J Wood Sci (2000) 46:175–179

NOTE

Shoji Ohga

Influence of wood species on the sawdust-based cultivation of *Pleurotus abalonus* and *Pleurotus eryngii*

Received: September 21, 1998 / Accepted: May 11, 1999

Abstract Mycelial growth and fruit body formation of *Pleurotus abalonus* and *P. eryngii* cultured on various sawdust-based substrates from different wood species were investigated. Growth on *Cryptomeria japonica* substrate resulted in good mycelial growth and a high yield of fruit bodies. *Larix kaempferi* substrate was unsuitable for the cultivation of these mushrooms. The fruit body formation rate correlated with mycelial growth from all the wood species tested. Although differences were found for mycelial growth and fruit body formation on various wood species, there were no wood species that were completely unsuitable except *L. kaempferi*. These results show that a wide range of wood species can be used for the cultivation of *P. abalonus* and *P. eryngii*.

Key words *Pleurotus abalonus* · *Pleurotus eryngii* · Sawdust-based cultivation · Mycelial growth · Fruit body formation

Introduction

Pleurotus mushrooms are generally called oyster mushrooms because the pileus or cap is shell-like, spatulate, and tongue-shaped and the stipe is eccentric or lateral. They belong to Pleurotaceae, Agaricales. *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer is one of the best known of the oyster mushrooms; it is an edible species widely cultivated in Europe, Japan, and China. An edible species of *Pleurotus* has recently been cultivated in Japan. These two new cultivated mushrooms are *P. abalonus* Han, Chen et Cheng and *P. eryngii* (D. C. ex Fr.) Quel.

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

Pleurotus abalonus, found in Taiwan in warm seasons, is characterized by its black-headed coremioid imperfect state that is seen on the edges and faces of the lamellae and is produced abundantly in all cultures.[1] *Pleurotus eryngii* originated in Europe and Central Asia.[2] Both of these edible mushrooms have an excellent texture and taste. The production of these species is rapidly increasing in every district, and they compete with other mushrooms in the market as a regular commodity in Japan. They are now produced from sawdust-based cultures consisting of sawdust and various other nutritional supplements. Mycelial growth and fruit body formations of mushrooms are greatly affected by wood species and quality. Although mycelial growth and fruit body formations have been studied for *P. abalonus*[3] and *P. eryngii*,[4] little work has been done concerning the suitability of various wood species for the cultivation process. This paper describes the suitability of various wood species for use as a substrate for *P. abalonus* and *P. eryngii* sawdust-based cultivation.

Materials and methods

Microorganisms

Strains used in this study were stock cultures maintained on potato dextrose agar medium (PDA, Difco Laboratories, Detroit, MI, USA). *Pleurotus abalonus* and *P. eryngii* were isolated from fruit bodies obtained from commercial sources in Fukuoka, Japan in 1996. A 5 mm diameter plug from agar plates was used as a inoculum for the experiments on petri dishes. Sawdust spawn was used as an inoculum for the cultivation tests carried out in plastic bottles. The sawdust spawn was prepared by inoculation of *Fagus crenata* Blume sawdust and wheat bran (3:1, v/v).

Sawdust

Five species of broad-leaved tree (*Quercus serrata* Thunb., *Q. acutissima* Carr., *Q. mongolica* var. *grosserrata* Rehd. et

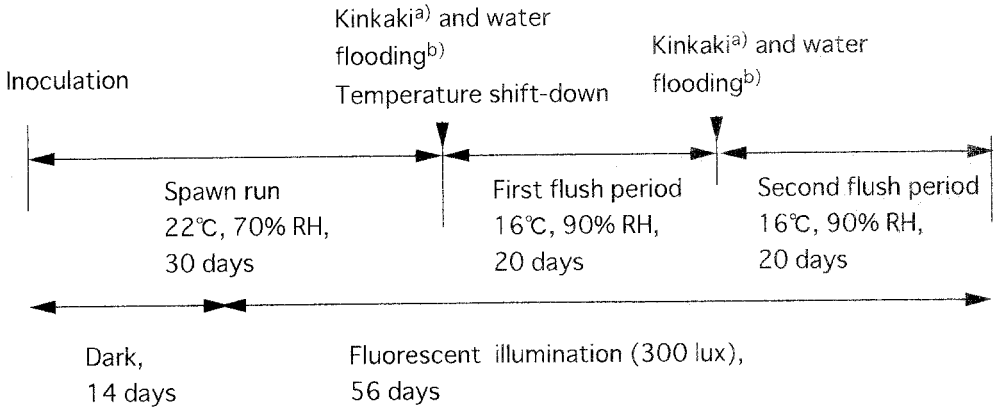

Fig. 1. Culture process of *Pleurotus abalonus* and *Pleurotus eryngii* in sawdust-based substrate. [a]Removal of both spawn and the uppermost layer of the medium. [b]Water flooding (13°C) for 1 day and draining

Wils., *Acer mono* Maxim., *Betula platyphylla* var. *japonica* Hara) and three species of conifer (*Cryptomeria japonica* D. Don, *Larix kaempferi* Carr., *Abies sachalinensis* Masters) were felled in Kyushu University forests in 1996. *Pseudotsuga menziesii* Franco and *Shorea* spp. were obtained from a mill yard in Fukuoka, Japan, in 1996. The sawdust was prepared with a Wgran machine (Morishita Co., Wakayama), and screened through 20–48 mesh.

Culture conditions

Two types of sawdust-based culture were prepared using petri dishes for mycelial growth tests and plastic bottles (Takeda Co., Nagano) for the fruit body formation test. One hundred grams of the mixture (wood sawdust/wheat bran 5:1, v/v; moisture content 65%) was placed in and then compressed in a flat-bottomed 85 mm diameter high-form petri dish that provided 20 mm depth of medium. The 800-ml plastic bottle containing 500 g of the appropriate sawdust and wheat bran (of the same formulation used for the petri dish experiments) was used for sawdust-based cultivation. The medium was sterilized by autoclaving at 120°C for 30 min and then allowed to cool to room temperature. An agar plug (ø5 mm) or sawdust spawn (10 g) was inoculated at the center of the petri dish medium and the plastic bottle substrate, respectively.

The culture process is shown in Fig. 1. The cultures were incubated at 22°C for vegetative mycelial growth. All were cultured in the dark during the early phase of fungal growth until 14 days after inoculation and were then exposed to 300 lux intensity continuously with cool-white fluorescent illumination. Mycelial growth (colony diameter) was measured in the petri dish cultures at day 10 after inoculation. The plastic bottle cultures were incubated during vegetative mycelial growth until day 30. Fruiting stimulation, kinkaki (removal of both spawn and the uppermost layer of the medium), and temperature shift-down (13°C water flooding during 1 day) were done to the 30-day-old plastic bottle cultures. The bottles were transferred to a growing room maintained at 16°C. Misting was provided to maintain 90% RH in the production room. Fruit body formation of bottle cultures was obtained during days 15–20 (first flush) and days 35–40 (second flush) after placement in the production room. Mature fruit bodies were picked from the substrate and weighed during the first and second flush periods (Fig. 2). Eighty bottles were tested for each sawdust species.

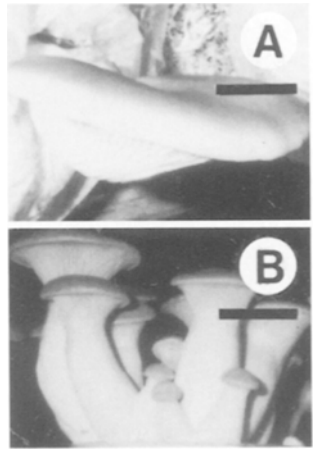

Fig. 2. Fruit bodies of *P. abalonus* (**A**) and *P. eryngii* (**B**) on *C. japonica* sawdust-based substrate. This photograph shows the first flush on day 45 after inoculation. Fruit bodies were picked from the substrate at this stage, with the pileus open and the gills more than 80% exposed. Bars 2 cm

Determination of glucosamine and ergosterol

Glucosamine and ergosterol contents were measured to estimate the mycelial quantity in the various substrates just before fruiting treatments. These methods are useful indicators for judging mycelial quantity in the sawdust-based substrate of *Lentinula edodes*.[5] Samples were taken from triplicate cultures. Unless indicated, the chemicals used were of analytical reagent grade and were purchased from Wako Pure Chemicals (Osaka).

Chitin was assayed by the method of Braid and Line.[6] To obtain fungal chitin hydrolysis to *N*-acetyl glucosamine, 1 g of the dry substrate (30 days old) was incubated with 5 ml of 5 N HCl at 80°C for 20 h. Samples were then filtered, and 2.0-ml aliquots were diluted with 10 ml of distilled water and then added to glass columns containing Dowex 50 W-X8, 60–80 mesh (Bio-Rad, Richmond, CA, USA) strongly acidic cation-exchange resin (hydrogen form). Colorimetric assays were done using 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) and iron(III) chloride; the re-

sulting color was measured with a Beckman DU-64 (Fullerton, CA, USA) spectrometer set at 630nm.

The ergosterol extraction procedure was modified from Seitz et al.[7] Saponification of 1g dry substrate (30 days old) was carried out with 20ml methanol, 10ml ethanol, and 4g KOH and incubated at 40°C for 90min. The solution was centrifuged for 10min at 5000rpm. The supernatant was diluted with 20ml distilled water and 20ml hexane and mixed for 5min. The hexane extract was evaporated under vacuum at 30°C. The dry residue was dissolved in 2ml of methanol. Ergosterol was measured by high-performance liquid chromatography (HPLC) using a reversed-phase system consisting of a μ-Bondapak-C_{18} column (Waters 626 LC System: Millipore Co.) and 100% HPLC-grade methanol. Ergosterol was detected by absorption at 282nm. At a flow rate of 1.0ml/min, ergosterol was eluted at 9min.

Results and discussion

Mycelial growth

Mycelial growth of *P. abalonus* and *P. eryngii* is shown in Fig. 3. *Larix kaempferi* medium gave poor mycelial growth of both *P. abalonus* and *P. eryngii*. *Pseudotsuga menziesii* and *Shorea* spp. media showed limited mycelial growth of *P. eryngii*. *Cryptomeria japonica* was the most suitable wood species for the mycelial growth stage of *P. eryngii* and *Q. mongolica* var. *grosserrata* for *P. abalonus*. Mycelial quantity was evaluated by measuring the glucosamine and ergosterol content (Fig. 4). It was clearly shown that the mycelial biomass was extremely low on the *L. kaempferi* sawdust-based substrate. *Cryptomeria japonica* was judged a suitable wood species for mycelial growth on *P. abalonus* and *P. eryngii*.

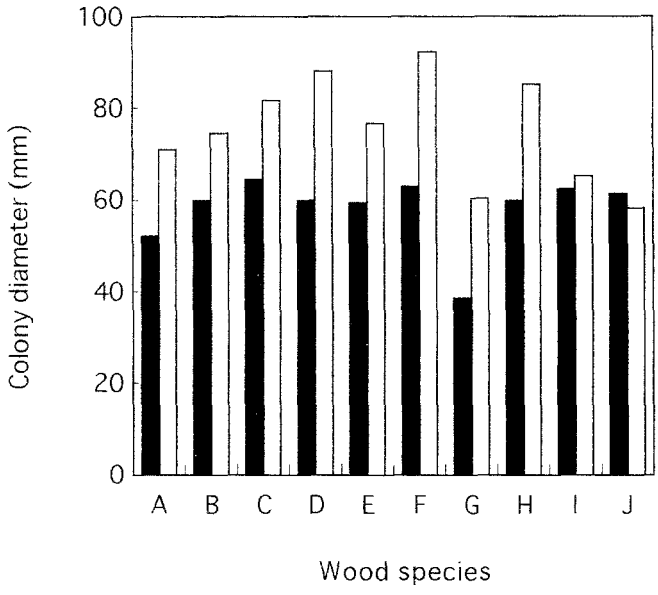

Fig. 3. Mycelial growth of *P. abalonus* and *P. eryngii* on the sawdust-based substrate. *Filled bars*, *P. abalonus*; *open bars*, *P. eryngii*. Wood species are as follows: *A*, *Q. serrata*; *B*, *Q. acutissima*; *C*, *Q. mongolica* var. *grosseserrata*; *D*, *A. mono*; *E*, *B. platyphylla* var. *japonica*; *F*, *C. japonica*; *G*, *L. kaempferi*; *H*, *A. sachalinensis*; *I*, *P. menziesii*; *J*, *Shorea* spp. This figure shows the results of 10 days of incubation for mycelial vegetative growth. The data are the average from five petri dishes

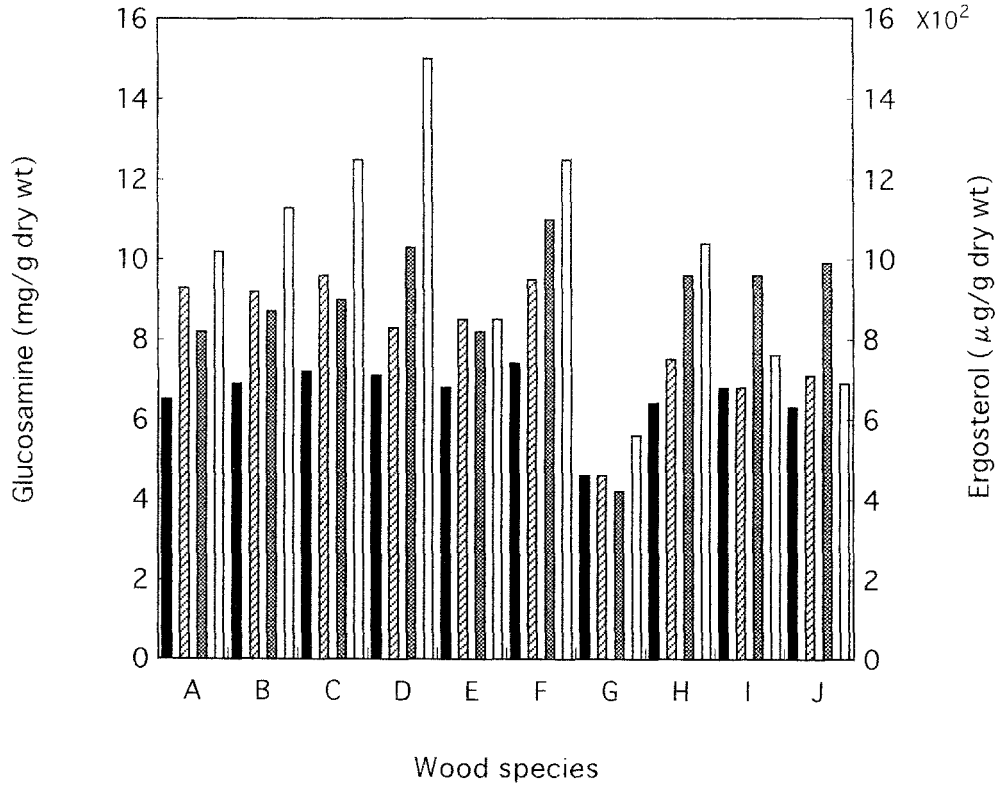

Fig. 4. Mycelial quantity of *P. abalonus* and *P. eryngii* on the sawdust-based substrate. See legends in Fig. 3 for wood species. *Filled bars*, glucosamine of *P. abalonus*; *cross-hatched bars*, ergosterol of *P. abalonus*; *shaded bars*, glucosamine of *P. eryngii*; *open bars*, ergosterol of *P. eryngii*

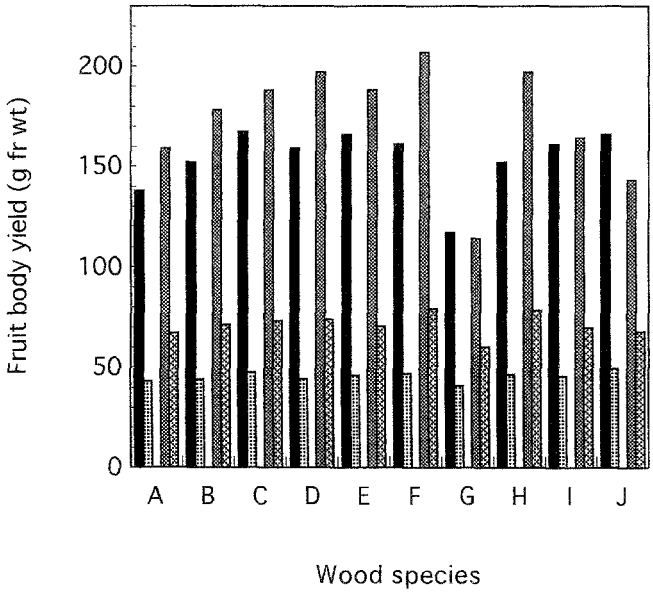

Fig. 5. Fruit body formation of *P. abalonus* and *P. eryngii* on the sawdust-based substrate. See legends in Fig. 3 for wood species. *Filled bars*, first flush of *P. abalonus*; *dotted bars*, second flush of *P. abalonus*; *shaded bars*, first flush of *P. eryngii*; *cross-hatohed bars*, second flush of *P. eryngii*. The data shown in the figure are the average for 80 bottles of the various wood species

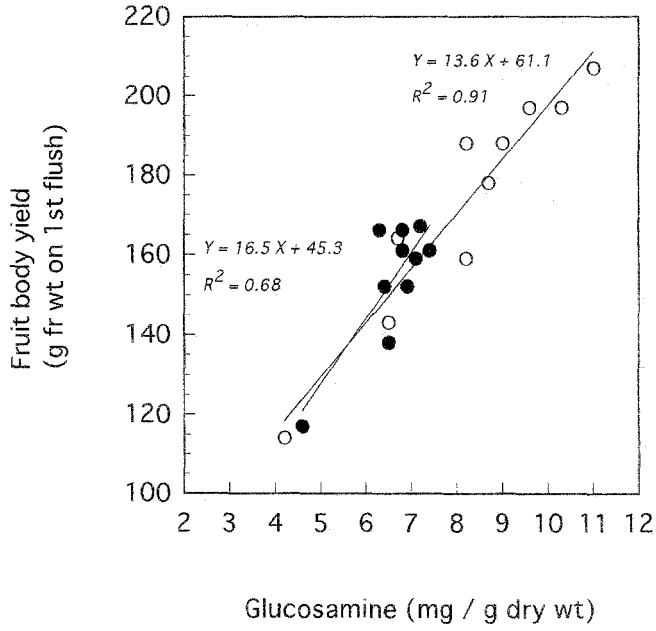

Fig. 6. Relation between glucosamine content and fruit body yield. *Filled circles*, *P. abalonus*; *open circles*, *P. eryngii*

Fruit body formation

Fruit body formation of *P. abalonus* and *P. eryngii* is shown in Fig. 5. *Quercus mongolica* var. *grosserrata*, *B. platyphylla* var. *japonica*, and *Shorea* spp. were more suitable for fruit body formation of *P. abalonus*. *Cryptomeria japonica*, *A. mono*, and *A. sachalinensis* were suitable for fruit body formation of *P. eryngii*. *Larix kaempferi* was unsuitable for the production of either species of mushroom. Sawa has tested the suitability of 18 wood species on *P. eryngii* culture and reported that *C. japonica* was useful for the sawdust-based cultivation.[4] The rapidity of primordium initiation period after fruiting stimulation was obviously in that the faster-growing substrate with dense mycelial quantity resulted in earlier fruit body formation.

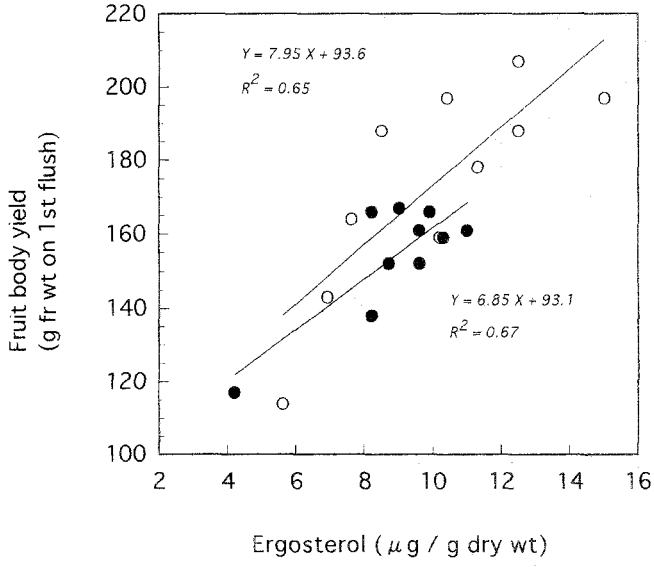

Fig. 7. Relation between ergosterol content and fruit body yield. *Filled circles*, *P. abalonus*; *open circles*, *P. eryngii*

Relation between mycelial growth and fruit body formation

Fruit body formation was directly reflected by the amount of mycelial growth. Larger yields of fruit bodies were obtained from those substrates that had a greater mycelial biomass (Figs. 6, 7). The methods for measuring glucosamine and ergosterol content were useful for estimating the fruiting potential on all the wood species. In *Lentinula edodes*, the content of ergosterol on fruiting substrate was especially higher compared with nonfruiting substrate at the fully colonized stage prior to fruiting. These two components were increased rapidly on the fruiting substrate with fruit body development and were considered to be a convenient way to judge culture maturity and fruiting potentials.[8] Fruiting potential could be judged from the mycelial growth stage prior to fruit body formation.

Acknowledgment I am grateful to Shozo Sawa (Aichi Prefecture Forest Research Institute) for useful discussions.

References

1. Neda H, Furukawa H (1987) *Pleurotus abalonus* Han, Chen et Cheng, a newly cultivated mushroom in Japan (in Japanese). Trans Mycol Soc Jpn 28:69–73

2. Dickinson C, Lucas J (1979) The encyclopedia of mushrooms. Orbis, London, p 238
3. Kinjyo K, Yaga S (1988) Study on the cultivation culture media of Basidiomycetes V (in Japanese). Mokuzai Gakkaishi 34:48–53
4. Sawa S (1996) Cultivation characteristic of *Pleurotus eryngii* (in Japanese). Bull Aichi For Res Cent 33:41–46
5. Ohga S (1995) Sawdust-based cultivation of *Lentinus edodes* and degree of culture maturity (in Japanese). Mushroom Sci Biotechnol 2:1–13
6. Braid GH, Line MA (1981) A sensitive chitin assay for the estimation of fungal biomass in hardwoods. Holzforschung 35:10–15
7. Seitz LM, Sauer DB, Burroughs R, Mohr HE, Hubbard JD (1979) Ergosterol as a measure of fungal growth. Phytopathology 69:1202–1203
8. Ohga S, Donoghue JD (1998) Evaluation of methods for determining microbial activity and maturity of sawdust-based cultures of shiitake (*Lentinula edodes*). Mushroom Sci Biotechnol 6:115–123