J Wood Sci (1999) 45:337–342

ORIGINAL ARTICLE

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Effect of water potential on fruit body formation of *Lentinula edodes* in sawdust-based substrate

Received: June 2, 1998 / Accepted: December 11, 1998

Abstract The influence of substrate water potential (ψ) on the growth and fruiting of three genotypes of shiitake (*Lentinula edodes*) was investigated. A slight reduction of ψ (−0.5 MPa) stimulated mycelial and colony growth on liquid, agar, and sawdust-based substrates. *L. edodes* has been found to grow well at a ψ around −0.5 MPa, which corresponds to a moisture content around 55%. A small decrease in ψ at the final vegetative growth phase had positive effects on flush quantity. The substrate ψ was significantly affected by the interaction between genotypes and spawn run time. The ψ of well-colonized mature substrate was −0.7 MPa before and −4.0 MPa after the fruiting. The ψ rose again to −0.7 MPa during rapid absorbance of water by soaking, and this rise was repeated during the second and third flushes. It is suggested that the water-holding capacity of a substrate is related to culture maturity. Excellent water-providing capacity (higher ψ) is expected in the substrate of well-matured cultures with a high density of mycelial colonization.

Key words Shiitake · *Lentinula edodes* · Water potential · Sawdust-based cultivation · Strain variety

Introduction

Water availability during shiitake [*Lentinula edodes* (Berk.) Pegler] cultivation is probably the most important factor influencing growth and fruit body production. Water potential (ψ) provides an accurate measure of available water. It is predominantly the sum of osmotic (ψ_π), matric (ψ_m), and turgor (ψ_p) potentials and is measured in pascals. Microbial growth is determined not by actual total water content but by the sum of the components of its ψ_w. Spore germination and mycelial growth of basidiomycetes are known to be more sensitive to changes in ψ_w than lower fungi. Wood decay basidiomycetes have optimum growth between −0.1 and −0.7 MPa in vitro.[1] The influence of ψ on hyphal growth has been examined for various fungi, including the wood-rotting fungi *Serpula lacrimans* and *Armillaria mellea*,[2] vesicular-arbuscular mycorrhizal fungi,[3] and the edible fungi *L. edodes* and *Agaricus bisporus*.[4–7] Kalberer demonstrated the mechanism and regulation of water uptake and translocation in *A. bisporus*.[4] Moreover, the effect of ψ on mycelial morphology was described in *Stropharia caerulea*.[8]

The sensitivity or tolerance of *L. edodes* strains to both osmotic and matric components can be determined. Studies on the growth of *L. edodes* demonstrated that it grows optimally at −0.5 MPa ψ_w.[6] It was found to be highly sensitive to changes in total ψ_w, with no growth occurring at −2 MPa. *L. edodes* is the second most important of the cultivated mushrooms in the world and is the major mushroom species grown on wood substrates. Cultivation of shiitake on logs is a well-established industry, especially in Japan. It is cultivated on either *Quercus* logs or lignocellulosic particle sawdust in plastic bags. Recently, there has been an increase of interest in growing *L. edodes* on sawdust-based substrates.[9,10]

An attractive feature of edible cultivated mushrooms is that they can utilize a large variety of agricultural waste products and convert the lignocellulosic biomass into food of high quality, flavor, and nutritive value. The advantages of producing shiitake on sawdust-based substrate over producing it on bed logs include the shorter time required to complete a growing cycle and greater biological efficiency. The major disadvantage is the relatively higher initial investment cost of production.[11] The traditional log method requires more than 1 year for fruit bodies to appear, but a spawn run of less than 3 months suffices to obtain the first fruiting flush on sawdust-based substrates. The nutritional

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Part of this paper was presented at the 46th annual meeting of the Japan Wood Research Society, Kumamoto, April 1996

requirements and limits of the physical environment for mycelial growth and fruiting have been investigated for *L. edodes*. Considerable effort is now being directed toward the elucidation of factors contributing to fruit body yield and size variation when *L. edodes* is grown on synthetic substrates. Various factors have been reported or implicated to be conducive to the vegetative development and fructification in *L. edodes*.[12–15]

An understanding of the range of water requirements for optimal fruiting yield is needed. Water in a substrate is usually measured under oven-dried conditions, though ψ is unknown in a biological sense. Within the sawdust-based substrate the availability of water to the mycelia is affected by two main forces: ψ_m and ψ_π. The ψ_m is a result of forces associated with the interfaces between air and the sawdust-based matrix, and ψ_π is a result of the presence of solutes within the water. In this study the effect of ψ_π and ψ_m on in vitro growth and fruiting of a variety of spawn genotypes were examined. The purpose of the present investigation was to determine the effects of ψ on fruiting of *L. edodes*.

Materials and methods

Strains

This study compared ψ relations of three genotype strains of *L. edodes* developed for use in different climatic conditions: KS-9 (wide-range weather), KS-58 (warm weather), and KS-24 (cold weather). These strains are maintained in the Kyushu University Forests culture collection. Cultures were maintained on potato dextrose agar (PDA) in 9-cm petri dishes.

Mushroom culture

Growth studies were performed on a malt-yeast-peptone (MYP) medium, consisting of 7 g malt extract, 0.5 g yeast extract, 1 g peptone, and 1 l distilled water. For solid medium 15 g of agar was added. The ψ of the medium was controlled with KCl or polyethylene glycol 4000 (PEG). The media were centrally inoculated with a 5-mm agar disc from the margin of a growing colony of each strain. Cultures were grown on a sawdust-based substrate consisting of *Quercus mongolica* sawdust (70% dry wt), wheat bran (10%), rice bran (10%), and corncob meal (10%), with water added to give a final moisture content of 60%. Polypropylene bags were filled with the medium (1.2 kg wet wt), autoclaved at 120°C for 1 h, cooled, and inoculated with 10 g sawdust spawn. The bags were then capped and placed in a controlled environment for incubation. The bags were incubated at 20°C to promote vegetative growth for 70, 75, 80, 85, and 90 days, respectively. At the end of the respective period the plastic bags were removed, and the colonized substrates were transferred to a production room in which the temperature was maintained at 17°C and the relative humidity (RH) at 90% throughout the experiment. Sufficient air changes were maintained to keep CO_2 levels below 2000 ppm. The first flush occurred from days 10 to 15, the second flush from days 35 to 40, and the third flush from days 60 to 65. All fruit bodies were picked, counted, weighed, and dried at 50°C overnight. The soaking treatment was done 30 days (I) before the second flush and 55 days (II) before the third flush by immersing the colonized substrates in water overnight. The ψ was measured prior to soaking (after the flushing period and picking of the fruit bodies) and after the soaking treatment.

Measurement of ψ

Thermocouple psychrometry, a Wescor HR-33T microvoltmeter coupled to a C-52F sample chamber, was used to determine the ψ of agar and sawdust-based substrate samples. The thermocouple junction was cooled for 15 s, and an equilibrium time of 30 s was used for each sample. All measurements were made in the dew point mode after calibration with a series of Whatman filter disks dipped in NaCl solutions of known potentials. The sawdust-based samples were sealed for 10 min into a Decagon SC-10 sample chamber for equilibration. The moisture content of sawdust-based substrate was determined by oven-drying methods at 105°C.

Growth measurement

The mycelium was separated from the medium by filtration through a weighed glass extraction thimble; it was then washed and dried at 105°C for 16 h and cooled in a desiccator, and the thimble was then reweighed. As for the solid media (agar and sawdust), colony radial growth was determined by measuring colony diameters with a slide caliper. Because of the deviations from absolute circularity, the largest and smallest diameters of the colonies were measured and the mean taken.

Results

Effect of ψ on mycelial growth

The effect of varying osmotic potential on the vegetative growth of three genotypes of *L. edodes* was investigated using two solute systems. The MYP medium was modified by adding the ionic solute KCl and matrically by adding PEG 4000 in the range −0.25 to −5.0 MPa ψ. When various amounts of water were added to the sawdust-based substrate, ψ was not linearly related to the amount of moisture (Fig. 1). A decrease from 80% to 63% moisture corresponded to a decrease in ψ of approximately 0.006 MPa per 1% reduction. Between 60% and 35% the decrease is close to 0.08 MPa per 1% reduction in moisture content.

The mycelium grew well on all culture media (i.e., liquid, agar, and sawdust-based substrate) at −0.5 MPa (Figs. 2–4). Mycelial growth was stimulated on all culture media with slightly more negative ψ than the control, although a further

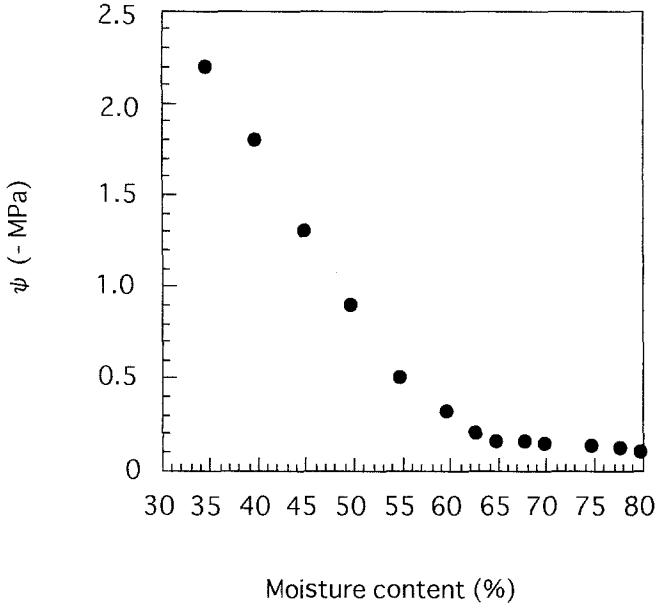

Fig. 1. Relation between moisture content and ψ in sawdust-based substrates. The data were obtained prior to spawn inoculation

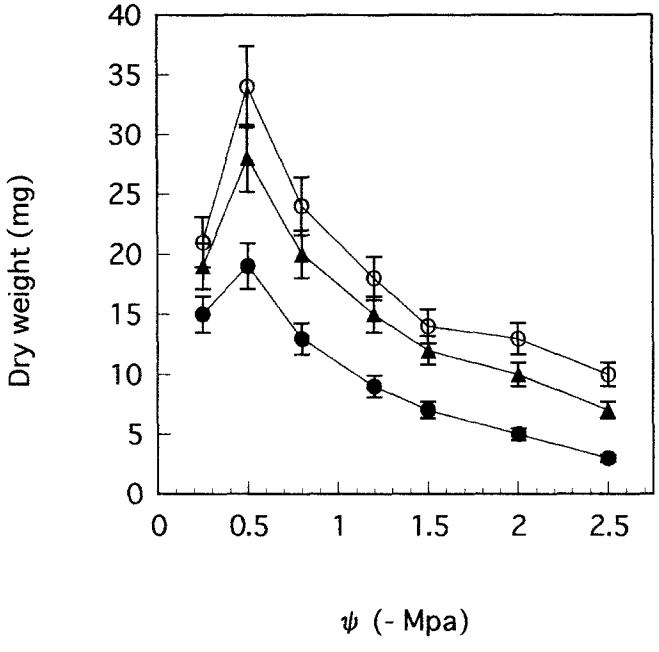

Fig. 2. Dry weight of mycelium of *Lentinula edodes* after 14 days on liquid media of different ψ produced by PEG 4000. Strain types are as follows: *filled circles*, KS-24: cold weather; *open circles*, KS-58: warm weather; *triangles*, KS-9: wide-range weather. Data are expressed as means ± standard deviations of four replicates

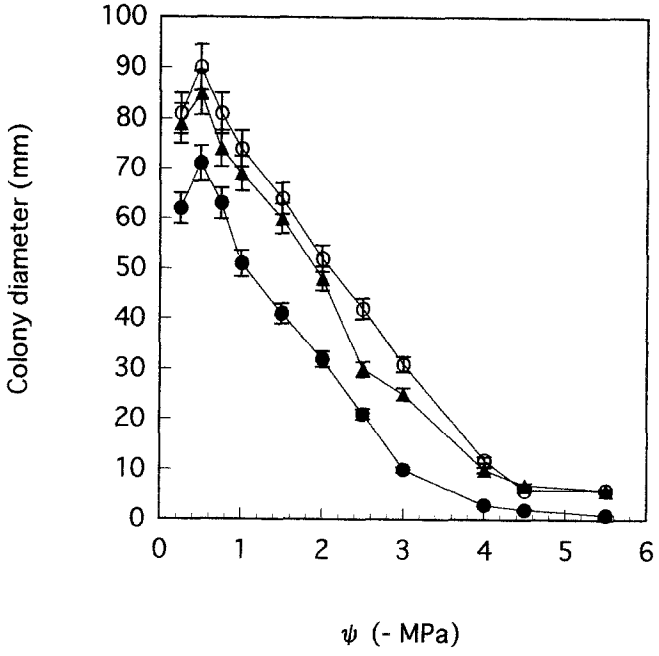

Fig. 3. Colony diameter of *Lentinula edodes* after 10 days on agar media of different ψ produced by KCl. Strain types are the same as in Fig. 2. Data are expressed as means ± standard deviations of four replicates

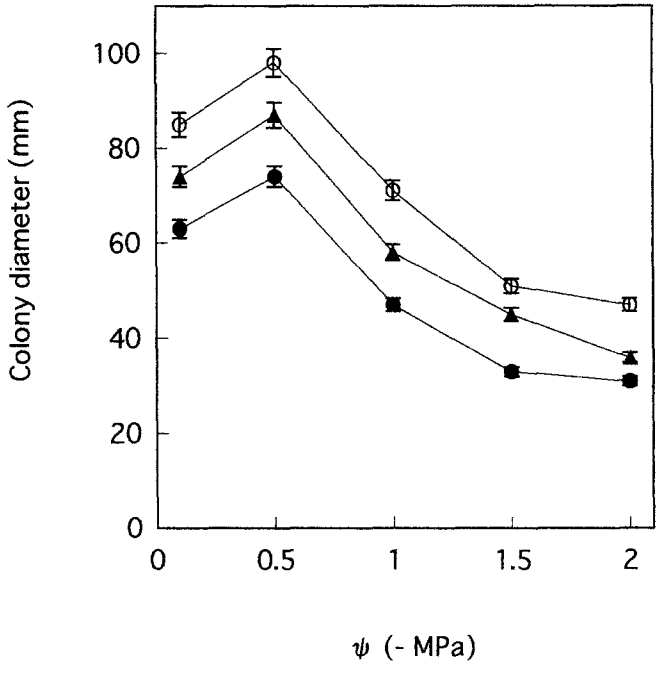

Fig. 4. Colony diameter of *Lentinula edodes* after 10 days on sawdust-based substrates of different ψ at different moisture contents. Strain types are the same as in Fig. 2. Data are expressed as means ± standard deviations of four replicates

decrease resulted in a reduction of growth. The mycelial extension rate of *L. edodes* peaked with decreasing ψ at around −0.5 MPa; it then decreased and ceased growing at −5.5 MPa. KS-58, a strain belonging to the warm-weather type, revealed the best growth on every ψ level of the three cultures. The extension rate of the KS-24 strain (cold-weather strain) were less than those for strains KS-58 and KS-9. The strain types responded significantly differently to the sawdust culture technique. The three strains KS-24, KS-58, and KS-9 colonized fully in the substrate within 50, 30, and 35 days, respectively.

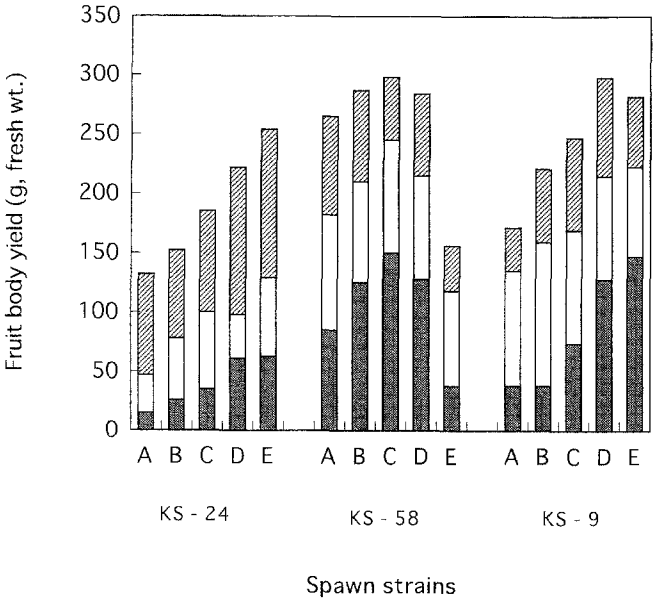

Fig. 5. Fruit body yield for three flushes of *Lentinula edodes* cultivated on sawdust-based substrates. *Shaded bars*, 1st flush; *open bars*, 2nd flush; *hatched bars*, 3rd flush. Spawn-run times for vegetative mycelial growth stage are as follows: *A*, 70; *B*, 75; *C*, 80; *D*, 85; *E*, 90 days

A linear positive correlation between ψ (−0.5 to −2.0 MPa) and mycelial growth was observed on the sawdust-based substrate of various moisture contents (55%–35%) (Figs. 1, 4). *Lentinula edodes* has been found to grow well at ψ around −0.5 MPa, which corresponds to a moisture content of 55%. In high-moisture-content substrates with high ψ, excess free available water inhibited mycelium expansion.

Effect of ψ on fruiting

Although all strains produced fruit bodies, the biological efficiency (fresh weight of fruit body/dry weight of substrate multiplied by 100%) varied from 27% to 50% (KS-24), 30% to 58% (KS-58), and 33% to 61% (KS-9) for the spawn-run time (period of vegetative growth) (Fig. 5). Significant differences in biological efficiency were found among the three strains evaluated during the first, second, and third flushes over a 160-day production period. The ψ was −0.7 to −1.2 MPa after the soaking treatment (Fig. 6). The substrate ψ was significantly affected by the interaction between genotypes and spawn run time. There was a marked decrease of ψ in the sawdust-based substrate after each flush. The ψ decrease was larger in strain KS-58 (with higher yield) than in strain KS-24 (with lower yield). As shown in Fig. 7, a clear relation was found between the ψ and fruit body yield in this study. The sawdust-based substrate, which has a higher ψ, resulted in higher fruiting ability. Large, excellent quality fruit bodies were harvested even in the third flush substrates, which maintained sufficient water and suitable ψ range (Fig. 8).

Discussion

Mycelial growth of *L. edodes* as a function of the ψ of the medium is qualitatively similar to that observed for other fungi in that there is stimulation as ψ decreased slightly but inhibition with a further decrease.[16] *Lentinula edodes* has been found to grow well at ψ around −0.5 MPa, which corresponds to a moisture content of 55%. High moisture content with high ψ–and thus excess free available water–inhibited mycelial growth. This phenomenon may be due to a lack of oxygen, producing insufficient gas exchange. The adsorption curve may be helpful for substrate preparation, because if the optimum ψ for a particular fungus is known, the quantity of water required to provide that ψ can easily be determined.

Fruit body initiation involves a change in the mycelial growth pattern from the vegetative to the reproductive phase, resulting in the production of fruit bodies.[17–19] Fructification is initiated by a combination of various environmental factors including CO_2 concentration, airflow rate, temperature, humidity, and light intensity. The timing and method for fructification has a major effect on mushroom yield and quality. Sporophore initiation is brought about once the mycelium has reached a high degree of maturity.[20]

The strain of *L. edodes* also affects many parameters associated with culture maturity, including fruit body size, cap color, and dry matter content. It has been reported that mycelial growth, primordium formation, and productivity vary with different genotypes on sawdust-based substrates. A significant genotype and spawn run time interaction was observed for crop yield.[11,13] Strains of *L. edodes* are characterized on the basis of fruiting temperature, that is, cold weather, warm weather, and wide-range weather.

High humidity levels are needed during fruiting to reduce evaporation from the substrate, thereby promoting primordia formation. The better growth of basidiocarps can be correlated with high atmospheric humidity; and mycelial growth changes markedly with the substrate's moisture content. It is generally assumed that the water-holding capacity of a substrate is related to the culture age. Excellent water-providing capacity (high ψ) is expected in the substrate of the well matured late stage with a high density of mycelial colonization. The mycelium of *L. edodes* has high water-absorbing potential, which results in a high moisture content in the colonized substrate. Pinning and pinhead development require higher ψ levels to encourage free water transport from the culture to the primordium. The lower ψ of the substrate requires more energy consumption for water uptake by the fungus. To obtain good yields of fruit bodies as well as good fruit body quality, the ψ of the substrate must be high and constant during fruit body growth.

Water can be moved by hydrostatic pressure and by ψ gradients. The ψ of a cell is the sum of the osmotic potential (ψ_π) and turgor pressure. The difference in ψ between the substrate and the mycelial cells strongly influences water uptake by the mycelium. The ψ of the substrate depends on the concentration of solutes in substrate water. This concen-

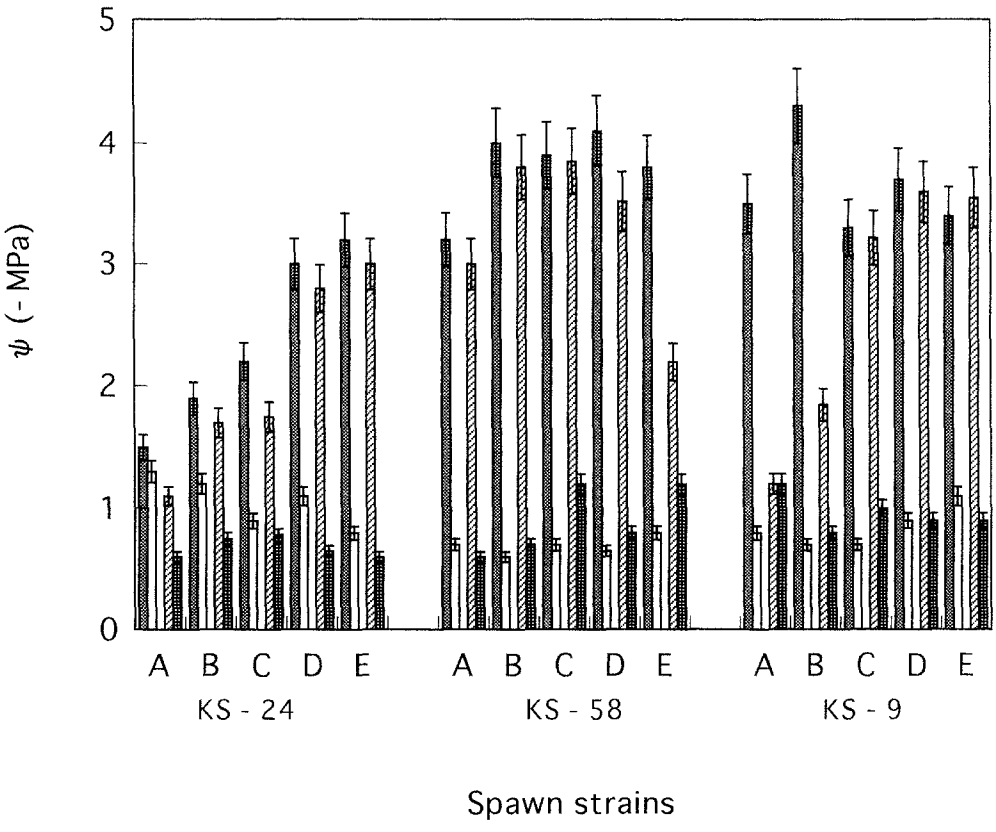

Fig. 6. Effect of soaking treatment on ψ in sawdust-based substrate. *Shaded bars*, before soaking of second flush; *open bars*, after soaking of second flush; *cross-hatched bars*, before soaking of third flush; *vertical/horizontal hatched bars*, after soaking of third flush. Data are expressed as means ± standard deviations of four replicates

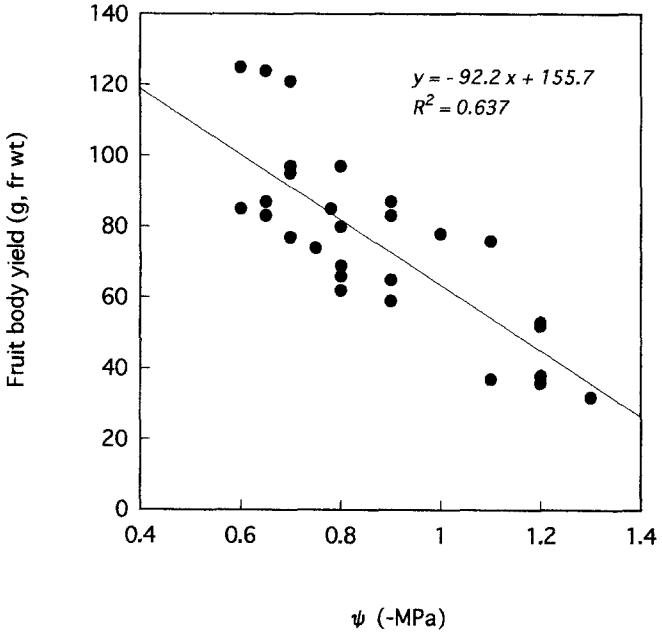

Fig. 7. Relation between ψ and fruit body yield in the sawdust-based substrate. The ψ of various substrates were measured after soaking, just before fruiting. The strain types were KS-24, KS-58, and KS-9

Fig. 8. Fruiting of the KS-24 during the third flush on sawdust-based substrate cultivated for 90 days for vegetative mycelial growth. Fructification responded to a suitable substrate ψ (−0.6 MPa after soaking)

tration is influenced by substrate additives and by solutes formed by the mushroom mycelium. Production of a wide range of extracellular hydrolytic and oxidative enzymes by *Agaricus bisporus* has been demonstrated. These enzymes are capable of degrading various constituent biopolymers of compost.[21] The metabolism of mycelium in such substrates metabolically produces water via hydrolysis of dry matter. This metabolism results in a rise in ψ around the primordium. These hydrolysis products act as solutes to decrease ψ in a substrate. The higher concentration of such solutes in the external matrix, the more difficult it is for fungal cells to build up a water supply to the external solutions. Breakdown products of the substrate produced by mycelial extracellular enzymes and excreted metabolites can increase the concentration of solutes in the substrate, lower its osmotic potential, and make water uptake more difficult for the cells.

A ψ gradient in the hyphae with the lower potential in the fruit bodies could be the basis for a translocation mecha-

nism of pressure-driven mass flow. In the fruit bodies, a low potential could be generated osmotically and by transpiration.[4] Water uptake by a mycelium depends on the ψ difference between the cells of the mycelium and the external solution, the mass of the mycelium, and the matric potentials in the substrate. Only a small percentage of the total water content of the substrate is available to an *L. edodes* mycelium for growth; the rest is bound to the chemical structure of the substrate and is unavailable. Matrix binds water to the substrate particles more strongly in the case of the low moisture content of sawdust-based substrate. In the sawdust-based substrate this factor exerts a major effect on water availability to *L. edodes* growth and fruiting. Small decreases in ψ at the final vegetative growth phase had positive effects on flush quantity. Free water resulting from high ψ during the fruiting process resulted in a good water supply to the primordium for further development.

The fruit body of *L. edodes* contains more than 90% water. Water present in the fruit bodies is extracted from the substrate. The moisture content and ψ of the substrate decrease markedly during growth of a high-yielding flush. Therefore, the water supply strongly influences both the quality and quantity of sporophores produced. The mycelium takes a major fraction of its water for fruit body formation from the substrate. Mycelial cells can absorb water only if their osmotic potential is lower than the ψ of the substrate. Soaking treatment contributes the necessary increase in substrate ψ. Soaking the substrate in water between flushes is essential to optimize production. Misting alone is not sufficient for primordia formation because of the low ψ after the picking of fruit bodies. Water uptake by the fungus is high during fruit body development. Mushroom cultures lose water by the harvesting of fruit bodies and by evaporation and transpiration from the surface of the substrate.

The immersion of substrate is common practice in industry to supply the moisture lost during the production period and to initiate the fruiting process. Water supply to the substrate markedly affects the quantity and quality of the harvested crop.

Acknowledgments I am grateful to various staff members of JA Ashoro, Hokkaido, including T. Hoshi, S. Mori, Y. Hamashita, M. Yoshida, T. Uchida, and A. Akegawa; and to the Aisawa Mushroom Farm for the opportunity to use their laboratory and growing houses. I thank H. Tanaka, H. Mori, and N. Yamauchi of my university and K. Ohga who helped with technical assistance above and beyond the call of duty. Part of this research was supported by a Grant-in-Aid for Scientific Research (09660188) from the Ministry of Education, Science, Sports, and Culture, Japan.

References

1. Boddy L (1983) The effect of temperature and water potential on growth rate of wood rotting basidiomycetes. Trans Br Mycol Soc 80:141–149
2. Eamus D, Jennings DH (1984) Determination of water, solute and turgor potentials of mycelium of various basidiomycete fungi causing wood decay. J Exp Bot 35:1782–1786
3. Tommerup IC (1984) Effect of soil water potential on spore germination by vesicular-arbuscular mycorrhizal fungi. Trans Br Mycol Soc 83:193–202
4. Kalberer PP (1987) Water potentials of casing and substrate and osmotic potentials of fruit bodies of *Agaricus bisporus*. Scientia Hortic 32:175–182
5. Badham ER (1989) Influence of water potential on growth of shiitake mycelium. Mycologia 81:464–468
6. Ohga S (1990) Growth rate of mycelium of shiitake, *Lentinus edodes*, in relation to water potential of medium. J Fac Agr Kyushu Univ 34:413–420
7. Magan N, Challen MP, Elliot TJ (1995) Osmotic, matric and temperature effects on in vitro growth of *Agaricus bisporus* and *A. bitorquis* strains. In: Elliott TJ (ed) Science and cultivation of edible fungi. Balkema, Rotterdam, pp 773–780
8. Donnelly DP, Boddy L (1997) Development of mycelial systems of *Stropharia caerulea* and *Phanerochaete velutina* on soil: effect of temperature and water potential. Mycol Res 101:705–713
9. Miles PG, Chang ST (1997) Mushroom biology. World Scientific Publishing, New York
10. Ohga S, Kitamoto Y (1997) Future of mushroom production and biotechnology. Food Rev Int 13:461–469
11. Royse DJ, Bahler CC (1986) Effects of genotype, spawn run time, and substrate formulation on biological efficiency of shiitake. Appl Environ Microbiol 52:1425–1427
12. Diehle DA, Royse DJ (1991) Effect of substrate heat treatment on biological efficiency (BE) and size of a selected line of *Lentinula edodes*. In: Maher MJ (ed) Science and cultivation of edible fungi. Balkema, Rotterdam, pp 517–521
13. Ohga S, Roozendael FV, Aspinwall M, Miwa M (1992) Yield and size response of the shiitake mushroom, *Lentinus edodes*, depending on incubation time on sawdust-based culture. Trans Mycol Soc Jpn 33:349–357
14. Donoghue JD, Denison WC (1995) Shiitake cultivation: gas phase during incubation influences productivity. Mycologia 87:239–244
15. Zhang YF, Molina FI (1995) Strain typing of *Lentinula edodes* by random amplified polymorphic DNA assay. FEMS Microbiol Lett 131:17–20
16. Clarke RW, Jennings DH, Coggins CR (1980) Growth of *Serpula lacrimans* in relation to water potential of substrate. Trans Br Mycol Soc 75:271–280
17. Wood DA, Goodenough PW (1977) Fruiting of *Agaricus bisporus*: changes in extracellular enzyme activities during growth and fruiting. Arch Microbiol 114:161–165
18. Leatham GF (1985) Extracellular enzymes produced by the cultivated mushroom *Lentinus edodes* during degradation of a lignocellulosic medium. Appl Environ Microbiol 50:859–867
19. Ohga S (1992) Comparison of extracellular enzyme activities among different strains of *Lentinus edodes* grown on sawdust-based cultures in relationship to their fruiting abilities. Mokuzai Gakkaishi 38:310–316
20. Ohga S (1995) Sawdust-based cultivation of *Lentinus edodes* and degree of culture maturity (in Japanese). Mushroom Sci Biotechnol 2:1–13
21. Wood DA, Allan M, Claydon N, Dudley K (1990) Regulation and properties of *Agaricus bisporus* endocellulase. In: Proceedings of the International Union of the Microbiological Society Congress, Osaka, p 586