韓 國 菌 學 會 誌 第 12 卷 第 1 號
Kor. J. Mycol. Vol. 12, No. 1, 15~19, 1984

Effect of Temperature, pH, Carbon and Nitrogen Nutritions on Mycelial Growth of *Pleurotus sajor-caju* (Fr.) Sing. and *Pleurotus ostreatus* (Fr.) Quél.

Seung Joo Go, Chang Hyun You and Yong Hwan Park
Institute of Agricultural Sciences, O.R.D. Suweon 170, Korea

여름느타리버섯과 느타리버섯의 菌系 生長에 影響을 미치는 몇가지 要因에 관한 試驗

高 昇 柱·柳 昌 鉉·朴 容 煥
農村振興廳 農業技術研究所

Abstract: Some factors affecting mycelial growth of *Pleurotus sajor-caju* and *P. ostreatus.* were investigated. The optimal temperature and pH of the medium for mycelial growth of both the species were 25 to 30°C, and 5 to 6, respectively. The effect of carbon and nitrogen sources on mycelial growth of the species was studied using modified Waksman's special medium as the basal medium. Generally, the disaccharides and polysaccharides showed good effect for mycelial growth of *P. sajor-caju*, and the polysaccharides were superior to the other classes of carbon sources for mycelial growth of *P. ostreatus.* Generally the organic nitrogen compounds showed good mycelial growth of both the species. The mycelial growth of the species were favored by the ammonium nitrogen more than by the nitrate nitrogen source. Asparagine was superior to the other kinds of amino acid. Six percentage of glucose and 0.6% ammonium tartarate was optimum for mycelial growth of *P. sajor-caju* whereas the optimum concentration for the *P. ostreatut* was 4% glucose and 0.4% ammonium tatarate.

Keywords: Basidiomycete, *Pleurotus sajor-caju, P. ostreatus,* Mycelial growth, Optimal temperature, Optimal pH, Carbon sources, Nitrogen sources.

Pleurotus sajor-caju has been well known in South East Asia and India for its unique flavors and testy. Jandaik and Kapoor (1976) reported that the edible mushroom was potentially a valuable source of food and fodder protein. It had about 47.93 percent of protein which contains all essential amino acid.

The mushroom had been introduced for the artificial cultivation by Jandaik in 1974. Since then, several lines of works including improvement of substrates and chemical analysis of the mushroom have been done by some of Indian scholars (Bano and Rajarathnam, 1982; Jandaik and Kapoor, 1974, 1976; Sivarakasam and Kandaswamy, 1981). But physiological study of the mushroom has been received little attention in spite that was very important factors for cultivation of the mushroom.

P. sajor-cajus is favorable to cultivate in the tropical area for its fruiting temperature which is formed 10 to 30°C (Jandaik, 1974). On the other hand, *P. ostreatus* common grey oyster mushroom which is formed fruit body 5 to 15°C has been cultivatedd in the temperate zone (Park, Go, and Kim, 1975; Hash

imoto, 1974; Zadrazil, 1974). This common oyster mushroom has been cultivated during spring and autumn seasons in Korea. But, there wasn't any cultivated oyster mushroom species in summer season in the country due to high temperature more than 20°C up to now. So that *P. sajor-caju* was selected to suitable oyster mushroom species for summer season in Korea by the authors. The objective of this study was to find out some physiological conditions affecting on mycelial growth of *P. sajor-caju* and to campare with those of *P. ostreatus.*

Materials and Methods

Fungal material

Pleurotus sajor-caju used in this experiment was from Dr. Lee(1979). *P. ostreatus* has been maintained at the Institute of Agricultural Sciences as ASI 2018. These cultures were grown on potato sucrose agar medium and were kept in the refrigerator at 5°C.

Effects of Temperature and pH

The temperature experiments were made 5 to 40°C at 5°C intervals. The experiment was replicated four times using electronic coolnics Model CTG 520 Yamato. The mycelial growth were evaluated by linear growth in a petri-dish containing 20ml of potato sucrose agar medium after incubating for 7 days. The inoculum of the cultures were grown on potato sucrose agar medium at 25°C for 7 days and were punched the medium containing the mycelia at 5mm in diameter.

The medium used in the experiment was consisted of 20g sucrose, 4g yeast extract, 3g K_2HPO_4, 0.5g $MgSO_4$ $7H_2O$, 20g agar and 1,000ml distilled water. The pH of each medium was adjusted from 4 to 9 with citrus phosphates buffer solution.

Effecs of Nutrition

The effect of carbon and nitrogen sources on mycelial growth of the species were tested using modified Waksman's special medium (Booth, 1971) as the basal medium with the test substaces. Nine carbon sources tested were listed below 1) Monosaccharide; D-glucose, D-fructose, D-xylose 2) Disaccharides; sucrose, maltose, cellobiose 3) Polysaccharides; starch, a-cellulose, 4) Organic acid; citric acid. One of these carbon sources was replaced with the carbon sources in Waksman's spe cial medium. To evaluate nitrogen sources, the nitrogen source in the basal medium was replaced with one of eight nitrogen sources listed as following; 1) Amino acid; asparatic acid, methionine, asparagine, 2) Inorganic nitrogen; ammonium tartrate sodium nitrate, 3) Organic nitrogen compoands; peptone, yeast extracts, malt extracts. All of the nitrogen sources were adjusted by 0.4g of nitrogen content per liter of the basal medium. The basal medium without a carbon or nitrogen source severed as the control for each of the experiment. The first grade of the reagent was used to make media. The reagents were products of Sigma chemical Co. or Difco Lab.

To make inoculum for the experiments, the mycelia of the species were washed with sterlized water by three times and the mycelia was cut with blender at 7500 RPM for three minutes after submerged culture for 7 days. The suspended mycelia were collected into 100ml of sterlized water in 250 Erenmyer flask. The inocula were inoculated by 1ml of the mycelial suspension into 250 Erenmyer flask containing 50ml of media tested. The mycelia after incubating stationary at 25°C for 14 days were washed using fine sieve with tapwater to remove the residual substrates and gradually dried up to 60°C for 10 hours.To find out the rational composition of the carbon and nitrogen sources in the medium, glucose and ammonium tartarate were used as the carbon and nitrogen sources at different ratios to the basal medium, respectively.

Results and Discussion

Temperature and pH

The mycelia of *Pleurotus sajor-caju* and *P. ostreatus* grew 5 to 35°C, but no growth occurred at 40°C. As shown Fig. 1, the mycelial growth of the species was gradually increased following the temperature increase up to 25 in *P. sajor-caju* or 30°C in *P. ostreatus* and were decreased sharply in both the species over 30°C. The optimal growth of both the species was between 25 to 30°C. However, the growth rates of mycelia of both the species were

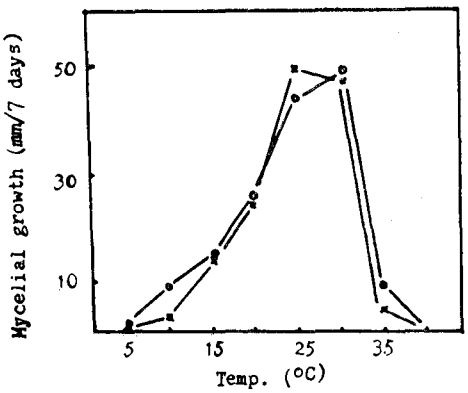

Fig. 1. Influence of incubating temperature on mycelial growth of *P. sajor-caju*(×···×) and *P. ostreatus* (○···○) on PSA medium.

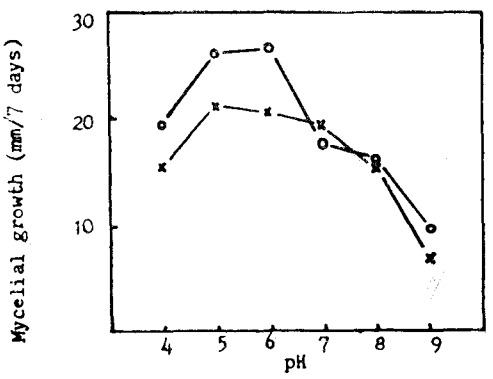

Fig. 2. Influence of pH of the medium on mycelial growth of *P. sajor-caju* (×···×) and *P. ostreatus* (○···○) on the solid medium adjusted the pH by citratic phosphate buffer.

stunted at the temperature below 15°C and over 35°C.

Although their fruiting temperatures were different, their average of mycelial growths in the temperature experiment were similar to each other. In this experiment, the results from *P. ostreatus* were obtained similar to that of Zadrazil (1974), but no authors have attentioned the mycelial growth of *P.sajor-caju.* Zadrazil (1974) reported that *Pleurotus erygii* and *P. cornucopisae* reached their optimal growth at 25°C *P. ostreatus*, and *P. florida* at about 30°C.

The mycelial growth of the *P. sajor-caju* and *P. ostreatus* occurred in all range of pH experiments (see Fig. 2). The optimal growth of both the species was obtained between pH 5 and 6. However, the average of mycelial growth of *P. sajor-caju* was a little lower than that of *P. ostreatus* on the media pH 4 to 6, but it was similar to each other over 7.

The mycelia of the species at lower pH in themedium were grown vigorously but at higher pH were grown poorly. The results obtained from the experiment showed a similar trend to that of Hashimoto (1974) Hong (1978) and Zadrazil (1974) in *P. ostreatus.* They examined the effects of pH on the mycelial growth by submerged culture with pH 3 to 7, not over 7. Their results of the experiment were that the mushroom has one optimal growth curve,whereas the authors have some of experiences that the cultures showed twice optimal growth curve one was below pH 7 and the other one was over 7 when the pH were examined over 7 insubmerged culture. The reasons were not found yet. Therefore, the authors presented the data from only solid culture here.

Carbon and Nitrogen Sources

The mycelia of *P. sajor-caju* and *P. ostreatus* grew on all the media tested carbon sources (Table I). As shown Table 1, generally the disaccharides and polysaccbarides were the best favorite sources as the carbon source for the mycelial growth of *P. sajor-caju*, and the polysaccharides were superior than the others kinds of sources for the *P. ostreatus.* However, citric acid as the organic acid source showed poor results in both the species. The results suggested that the two cultures were a little different for their utilization of the carbon sources. Cellobiose, starch and maltose were the best carbon sources as sole carbon source for *P. sajor-caju*, and a-cellulose, starch were the best one for the *P. ostreasvs.*

Yusef and Allen(1967) reported that *P. ostreatus* utilized dextrin and fumaric acid best. But mannose galactose and maltose supported very little growth. This result was quite different from our results. However, the results from Hashimoto and Takashi (1974) and Hong (1978) were similar to that of our results. The reason of differences bestween the result of Yuself and Allen and those of our results might be due to different strains each other to be used.

Table I. Effect of carbon source on mycelial growth of *P. sajor-caju* and *P. ostreatus.*

Carbon source	Dry weight(mg/14 days)	
	P. sajor-caju	*P. ostreatus*
Monosaccharides		
D-Glucose	81	96
D-Fructose	63	108
D-Xylose	50	48
Disaccharides		
Sucrose	74	64
Maltose	124	75
Cellobiose	127	85
Polysaccharides		
Starch	124	123
a-Cellulose	101	143
Organid acid		
Citric acid	35	21
None	32	24

Medium: Glucose in the Wakman's special medium as the basal medium was replaced with carbon sources tested.

Table II. Effect of nitrogen source on mycelial growth of *P. sajor-caju* and *P. ostreatus.*

Nitrogen source	Dry weight(mg/14 days)	
	P. sajor-caju	*P. ostreatus*
Amino acid		
Asparatic acid	78	30
Methionine	39	55
Asparagine	116	71
Inorganic nitrogen		
Ammonium tartrate	106	87
Sodium nitrate	25	36
Organic nitrogen compounds		
Peptone	169	92
Yeast extracts	366	230
Malt extracts	215	61
None	41	34

Medium: Peptone in the Waksman's special medium as the basal medium was replaced with nitr ogen sources tested.

Because Yusef mentioned their strain could make oidial mass on the media made with glycine, but our strain couldn't make a oidial mass on the media cont ained 1% of glycine and sesame-oil.

The results that *P. sajor-caju*, favored cellobiose, starch, maltose and a-cellulose as its carbon source suggested that many agaric wastes can be utilized for the substrate of the mushroom in commercial scale (Bano, Z. and S. Rajarathnam, 1982; Jandaik, C. and J. N. Kapoor, 1974; Sing. R. R., 1983; Sivaprakasam, K. and T. K. Kandaswamy, 1981).

Most organic nitrogen compounds showed better mycetlial growth of both the species than did Amino acid as sole scource or inorganic nitrogen (Table II). Yeast extracts, malt extracts and peptone were the best for the *P. sajor-caju*, whereas the yeast extracts was the best for the *P. ostreatus* but malt extracts and peptone were not much as good as *P. sajor-caju.*

Asparagine was better nitrogen source than asparatic acid and methionine. Asparatic acid in *P. ostreatus* and methionine in both the species were unfavorable. Acidic amino acid makes low pH in the liquid medium and the reason of poor growth of the mushrooms might be due to the low pH (Hashimoto, 1974).

Sulfuric acid like methionine was not much utilized for the growth, because the mushrooms were constituted of sulfuric amino acid at low levels. The mycelial growth of the species promotive with ammonium tartrate as inorganic nitrogen source but was poor with sodium nitrate. This result suggested the ammonium form of nitrogen source was better than that of

Table III. Effect of ratio of glucose and ammonium tartrate on mycelial growth of *P. sajor-caju*(S) and *P. ostreatus*(O).

Glucose percentage	Species	Ammonium tartrate percentage			
		0.1	0.2	0.4	0.6
1	S	+	++	++	++[a)]
	O	+	++	++	+
2	S	++	+++	+++	++
	O	+	++	++	++
4	S	+	+++	++++	++
	O	+	+++	++++	++
6	S	+	++	+++	++++
	O	+	+	++++	++

a) Mycelial dry weight (mg/7 days) below 30; +, 31-60; ++, 61-90; +++, Over 90; ++++

nitrate form for the mycelial growth of *P. sajor-caju* and *P. ostreatus*. The result were corresponding to that of Hashimoto (1974).

The mycelia of *P. sajorcaju* grew at any glucose concentration and ammonium tartrate and grew better with increase of glucose percentage and ammonium tartrate synchronously up to 6 and 0.4 percent, respectively (Table III). Six percentage glucose and 0.6 percentage ammonium tartrate was optimal C/N ratio for the mycelial growth of *P. sajor-caju*, whereas the optimal ratio for the *P. ostreatus* was 4% glucose and 0.4% ammonium tartrate. However the mycelial growth of both the species at below 1% glucose and 0.1% ammonium tartrate was not suitable. As shown in the Table III, *P. sajor-caju* was favorable at higher concentration of glucose and ammonium tartrate than of *P. ostreatus*, therefore it might be better to add the nutrional supplements for the cultivation of *P. sajor-caju* in practical scale.

From the above results, the vegetative growth properties of *P. sajor-caju* were similar to those of *P. ostreatus*, their fruiting characters were different.

摘 要

여름느타리버섯 및 느타리버섯의 菌絲生長 適溫은 25~30°C이며 培地의 最適 pH는 5~6이였다. 여름느타리버섯은 二糖類 및 多糖類가, 느타리버섯은 多糖類가 菌絲生長에 가장좋은 炭素給源이었으며 窒素給源으로는 yeast extract等의 複合 有機態窒素가 單一 amino 酸 및 無機態窒素보다 좋았다. 單一 amino酸 中 asparagine이 가장 좋았으며 無機態窒素給源 中 ammonium 態 窒素가 Nitrate態 窒素보다 菌絲生長量이 많았다. 菌絲生長에 알맞는 glucose와 ammonium tartrate의 比는 여름느타리버섯이 各各 6% 및 0.6%, 느타리버섯이 4% 및 0.4%였다.

References

Bano, Z. and Rajarathnam, S. (1982): Studies on the cultivation of *Pleurotus sajor-caju*. *The mushroom J.* **115**: 243~245.

Booth, C. (1971): *Fungal Cultural Media* ed. C. Booth, *Methods in microbiology*, Vol. 4. Academic press. London and New York.

Hashimoto, K. and Takashi, Z. (1974): Studies on the growth of *Pleurotus ostreatus*' *Mushroom Science* **9** (part 1): 585~593.

Hong, J. S. (1978): Studies on the physiochamical properties and the cultivation of oyster mushroom (*Pleurotus ostreatus*). *J. of Korean Agr. Chem. Society* **21**, 150~184(In Korean).

Jandaik, C. L. (1974): Artificial cultivation of *Pleurotus sajor-caju*. *The mushroom J.* **27**: 405.

Jandaik, C. L., and Kapoor J.N. (1974): Studies on cultivation of *Pleurotus sajor-caju* (Fr.) Singer. *Mushroom Science* **9** (part 1): 667~672.

Jandaik, C. L., and *Kapoor J. N.* (1976). Amino acid composition of mushroom *Pleurotus sajor-caju* (Fr.) Singer. *The mushroom J.* **41**: 154~156.

Lee, E. R. (1979): Studies on the possibility of oak mushroom (shiitake) cultivation on ban oak (*Quercus incana*) of India. *Kor. J. Mycol.* **6**: 29~33.

Park, Y. H., Go, S. J. and Kim, D. S. (1975). Studies on the cultivation of oyster mushroom, *Pleurotus ostreatus* (Fr.) Quel. Using Rice straw as growing substrates. 1. Experiment on the development of growing substrates. *The research reports of the offices of Rural Development* **17** (Soil science, Fertilizer. Plant protection and Micrology): 103~107 (In Korean).

Singh, R. P. (1981): Cultivation of *Pleurotus sajor-caju* (Fr.) Sing. Mushroom. *Mushroom Science* **11** (part 1): 667~673.

Sivaprakasam, K. and Kandaswamy, T. K. (1981): Waste materials for the cultivation of *Pleurotus sajor-caju*. *The Mushroom J.* **101**: 178~179.

Yusef, H. M. and Allam, M. E. (1967): The carbon and Nitrogen nutrogen nutrition of certain fungi. *Canadiun Journal of Microbiology.* **13**: 1097~1105.

Zadrazil, F. (1974): The ecology and industrial prod uction of *Pleurotus ostreatus*, *P. florida*, *P. cornucopiae*, and *P. eryngii*. *Mushroom Science* **9** (part 1): 621~652.

〈Received February 10, 1984〉