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# Effect of Rice Bran on the Production of Different King Oyster Mushroom Strains During Bottle Cultivation[1]

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**Abstract:** The effect of rice bran supplementation on the production of two king oyster mushroom strains was investigated in order to recommend the optimum and economic amount of rice bran for growing the mushroom to Pleurotus mushroom growers in Taiwan. The average yield, biological efficiency (B.E.) and production efficiency (P.E.) of the strain ATCC 36047 increased significantly with the supplementation of rice bran to substrate from 0 to 47.95% by weight of dry matter. The B.E. and P.E. of the strain Holland 150 reached the highest level when the rice bran was added up to 38.08%, but decreased significantly when the supplementation was up to 47.95%. These results show that different king oyster mushroom strains response differently to the supplementation amount of rice bran and might indicate that the strain ATCC 36047 necessitate a great supply of some ingredients in rice bran to increase its B.E. and P.E. In consideration of the capacity of cropping room, productivity of cultivation spawn and mushroom production cost the most optimum and economic amout of rice bran in substrate would be about 48% and 38% for the strains ATCC 36047 and Holland 150 respectively..

**Key words**: Rice bran, Production, King oyster mushroom, Bottle cultivation

## INTRODUCTION

In the recent years the king oyster mushroom, *Pleurotus eryngii* (DC.:Fr.) Quél., has been commercially grown in Taiwan and Japan, and on a small scale in U. S. A. due to its excellent texture of both pileus and stipe[2, 4, 8, 9]. In the former two countries bottle cultivation technology is the most popularly adopted for growing this new Pleurotus species owing to its suitability for a high degree of automatic operations during cultivation. Various basic raw materials or nutritive supplements have been tried to cultivate or to increase the productivity of this mushroom[3, 5, 6, 7, 9, 10]. However, rice bran is the most popular and available organic additive in the substrates for growing a number of edible mushrooms in Taiwan. In order to know the most optimum and economic amount of rice bran in the substrate for the cultivation of the king oyster mushroom, investigation on

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the effect of rice bran on the production of this mushroom was conducted and described.

## MATERIALS AND METHODS

### Strains of the king oyster mushroom

Strains ATCC 36047 and Holland 150 were used in this investigation. They were maintained on PDA for regular subculturing and stored in liquid nitrogen for a long term storage.

### Preparation of original and mother spawn

Sawdust from *Trema orientalis* (Linn.) Bume was mixed with 40% of rice bran by weight and the water content of the mixture was adjusted at ca. 63% and its acidity was adjusted at pH 6.5 with calcium carbonate. The mixture was then filled into 200ml flask and sterilized at 121 ℃ for 30 minutes. After cooling down to room temperature the sterilized substrate was inoculated with the mycelium on agar slant of each king oyster mushroom strain and incubated at 25 ℃ for about 25days. When the mycelium has grown throughout the sawdust substrate in flask, such sawdust culture is called original spawn which can be used to inoculate mother spawn. The mother spawn has the same ingredients of raw material as orignial spawn, however the substrate was filled in 1100 ml polypropane plastic bottle and sterilized at 121 ℃ for 45 minutes.

### Preparation of cultivation spawn for fruiting

Sawdust from *Trema orientalis* was used as basic raw material to which different amount of rice bran was added from 0 to 47.95% by weight of dry matter. The formulas of the substrates and the water contents of the substrates at inoculation and before cropping were shown in Table 1. Substrates were filled into autoclavable polypropane plastic bottles (1100ml) with an automatic filling machine and sterilized at 121 ℃ for 45 minutes. After cooling down to room temperature the sterilized substrates were inoculated with the mother spawns of the strains ATCC 36047 and Holland 150 respectively. The inoculated substrates in bottles were incubated at 18.5 to 20 ℃ for 46 days.

### Cropping

After incubation the top layer (ca. 1.5cm in depth) of substrate in each bottle was completely removed by

**Table 1.** The formulas for studying the effects of the supplementation of rice bran on the production of *Pl. eryngii* using bottle cultivation technology[z].

| Substrate.No. | Sawdust | | Rice bran | | Water content at inoculation (%) | Water content before cropping | |
|---|---|---|---|---|---|---|---|
| | FW (Kg) | DW (%) | FW (Kg) | DW (%) | | Holland 150 | ATCC 36047 |
| 1 | 255 | 100.00 | 0.0 | 0.00 | 73.68 | 74.52 | 73.92 |
| 2 | 255 | 90.70 | 7.6 | 9.30 | 72.76 | 74.04 | 73.64 |
| 3 | 255 | 81.26 | 17.1 | 18.74 | 70.40 | 71.76 | 71.68 |
| 4 | 255 | 71.68 | 29.3 | 28.32 | 69.20 | 70.20 | 70.52 |
| 5 | 255 | 61.92 | 45.6 | 38.08 | 68.00 | 70.08 | 69.24 |
| 6 | 255 | 52.05 | 68.3 | 47.95 | 68.12 | 69.36 | 69.80 |

[z] Sawdusts were derived from *Trema orientalis* and had been heaped for 4 months before use and its average water content was 75.70 % ◦ The average water content of rice bran was 12.30 % ◦ .Each substrate was added with 2.0kg of calcium carbonate. No.5and 6 substrates were added with 31.5 and 75.0Kg of tap water respectively..All components of each substrate were uniformly mixed mechanically. The substrates were filled into 1100 ml autoclavable bottles with an automatic filling machine. FW=Fresh weight, DW=Dry weight.

hand with a special metal spoon (bottle neck treatment, BNT). After bottle neck treatment bottles were capped again before moving to a cropping room. These bottles were uncapped after the appearance of pinheads or small mushrooms. The cropping room was controlled at 15.5 to 17.5 ℃ in temperature, at 85 to 95% of RH and at ca. 250 lux of light intensity. Carbon dioxide concentration was not monitored and controlled instrumentally, but fresh air was introduced intermittently into to the room with heat exchanger.

### Harvesting

Mushrooms in each bottle were harvested when the mushroom cap surfaces were flat to slightly up-rolled at the cap margins. One flush of mushrooms in each bottle was harvested. The harvested mushrooms were then weighed for calculating biological and production efficiencies.

### Determination of biological and production efficiencies

In order to calculate the biological efficiency (B.E.) and production efficiency (P.E.) the water contents of all the substrates at inoculation and at the end of incubation (before cropping) were measured. Biological and production efficiencies were calculated as following:

**Biological efficiency**(%)= (the weight of fresh mushrooms harvested ÷ substrate dry matter content before inoculation) X 100

**Production efficiency**(%) = (the weight of fresh mushrooms harvested ÷ substrate dry matter content before cropping) X 100

### Experimental design

The experiment was a completely randomized design with 6 replicates per formula of substrate. Each replicate consisted of 12 bottles of cultivation spawn.

## RESULTS AND DISCUSSION

The average yield, B.E. and P.E. of the strain ATCC 36047 increased significantly with the increase of rice bran in substrate from 0 to 9.30, 18.34, 28.32, 38.08 and 47.95% (Table 2). The yield of the strain Holland 150 reached the highest level when the rice bran was added up to 47.95%. This yield was not significantly different from that of the same strain when the supplementation was up to 38.08%. In addition the B.E. and P.E. of the same strain also reached the highest level when the rice bran was added up to 38.08%, but decreased significantly when the supplementation was up to 47.95% (Table 2). Such reduction in productivity may be due to the compactness or poor airation of the substrates, which then results in the inefficient utilization of the nutrients in the substrates. This can also be ascribed to the limitation of spaces or surfaces for the development of fruiting bodies. The above results show that different strains of King oyster mushroom strains response differently to the supplementation amount of rice bran in the aspects of average yield, B.E. and P.E. as reported by Visscher on other Pleurotus species[11]. The yield, B.E. and P.E of the strain ATCC 36047 were apparently lower than those of the strain Holland 150 when grown with the same formula of substrate except the one with the highest level of supplementation of rice bran. This might indicate that the strain ATCC 36047 necessitate a greater supply of some ingredients in rice bran to increase its biological and production efficiencies or its efficiency to utilize the nutrients of substrates is lower than that of the strain Holland 150. What compositions of the rice bran are most important to the productivity of the king oyster mushroom needs to be further investigated.

According to the above results the optimum amount of supplementation of rice bran in substrate for the strain ATCC 36047 would be apparantly about 48% by the weight of dry matter. In consideration the capacity

**Table 2.** Effect of supplementation of rice bran on the production of the two strains of *Pleurotus eryngii*, Holland 150 and ATCC 36047[z].

| Rice bran supplemented (%) | Yield(g/12bottles) | | Biological efficiency(%) | | Production efficiency(%) | |
|---|---|---|---|---|---|---|
| | Holland 150 | ATCC 36047 | Holland 150 | ATCC 36047 | Holland 150 | ATCC 36047 |
| 0.00 | 173.00 e | 67.67 f | 10.77 e | 4.25 f | 11.74 e | 4.50 f |
| 9.30 | 747.33 d | 471.83 e | 42.23 d | 26.54 e | 46.80 d | 28.94 e |
| 18.34 | 1444.00 c | 1022.00 d | 72.52 c | 50.85 d | 80.66 c | 55.46 d |
| 28.32 | 1790.17 b | 1350.50 c | 85.32 ab | 62.28 c | 92.49 ab | 69.24 c |
| 38.08 | 2117.33 a | 1809.33 b | 86.74 a | 73.97 b | 97.05 a | 79.86 b |
| 47.95 | 2198.00 a | 2207.00 a | 81.15 b | 82.68 a | 87.91 b | 90.00 a |

[z] The formulas of all substrates and the water contents of these substrates at inoculation and before fruiting were shown in Table 1. All plastic bottles with substrate were weighed and recorded both after inoculation and before cropping. All inoculated substrates were incubated at 18.5 ~ 20.5 ℃ for 46 days. Before cropping the top layer(ca. one cm deep) of the substrate in each bottle was removed completely by a special metal spoon. This treatment is called "bottle neck treatment(BNT)". The cropping room was controlled at 15.5 ~ 17.5 ℃ in temperature, 85 ~ 95 % in RH and ca 250 lux in light intensity from reducing temperature for fruiting to the end of crop. Fresh air was introduced with heat exchanger. Each formula of substrate had 6 replicates which were arranged by random, each replicate consisted of 12 bottles of cultivation spawn(1100 ml/bottle). Calculation of biological and production efficiencies are shown in text.
Means in the same column not followed by the same letter or letters are significantly different at the 5% probability level (Duncan's multiple range test).

of cropping room, productivity of cultivation spawn and production cost the most optimum and economic amount of supplementation of rice bran in substrate would be about 38% for the strain Holland 150 although both B.E. and P.E. of the strain were not significantly different between the supplementation of rice bran up to 28.32% and that up to 38.08%. The appearances of the fruiting bodies produced by the strain Holland 150 grown on the susbrates of all the six formulas were excellent with a long smooth stipe and a small cap being suitable for packing (Fig.2a-f). The fruiting bodies produced by the strain ATCC 36047 on the substrates with the supplementation of rice bran from 9.30 to 38.08% were also good for packing (Fig.1b-e), while those produced by the same strain on the substrate with 47.95% of rice bran were poor in quality with a large cap and an irregular stipe (Fig.1f). Large caps are easily to be broken during packing resulting in low quaulity of the product. This might be improved by reducing the content of rice bran in substrate to reduce the number of fruiting bodies in each bottle or by reducing light intensity of the cropping room to reduce the size of cap since light can promote the development of the caps of the king oyster mushroom[1, 12]. This might also be improved by properly increasing the concentration of carbon dioxide in the cropping room because high concentration of the gas promote the elongation of the stipe of the mushroom species[1, 12].

Since the yield, B.E. and P.E. of the two king oyster mushroom strains were very low when grown without rice bran it implies that nutrients for the development of the fruiting bodies of the mushroom species mainly come from the rice bran, but not from the sawdust. Because the yield, B.E and P.E.of the strain Holland 150 were apparently higher than those of the strain ATCC 36047 when grown without rice bran it indicates that the strain Holland 150 has a greater ability to decompose the components of sawdust. The differences in the production and acitivities of the enzymes of the two king oyster mushroom strains for degradation of sawdust need to be studied in detail. Such information would be helpful to the breeding of the mushroom species.




**Fig.1a-f.** Fruiting bodies of the strain ATCC 36047 grown on the substrates which constisted of 0, 9.30, 18.34, 28.32, 38.08 and 47.95% of rice bran in order.




**Fig.2a-f.** Fruiting bodies of the strain Holland 150 grown on the substrates which constisted of 0, 9.30, 18.34, 28.32, 38.08 and 47.95% of rice bran in order.

Theoretically it is possible to increase the yield, B.E. and P.E. of the king oyster mushroom by lengthening the incubation time of cultivation spawn. However, practically it is necessary to take into account of the efficient application of growing facilities and energy cost of the lengthened incubation as well as the economic value of the increased yield.

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# 米糠對不同杏鮑菇品系瓶栽自動化生產之影響[1]

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## 摘要

爲向本省菇農推薦杏鮑菇瓶栽自動化生產最適與最經濟之米糠用量，遂進行本試驗研究。研究結果顯示，當基質內之米糠量由0增至47.95%時，杏鮑菇ATCC 36047品系之產量、生物與產菇效率均隨米糠量增加而顯著提高；至於Holland 150品系之生物與產菇效率則當米糠填加至38.08%時，達到最高，若米糠量增至47.95%時，則反而顯著下降。此結果顯示不同杏鮑菇品系對米糠填加量之反應不同，其中ATCC 36047品系較Holland 150品系需要更多米糠之某種成份以發揮其最大之生物與產菇效率。考量杏鮑菇栽培庫之容量、每瓶栽培用菌種之生產力及杏鮑菇之生產成本，杏鮑菇瓶栽自動化生產時最適及最經濟之基質米糠建議使用量，對ATCC 36047及Holland 150兩品系分別爲約基質乾重之48%與38.08%。

**關鍵詞**：米糠、杏鮑菇、瓶栽自動化、生產。

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