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## Growth Rate of Mycelium of Shiitake, *Lentinus edodes*, in Relation to Water Potential of Medium

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The growth rate of mycelium of shiitake, *Lentinus edodes* (Berk.) Sing., as a function of the water potential ( $\psi$ ) of the medium, whether it be agar or liquid, is qualitatively similar to that observed for other fungi in that there is a stimulation of the growth rate as  $\psi$  is decreased slightly but as  $\psi$  is decreased further, there is an actual inhibition of the growth rate. A slight reduction in  $\psi$  stimulated the mycelial growth of *L. edodes*, where a maximum growth rate occurred at -0.5 MPa and there was no growth at -5.3 MPa on a KCl-adjusted agar medium. The  $\psi$  of sawdust substrate cultivating shiitake mushroom, especially in terms of physiologically available water, may be more important than the fresh-weight moisture content expressed as a percentage of weight. And oxygen availability might be a factor in the mycelial growth.

### INTRODUCTION

The influence of the water potential ( $\psi$ ) of substrates on fungal growth has received increasing attention in recent years (Dix, 1985 ; Eamus and Jennings, 1986 ; Kuthubutheen and Webster, 1986 ; Al-Hamdani and Cooke, 1987 ; Badham, 1989). It is widely known that adequate water is required for growth of mushrooms in nature and under cultivation.

The growth of some basidiocarps are correlated with high atmospheric humidity (Badham, 1985). Transpiration and growth of a mushroom were significantly correlated with the humidity of the air. Most research on the influence of humidity on mushroom growth has been conducted with commercially important species, that is ; on *Agaricus bisporus* (Edwards, 1978), on *Pleurotus ostreatus* (Zadrazil, 1978), and on *Volvariella volvacea* (Chang and Miles, 1989).

Although *L. edodes* is the second most important commercially cultivated mushroom in the world, there is little information on its water relationships (Badham, 1989). Growth of mycelium is correlated more significantly with substrate moisture content than atmospheric humidity. Wood-decaying basidiomycetes appear to be particularly sensitive to a decreased  $\psi$  of the substrate (Boddy, 1983 ; Dowson et al., 1989).

Measurement of water in a substrate is usually performed under overdrying methods, though water availability is unknown in a biological sense. Within sawdust substrate the availability of water to the mycelia is affected by two main forces, matric potential ( $\psi_m$ ) and osmotic potential ( $\psi_s$ ).  $\psi_m$  is a result of forces associated with the interfaces between air and the sawdust matrix, and  $\psi_s$  is a result of the presence of solutes within the water. The sum of  $\psi_m$  and  $\psi_s$  is termed  $\psi$ .

The aim of this paper is to provide data on the effect of  $\psi$  on the growth of *L. edodes* mycelium.

## MATERIALS AND METHODS

### Organism and culture

The strain, *Lentinus edodes* (Berk.) Sing., was isolated from the collection of the Institute for Fermentation Osaka (IFO 7123). It was grown in 90 mm Petri dishes filled with a potato-dextrose agar (PDA, Difco) medium. A 4 mm diam plug was used for inoculation. Cultures were incubated at 25°C.

### General methods

#### Agar plates

The  $\psi$  of the PDA agar medium was adjusted to desired values by adding appropriate molal concentrations of sucrose, glycerol, NaCl, KCl, or a salt mixture comprised of NaCl, KCl, and Na<sub>2</sub>SO<sub>4</sub> in a molal ratio of 5 : 3 : 2 (Ioannou *et al.*, 1977). Then 90 mm diam Petri dishes were filled to a depth of 5 mm with the adjusted media. Each dish was inoculated with a 4 mm diam plug from the mycelial front margin an active young colony growing on agar, used to maintain cultures.

#### Liquid medium

Studies on liquid media were performed on a medium consisting of 7 g malt extract, 1 g peptone, 0.5 g yeast extract, and 1 L distilled water (Koske and Tessier, 1986). Polyethylene glycol (PEG) 4000 of a large molecular weight was used to adjust  $\psi$  of the liquid media such as in previous microbial studies (Mexal *et al.*, 1975 ; Brownell and Schneider, 1985). Liquid media were prepared in 100 ml Erlenmeyer flasks, each containing 20 ml of medium.

#### Sawdust media

Wood powder was prepared from freshly cut beech, *Fagus crenata* Bl. with Wiley mill, and it was separated into several particle sizes using a sieve shaker. For example, sawdust grade 20-48 contains particle sizes between 20 mesh and 48 mesh of the U. S. standard sieve size.

For matric control of  $\psi$ , various amounts of distilled water were added to the dry sawdust medium. This medium was composed of 20 g (dry weight) of wood powder and 4 g of rice bran. Approximately 24 g (dry weight) of sawdust mix was filled and compressed into 90 mm Petri dishes and a cavity having the size of the inoculant plug was made in the center. One spawn plug, a small cylinder of wood colonized by mycelium of *L. edodes*, was inoculated into the cavity.

### Psychrometric techniques

The  $\psi$  of each medium was determined by thermocouple psychrometry using a Wescor HR-33T microvoltmeter coupled to a C-52SF sample chamber. All measurements were made in the dew point mode, after calibration with a series of NaCl solutions of known potentials.

To determine  $\psi$  of the agar medium, disks having 4 mm diam and 2-3 mm thick

were used for each sample and an equilibrium time of 30 sec as well as a cooling of the thermocouple junction time of 15 sec were given. The apparatus was calibrated using Whatman antibiotic assay filter disks which had been left to soak in solutions of NaCl of known molality, any excess solution being quickly drained off. Measurements of  $\psi$  were made at 25°C and converted to values at 20°C using tables (Lang, 1967).

The sawdust samples of approximately 0.1 g were picked up from the medium and within 10 min sealed into the sample chamber to allow for equilibrium. The same method was also applied for agar. The moisture content of the sawdust medium was determined by oven-drying methods. About 1.0 g was weighed into a weighing bottle and left for 16 h at 105°C, followed by reweighing after cooling in a desiccator.

### **Growth measurement**

Colony radial growth on solid media, agar or sawdust, were determined by measuring colony diameters with a slide caliper. Due to deviations from absolute circularity, both the largest and smallest diameters of the colonies were measured, and the mean was taken. Mycelial biomass grown on a sawdust medium was qualitatively determined by measuring the surface whiteness of the medium (Ohga, 1989). As for liquid media, the mycelium was separated from the medium by filtration through a weighed glass extraction thimble, then it was washed and dried at 105°C for 16 h and then cooled in a desiccator ; the thimble was then reweighed.

## **RESULTS AND DISCUSSION**

### **Growth of fungi at varying media with different $\psi$**

#### **Agar plates**

Figure 1 shows the significant correlation between  $\psi$  and the growth of *L. edodes* mycelium. The rate of growth of mycelium coming from an agar block inoculum was tested as a function of  $\psi$  of the agar. The basal medium, which has no solutes, has a  $\psi$  of -0.24 MPa. At -0.5 MPa, however, the growth rate of the mycelium with different solutes is greater than without solutes. At values below -0.5 MPa, the growth rate declines in all instances with decreasing  $\psi$ . It should be noted that maximum radial growth in agar adjusted with several solutes occurred at  $\psi$  below that of the nonadjusted basal medium. Below -0.5 MPa, the use of glycerol as a solute yielded much higher growth rates than other solutes. Generally growth with KCl tended to be higher than with mixed salts. NaCl as solute usually yielded the lowest curve and no growth occurred below -1.6 MPa whereas growth on other salts often continued until -5.3 MPa.

#### **Liquid medium**

Figure 2 gives the dry weight of *L. edodes* mycelium after 14 days growth on media of different  $\psi$  produced by varying the concentration of PEG 4000 in the medium. The pattern of growth is much like that on agar with greater growth as  $\psi$  is decreased slightly below that of the control but a decrease of growth when  $\psi$  is decreased still further, with little growth at -2.1 MPa. The findings of this study are in general agreement with other recent studies of the effects of  $\psi$  on the growth of natural litter- and wood-decomposing basidiomycetes (Harrower and Nagy, 1979 ; Clarke et al.,

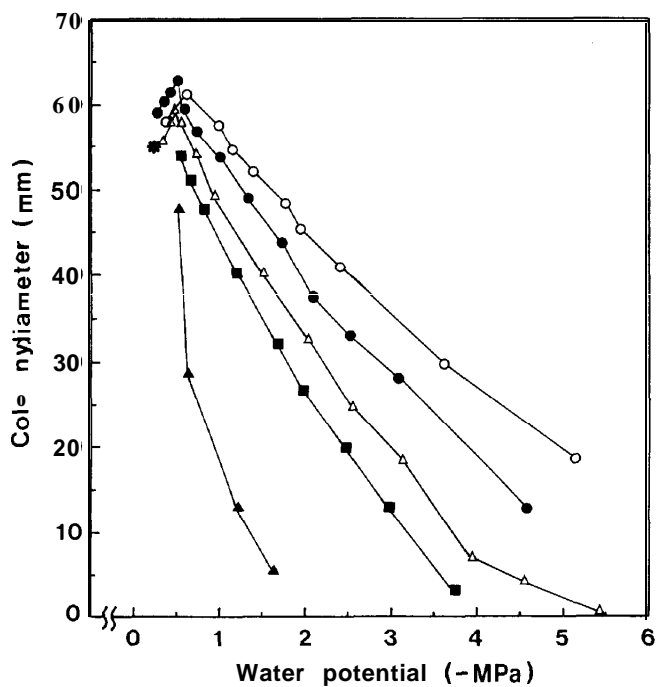


Fig. 1. Growth rate of mycelium from an agar plug inoculum of *Lentinus edodes* after 8 days over potato-dextrose agar medium of different water potential produced by following solutes : ○ : Glycerol, ● : Sucrose, △ : KCl, ▲ : NaCl, ■ : Salt mixture, \* : agar containing none of these solutes.

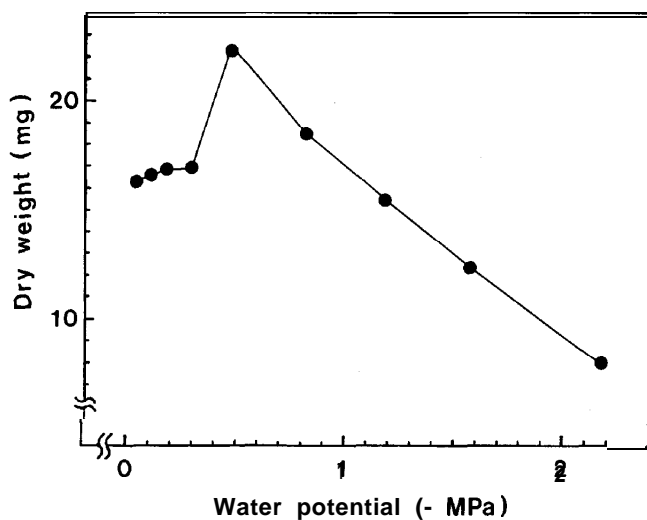


Fig. 2. Dry weight of mycelium of *Lentinus edodes* after 14 days on liquid media of different water potential produced by PEG 4000.

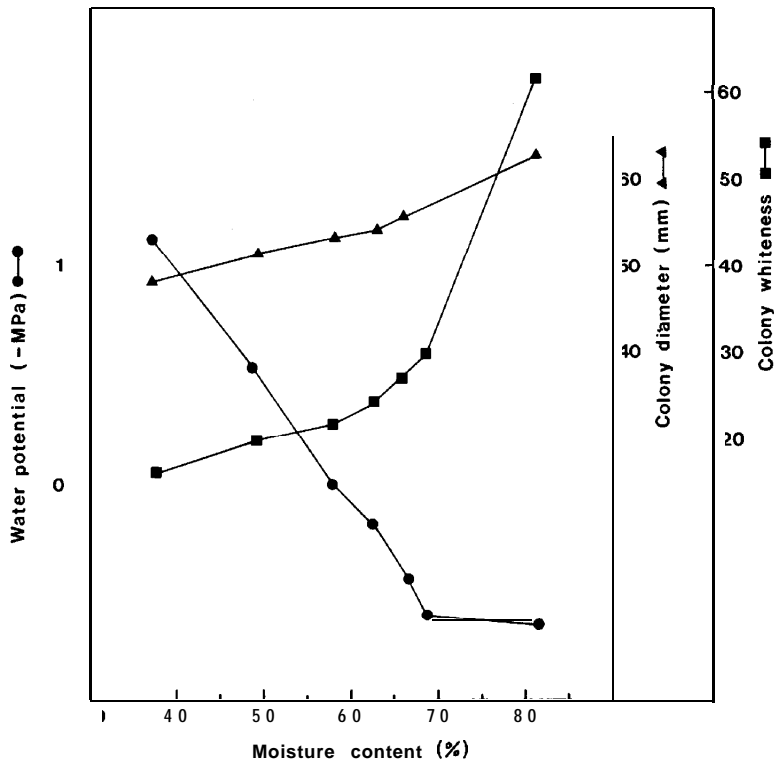
1980 ; Singh and Noah, 1989).

### Effects of moisture content and $\psi$ on mycelial growth in sawdust media

When various amounts of water were added to the supplemented sawdust substrates the  $\psi$  was not linearly related to percent moisture. Figure 3 gives results for colony diameter of *L. edodes* in relation to the initial moisture content of the substrates. The correlation is linear from 37% to 65% moisture. A marked increase of mycelial biomass was recognized on the substrate over 68% moisture, judging from surface whiteness of the medium.

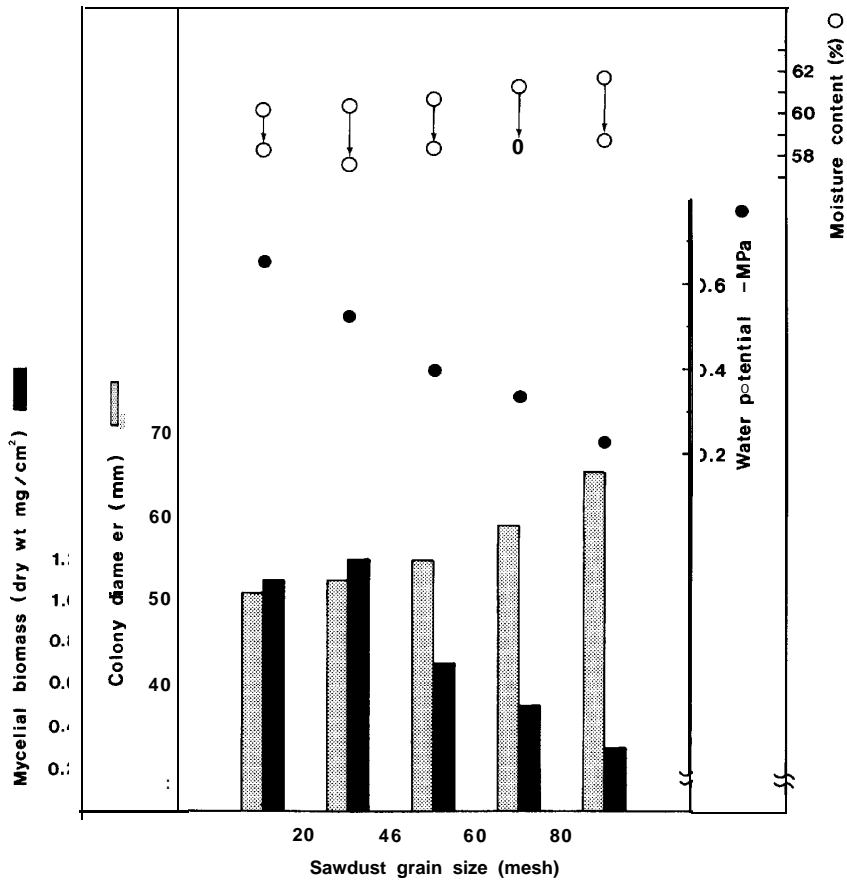
### Effects of sawdust grain size on mycelial growth in sawdust media

Differences in sawdust grain size will make many media with different  $\psi$ , especially in terms of physiologically available water, at the same moisture content when it is expressed as a percentage of weight (Fig. 4). Percentage of moisture always decreased during the course of the experiment, usually by 2-3%. Within the range of these experiments,  $\psi$  of the medium did not have a great influence on the transpiration rate.



**Fig. 3.** Growth of *Lentinus edodes* mycelium and water potential on supplemented sawdust substrate at different moisture content (incubated for 11 days.).

●: water potential, ▲: colony diameter, ■: colony whiteness



**Fig. 4.** Effect of sawdust grain size on mycelial development of *Lentinus edodes* and water potential of sawdust medium (incubated for 10 days.).

▨: colony radial extension, ■: mycelial biomass

The symbols ○→○ show decrease percentage of moisture content.

Rapid colony radial extension of *L. edodes* mycelia on the medium surface area was expected having sufficient free water with increasing  $\psi$ . But great mycelial biomass did not increase in the inner parts of these sawdust medium with decreased  $\psi$  (Fig. 4). This lower mycelial biomass might be due to the depletion of oxygen ( $O_2$ ) around that part of the mycelium in the medium which is submerged. The diffusion rate of  $O_2$  through water is much lower than through gas. Inadequate gas exchange, resulting in depletion of  $O_2$  and increase in carbon dioxide concentrations, probably was the primary cause of the inhibition observed in saturated and very wet substrate.

When the  $\psi$  of sawdust is decreased, the matrix force binds water to the medium particles even more strongly. In nature, this factor exerts the major effect of water availability to wood decaying fungi. It is important to note that *L. edodes* mycelium is more tolerant of water stress than other fungi and there is a stimulation effect to the

mycelial growth as  $\psi$  is decreased slightly whether the medium be agar, liquid or sawdust (as shown in Figs 1, '2 and 4).

According to the results of these experiments, *L. edodes* mycelium is capable of significant growth at substantial water deficiencies. Also, peak mycelial biomass is expected with a moisture content of a medium at about 60% and a decreased  $\psi$  of -0.5 MPa. Also, crude grain sawdust is suitable for adequate O<sub>2</sub> supply.

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