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The Role of the Rice Bran Employed in the Traditional Spawn Sawdust Medium

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전통적인 버섯재배지에서 사용되는 미강의 역할

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ABSTRACT: Metaboilc parameters were obtained from the measurment of productivities of carbon dioxide on the sawdust medium. The productivites of carbon dioxide obtained during fourteen days' incubation were employed for the fungal biomass, representing the fungal growth, and applied for understanding the physioligical parameters on the sawdust medium. The role of rice bran, commonly employed in the conventional spawn medium was speculated to be three kinds of nutrients of starch, nitrogen source, and and minerals. Biologically, the role of rice bran was considered to be the fast growing agents which led to prevention of other microorganisms.

KEYWORDS: Productivities of carbon dioxide, Lentinus, spawn cultivation

Fungal culture was divided into three different kinds of cultures; surface, liguid, and solid state cultures due to aerations or various substrates. Aeration, which is necessary in liquid cultivation, has been recently established with the increase of penicillin priduction. Shaking culture has been general with the fermentation industries. The physiological parameters obtained from shaking or liquid culture have been recognized in the studies of fungal physiology. Growth or morphogenesis of mushroom fungi have been progressed in the solid state culture, but not quite often obtained from liquid or shaking cultures (Matcham *et al.*, 1984). The parameters obtained in the liquid cultures were considered to be quite different from those in the solid state cultures.

Mushroom fungi have been mostly cultivated on the lignocellulosic complex (agricultural waste), such as straws, woods and so on (Kaneshiro, 1977; Streer, 1981). Natural decomposition of straws or woods involved in the fungal flora has been important in revegetation of economical plants in the agricultural and forestry fields (Blanchette, 1978). Also, commercial production of mushroom has been employed by the solid state media, so called "composite" (Stametes, 1983). Fungal growth on the above mentioned solid state media was determined by measuring substrate loss (Kaneshiro, 1977; Lindenfelser *et al.*, 1978; Lundquist, 1977), chitin analyses (Gurusiddaiah *et al.*, 1978), metabolite production (Hiroi and Eriksoon, 1976; Lindenfelser *et al.*, 1979), wieght loss (Reid, 1979; 1985), and $^{14}CO_2$ production (Crawford and Sutherland, 1979; Hackett *et al.*, 1978; Marinucci and Bartha, 1979) in the solid state media. Little has been known for fungal physioligy in the solid state cultures.

Mushroom productions of shiitake, oyster musgroom, *Ganoderma,* and other mushrooms are more active in Korea than in other European cou-

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ntries and also gradually increased every year. The medium employed for mushroom productions as the above consisted of an oak or popular sawdust and an agricultural wastes in the solid state medium (RDA, 1987). The parameters involved in the solid state medium would be considered to be quite different from those in the shaking medium. This is subjected to determine the parameters obtained from and also to find the role of rice bran on the solid solid state culture by using carbon dioxode productivities instead of biomass measurements.

Materials and Methods

Fungi employed

Lentinus edodes and *Pleurotus ostreatus* are common fungi cultivated in Korean mushroom industries: *L. edodes* (line 15-23) was obtained from the Laboratory of Applied Forestry Research Institute in Forestry Administrations (Seoul) and *P. ostreatus* from Laboratory of Applied Mycoloy at Agricultural Science Institute, R. D. A. (Suweon 440-707). Both fungi were stored on potato dextrose agar and transferred in complete media agar before inoculation in the sawdust medium (Raper and Raper, 1972).

Sawdust medium

The recipe of an organic sawdust medium used for the spawn culture was employed in this work and modified as follows (RDA, 1987; Stametes, 1983) : 50 gm sawdust of *Quercus acutissima* for *L. edodes* or *Popular deltodies* for *P. ostreatus*, 12.5 gm bran of rice *(Oryzae sativa* var. Yushin), and 80 m*l* of distilled water including with the mineral solution were mixed and placed at a 500 m*l* Erlemeyer flask and autoclaved with a cotton plug. The mineral solution of 2.0 gm $MgSO_4 \cdot 7H_2O$, 4.0 gm K_2HP0_4, 0.6 gm KH_2PO_4, 4.0 gm $CaCO_3 \cdot 2H_2O$, 0.04 gm $FeCl_3 \cdot 2H_2O$, 0.04 gm $FeCl_3 \cdot 2H_2O$, 4.0 gm $(NH_4)_2SO_4$ and 0.04 gm thiamine HCl were mixed in a liter of the distilled water (called "Minerals"), stored, and added to the above sawdust medium with the 20 m*l* (Ginterova and Janotkova, 1975; Molina and Palmer, 1980). One gram of Difco peptone or Difco yeast extracts was employed instead of ammonium sulfate as a nitrogen source. A flask including the above ingredients and inoculated was placed at 25℃ incubators and measured for carbon dioxide priduction for 30 min. Here, the organic sawdust medium commonly used as a spawn culture (starter culture) was developed by Laboratory of Applied Mycoloy at Agricultural Science Institute, R. D. A. (Suweon 440-707) and refered to "sawdust medium".

Measuremnet of carbon dioxide

Two kinds of stoppers were employed to seal an Erlenmeyer flask containing the cultured sawdust medium ; the red rubber septum was attached with the black rubber (#9 or #10) for collecting air samples by a ten μ*l* syringe. The cultured flasks were placed on the clean bench (a bacteria-free chamber) and sucked by a vacuum to remove the metabolic carbon dioxide. After then, the cultured flask was filled with the septum previously mentioned on the top instead of the cotton plug and incubated at 25℃ for 30 min. The five μ*l* of the air in the cultured flask was collected and used to measure the carbon dioxode productivities. A six feet stainless column filled with Porapak Q 80/100 mesh were set up in the gas chromatography medeled "HP-5890". The amount of carbon dioxide was measured by a thermal conductivith detector at 100℃ (with reference of He gas at the flow rate of 20 m*l*/min) and with a carrier gas of He at te flow rate of 30 m*l*/min. The above column was programmed at the isotermal cinditions (50℃).

Results and Discussion

Cultivations

Two fungi of *L. edodes* and *P. ostreatus* were grown in the solid state by using the different sawdusts of oak and popular trees. The productivities of carbon dioxide were measured and represente as the fungal growth (would be mentioned again, later), after the fungus were inoculated and incubated on the solid state culture (Table I and Fig. 1). Two fungal growths on the different sawdust media were variable, depending on the different sawdusts (Table I); The fungus of *L. edodes*

Table I. Carbon dioxide productivities of edible mushrooms grown on the different sawdust media.

Edible fungi[b]	14 days' incubations (μl/min)[a]	
	Oak sawdust	Poplar sawdust
Lentinus edodes	237.9± 20.2	155.6± 23.2
Pleurotus ostreatus		
Pl-2021	396.4± 34.1	684.2± 20.1
Pl-2029	—	336.7± 31.0

[a]The mixtures of sawdust 50.0 gm, rice bran 12.5 gm and the distilled water 80 m*l* was placed on a 500 m*l* Erlemeyer flask with cotton plug and inocubated at 25℃ for 14 days.
[b]*L. edodes* (L-23) was obtained from the Forestry Research Institute in Forestry Administrations (Seoul) and the isolate of *P. ostreatus* from Laboratory of Applied Mycoloy at Agricultural Science Institute, R. D. A. (Suweon 440-707).

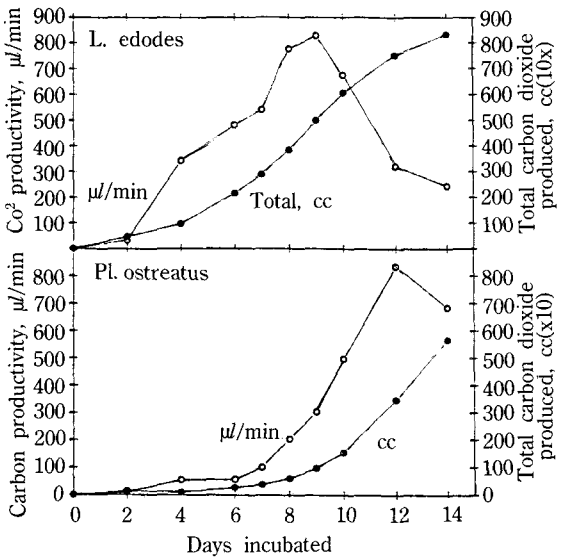

Fig. 1. The productivities and total productions of carbon dioxide on different wood-sawdust media in solid state culture for fourteen days' incubations.

showed better growth on the oak sawdust than on the popular sawdust medium, whereas the fungus of *P. ostreatus* better growth on popular sawdust than on the oak sawdust. The fungus of *L. edodes* produced the carbon dioxide with a gradual increase and showed a high peak of productivities of carbon dioxide at the nine days' incubation (Fig. 1). After then, it slowly produced the carbon dioxide with a gradual decrease until twelve days. The fungus of *P. ostreatus* slowly produced the carbon dioxide until six days' incubation, and abruptly produced the carbon dioxide until twelve days' incubation. At the twelve days' cultivation, it produced highest amounts of carbon dioxide and then produced carbon dioxide with a decrease. As based on these experiments, the eight days' productivities of carbon dioxide were selected for a typical value representing the growth rate of the fungus, *L. edodes*. In other words, after these figures, the values shown in all tables (Table II to V and Fig. 2) were based on this eight-day incubation. The fungus of *L. edodes* was also selec-

Table II. Carbon dioxide productivities of *Lentinus edodes* on the sawdust medium according to the different nitrogen sources.

Nitrogen sources[a]	CO_2 Productivities (μl/min)[b]
Rice bran	772.3
Only sawdust	197.1
Ammonium sulfate	298.9
Peptone	346.4
Yeast extracts	366.9

[a]*Lentinus edodes* was cultivated by the solid state culture. The mixture of oat sawdust (50 g) and rice bran (12.5 g) hydrated with the 80 m*l* of distilled water per a 500 m*l* Erlemeyer flask was autoclaved at 126℃ for 20 min. Ammonium sulfate (80 mg), peptine (1 gm) and yeast extracts (1 gm) were replaced with the rice bran per flask.
[b]LSD (df=15) at 95% confidence level was 83.2 μl/min of carbon dioxide productivities.

Table III. Carbon dioxide productivities of *Lentinus edodes* on the sawdust media according to addition of glucose.

Nitrogen sources[a]	Glucose added (μl/ml)[b]	
	Without	With
Ammoniun sulfate	298.9	277.9
Peptone	346.4	318.9
Yeast extract	366.7	438.3

[a]Nitrogen sources were added as previously described in Table II. LSD (df=15) at 95% confidencd level was 83.2 μl/min.
[b]The glucose (2 gm per flask) was added and mixed.

Table IV. Carbon dioxide productivies of *Lentinus edodes* on the sawdust media according to addition of starch.

Additives[a]	Water-soluble starch added (μl/min)[b]	
	Without	With
Rice bran	833.4	–
Mineral[c]	266.6	308.1
Mineral+N	369.3	710.4
Mineral+N+Y	404.5	819.5

[a]Additives described in Table II.
[b]Water-soluble starch (10 gm per flask) added. LSD (df=15) at 95% confidence level was 99.7 μl/min.
[c]Basic mineral solution added (see Materials and Methods). N and Y was represented as ammonium sulfate (80 mg) and yeast extracts (1 gm) added on oak sawdust per flask, respectively.

Table V. Carbon dioxide productivities of *Lentinus edodes* on oak sawdust media according to the mineral solution.

Nitrogen source[a]	Mineral added (μl/min)[b]	
	With	Without
Rice bran	924.2	934.3
Ammonium sulfate	717.0	557.5
Yeast extract	700.9	778.4

[a]Ammonium sulfate and yeast extract were replaced with rice bren as previously described at Table II.
[b]Minral solution was added and mixed (see Materials and Methods in detail). LSD (df=15) at 95% confidence level was 110.7 μl/min.

ted for further experiments.

Physiological tests

In the previous experiments, the mineral nitrogen source of NO_3 was conducted on a sawdust medium for the productivities of carbon dioxide, but not shown here. Three nitrogen sources indicated in Table III were replaced with the rice bran, which was commonly employed as an ingredient in the conventional sawdust medium. The productivities of carbon dioxide by fungus of *L. edodes* were different, depending on various additives of nitrogen source; The productivities of carbon dioxode on the sawdust medium added with peptone were measured to be similar to those with mineral nitrogen source, ammonium sulfate,

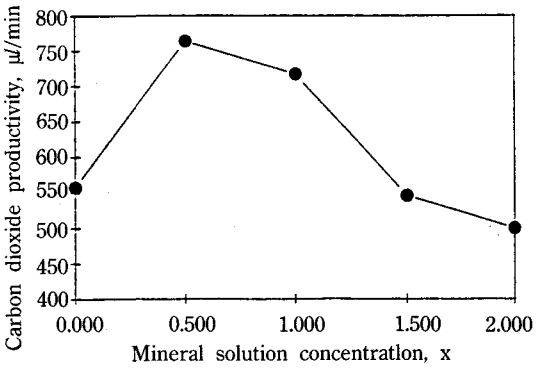

Fig. 2. The productivities of carbon dioxide on starch-sawdust medim with different concentrations of mineral solutions defined.

and also similar to those with an ingredient, yeast extracts. But, the productivities of carbon dioxide on the medium added with the rice bran were measured to be higher than those on the medium added with the other additives. The productivities of carbon dioxide on the medium added with a nitrogen source (peptone, yeast extracts, or mineral nitrogen source) were different from those on the medium without it.

In other experiments with the above results, the glucose, 2 gm per flask, was added to the sawdust medium. The addition of glucose did not affect any productivities of carbon dioxide shown in Table III. The additives of yeast extracts increased more productivities of carbon dioxide, disregarding of addition, than the other additives as a nitrogen source, but were similar at 95% confidence level under the statistic analysis. The water-soluble starch, 10 gm per flask, was replaced with the glucose, and their results were shown in Table IV. In the effects of starch on the sawdust medium, the productivities of carbon by the fungus of *L. edodes* were variable: The productivities of carbon dioxide on sawdust medium added with starch were similar to those without starch. The productivities of carbon dioxide on the sawdust media added with both starch and a nitrogen source quite increased rather than those with either of them. Effects of starch on the productivities of carbon dioxide by the fungus *L. edodes* were twice. In conclusion, the effects of starch, 10 gm per flask on sawdust medium previously

defined were not shown as based on the measurement of productivivties of carbon dioxide, but with additions of a nitrogen source.

Mineral nutritions

The additions of starch and a nitrogen source increased the productivities of carbon dioxide on the sawdust medium defined above. However, the effects of minerals, which would be utilized by the fungus of *L. edodes*, were not investigated. So, the formulae of "Minerals" solution added to the sawdust medium and previously defined, were applied to the sawdust medium (Table IV and Fig. 2). These experiments shown on Table IV and Fig. 2 were based on the sawdust medium defined above (the additions of ammonium sulfate or yeast extracts). The productivities of carbon dioxide on sawdust medium added with minerals including the rice bran or yeast extract, were less than those not included, but were similar to those without minerals at 95% confidence level. However, the productivities of carbon dioxide on sawdust-starch ammonium sulfate-medium added with minerals greatly increased more than those without minerals. As based on the results shown in Table IV, the following experiments shown in Fig. 2 were conducted. The productivities of carbon dioxide on sawdust-starch-ammounium sulfate-medium were high with 0.5 times addition of mimerals among the treatments of various additions of minerals. These works indicated that the sawdust medium, when added with the rice bran, did not need any minerals, but that the sawdust medium, when added with the pure starch, needed the mineral in cultivations of *L. edodes*.

Discussions

Solid state cultivations

The productivities of carbon dioxide shown in Table I were similar to those done by Lindenfelser (1978), as compared with *P. ostreatus* cutivations. His observations for the productivities of carbon dioxide on the wheat straw were conducted until 48 days' cultivations. Also, he measured the periodic peaks of productivities of carbon dioxide on *P. ostreatus* cultivations. Our measurements for the two fungi were only a fourteen days' cultivation. Serkmann (1971) and Ander (1979) indicated that wood was composed of lignin monomers of guaiacyl, syringyl, and hydroxyl phenol groups at the different ratio. In other words, the chemical nature of wood was various, depending upon the plant species. Also, the white rot fungi prodced the different intermediate of lignin compounds when cultivated on a sawdust or wood medium (Serkman, 1971). The different kinds of sawdust, such as an oak and a popular sawdust were considered to be a different lignocellulosic comtlex.

In the soild state culture, the fungal mycelia penetrated the wood in the spaces of lignoellulosic complex and were intermixed with the fibers of wood. Mycelia, as a fungal mass, were not separated from the lignocellulosic complex of wood. Thus, the fungal biomass were difficult to be measured in a sawdust medium. Several indicators representing the fungal growth were disscussed in the previous (Crawford and Sutherland, 1979; Hackett *et al.*, 1978; Marinucci and Bartha, 1979). Thus, the productivities of carbon dioxide were employed as an indicator of a fungal growth in this experiment. A cumulative productions of carbon dioxide (total, cc shown in Fig. 1) wer similar to the general pattern of growth curves in the biological systems; lag, exponential, stationary and death phases. The following equations, (1) and (2), were drived if assumed that the productivities of carbon dioxide is in proportion to the fungal mass

$$G = a\ D \qquad \text{(Equation-1)}$$

$$p = dG/dt = a(dD/dt = \text{growth rate}) \qquad \text{(Equation-2)}$$

here, G = cumulative production of carbon dioxide, cc.
D = dry weight of fungal mycelig, mg or g.
a = constant
p = productivities of carbon dioxiden, μl/min.

indicating the growth rate. The G (cc) and p (or dG/dt, μl/min) were represented in the cumulative productions of carbon dioxide and productivities of carbon dioxide, respectively. In the exeperime-

nts of *P. ostreatus,* the physiological parameters obtained in the solid state culture were compared with those obtained in liquid culture (Hashimoto, 1974). The results indicated the different values of physiological parameters obtained in the two different fungal cultures. The prductivities of carbon dioxide in the solid culture, although it was an indirect value for the fungal biomass, were an important paramater for the fungus of *L. edodes* under the same environments.

Physiological tests

Two kinds of mineral nitrogen source, NO_3 and $(NH_4)_2SO_4$, were applied to the fungus of *L. edodes.* On fungal cultivation, ammonium sulfate was compared with the organic nitrogen sources, peptone and yeast extracts, when these compounds were added to the sawdust medium. Additions of a mineral mineral nitrogen source, $(NH_4)_2SO_4$, stimulate the productivities of carbon dioxide on the sawdust medium with other organic nitrogen source. The fungus of *L. edodes* was considered to utilize the nitrogen sources at the growth in sawdust medium. In other words, the nitrogen source was considered to be needed in only sawdust containing medium for the fungal growth. Therefore, the fungal growth of *L. edodes* was considerably limited without any additions of starch when the productivities of carbon dioxide shown in Table II were compared with those shown in Table IV. The utilization of nitrogen sources indicated to be a synergetic effects with additions of starch, but not with additions of glucose. It was speculated that a nitrogen source is needed and causes a synergetic effect with the sterch for the edible fungal cultivations.

The minerals mentioned above was not required in the conventional spawn medium containing the rice bran, but in the semi-synthetic medium previously discussed. The additions of minerals was toxic on the conventional sawdust media, but stimulated the fungal growth on semi-synthetic sawdust medium containing the oak sawdust, starch, ammonium sultfate. The oraganic compounds, the rice bran and yeast extracts, were considered to contain minerals including the unidentified growth factors, and also to contain the minerals enough to fungal growth. The analysis table of rice bran or yeast extracts indicated enough amounts of minerals for fungal growth without any additions of minerals in the food or feedstuff analysis or Difco manuals. If correct in my experiment, the natural oak wood did not contain the minerals for the edible fungus enough to grow on. Based on this experiment, several ingredients mentioned above should be needed for a spawn sawdust medium if the rice bran was not employed.

Role of rice bran in spawn medium

The amounts of sawdust to be ulitized by the fungus of *L. edodes* were first speculated in the sawdust medium before. It was also questionable if the fungus utilizes the lignocellulosic compounds in ths sawdust medium for fourteen dyas' cultuvations. Lindenfelser (1975) showed that the fungus produces several periodic peaks of productivities of carbon dioxide on wheat straw medium. Additions of starch were reported to inhibit the decompositions of lignin in the pollution experiments (Hackett *et al.*; Lunquist *et al.*, 1977; Reid, 1985). Based on these previous studies, the additional productivities of carbon dioxide on the sawdust media (341.1 or 415.0 μl/min difference between the starch addition and no addition to the sawdust medium) was considered to be degraded from the starch, but not from the lignocellulosic compounds of wood. Based on this result, the rice bran was speculated to play a role a role in nitrogen source, minerals, starch and so on. The rice bran, as a source of strach, was speculated to stimulate the fungal growth and to prevent the contamination from other microorganisms, at least, under the lag phase or the initial stage. The rice bran, as a nitrogen source or minerals was also speculated to contribute to fungal growth as the essential nutrient elements on the sawdust media.

摘 要

톱밥배지에서 생성되는 탄산까스를 정량하여 생리실험을 하였다. 14일 배양 기간에서, 탄산까스생산량을 균의 성장을 표시하는 균의 무게성장으로 간주하였으며, 이는 톱밥배지에서 균의 생리 para-

meter 측정에 응용하였다. 일반적으로 종균 배양에 흔히 사용되는 미강의 역활로서, 미강은 전분, 질소 영양분, 및 금속영양분으로 사용되고 있다. 특히, 생물학적으로 미강은 종균배양에서 빠른 속도의 성장으로 다른 미생물의 오염을 방지하는 역할을 하는 것으로 사려된다.

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