J Wood Sci (1999) 45:431–434


ORIGINAL ARTICLE

Shoji Ohga

# Evaluation of maturity by use of pH indicators in sawdust-based cultures of *Lentinula edodes*

Received: July 8, 1998 / Accepted: December 14, 1998

**Abstract** Colorimetric pH indicators were examined for their use in judging the culture maturity of sawdust-based cultures of shiitake, *Lentinula edodes* (Berk.) Pegler. Bromophenol blue (BPB) was the preferred indicator for judging culture maturity. Culture pH changes were detected as changes in color from bluish purple to yellow by direct spraying. The pH values of such cultures varied from 6.3 before inoculation to 4.0 during the fruiting stage. Culture maturity assessment can be used to control fruit body flush timing. Measurement of BPB staining in *L. edodes* sawdust-based cultures showed a good correlation with fruit body yield of the first flush. This method was concluded to be of value for detecting fruiting potential and therefore may be useful for evaluating flush timing for *L. edodes* cultivators using sawdust-based methods.

**Key words** Shiitake · *Lentinula edodes* · Bromophenol blue · Culture maturity · Sawdust-based culture

## Introduction

Shiitake, *Lentinula edodes* (Berk.) Pegler, cultivation on sawdust-based substrates consists of two phases—first vegetative growth and then fruit body formation—totaling about 120 days. These two phases differ in the physiology of their culture conditions. The cultures are contained in polypropylene bags during the mycelial vegetative phase; the bags are then removed, and the cultures are placed under fruiting conditions. Various culture conditions, including moisture content, water potential,[1] strain types,[2] and substrate constitutions,[3] influence mycelial growth and subsequent culture maturity. The gas atmosphere composition also affects the maturity.[4,5] The length of the vegetative stage[6,7] and various enzyme activities[8] can also directly affect the fruiting capacity.

The timing of the transfer operation of the culture from the vegetative growing chamber to the fruiting chamber is one of the most important stages influencing the fruit body formation rate. This process must be done according to the degree of culture maturity. Although accurate assessment of the maturity of sawdust-based cultures is necessary for controlling the timing to induce fruit body flushing, the maturity of the culture is usually judged by the experience of the grower. A method for evaluating culture maturity is essential for high yields of fruit body production and excellent quality of fruit bodies.

Certain chemicals, including pH indicators, have been found to be of use for determining wood pH and species of board and for assessing wood decay. A method was developed to determine the pH of wood surfaces using colorimetric indicators.[9] Use of a chemical indicator provides a quicker, simpler method of distinguishing spruce and fir boards.[10] Color indicators were considered suitable for use in detecting internal decay in pine poles.[11] Various colorations produced by chemical reagents were investigated on log wood used for *L. edodes* cultivation.[12] It has been shown that bromophenol blue (BPB) is the most useful reagent for assessing log wood maturity. The purpose of this study was to find a colorimetric method using a pH reagent that would rapidly measure the mycelial biomass of the culture and culture maturity so we could predict the fruiting capacity in *L. edodes* in sawdust-based cultures.

## Materials and methods

### Strains

Twelve strains of *L. edodes* belonging to three fruiting types were used (they were maintained in the Kyushu University Forests culture collection). Strains KS-5, KS-9, KS-10, and KS-46 are wide-range temperature types. Strains KS-43, KS-53, KS-58, and KS-60 are warm-weather types. Strains

S. Ohga (✉)
Research Institute of University Forests, Kyushu University, Sasaguri, Fukuoka 811-2415, Japan
Tel. +81-92-948-3116; Fax +81-92-948-3119
e-mail: ohgasfor@mbox.nc.kyushu-u.ac.jp

KS-6, KS-22, KS-24, and KS-50 are cold-weather types. All of them are used extensively in Japanese commercial cultivation of *L. edodes*.

### Culture media and growth conditions

Cultures were grown on a sawdust-based substrate consisting of *Quercus mongolica* sawdust (70% dry wt), wheat bran (10%), rice bran (10%), and bean curd refuse (10%), with water added to give a final moisture content of 60%. Polypropylene bags were filled with medium (1 kg wet wt) and then were autoclaved at 120°C for 1 h, cooled, and through spawn inoculated with 10 g sawdust spawn. The bags were then capped and placed in a controlled environment for incubation. The bags were incubated for the vegetative growth period at 20°C for 90 days and then transferred to the production room at 17°C for fruit body initiation and development. Fruit body formation for the first crop continued for 30 days. Twenty cultures were tested for each of the 12 strain types.

### Coloration produced by spraying with pH indicators

The pH indicators used were BPB (which is yellow at pH 3.0 and bluish purple at pH 4.6), bromocresol green (BCG) (yellow at pH 3.8 and blue at pH 5.4), and the combined indicator methyl orange + indigo carmine (MO + IC) (changes from red to yellow at pH 4.1). All reagent concentrations were 1 g/l in 95% ethanol solution except for IC, which was 2.5 g/l in distilled water. The pH indicators were sprayed directly from a handheld spray bottle to the cut surfaces of sawdust-based cultures. The immediate color change and that after 30 min were the most useful. Color change was recognized immediately by the naked eye and was then measured with a Minolta CR-200 colorimeter.[12] The color was also determined by Muncell numbers with the use of a color chart. The color phase can be obtained by the L*a*b* mode (CIE-1976): a* value, reddish; b* value, yellowish. The ΔE*ab values were obtained as follows: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The pH value of cultures were measured on sawdust-based culture mixed 1:20 (w/w) with deionized water. All test cultures were prepared at least in triplicate.

## Results and discussion

### Coloration with BPB

The maturity of the sawdust-based cultures was determined by staining with BPB solution. BPB was the preferred indicator and when used at a 0.1% concentration produced various-intensity yellowish b* values. Using 95% ethanol as a solvent for the BPB reagent produced better coloration than distilled water. The indicator solution was easily applied with a handheld spray bottle.

As shown in Fig. 1, the b* value of the yellowish intensity increased with culture maturity. BPB on the sawdust-based culture was purple during the early stage day 30 and then steadily increased with a b* value to yellowish with further of incubation periods and finally revealed a rapid increase at the fruiting stage from days 90 to 120. Color differences are indicated by the value of ΔE*ab in Fig. 2. Although

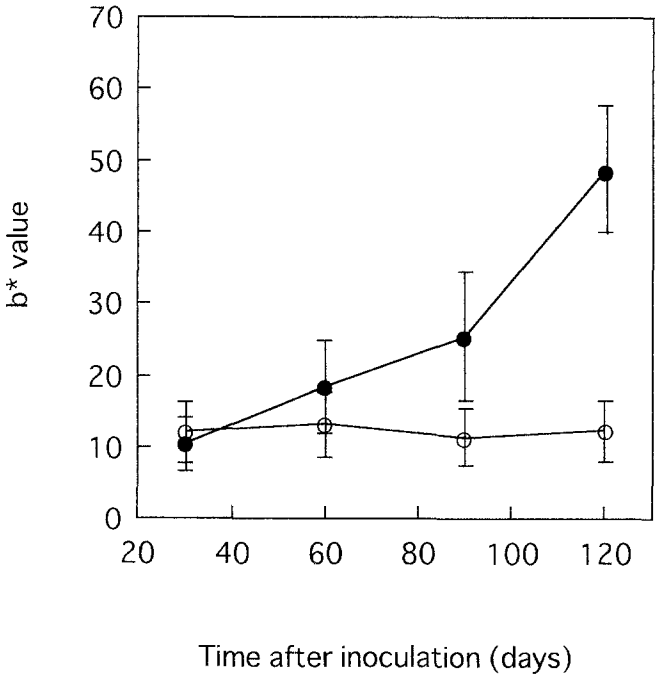


**Fig. 1.** Changes of b* value with coloration by bromophenol blue (BPB) staining on the sawdust-based culture of *Lentinula edodes*. *Filled circles*, staining; *open circles*, nonstaining. The values are means ± standard deviations of 12 strain types, which were tested in triplicate

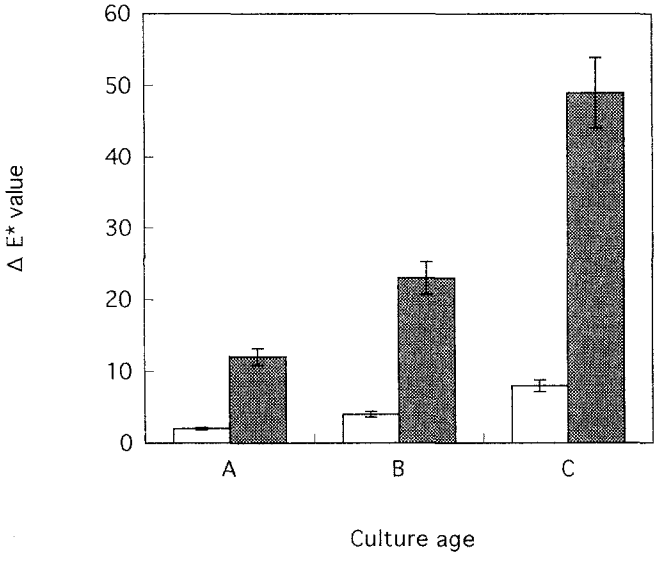


**Fig. 2.** ΔE*ab values of sawdust-based culture of *L. edodes* staining with and without BPB. *Filled bars* staining; *open bars*, nonstaining. The ΔE*ab values indicates the degree of color differences between the various ages of cultures: *A*, 30 and 60 days; *B*, 60 and 90 days; *C*, 90 and 120 days. ΔE*ab values were calculated as follows: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. This value can also be calculated automatically with a colorimeter. The values are means ± standard deviations of 12 strain types, which were tested in triplicate

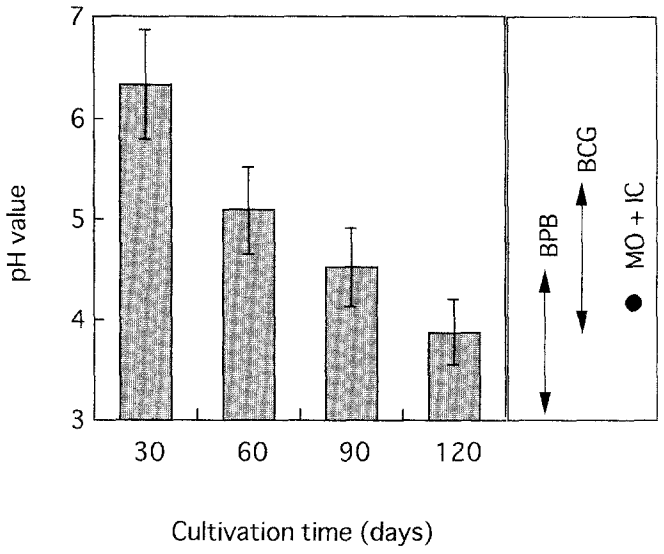


**Fig. 3.** pH values of cultures at various ages. Bromophenol blue (*BPB*) is yellow at pH 3.0 and bluish purple at pH 4.6. Bromocresol green (*BCG*) is yellow at pH 3.8 and blue at pH 5.4. The combined indicator methyl orange + indigo carmine (*MO + IC*) changes color at pH 4.1 (acid: red; alkaline: yellow). The values are means ± standard deviations of 12 strain types, which were tested in triplicate

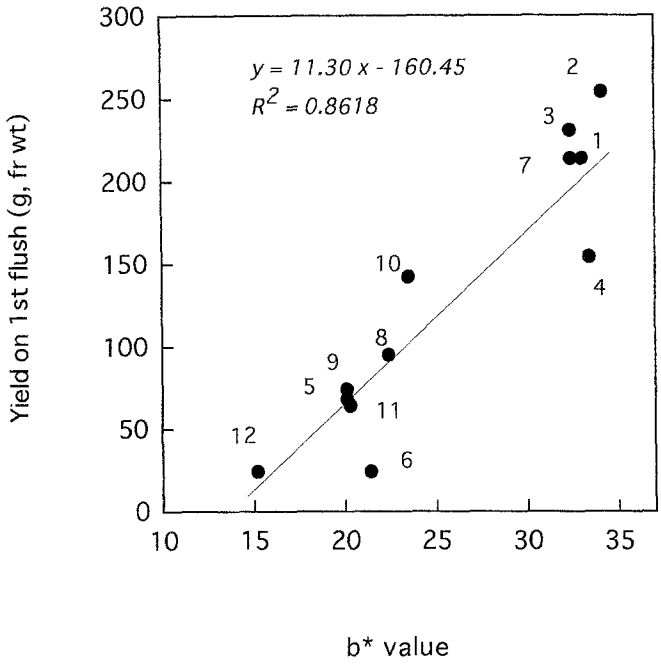


**Fig. 4.** Relation between b* values and fruit body yield on the first flush. The b* values were measured on day 90 just before fruit body initiation. Strain types according to temperature preference are as follows: wide-range strains (1, KS-5; 2, KS-9; 3, KS-10; 4, KS-46); warm-weather strains (5, KS-43; 6, KS-53; 7, KS-58; 8, KS-60); cold-weather strains (9, KS-6; 10, KS-22; 11, KS-24; 12, KS-50)

differences were not distinguished in treated cultures, BPB spraying treatment was useful for evaluating a culture maturity increase with incubation days. In relation to surface drying of the sawdust cultures, it was determined that a freshly prepared culture surface was not ideal for producing the best color reaction. Factors that improved the colorimetric reaction could be produced by drying the culture cut surface. One such factor was moisture content, which can affect pH values.[9] Read et al.[9] reported a change in the pH of western red cedar from 4.3 to 5.3 as the moisture content was varied from 20% to 70%; for western hemlock they found a change from 4.0 to 4.8 as the moisture content increased from 10% to about 55%.

This change in coloration reflects the culture pH drop associated with various metabolic products. The initial pH of the culture was around 6.0 just after autoclaving, and the pH of the culture changed from 5.0 to 4.0 (Fig. 3). *Lentinula edodes* produces metabolic products, and acids accumulate in the growth cultures. The production of such acids and the consequent lowering of the pH of the colonized culture was used to evaluate culture maturity. The present study utilized this reaction with the color indicator BPB.

### Relation between b* value by BPB staining and crop yield

The relation of the b* values and fruit body yield rate in the first crop was positive (Fig. 4). The fruit body yield was estimated by the test of coloration on spraying the BPB indicator. The high b* value with BPB spraying correlated with culture maturity, and this high value is also an indicator of the high quantity of fruit body formation. The colorimetric method was useful for determining the culture maturity for fruiting. In conclusion, BPB is considered a suitable reagent for detecting fruiting potential of sawdust-based cultures of *L. edodes.*

## References


1. Ohga S (1990) Growth rate of mycelium of shiitake, *Lentinus edodes*, in relation to water potential of medium. J Fac Agric Kyushu Univ 34:413–420
2. Ohga S (1992) Adaptability of *Lentinus edodes* strains to a sawdust-based cultivation procedure. Mokuzai Gakkaishi 38:301–309
3. Ohga S (1990) Effect of nutritional component on mycelial growth and fruit body yield of the shiitake mushroom, *Lentinus edodes*, on sawdust substrate. J Fac Agric Kyushu Univ 34:405–412
4. Leatham GF, Stahmann MA (1987) Effect of light and aeration on fruiting of *Lentinula edodes*. Trans Br Mycol Soc 88:9–20
5. Donoghue JD, Denison WC (1995) Shiitake cultivation: gas phase during incubation influences productivity. Mycologia 87:239–244
6. Royse DJ (1985) Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. Mycologia 77:756–762
7. 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
8. 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
9. Read DW, Wong PY, Eade BD (1969) Determination of wood pH with indicators. Pulp Paper Mag Can 70:59–64
10. Kutscha NP, Lomerson JT, Dyer MV (1978) Separation of eastern spruce and balsam fir by chemical methods. Wood Sci Technol 12:293–308
11. Eslyn WE (1979) Utility pole decay: detection in pine by color indicators. Wood Sci Technol 13:117–126
12. Ohga S (1985) Biotechnological studies of edible mushroom cultivation. V. Determination of the degree of maturation in shiitake bed-logs using color indicators (in Japanese). Mokuzai Gakkaishi 31:772–778