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Vegetative Growth of Four Strains of *Hericium erinaceus* Collected from Different Habitats

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Vegetative growth of four different strains of *Hericium erinaceus* was observed. The temperature suitable for optimal mycelial growth was determined to be 25°C, with growth observed in the extend temperature range of 20~30°C. The different strains of this mushroom showed distinct pH requirements for their optimum vegetative growth, with the most favorable growth observed at pH 6. Considering vegetative mycelial growth, PDA, YM, Hennerberg, Hamada, and Glucose peptone were the most favorable media, and Czapek Dox, Hoppkins, Glucose tryptone, and Lilly were the most unfavorable media for these mushroom strains. With the exception of lactose, most of the carbon sources assayed demonstrated favorable vegetative growth of *H. erinaceus*. For mycelial growth, the most suitable nitrogen source was alanine and the most unsuitable was histidine. Oak sawdust medium supplemented with 10~20% rice bran was the best for mycelial growth of the mushroom.

KEYWORDS : Culture media, *Hericium erinaceus*, pH, Temperature, Vegetative growth

Hericium erinaceus is an edible and medicinal mushroom belonging to Hericiaceae, Basidiomycota. This mushroom is delicious with a subtle citrus-floral flavor and a musky delicate smell. *H. erinaceus* is known as a forest dweller, and this so-called 'lion's mane' grows off wound scars or recently fallen hardwoods, mostly oak. It can be easily distinguished by its thin tendrils protruding from a white rubbery center. The flesh is white to off-white, slightly translucent, and rubbery. The base can sometimes be very strong and difficult to remove from the host tree without a sharp knife (Kuo, 2003). *Hericium* spp. may have important physiological functions in humans, including antioxidant activities, the regulation of blood lipid levels, and reduction of blood glucose levels (Wang *et al*., 2005). The polysaccharides from this fungus may have cytostatic effects on gastric ulcers, esophageal cancer, hepatitis, and skin cancer (Mizuno, 1999; Mizuno *et al*., 1992). Ying *et al*. (1987) also reported that pills of this mushroom are used in the treatment of gastric ulcers and esophageal carcinoma. Hot water extracts from the mycelia of several *Hericium* spp. has been used in sports drinks named 'houtou' that were used in the 11th Asia Sports Festival (1990) and contributed to the to promoted digestion and general vigor of the Chinese players. Therefore, it can be anticipated that this mushroom is in use as a health food in Asian countries and will also become an important research subject.

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Based on these reports, a study has been conducted on the vegetative growth for 4 strains of *H. erinaceus*. The different environmental and nutritional factors were assayed to determine the optimal culture conditions for mycelial growth for this fungus.

Materials and Methods

Culture collection and use. The mycelial culture of 4 strains of *Hericeum erinaceus* originating from Korea (IUM0217 and IUM2876), Japan (IUM1128), and China (IUM3271) were obtained from the Culture Collection and DNA Bank of Mushrooms (CCDBM) in the Department of Biology, University of Incheon, Korea. To facilitate the study, strains of *H. erinaceus* were transferred to potato dextrose agar (PDA) plates and incubated at 25°C under dark conditions until they showed full growth and then kept at 4°C for further use. Unless otherwise stated, all tests were performed at least 4 times.

Effect of temperature and pH on the vegetative growth. To screen for the optimum temperature for mycelial growth of the mushrooms, 5 different temperatures (15, 20, 25, 30, and 35°C) were assayed. A 5 mm diameter agar plug was removed from 10-day-old cultures grown on PDA and placed in the centre of a new plate filled with 20 *ml* of PDA. The medium was adjusted to pH 6 and incubated for 10 days at 15, 20, 25, 30, or 35°C. Radial growth of mycelia on each Petri dish was

Table 1. Media and their compositions used in this study

Composition	Media (g/*l*)									
	Cza	Ham	Hen	Hop	GP	GT	Lil	MC	PDA	YM
Agar	20	20	20	20	20	20	20	20	20	20
Asparagine							2			
Dextrose		10							20	10
Ebiose		5								
Hyponex		3								
Glucose			50	10	10	5				
Malt-extract					15			20		3
Maltose							10			
Peptone					10			2		5
Potatoes									200	
Sucrose	30									
Triptone						10				
Yeast-extract		3			10	3		2		3
$NaNO_3$	3		2							
K_2HPO_4	1							1		
$MgSO_4$	0.5		0.5	0.5			0.5	0.5		
KCl	0.5									
$FeSO_4$	0.01									
$CaCl_2$			0.1							
KH_2PO_4			1	0.1			1	0.5		
KNO_3			2	2						

Cza: Czapek's, Ham: Hamada, Hen: Hennerberg, Hop: Hoppkins, GP: Glucose peptone, GT: Glucose tryptone, Lil: Lilly, MC: Mushroom complete, PDA: Potato dextrose agar, and YM: Yeast-malt extract.

measured in 3 directions and the average value was calculated from these measurements. To calculate the final mean value of mycelial growth for each strain, 4 replications were performed. For assaying the effect of pH, a 5 mm diameter agar plug was removed and placed as above. The medium was adjusted to pH 5, 6, 7, 8, or 9 with the addition of 1 N NaOH or HCl, and incubated for 10 days at 25°C. The measurement of mycelial growth was performed following same technique as the optimum temperature tests.

Effect of culture media on vegetative growth. Ten different culture media (Czapek Dox, Hamada, Hennerberg, Hoppkins, Glucose peptone, Glucose tryptone, Lilly, Mushroom complete, PDA, and YM) were prepared to investigate the mycelial growth of the strains (Table 1). The media were adjusted to pH 6 before autoclaving. All culture media were inoculated as above. After 10 days of incubation at 25°C, measurements for mycelial growth were performed with same method as above.

Effect of carbon and nitrogen sources on vegetative growth. To screen for carbon and nitrogen sources favorable for mycelial growth of the selected mushroom strains, tests were performed using basal medium (Sung *et al.*, 1993) supplemented with each of 10 carbon (Dextrin, Fructose, Galactose, Glucose, Lactose, Maltose, Mannose, Sorbitol, Sucrose, and Xylose) or 10 nitrogen (Alanine, Ammonium acetate, Ammonium phosphate, Arginine, Calcium nitrate, Glycine, Histidine, Methionine, Potassium nitrate, and Urea) sources, separately. The basal medium was composed of $MgSO_4$ 0.05 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, thiamine-HCl 120 μg, agar 20 g, and 1000 *ml* of distilled water. To screen for carbon sources favorable for mycelial growth, each carbon source along with 5 g of peptone was added to the basal medium separately, at a concentration of 0.1 M per 1000 *ml*, and mixed thoroughly (Shim *et al.*, 1997). The basal medium that was used for screening favorable nitrogen sources was made as described by Sung *et al.* (1993). Each nitrogen source along with 20 g of glucose was added to the basal medium at a concentration of 0.02 M. In all cases, the basal medium was adjusted to pH 6 before autoclaving. To measure the colony diameter on the media, all plates were incubated for 10 days at 25°C. Radial growth of mycelia was measured following the same method described above.

Effect of rice bran additives in sawdust medium on vegetative growth. Oak (*Quercus variabilis*) sawdust and rice bran were purchased from a Yongsan Sawdust Company, Namwon city, Jeonbuk, Korea. To determine suitable additives for vegetative growth in sawdust medium, sawdust and rice bran were used at the ratios of 70 : 30, 80 : 20, 90 : 10, or 100 : 0% (v/v), respectively. Calcium carbonate ($CaCO_3$) was added to the medium at 2% and the moisture content was adjusted to 65~70%. The medium was then pored into round glass columns

(2 × 22 cm) and steam-sterilized for 90 minutes at 121°C. The sawdust medium was inoculated with 4 strains of *H. erinaceus* in the glass columns, separately. A 5 mm agar plug was removed with a cork borer from 7-day-old cultures of *H. erinaceus* and placed on the top surface of the sawdust medium in the glass column. The columns were incubated for 35 days at 25°C under dark conditions. The mycelial growth of *H. erinaceus* in oak sawdust medium supplemented with rice bran was measured after 35 days of incubation. A suitable additive ratio was selected.

Results and Discussion

Effect of temperature on vegetative growth. The temperature most suitable for the mycelial growth of the tested mushroom strains was determined to be 25°C and the lowest (sometimes no growth) mycelial growth rates were recorded at 15 and 35°C. The mycelial growth of IUM1128 was similar (67 mm) at 20 and 25°C. The extend range of temperatures was 20~30°C for vegetative growth of *H. erinaceus* (Fig. 1). Jayasinghe *et al.* (2008) studied the effect of temperature on the mycelial growth of 8 strains belonging to *Ganoderma lucidum* and found suitable growth at 25°C with an extended range of 20~30°C. Shim *et al.* (2003) reported that the mycelial growth of *Paecilomyces fumosoroseus* had been expedited gradually in proportion to the rise in temperature and was most suitable at 25°C. Even though the mycelial growth of *P. fumosoroseus* was favorable over the range of 20~25°C and had been expedited in proportion to the rise of temperature, the mycelial growth of this strain appeared to be suppressed at temperatures higher than 30°C.

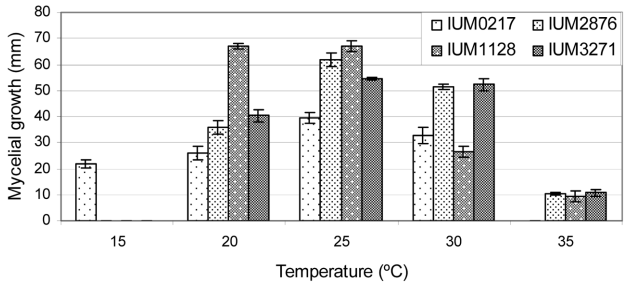

Fig. 1. Effect of temperature on the mycelial growth of 4 strains of *H. erinaceus*. Mycelial growth was measured (n = 4) after 10 days of incubation on PDA.

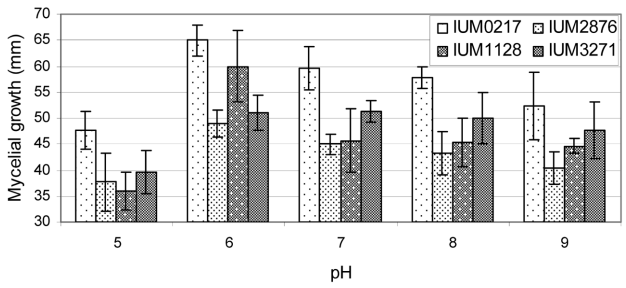

Fig. 2. Effect of pH on the mycelial growth of 4 strains of *H. erinaceus*. Mycelial growth was measured (n = 4) after 10 days of incubation at 25°C on PDA.

Effect of pH on the vegetative growth. The pH values most suitable for the favorable growth of *H. erinaceus* was observed in the range of 5~9 and the best was pH 6. In the case of IUM3271, similar mycelial growth was observed at pH 6, 7, or 8. The other pH values also showed good mycelial growth, and pH 9 was better than pH 5 for the growth of the different strains of *H. erinaceus* (Fig. 2). Imtiaj *et al.* (2008) assayede the pH requirements for the vegetative growth of 10 strains of *Schizophyllum commune* and reported that pH 5 was the best. A decreased mycelial growth for this strain was found when the pH value was gradually increased to pH 9. Shim *et al.* (2005) revealed that pH 7 is the most suitable for the optimal growth of *M. procera.* Shim *et al.* (2003) shown that the optimal pH of *Paecilomyces sinclairii* was 8. This result suggested that different species of mushrooms demonstrate optimal mycelial growth at different pH values.

Favorable culture media for vegetative growth. Ten different culture media were used to screen for the optimal mycelial growth of the 4 strains of *H. erinaceus*. According to mycelial growth, PDA, YM, Hennerberg, and Glucose peptone were the most suitable, and Czapek Dox, Hoppkins, Glucose tryptone, and Lilly were the most unfavorable media for mycelial growth. The strain IUM2876 showed faster growth in every medium relative to the other strains (Fig. 3). This result partially corresponded with that of *P. sinclairii* and *P. fumosoroseus,* which had been reported by Shim *et al.* (2005) to grow most favorably on PDA, YM, Mushroom complete, and Hamada media, whereas Czapek Dox and Glucose pep-

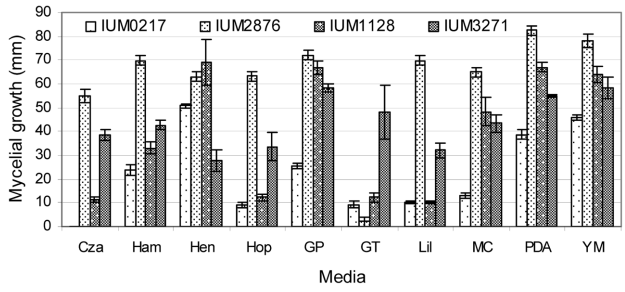

Fig. 3. Effect of media on the mycelial growth of 4 strains of *H. erinaceus*. Mycelial growth was measured (n = 4) after 10 days of incubation at 25°C. Cza: Czapek Dox, Ham: Hamada, Hen: Hennerberg, Hop: Hoppkins, GP: Glucose peptone, GT: Glucose tryptone, Lil: Lilly, MC: Mushroom complete, PDA: Potato dextrose agar, and YM: Yeast-malt extract.

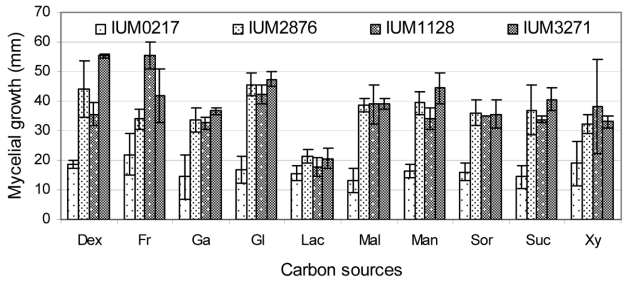

Fig. 4. Effect of carbon source on the mycelial growth of 4 strains of *H. erinaceus*. Mycelial growth was measured (n = 4) after 10 days of incubation at 25°C on basal medium. Dex: Dextrin, Fr: Fructose, Ga: Galactose, Gl: Glucose, Lac: Lactose, Mal: Maltose, Man: Mannose, Sor: Sorbitol, Suc: Sucrose, and Xy: Xylose. Each carbon source was added to the basal medium at the concentration of 0.1 M.

tone were most unfavorable for the mycelial growth of *M. procera*.

Effect of carbon sources on vegetative growth. Ten different carbon sources were assayed for optimal culture conditions. The mycelial growth of the strains used was best and mostly similar on dextrose, fructose, or glucose. Lactose showed very slow vegetative growth for all 4 strains (Fig. 4). Shim *et al.* (1997) assayed the effect of 19 carbon sources on the mycelial growth of *G. umbellata* and reported that growth was favorable on any carbon source used except salicin, cellobiose, and lactose. Shim *et al.* (2003) also found that dextrin was suitable for mycelial growth of *P. fumosoroseus,* which is equivalent to our findings.

Effect of nitrogen sources on vegetative growth. It was observed that the most suitable and unsuitable nitrogen sources were alanine and histidine for mycelial growth of *H. erinaceus*, respectively. Ammonium acetate, ammonium phosphate, arginine, and glycine demonstrated intermediate mycelial growth (Fig. 5). Imtiaj *et al.* (2008) assayed the requirement for nitrogen sources on the vegetative growth of 10 strains of *S. commune* and reported that calcium nitrate, glycine, potassium nitrate, and alanine were best, which is similar to our results with the exception of calcium nitrate. Shim *et al.* (2005) determined that glycine was the most favorable and histidine, arginine, and ammonium oxalate were the most unfavorable for the mycelial growth of *M. procera*.

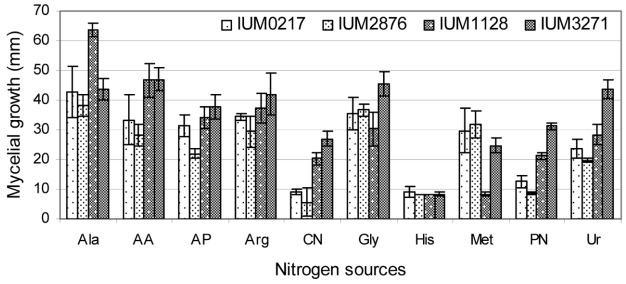

Fig. 5. Effect of nitrogen sources on the mycelial growth of 4 strains of *H. erinaceus*. Mycelial growth was measured (n = 4) after 10 days of incubation at 25°C on basal medium. Ala: Alanine, AA: Ammonium acetate, AP: Ammonium phosphate, Arg: Arginine, CN: Calcium nitrate, Gly: Glycine, His: Histidine, Met: Methionine, PN: Potassium nitrate, and Ur: Urea. Each nitrogen source was added to the basal medium at the concentration of 0.02 M.

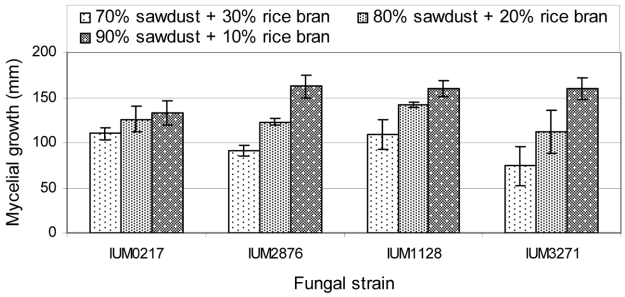

Fig. 6. Effect of oak sawdust medium supplemented with rice bran at different concentrations on the mycelial growth of 4 strains of *H. erinaceus*. Mycelial growth was measured (n = 4) after 35 days of incubation at 25°C.

Suitable additive ratio of rice bran in sawdust medium for vegetative growth. After 35 days of incubation in glass columns at 25°C, the mycelial growth of *H. erinaceus* was measured. The addition of 10~20% rice bran to sawdust medium showed the best vegetative mycelia growth for *H. erinaceus*. The sawdust that was supplemented with 10% rice brain showed the best mycelial growth (Fig. 6). No addition of rice bran (sawdust only) showed faster but very thin growth (data not shown) and 30% rice bran showed very slow and thick vegetative mycelial growth. It should be mentioned that rice bran is used as a nutrient source in the culture medium and an excess of rice bran can made it significantly harder. Therefore, 10% rice bran was chosen to supplement the sawdust media to produce fruiting bodies of *H. erinaceus*. Rew *et al.* (2004) showed that the mycelial growth of *Phellinus baumii* was the best on sawdust mixed with 20% rice bran. Lee *et al.* (2007) determined that sawdust medium supplemented with 20~30% rice bran was good for forming fruiting bodies of *Oudemansiella mucida*.

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