STUDIES ON THE GROWTH OF *PLEUROTUS OSTREATUS*

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INTRODUCTION

Pleurotus ostreatus is one of the white rot fungi which attack both cellulose and lignin components of wood. This fungus grows on dead tree trunks and branches, and very rarely on living trees. This fungus has good flavor and is commonly eaten in Japan. BLOCK *et al.* (1958, 1959) demonstrated that this fungus can be readily grown on sawdust and other agricultural waste products. The purpose of the present study was to obtain accurate information on its nutritional requirements, with an idea that it might be grown on such materials as rice straw and newspaper, a cheap abundant waste.

MATERIALS AND METHODS

On liquid culture experiments

Pleurotus ostreatus was cultured in 100 m*l* Erlenmyer flasks with 20 m*l* of a medium which contained (per liter of distilled water); D-glucose, 20 g; peptone, 2 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2$, 0.1 g; thiamine hydrochloride, 100 μg. The ingredients were autoclaved together.

Inoculum was consisted of 1 m*l* of a blended macerate from fully grown culture and incubated at 27°C under stationary conditions for 10 days. The mycelial weights were obtained by filtering through a fine sieve, washing and drying it in an oven at 100°C for 15 hr before weighed, and also fruit-bodies were removed from mycelia, dried and weighed.

On solid culture experiments

The rice straw was chopped into 3–5 cm pieces and soaked in the 1% solution of sodium carbonate at 100°C for 30 min. The straw, washed with water, was supplemented with rice bran and kept up in 70% moisture. These prepared materials were put, 500 g in glass jars or 10 kg in trays (60×40×10 cm) which were covered with polyester sheets to prevent contamination and to retain moisture. The newspaper was cut into pieces and sufficient water was added to bring the moisture to approximately 70%.

The inoculating material was produced in the mixture of 100 g of pine sawdust, 20 g of rice bran, and 250 m*l* of water in 500 m*l* flasks.

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The mycelia developed in the substrate served as inoculum. The fresh material was impregnated with 0.5% of above inoculum.

The inoculated material was kept in a room temperature at 25°C.

After about 4 weeks the first fruit-bodies appeared on the surface of culture, and after some more days they were large enough for harvesting.

The bottom portion of the stems was cut off, and the weight of the fruit-bodies was recorded.

RESULTS

In the experiments of carbon utilization, the mycelia of *P. ostreatus* grew fast with mannose as a sole carbon source, followed by starch and glucose. Maltose, sucrose, fructose, mannitol and ethylalcohol were intermediate in their effect on the growth of the fungus, while pentose like xylose and arabinose were not so effective (Table 1).

TABLE 1. Effect of carbon sources on vegetative growth

Carbon source (2%)	Dry wt. of mycelium (mg/20 m*l*)
Mannose	181.5
Starch	175.8
Glucose	167.2
Maltose	144.0
Sucrose	119.0
Fructose	109.7
Mannitol	106.3
Ethanol	105.5
Galactose	97.3
Xylose	52.5
Arabinose	35.5
Citrate	39.4
Malate	47.0
Fumarate	46.8
Succinate	45.2
Oxalate	42.7
No carbon	9.5

Medium; Basal medium in which glucose is replaced with carbohydrates indicated.
Incubation; At 27°C for 10 days.

Also mycelia failed to grow on certain organic acids, but they stimulated mycelial growth in combination with glucose (Table 2).

The maximal growth was obtained with 0.1% of citric acid in basal medium (Table 3).

The fruit-bodies were formed on liquid culture when the culture was transferred in the light at 15±5°C after incubated in the dark at 27°C for 10 days. Fruiting was most profuse on fructose, sucrose, starch, and cellulose

TABLE 2. Growth of *P. ostreatus* in basal medium added with organic acids

Organic acid (0.1%)	Dry wt. of mycelium (mg/20 ml)
Citrate	186.5
Malate	180.2
Fumarate	176.1
Succinate	154.5
Oxalate	143.1
Tartrate	129.6
None	132.4

TABLE 3. Effect of sodium citrate concentration on vegetative growth

Citrate (%)	Dry wt. of mycelium (mg/20 ml)
1	164.4
0.5	169.3
0.25	178.2
0.1	183.5
0.05	148.8
0.01	131.4
0	132.4

Medium: Basal medium in which sodium citrate concentration is varied.

TABLE 4. Effect of carbon sources on fruiting

Carbon source (1%)	Mycelium (mg/20 ml)	Fruit-body (mg/20 ml)	Total wt. (mg/20 ml)	Final pH
Glucose	65.7	18.3	84.0	5.6
Fructose	50.1	31.2	81.3	5.2
Mannose	78.6	24.5	103.1	5.3
Sucrose	41.2	36.1	77.3	5.4
Maltose	60.3	19.3	79.6	5.0
Starch	77.2	28.6	105.8	5.8
Cellulose	—	27.0	—	5.2

Medium: Basal medium in which glucose is replaced with 1% carbohydrates indicated.

media, and less so on glucose and maltose media (Table 4).

To investigate good sources of nitrogen for *P. ostreatus*, the peptone in the culture solution was replaced by its nitrogen equivalent of other sources.

Organic sources of nitrogen supported good growth as did certain amino acids. Peptone, casamino acid, asparagine, serine, leucine, and alanine were favorable sources. Ammonium tartrate promoted less gorwth, followed by ammonium salts and nitrates. It must be considered that the organic nitrogen sources served carbon nutrition as well as nitrogen. Ammonium sulfate, like the other ammonium salts of inorganic acids, was not suitable as nitrogen sources due to low pH (Table 5).

Thiamine was required for growth, and its suitable concentration was approximately 100 $\mu g/l$. Addition of other vitamins did not influence growth (Table 6).

No growth occurred after 10 days at initial pH of 3.0. Although mycelial growth occurred in pH range of 4.0 to 8.0, fruiting was obtained with initial pH between 5.0 and 7.0, the most suitable pH value being about 5.5 (Tables 4, 7).

TABLE 5. Effect of nitrogen sources on vegetative growth

Nitrogen source (0.2 gN/l)	Mycelium (mg/20 ml)	Final pH	Amino acid (0.2 gN/l)	Mycelium (mg/20 ml)	Final pH
Peptone	157.4	5.6	Asparagine	135.9	5.4
Casamino acid	145.6	5.4	Serine	130.2	5.3
Ammonium tartrate	84.5	4.0	Leucine	129.2	4.9
Ammonium sulfate	47.3	3.4	Alanine	107.8	5.1
Ammonium chloride	45.4	3.4	Arginine	98.0	5.2
Ammonium nitrate	43.7	3.2	Glycine	95.2	5.2
Potassium nitrate	21.5	5.8	Phenylalanine	62.6	5.5
Calcium nitrate	12.2	5.6	Methionine	40.7	6.2
No nitrogen	10.3	6.0	Proline	17.4	8.8

Medium: Basal medium in which peptone is replaced with substances indicated.

TABLE 6. Effect of thiamine concentration and some vitamins on vegetative growth

Thiamine (μg/l)	Vitamin (μg/l)		Mycelium (mg/20 ml)
1,000	—	—	127.3
〃	Biotin	10	134.9
〃	Niacin	30	136.4
〃	Pyridoxine	50	114.0
〃	Pantothenic acid	50	123.5
〃	Riboflavin	50	136.1
500	—	—	121.1
100	—	—	134.1
10	—	—	109.3
1	—	—	88.4
0.1	—	—	93.8
0	—	—	50.4

TABLE 7. Effect of pH on vegetative growth

Initial pH	Final pH	Dry wt. of mycelium (mg/20 ml)
3.4	3.3	7.4
4.2	3.7	32.5
5.0	4.3	76.7
5.5	4.7	98.3
6.2	5.0	127.5
6.5	5.1	152.4
7.0	5.3	145.2
7.5	6.0	98.6

TABLE 8. Yield on rice straw and other wastes supplemented with rice bran

	Rice straw[a]	News-paper[a]	Rice hull[a]	Pine sawdust[a]
Spawning to first yield (day)	37.1	37.0	38.6	56.8
Wt. of first yield (g)	60.0	61.2	58.6	48.2
First to second yield (day)	11.7	9.0	14.7	13.0
Wt. of second yield (g)	32.8	28.6	26.7	20.0
Second to third yield (day)	17.2	25.7	—	—
Wt. of third yield (g)	21.8	23.3	—	—
Total yield[b] (g)	118.6	113.1	85.3	68.2
Yield %	23.7	22.6	17.0	13.6

a) Each experimental jar was filled with 500 g media.
b) Fresh wt. of mushroom based on moistened media.

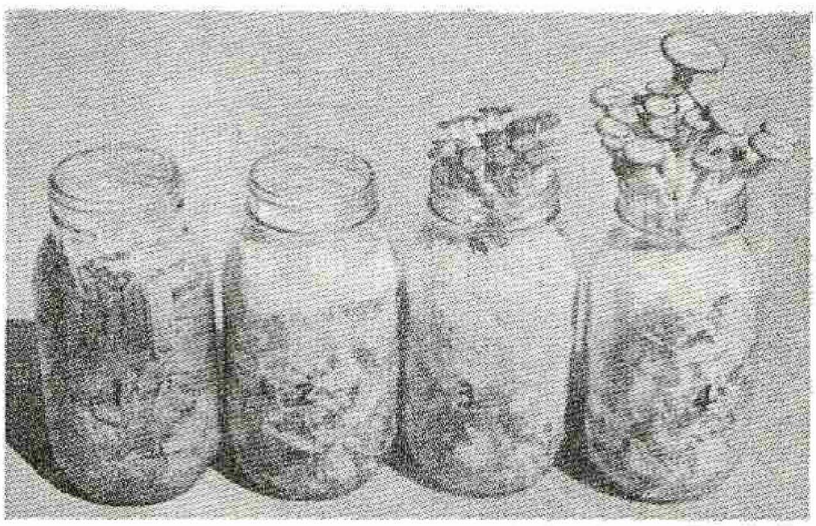

FIG. 1. Fruit-body development on glass jar using newspaper with rice bran. 1. At spawning. 2. After 25 days from spawning. 3, 4. Development of mushrooms.

When the mycelium of *P. ostreatus* spread through rice straw or other substrates supplemented with rice bran, the polyester sheets were removed and a spray of water was applied as necessary to keep the material moist but not wet. Usually, 5 to 7 days after removing the cover, the fruit-bodies began to appear as tiny coralline heads on the surface of the media and first crops were picked 3 to 5 days after the appearance of tiny heads.

The pine sawdust supplemented with rice bran gave the yield about 68.2 g whereas rice straw, newspaper, and rice hull gave 118.6 g, 113.1 g, and 85.3 g respectively.

The yields from rice straw and newspaper were equivalent to about 23% of the moistened media in glass jar (Table 8, Fig. 1).

On the larger scale cultivation in trays, there was also successful growth. The mycelia were grown on the tray at 25°C for a period of 20 to 25 days and good yield was obtained with an average 20% of the moistened media.

Rice straw contained around 0.86% of nitrogen. Its nitrogen contents were low, approximately one-third that of rice bran.

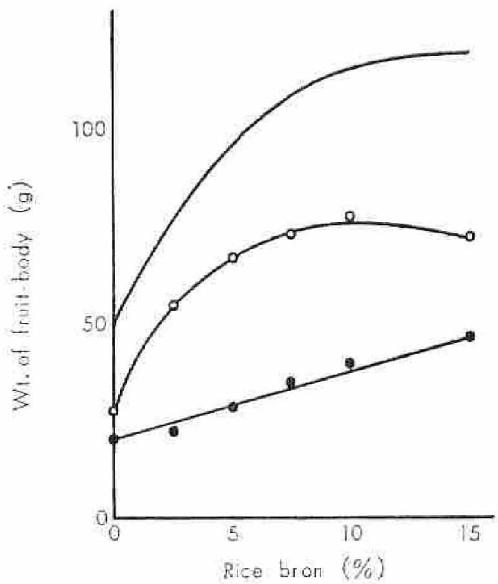

FIG. 2. Effect of rice bran on yield. ○-○ First yield. ●-● Second yield. —— Total yield.

Quantity of rice bran had considerable influence on the yield of fruit-bodies. As shown in Fig. 2, it was seen that the yield increased from 48 to 126 g as the quantity of rice bran was increased from 0 to 10% by weight. Further increasing the quantity of rice bran did not have much effect on the yield of fruit-bodies. Omission of supplement from rice straw media reduced the yield to 48 g.

Similarly, in the case of newspaper, rice hull and pine sawdust, mycelial growth was poor and no fruit-bodies produced when supplement was omitted from the substrate.

Through one series of tests, fruit-bodies was grown successfully on jars in unsterile conditions. The mycelia were grown on chopped rice straw, which was soaked in solution of sodium carbonate, washed with water and moistened up to 70% moisture content.

This material without supplement was impregnated with inoculum.

To ensure maximal growth of the mycelia on rice straw, large lumps of the inoculum were broken up and mixed thoroughly with fresh materials by hand. The inoculum used was about 10% of the materials.

The mycelia grew well under the above conditions but the material often became contaminated with molds to the detriment of the mycelia in the early stages.

During cultivations, however, the contaminants ceased to grow, presumably because of exhaustion of the soluble carbohydrates in the rice straw, and mycelia spread throughout the materials. A large quantity of inoculum assisted in promoting the growth of *P. ostreatus*. The yield on rice straw under unsterile conditions in glass jar gave average 18% of the moistened materials (Table 9).

TABLE 9. Yield in unsterile condition

Replicate[a]	First yield Days after inoculation	g	Second yield Days after first yield	g	Total yield (%)
1	35	75	16	34	109
2	36	65	15	28	93
3	37	48	12	36	84
4	37	52	13	35	87
5	37	51	13	28	79
Av.	36.4	58.2	13.8	32.2	90.4[b]

a) Each experimental jar was filled with 500 g media.
b) Wt. fresh mushroom × 100/Wt. moistened medium=18.0.

DISCUSSION

These results were obtained by growing mycelia of *P. ostreatus* on the monosaccharides mannose, glucose and fructose, disaccharides maltose and sucrose, the polysaccharides starch and cellulose as carbon sources. These saccharides occur naturally in rice straw.

Also mannitol and ethyl alcohol support growth, but xylose and arabinose, present in wood, did not give satisfactory support, even though *P. ostreatus* normally grow on dead or living trees.

Organic acids did not serve as a good carbon source but citrate, malate, fumarate and succinate stimulated mycelial growth in conbination with glucose.

COCHRANE (1958) stated that disaccharides and polysaccharides supported more fruiting than did simple hexoses. Similar results were obtained in the case of *P. ostreatus*. Sucrose, starch and cellulose were good carbon sources for fruiting, and also the effect of fructose was equal to sucrose.

Pleurotus ostreatus utilized organic nitrogen as nitrogen sources but ammonium salts and nitrate were not satisfactory as nitrogen sources. Possibly organic nitrogen sources were also metabolized as carbon sources, but normal growth did not occur when a carbon source was absent.

It has been observed that good yield can be obtained with rice straw and newspaper as substrate under sterile conditions.

Yield from rice straw and newspaper was twice, and also that from rice hull was perceptibly higher than that from pine sawdust supplemented with rice bran. It was found profitable to supply 10% of rice bran, which was not only a source of nitrogen but a source of carbohydrates and thiamine as well, and was most suitable for our purpose. The yield reached a maximum with increasing rice bran to 10% by weight, while further increase did not improve the yield significantly.

When using a large quantities of inoculum, the mycelium was grown successfully on moistened rice straw without supplement in unsterile conditions. Although the yields were low as compared with those in sterile conditions, it seems indicative for the practical production of mushroom.

SUMMARY

The results of attempt of *Peurotus ostreatus* growing on some abundant wastes in Japan is reported. When *P. ostreatus* was grown on chopped rice straw and newspaper as media respectively for fruiting with rice bran as the supplement, the yield and quality of fresh mushroom was satisfactory. The yield was about 23% in sterile conditions and about 18% in unsterile conditions of moistened media. The fungus was also grown in a liquid medium to obtain information on its nutritional requirements.

RÉSUMÉ

On rend compte ici du résultat des tentatives de culture de *Pleurotus ostreatus* sur certains sous produits très abondants au Japan. Des récoltes et une qualité satisfaisantes ont été obtenues sur des milieux à base de paille de riz hachée ou de papier journal, tous deux supplémentés avec du son de riz. Le rendement obtenu était en conditions stériles de 23% du milieu humide et en conditions non stériles de 18%. On a également fait croître le champignon sur milieu liquide pour mieux connaitre ses besoins nutritifs.

ZUSAMMENFASSUNG

Es wird über Versuche berichtet, *Pleurotus ostreatus* auf Abfallstoffen in Japan zu kultivieren. Die Ausbeute und Qualität der Frischpilze, die auf gehäckseltem Reisstroh bzw. Zeitungspapier mit Reiskleie als Aufwertung fruchteten, waren zufriedenstellend. Die Ausbeute betrug 23% unter sterilen und 18% unter insterilen Bedingungen bezogen auf das Feuchtsubstrat. Der

Pilz wurde auch in Flüssigkultur gezogen, um Informationen bezüglich der Nährstoffansprüche zu erhalten.

Bibliography

BLOCK, S. S., G. TSAO, and L. H. HAN. 1958. Production of mushrooms from sawdust. *J. Agr. Food Chem.* 6: 923–927.

——, G. TSAO, and L. H. HAN. 1959. Experiments on the cultivation of *Pleurotus ostreatus*. *Mushroom Science IV*: 309–325.

COCHRANE, V. W. 1958. *Physiology of Fungi* (John Wiley and Sons, New York): 524 pp.

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