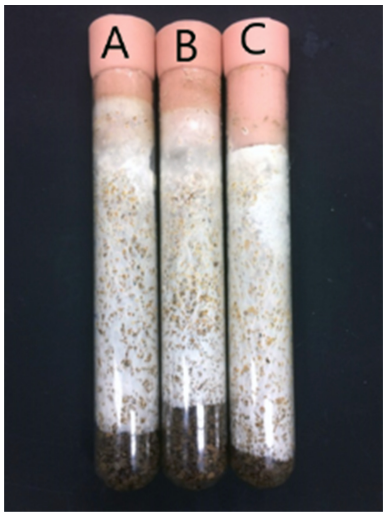

Fig. 1. Mycelial growth and density depending on the cooling temperature after sterilization. Mycelial growth of column A : 117 mm / 15 days at 15°C, B: 114 mm / 15 days at 20°C, C: 122 mm / 15 days at 10°C. Growth rate of mycelium was examined by 5 day intervals basis at 70% humidity and 22±1°C temperature.

Mycelial growth rate according to cooling temperature after sterilization

Mycelial growth in length (mm) was 122 mm / 15 days at 10°C and 117, 114 mm / 15 days at 15 and 20°C, respectively (Fig. 1).

Mycelial growth and contamination rate according to various temperature

The contamination rate of the sawdust media before inoculation was measured as 0, 4.5×10, 1.3×10^2 , 4×10^3 (cfu) at 5°C, 10°C, 15°C and 24°C respectively (Table 2).

According to Liao, Y. M.(1993), the number of sawdust medium contamination before inoculation at 24°C was 4×10^3 (cfu). The lower the temperature gets, the lower the degree of contamination takes place, while the higher the temperature gets, the higher the contamination rate becomes. Ideally, growing the mycelium should be kept in an ideal temperature range. Temperatures higher that this range may kill the mycelium and encourage growth of contaminants, and temperatures lower that this range may slow down colonization.

Table 2. Contamination rate depending on the temperature

	Temperature (°C)			
	5	10	15	24
Number of colony forming unit (cfu) per gram of sawdust	0	4.5x10	$1.3x10^2$	$4x10^3$

While the mycelium is growing it will generate a considerable amount of heat and can suffer harm if it is laced with too high of a temperature.

There is an average of 1.6×10^8 (cfu) of microorganisms in sawdust which has been piled for six months (Liao, Y. M. 1993). It should be used immediately after mixing sawdust in summer.

Mycelial growth rate and density by inoculated type of spawn

The growth rate and density of mycelium was best at 132 mm/15 days for sawdust, 127 mm for grain spawn and 99 mm/15 days for liquid spawn. (Fig. 2). The most stable mycelial growth rate and mycelial density were observed in sawdust spawn, while the liquid spawn

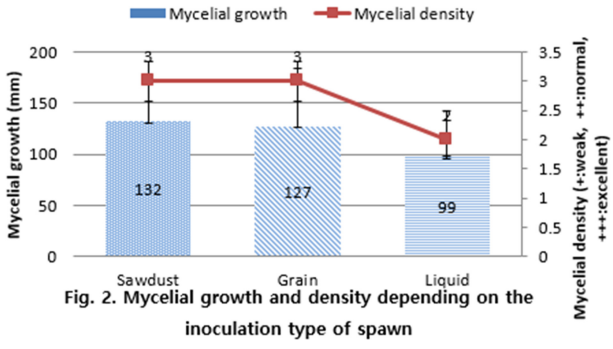

Fig. 2. Mycelial growth and density depending on the inoculation type of spawn

showed the lowest growth rate and mycelial density. It did not match the report of Kirchhoff, B. and Lelley, J. (1991). This is presumed to be the result of inoculation of immature liquid spawn. When using liquid spawn, moisture content of the medium must be adjusted from 55% to 50%.

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