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# Effect of Fermented Sawdust on *Pleurotus* Spawn

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**A method of spawn making procedures through the application of fermented sawdust for the purpose of avoiding contamination by undesirable fungi in the course of *Pleurotus ostreatus* mycelial growth was evaluated. Of three kinds of supplements, rice bran was the most effective to raise temperature up to 70°C. Mycelial activity and density was more considerably improved in the case of using fermented sawdust supplemented with rice bran than the case of non-fermented sawdust. Primordia of *Pleurotus ostreatus* were formed on fermented sawdust. The substrate of fermented sawdust showed potential to prevent the growth of *Trichoderma* sp. which caused a symptom on mushroom mycelium, whereas there was nothing to inhibit the growth of *Trichoderma* sp. during 30 days after inoculation in non-fermented sawdust.**

**KEYWORDS: Fermented sawdust, Oyster mushroom, *Pleurotus ostreatus*, Primordia, Sawdust spawn, *Trichoderma* sp.**

In spawn industry the process of commercial spawn making should be practised to avoid contamination by other microorganisms and assure the purity of spawn cultures under the constant vigilance and careful maintenance (Ivanovich-Biserka, 1972). Much attention should be paided to the spawn during incubation and spawn production (Fritsche, 1981). The modern systems of mass production have been established and purely cultured spawns have become available.

There are a few kinds of spawns in button mushroom but grain spawn is usually distributed worldwide. The *Agaricus* spawn making is based on formula as follows; 10 kg of wheat grains (Manitoba) in 15 liters water are boiled for 15 minutes (Lemke, 1972) and then mixed with 120 g of gypsum ($CaSO_4 \cdot 2H_2O$) which prevents the grains from sticking together and 30 g of $CaCO_3$ which adjusts pH 6.5 to 6.7 (Stoller, 1962), and the grain is bottled in 1 liter milk bottle (350~400 g/bottle), which is finally sterilized at 121°C for 2 hours at 15 lb steam pressure (Chang, 1978).

Sawdust spawn has been widely applied to cultivated edible and medicinal mushrooms, especially Shiitake mushroom is produced by using plug spawn and sawdust spawn which is composed of a mixture of sawdust and bran (4:1) (Paul and John, 1990). *Pleurotus ostreatus* was inoculated on the sawdust (Block *et al.*, 1959). Oyster mushroom produced in Korea is estimated by 70% of total mushroom products. Most of spawn companies produce oyster mushroom spawn using poplar sawdust with a mixture of 20% rice bran. The sawdust spawn material mixed with water is weighted 700~750 g in the 1000 m*l* bottle, which is sterilized at 121°C for 90 minutes at 15 lb steam pressure.

Sawdust spawn will not keep as long as plug spawn because the available nutrients in the sawdust are run down sooner. The safe storage period of spawn is affected by temperature. Spawn will last for one or two months at cool room temperatures (Paul and John, 1990). To assure high quality spawn, sterile conditions and stringent quality control are required throughout the process of sawdust spawn, and the mycelium in the spawn should vigorously digest the medium without undesirable microorganisms. However, many failures can be traced to poor-quality spawn and weak strains in the oyster mushroom farms. Green moulds usually take place during mycelial growth in the spawn bottle. It is suggested in this paper that fermentation technique is applicable to make a sawdust spawn of high quality and solve the problems related in spawn management.

## Materials and Methods

**Culture.** *Pleurotus ostreatus* (ASI 2504) obtained from breeding lab. Div. of Applied Microbiology National Institute of Agricultural Science and Technology (NIAST, RDA Suwon 441-707) was maintained on potato dextrose agar (PDA) at 25°C. *Trichoderma* sp. was isolated from oyster mushroom growing bed.

**Preparation of mixed sawdust and fermentation.** Poplar sawdust and pine sawdust were mixed at the proportion of 50% and 50% (v/v), and 20% of rice bran added. Water was fully applied to the mixed sawdust over 90% and stacked outside for 6 days, which reached the first turning-up point, and then stacked for 3 days, which was second turning-up point, and finally stacked for 7 days. At

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every turning-up process, water was applied to adjust water content of sawdust.

**Making spawn.** The fermented sawdust material was bottled in the 1,000 m*l* polypropylene bag, and sterilized at 121°C for 90 min under 15 lb steam pressure. 12 hours after sterilization, *Pleurotus ostreatus* (ASI 2504) was inoculated in the clean bench (Dasol DS-70D, Korea). And then the spawn bottles were incubated at 25°C for 20 days in the incubating room.

**Heating treatment and inoculation of *Trichoderma* sp.** The fermented or the non-fermented sawdusts containing rice bran 20% were heated in the fermentation room at 65°C for 5 days. The sawdusts were put in the polypropylene cap bottles with the weight of 700 g. *Trichoderma* sp. grown on PDA (Potato Dextrose Agar) was inoculated in the each sawdust bottle, and then those were incubated in the growth chamber (Forma Scientific Model 3158-S/N 38229-1804, Japan) for 30 days.

**Mycelial growth in column.** The column of 2.5 cm in diameter and 20 cm long was filled with the fermented sawdust and the non-fermented sawdust, respectively. *Pleurotus ostreatus* (ASI 2504) was inoculated in the clean bench (Dasol DS-70D, Korea) 12 hours after sterilizing the column at 121°C for 15 min under 15 lb steam pressure in the autoclave (Hanshin Medical Co., Model HS-60, Korea). The inoculated columns were put at 25°C for 20 days in the incubating room.

## Results and Discussion

**Changes of temperature in sawdust stack added by supplements.** Three kinds of supplements, those were rice bran, tobacco waste, or oriental medicine waste, were treated to the sawdust to raise temperature, respectively. Rice bran was found to be the most effective supplements of them. Temperature of the sawdust stack added with rice bran increased up to 70°C on 6th day, whereas those of control treatment with just sawdust slightly fluctuated at around 22 to 27°C without any temperature changes in sawdust stack. Treatment of tobacco waste increased up to 47°C in 12th day, and oriental medicine waste was up to 45°C in 12th day (Table 1). It was assumed that rice bran was most effective substrates to elevate the fermentation temperature, because it contained nutritious materials, such as carbohydrates, minerals and nitrogenous compound. In case of *Agaricus bisporus*, supplements for activating fermentations, such as horse, pig and chicken manure etc. (Chang, 1978), have been used.

**Table 1.** Temperature changes in the sawdust stack added by supplements

| Supplements | Days of stacking | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| Rice bran | 55 | 65 | 70 | 60 | 61 | 59 | 58 | 58 | 58 |
| Tobacco waste | 30 | 37 | 43 | 38 | 45 | 47 | 44 | 28 | 30 |
| Oriental medicine waste | 30 | 37 | 40 | 43 | 38 | 45 | 33 | 35 | 30 |
| Control | 27 | 27 | 26 | 26 | 22 | 26 | 25 | 22 | 22 |

Periods: 01. 9. 20-10. 08

**Mycelial growth in spawn bottle.** Mycelial lumps were formed under the lid in each spawn bottle, respectively, which was the fermented or non-fermented sawdust, and the appearance of the bottle took no differences between them (Fig. 1a, b). However, mushroom mycelium was more dense and active in the fermented sawdust (Fig. 1d and Table 2) than in the non-fermented sawdust (Fig. 1c and Table 2). To achieve the more rapid colonization of mycelium, rice bran was added to sawdust (Paul and John, 1990). Color of the fermented sawdust was dark brown when fermentation was finished, but that of the non-fermented sawdust was pale yellow. Farmers hope that mushroom spawn should definitely be healthy and vigorous, because spawn of more rapid growth could compete more actively against pathogenic fungi. Therefore, it is supposed that the fermented sawdust was the best substrate for the rapid growth of mycelium, because it contained a plenty of fermentation product produced by microorganisms like Actinomycete. So it was recommended that the fermented sawdust spawn be widely distributed and applied to the oyster mushroom spawn producers.

**Fruit body formation in column.** Oyster mushroom mycelium (ASI 2504) inoculated in column was well grown in the fermented sawdust showing dense mycelial growth compared to the non-fermented sawdust. Primordia of *Pleurotus ostreatus* were formed in the column packed with fermented sawdust (Fig. 2b and Table 2), whereas those were not produced in the column packed with non-fermented sawdust (Fig. 2a and Table 2). Mushroom primordia unexpectedly produced in the small column indicate that the fermented sawdust contains much nutrient sources easily utilizable by the mycelium.

**Antibiotic reaction.** *Trichoderma* sp. was severely inhibited on the fermented sawdust. The fermented sawdust was still unchanged without contamination by any other fungi, 30 days after inoculation of *Trichoderma* sp. (Fig. 3b, d and Table 2). It was thought that antibiotics produced by microorganisms in the fermented sawdust strongly inhibited development of *Trichoderma* sp. On the contrary, *Trichoderma* sp. was full grown and took up the whole substrate on non-fermented sawdust (Fig. 3a, c and Table 2). It implies that *Trichoderma* species could utilize

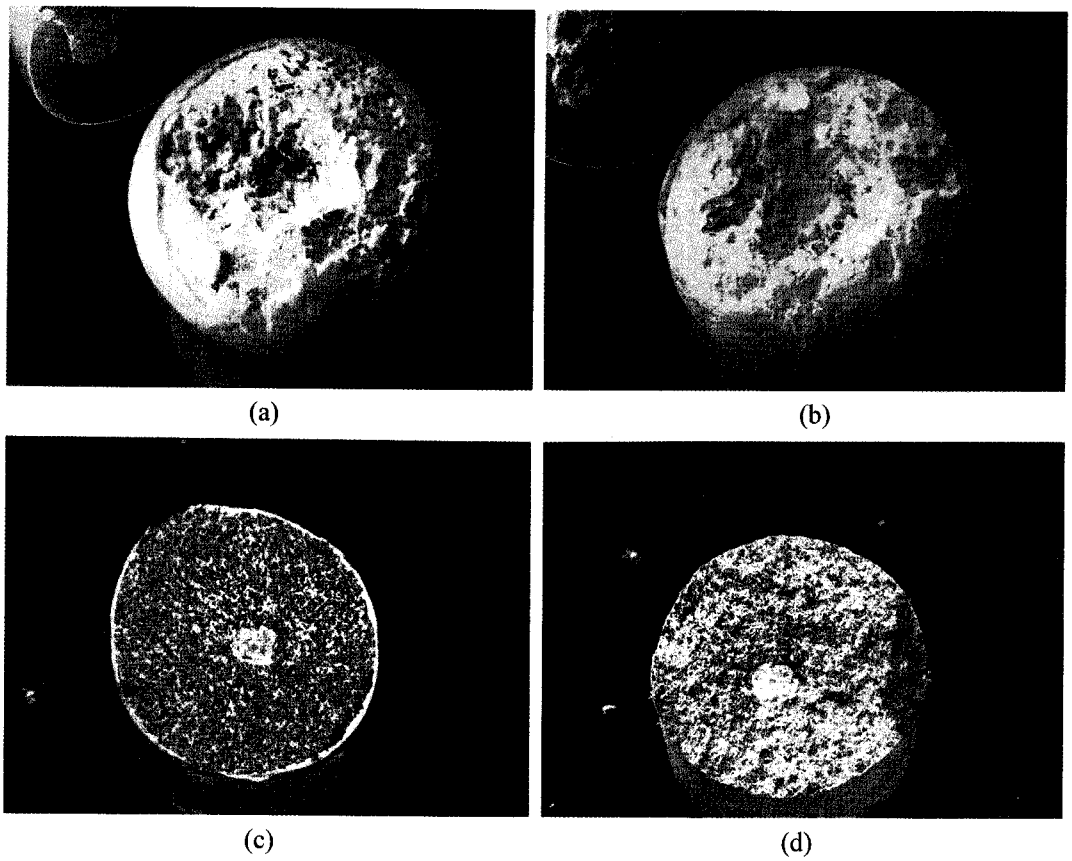


**Fig. 1.** Comparison of mycelial growth of *Pleurotus ostreatus* in the spawn bottle. a, Upper side of the non-fermented sawdust spawn bottle; b, Upper side of the fermented sawdust spawn bottle; c, Middle of the non-fermented sawdust spawn bottle; d, Middle of the fermented sawdust spawn bottle.

**Table 2.** Effects of fermented sawdust spawn on mycelial growth, primordia formation and disease incidence

| | Treatment of sawdust | |
|---|---|---|
| | Fermented | Non-fermented |
| Mycelial growth and density of *P. ostreatus* in the spawn bottle | +++++[a] | +++ |
| Primordia formation of *P. ostreatus* in column (%) | 100 | - |
| Inhibition of *Trichoderma* sp. infection (%) | 100 | - |

[a]+++++, excellent ; +++, normal.

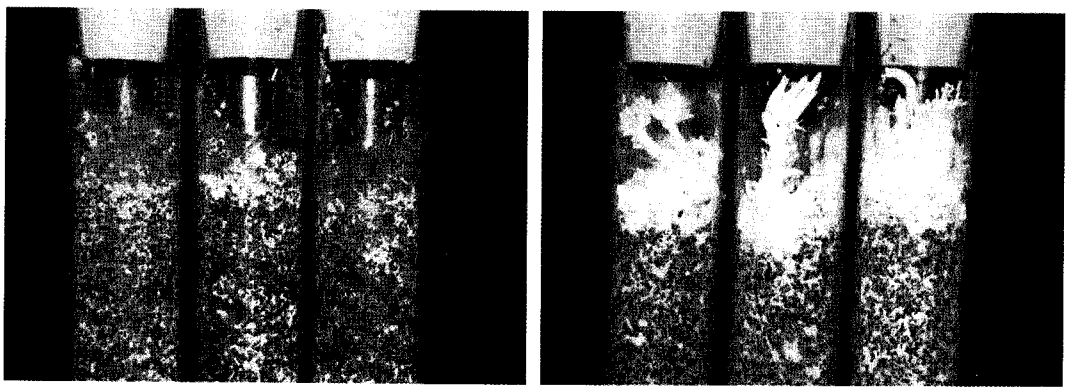


**Fig. 2.** Primordia formation of *Pleurotus ostreatus* in the column filled with sawdust substrate 30 days after inoculation of mushroom spawn. a, Non-fermented sawdust substrate; b, Fermented sawdust substrate.

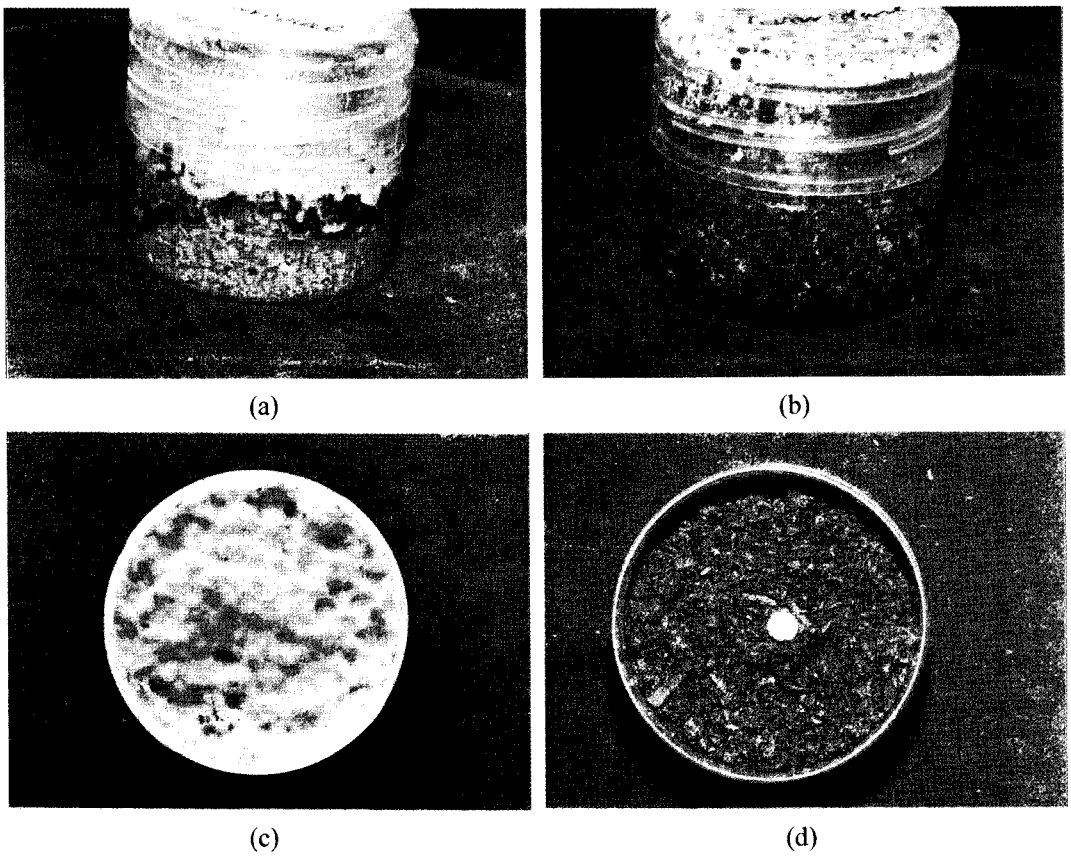


**Fig. 3.** Infected sawdust substrate 30 days after inoculation by *Trichoderma* sp. a, Outside of the bottle inoculated with *Trichoderma* sp. on the non-fermented sawdust substrate; b, Outside of the bottle inoculated with *Trichoderma* sp. on the fermented sawdust substrate; c, Inside of the bottle inoculated with *Trichoderma* sp. on the non-fermented sawdust substrate; d, Inside of the bottle inoculated with *Trichoderma* sp. on the fermented sawdust substrate.

cellulose of sawdust on the point of view that the non-fermented sawdust was infested by artificially inoculated *Trichoderma* sp. *Trichoderma* species degrade hemicellulose of hardwood and softwood by secreting hemicellulase (Harman and Kubicek, 1998). Also, *Trichoderma* attacks logs and Shiitake mushroom mycelium (Paul and John, 1990). Therefore, *Trichoderma* species cause economic losses in mushroom production worldwide. Kneebone and Merek (1959) reported that *Trichoderma* sp. secreted a toxin into the casing, which caused sunken brown lesions on mushrooms. Based on the inhibition of *Trichoderma* sp. in the fermented sawdust, we expect that this method would be prevalent to make oyster mushroom spawn industry to be more safe system in Korea, because the growth of *Trichoderma* sp. was inhibited in the fermented sawdust. Further studies are required in detail for the reason why the harmful fungi couldn't grow in the fermented sawdust.

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